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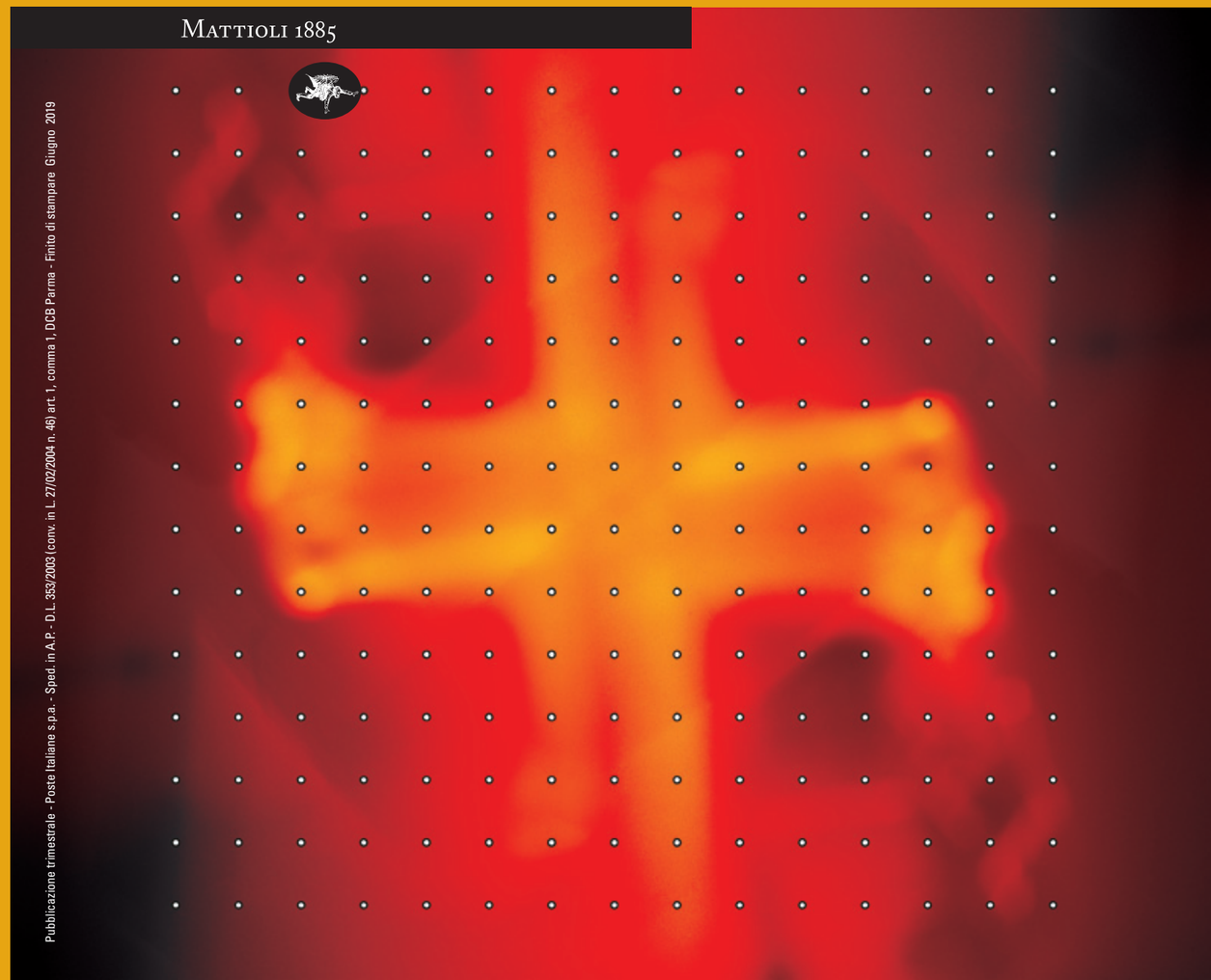

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Review

Protein-losing enteropathy after Fontan operation

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Summary. Protein-losing enteropathy (PLE) is a rare but severe complication of Fontan operation with high mortality and recurrent rates. It often develops 1-9 years after Fontan operation. This disorder often leads to chronic malnutrition, global immune dysfunction and failure to thrive in addition to peripheral edemas. PLE is often associated with increased chronic inflammatory biomarkers, such as C-reactive protein, complement fragments and tumor necrosis factor-α, etc., and decreased acute inflammatory biomarkers, for instance, lymphocytes. Abnormal fecal α_1-antitrypsin clearance is the golden diagnostic standard of PLE. The management strategies include medical, surgical and interventional therapies. However, none of the proposed treatment shows a specific efficacy to PLE after Fontan operation. Management of choice for PLE relies on the substantial response of the patient to the underlying regimen, and the patients may eventually warrant heart transplantation. The PLE-related mortality is high with cardiac dysfunction, severe infection and multiorgan failure being the major causes of death. Attempts to diminish Fontan failure and seek for effective therapeutic regimen for PLE after Fontan operation are research works of next step.

Key words: alpha 1-antitrypsin, Fontan procedure, protein-losing enteropathies

Introduction

Erosive and non-erosive gastrointestinal disease and increased interstitial pressure can be the underlying causes of protein-losing enteropathy (PLE). It has been recognized that a number of cardiac diseases may also lead to PLE (1). The most frequent cardiac cause of PLE is Fontan operation. The Fontan procedure is performed for the patients with a functional single ventricle by restructure the heart to allow supplying enough blood to the pulmonary circulation (1).

PLE is a rare but severe complication of Fontan operation with a very high mortality rate, and reoccurrence is also frequent (2). In a multicenter retrospective study including 3,029 Fontan operations, the incidence of PLE in the survivors was 3.7% and the incidence of PLE relative to the total number of Fontan operations in each center varied between 0% and 25% (3). In subsequent clinical studies with smaller patient populations, the incidence of PLE after Fontan operation was 5.5-12% (4, 5). The mechanisms of PLE after Fontan operation are poorly understood, and all the proposed therapeutic regimens only obtained limited success. In order to discuss the disputes over the PLE after Fontan operation, a review is made herewith.

Time of onset

Pundi *et al*. (6) reported that the mean age of patients at Fontan operation was 11.2 ± 8 years and the mean interval from Fontan operation to the diagnosis of PLE was 8.1 ± 7.9 years. Yu *et al*. (7) described that the median interval from Fontan operation to PLE development was 2.2 years. Factors relating to the time of PLE development based on univariate analysis were pulmonary vascular compliance, postoperative central venous pressure, and durations of Intensive Care Unit

stay, hospitalization and chest tube indwelling, whereas multivariate analysis showed that only pulmonary vascular compliance remained significant (7). Pundi *et al*. (6) reported that the mean interval from Fontan operation to onset of PLE by types of Fontan was 9.0 ± 8.7 years for atriopulmonary connections, 6.5 ± 4.7 years for lateral tunnels, 4.9 ± 3.1 years for extracardiac conduits and 6.6 ± 8.7 years for other types of Fontan operation. As reported by Lin *et al*. (5), the interval was 4.3 ± 3.4 (range, 1.3-9.4) years for atriopulmonary anastomosis in six patients, 6.4 and 9.7 years for two patients with lateral tunnel total cavopulmonary connection and 1.2 years for a single case of Kreutzer procedure.

Clinical manifestations

Most frequently, the onset of PLE is heralded by a new onset of pleural effusion, ascites, or peripheral edema (8). Most patients had edema (79%) and effusions (75%) (3). In PLE, protein requirements may increase to 2.0-3.0 g/kg/day to achieve positive protein balance in comparison to a normal requirement of 0.6-0.8 g/kg/day (1).

Ostrow *et al*. (9) reported that over one-third of the patients showed elevation of inflammatory markers including tumor necrosis factor-α and C-reactive protein (9). Rychik (10) observed that C-reactive protein was >1 mg/dL in 35% and >3 mg/dL in 16% of all patients with PLE after Fontan operation. Table 1 shows biomarker changes in this patient setting.

Although C-reactive protein, tumor necrosis factor-α, brain natriuretic peptide and angiotensin II levels were elevated; however, they had no correlation with abnormal enteric protein loss (9). Instead, there might be a severe decrease of $CD4^+$ lymphocytes (16). Apparent selective loss of $CD4^+$ lymphocytes could lead to reversal of the CD4/CD8 ratio (12).

Mechanisms

Impaired protein glycosylation may contribute to PLE. Patients with a deficiency in phosphomannose isomerase had periodic PLE, which could be resolved when glycosylation disorders were handled properly by daily supplements of mannose (17).

Several immunological alterations of such patients after PLE are similar to those found in patients with PLE of other etiologies. Muller *et al*. (18) reported the preferential loss of $CD3^+$ and $CD4^+$ cells into the gastrointestinal tract in patients with constrictive

Table 1. Biomarker changes in patients with protein losing enteropathy after Fontan operation

Change	Biomarker	
	Blood/serum (9-14)	**Intestinal mucosa (15)**
Decreased	Protein, γ-globulin & immunoglobulin G; Lymphocyte count; $CD4^+$ lymphocytes; CD4/CD8 ratio; Thyroid stimulating hormone	
Increased	α_1-globulin, α_2-globulin & β-globulin; Neutrophil; Natural killer cells; C-reactive protein; Complement fragment C3d; Tumor necrosis factor-α; Brain natriuretic peptide; Angiotensin II; Interleukin-8	Interferon-γ

pericarditis. PLE after total cavopulmonary connection was associated with areduced immunoglobulin G production, which was possibly a result of reduced B cell differentiation or the absence of costimulatory T helper cells. However, B cells of PLE patients may still be stimulated to produce immunoglobulin G as evidenced by *in vivo* corticoid administration (19). The passive lymph loss secondary to an increased central venous pressure could not explain the selective loss of $CD4^+$ lymphocytes, suggesting that the disturbance of the immune system could initiate local cytokine network disturbance of the gut and perturbe its homeostasis and immune defense. This process could affect the structural integrity and patency of the intestinal wall, thus triggering PLE (16). As already observed, PLE after total cavopulmonary connection was accompanied by signs of an acute inflammatory response and a dramatic loss of T cells, in particular of the αβ TCR^+ $CD4^+$ subtype (13).

Chronic venous congestion causes the lymphatics to decompress into the pleural or abdomen cavity. Under such circumstances, loss of proteins and lymphocytes and an inflammatory response leads to chronic malnutrition (20). Some authors suggested that mesenteric hypoperfusion would be a possible trigger for the development of the PLE as a result of an increased mesenteric vascular resistance (21). The pathophysiology of PLE after Fontan operation and its sequelae are shown in Figures 1 and 2 (9-11, 22). In general, factors leading to impairment to the enteric immune defense system might serve as a trigger of PLE (11).

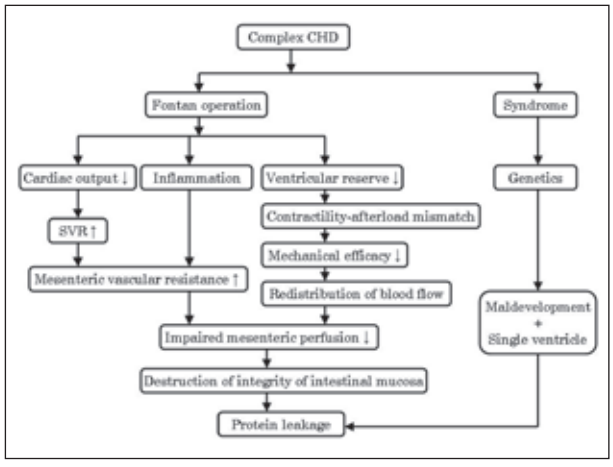

Figure 1. The pathophysiology of protein-losing enteropathy after Fontan operation. CHD: congenital heart defect; SVR: systemic vascular resistance.

Risk factors

Perioperative factors associated with development of PLE included longer cardiopulmonary bypass time, increased left atrial pressure after the operation, longer length of hospital stay, and presence of postoperative renal failure (8). It is possible that the association between infection and PLE might be a manifestation of an impaired immune system during the early development of PLE, rather than a causal factor (11).

In fact, several issues may be related to the occurrence of PLE and these may include pulmonary artery stenosis, Fontan conduit stenosis, major systemic-to-pulmonary artery collaterals, residual significant pulmonary forward flow, single ventricle dysfunction and atrio-ventricular valve dysfunction (23).

Diagnosis

The gold standard test for diagnosis of PLE is an abnormal fecal α_1-antitrypsin clearance (24). Normal α_1-antitrypsin clearance is typically <30 mL cleared/24 hours. Single spot analysis of stool concentration of α_1-antitrypsin values exhibited a correlation with the clearance values and could be used for the diagnosis of PLE in the absence of a 24-hour stool collection (25). The cut-off values of fecal α_1-antitrypsin clearance and fecal α_1-antitrypsin concentration were defined as >27 mL/24 hours and >54 mg/dL, respectively (25). The diagnostic criteria for PLE were, serum albumin level <3 g/dL, total protein <5 g/dL and elevated stool α_1-

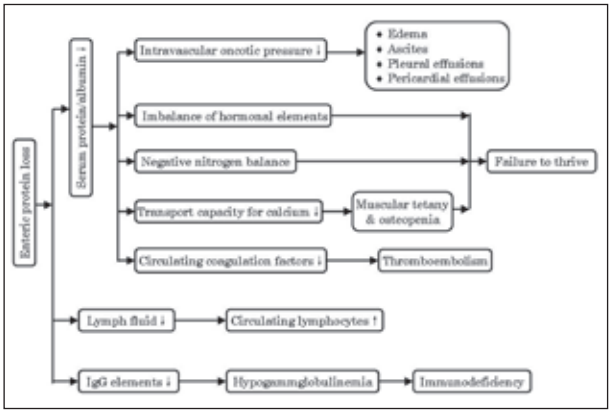

Figure 2. The sequelae of protein-losing enteropathy after Fontan operation. IgG: immunoglobulin G.

antitrypsin clearance (26). Besides, lymphangiectasia could be noted in patients undergoing gastric and small-bowel biopsies (11, 26). Bidirectional endoscopy could be applied for random biopsies of the duodenum and colon (1). Lymphangiectasia of the intestinal tract secondary to lymphatic hypertension could frequently be disclosed in PLE patients (6).

Treatment

Medical management

Supportive treatment: The current medical management of PLE consists of diet modification, albumin and (or) γ-globulin infusion, diuretics and corticosteroids, none of which have been entirely successful. The use of intermittent intravenous infusions of albumin is generally unhelpful in the long run (1). Anti-inflammatory therapy can at least temporarily reduce tumor necrosis factor-α levels (27).

Heparin: Substitution of heparin can reverse protein loss by protecting the intact cellular membrane of the intestinal mucosa (27). Recently, heparin therapy was reported to improve symptoms of PLE patients and essentially prevent enteric protein loss within 3 weeks of administration (28), so was a high dose subcutaneously unfractionated heparin (25,000 units/day) in addition to warfarin (12). Long-lasting relief has been reported by systemic administration of unfractionated heparin, but induction of severe osteoporosis may limit its long-term use (29). Intravenous heparin may be useful to decrease fluid secretion and protein exudation from the bowel.

Prednisolone: Prednisolone 2 mg/kg/day in children, or 25-60 mg twice a day in adolescent patients with a slow tapering regimen over five to six months is a preferred regimen (30). The membrane stabilizing effect of prednisone leads to a rapid cessation of enteric protein loss from the gut and the peripheral vasculature, with quick subsidence of a swelling with subsequent recovery of albumin and protein concentrations (30).

Octreotide: Patients with PLE after Fontan operation received intramuscular octreotide therapy (10-20 mg/month) for a period of 14-28 months have achieved a promising effect by symptomatic improvement and significantly reduced serum albumin, total protein and α_1-antitrypsin levels (31, 32). However, the mechanism of this drug in treating PLE is poorly understood and warrant further elaborations.

Sildenafil: The effect of sildenafil is likely to be due to a combination of actions including a reduction of pulmonary vascular resistance, dilating the mesenteric vessels and increasing mesenteric arterial flow (33). In a pediatric patient with PLE after Fontan operation, a trial of sildenafil at 0.5 mg/kg/dose, 4 times a day, with a rapid increase to a maximum of 1.5 mg/kg/dose was given. Her fecal α_1-antitrypsin levels returned to a normal value 6 weeks after oral sildenafil treatment (33). Maeda *et al*. (34) prescribed sildenafil in 3 young patients who underwent total cavopulmonary connection for single ventricle or double outlet right ventricle at a dose of 30 mg/day or 0.5-1 mg/kg/day titrating up to 4-8 mg/kg/day. Ascites in the patients were eventually resolved.

Budesonide: John *et al*. (35) reported treatment with controlled release budesonide was started at 9 mg daily for all patients with PLE after Fontan operation. The dose in 5 patients was weaned within 6-9 months to 3 mg daily or 3 mg every other day. The effect time was 6 months for serum albumin recovery or symptomatic improvement after the start of therapy. However, side effects of oral budesonide may limit its clinical use. Therefore, one must take caution with the use of this drug, and weaning and discontinuation is indicated if severe side effects are noted within 3-6 months.

Spironolactone: Spironolactone, a nonselective aldosterone receptor antagonist, has become an important adjuvant therapy for treatment of congestive cardiomyopathy. Ringel and Peddy (29) applied a high-dose spironolactone in three children who developed PLE after Fontan operation and achieved remission.

Surgical treatment

Fenestration: Late fenestration is an effective means of alleviating serious morbidity from effusions or PLE after Fontan operation. Fenestration of the interatrial septum should be considered in patients with a poor clinical result after Fontan operation, before proceeding to Fontan take-down or heart transplanta-

tion. Fenestration techniques included blade/balloon septostomy, stent placement, Amplatzer-fenestrated atrial septal defect device and balloon dilation of previous stent. Vyas *et al*. (36) reported that the size of the fenestration that they created was 5.2 ± 1.1 mm. After fenestration, cardiac index increased significantly, and reduction of ascites and edema was noted after 9 of the 16 procedures. Rychik *et al.* (26) reported that late creation of a surgical fenestration was performed in 9 patients, with 5 of them being PLE after lateral tunnel-type Fontan operation, and 3 having normalization of serum proteins and resolution of symptoms at 2-6 weeks. They also noted the relation between resolution of symptoms and the extent of right-to-left shunt created.

Fontan revision: Fontan revision should be considered for the patients with a previous atrio-pulmonary Fontan. In this way, a more conventional, more streamlined and energy efficient, extracardiac conduit-type Fontan is constructed. Theoretically, such patients have improved hemodynamics and increased cardiac output after a Fontan revision (37).

Intestinal resection: Resection of affected segments in lymphangiectasia may control symptoms and intestinal protein loss, and the serum proteins and immunoglobulins stabilized (38). Connor *et al*. (31) performed ^{99m}technetium-dextran scintigraphy to assess the extent of intestinal protein loss and a resection of localized intestinal lymphangiectasia was performed in a 14-year-old girl. She has remained well postoperatively, with normal bowel habit and no evidence of short bowel syndrome, reaccumulation of ascites, or pleural effusions.

Interventional procedures

Percutaneous fenestration: Mertens *et al*. (3) reported that three types of interventional procedures were performed in 13 patients with PLE: a balloon dilation with or without stenting of a stenosis on the Fontan-type connection or of a stenosis on the pulmonary artery (*n*=9), percutaneously creating a fenestration in the intraatrial septum (*n*=5) and systemic–pulmonary artery collateral occluded with coils (*n*=1). A marked effect was observed on the PLE symptoms in most of these patients.

Atrial pacing: In patients with post-Fontan PLE with sinus node dysfunction, complete resolution of PLE was observed within three weeks of placement of an epicardial single-chamber pacemaker. This result heralded that atrial pacing could be an alternative treatment regimen for patients with PLE after Fontan operation (39).

Prognosis

The prognosis of patients with PLE after Fontan operation has been improved steadily. A retrospective study on a large patient population revealed that the 10-year survival of these patients was 77% (40). Pundi *et al*. (6) reported that the overall mortality in the PLE cohort was 72% over 7 ± 7.4 years of follow-up, and the the overall freedom from PLE at 10, 20, and 30 years after the Fontan operation was 92%, 89% and 83%, respectively. In Fontan patients with PLE, freedom from death or the transplantation was significantly decreased compared to those without PLE (2). The death causes known in 36 patients with PLE after Fontan operation were congestive heart failure, sepsis and multiorgan failure, unrelated to PLE (6).

Conclusions

PLE is a rare but severe complication of Fontan operation with high mortality and recurrent rates. This disorder often leads to chronic malnutrition and global immune dysfunction in addition to peripheral edemas. Abnormal fecal α_1-antitrypsin clearance is the golden standard for the diagnosis of PLE. The management strategies include medical, surgical and interventional therapies. However, none of the proposed treatment show specific efficacy to some patients with PLE, and thus they may eventually warrant heart transplantation. The PLE-related mortality is high with cardiac dysfunction, severe infection and multiorgan failure being the major causes of death. Attempts to diminish Fontan failure and seek for effective therapeutic regimen for PLE after Fontan operation are research works of next step.

References

1. Umar SB, DiBaise JK. Protein-losing enteropathy: case illustrations and clinical review. Am J Gastroenterol 2010;105(1):43-9; quiz 50.
2. Kramer P, Peters B, Schubert S, Photiadis J, Berger F, Ovroutski S. MP3-16 Treatment strategies for protein-losing enteropathy in Fontan patients. www.uni-kiel.de/aepc/aepcAbstractsFinalPrint/MP3_16fin.pdf.
3. Mertens L, Hagler DJ, Sauer U, Somerville J, Gewillig M. Protein-losing enteropathy after the Fontan operation: an international multicenter study. PLE study group. J Thorac Cardiovasc Surg 1998;115(5):1063-73.
4. Kwok LSK, Cheung YF, Yung TC, Chau AKT, Chiu CSW. Protein-losing enteropathy after fontan procedure. HK J Paediatr (new series) 2002;7:85-91.
5. Lin WS, Hwang MS, Chung HT, Chu JJ, Lai MW, Yang JS. Protein-losing enteropathy after the Fontan operation: clinical analysis of nine cases. Chang Gung Med J 2006;29(5):505-12.
6. Pundi KN, Johnson JN, Dearani JA, Pundi KN, Li Z, Hinck CA, et al. 40-Year follow-up after the Fontan operation: long-term outcomes of 1,052 patients. J Am Coll Cardiol 2015;66(15):1700-10.
7. Yu JJ, Yun TJ, Yun SC, Im YM, Lee SC, Shin HJ, et al. Low pulmonary vascular compliance predisposes post-Fontan patients to protein-losing enteropathy. Int J Cardiol 2013;165(3):454-7.
8. Feldt RH, Driscoll DJ, Offord KP, Cha RH, Perrault J, Schaff HV, et al. Protein-losing enteropathy after the Fontan operation. J Thorac Cardiovasc Surg 1996;112(3):672-80.
9. Ostrow AM, Freeze H, Rychik J. Protein-losing enteropathy after fontan operation: investigations into possible pathophysiologic mechanisms. Ann Thorac Surg 2006;82(2):695-700.
10. Rychik J. Protein-losing enteropathy after Fontan operation. Congenit Heart Dis 2007;2(5):288-300.
11. Lenz D, Hambsch J, Schneider P, Häusler HJ, Sauer U, Hess J, et al. Protein-losing enteropathy in patients with Fontan circulation: is it triggered by infection? Crit Care 2003;7(2):185-90.
12. Chee R, Thomas MJ, Powell RJ. Immune deficiency secondary to protein-losing enteropathy after Fontan procedure for tricuspid atresia. CPD Bulletin Immunol Allergy 2003;3(1):23-4.
13. Bocsi J, Lenz D, Sauer U, Wild L, Hess J, Schranz D, et al. Inflammation and immune suppression following protein losing enteropathy after Fontan surgery detected by cytomics. Transfus Med Hemother 2007;34(3):168-75.
14. Mainwaring RD, Lamberti JJ, Carter TL Jr, Nelson JC. Reduction in triiodothyronine levels following modified Fontan procedure. J Card Surg 1994;9(3):322-31.
15. Shimizu T, Nagata S, Fujii T, Takahashi K, Kishiro M, Ohkubo M, et al. Enhanced production of interferon-gamma as a possible cause of protein-losing enteropathy after modified Fontan operation. J Pediatr Gastroenterol Nutr 2003;37(4):504-7.
16. Koch A, Hofbeck M, Feistel H, Buheitel G, Singer H. Circumscribed intestinal protein loss with deficiency in CD4+ lymphocytes after the Fontan procedure. Eur J Pediatr 1999;158(10):847-50.
17. Damen G, de Klerk H, Huijmans J, den Hollander J, Sinaasappel M. Gastrointestinal and other clinical manifestations in 17 children with congenital disorders of glycosylation type Ia, Ib, and Ic. J Pediatr Gastroenterol Nutr 2004;38(3):282-7.
18. Müller C, Wolf H, Göttlicher J, Zielinski CC, Eibl MM. Cellular immunodeficiency in protein-losing enteropathy. Predominant reduction of $CD3^+$ and $CD4^+$ lymphocytes. Dig Dis Sci 1991;36(1):116-22.
19. Zellers TM, Brown K. Protein-losing enteropathy after the modified fontan operation: oral prednisone treatment with biopsy and laboratory proved improvement. Pediatr Cardiol 1996;17(2):115-7.
20. Setty SP, Herrington CS. Fontan procedure: old lessons and new frontiers. Expert Rev Cardiovasc Ther 2006;4(4):515-21.
21. Rychik J, Gui-Yang S. Relation of mesenteric vascular resistance after Fontan operation and protein-losing enteropathy. Am J Cardiol 2002;90(6):672-4.
22. Ozawa H, Ueno T, Iwai S, Kawata H, Nishigaki K, Kishimoto H, Sawa Y. Contractility-afterload mismatch in patients with protein-losing enteropathy after the Fontan operation. Pediatr Cardiol 2014;35(7):1225-31.
23. Butera G, Marini D, MacDonald ST. Protein-losing enteropathy resolved by percutaneous intervention. Catheter Cardiovasc Interv 2011;78(4):584-8.
24. Florent C, L'Hirondel C, Desmazures C, Aymes C, Bernier JJ. Intestinal clearance of α_1-antitrypsin. A sensitive method for the detection of protein-losing enteropathy. Gastroenterology 1981;81(4):777-80.
25. Fujii T, Shimizu T, Takahashi K, Kishiro M, Ohkubo M, Akimoto K, et al. Fecal α_1-antitrypsin concentrations as a measure of enteric protein loss after modified fontan operations. J Pediatr Gastroenterol Nutr 2003;37(5):577-80.
26. Rychik J, Rome JJ, Jacobs ML. Late surgical fenestration for complications after the Fontan operation. Circulation 1997;96(1):33-6.
27. Tárnok A, Bocsi J, Lenz D, Janousek J. Protein losing enteropathy after Fontan surgery – clinical and diagnostical aspects. Transfus Med Hemother 2007;34:164-7.
28. Donnelly JP, Rosenthal A, Castle VP, Holmes RD. Reversal of protein-losing enteropathy with heparin therapy in three patients with univentricular hearts and Fontan palliation. J Pediatr 1997;130(3):474-8.
29. Ringel RE, Peddy SB. Effect of high-dose spironolactone on protein-losing enteropathy in patients with Fontan palliation of complex congenital heart disease. Am J Cardiol 2003;91(8):1031-2, A9.
30. Therrien J, Webb GD, Gatzoulis MA. Reversal of protein losing enteropathy with prednisone in adults with modified

fontan operations: long term palliation or bridge to cardiac transplantation? Heart 1999;82(2):241-3.
31. Connor FL, Angelides S, Gibson M, Larden DW, Roman MR, Jones O, et al. Successful resection of localized intestinal lymphangiectasia post-Fontan: role of ^{99m} technetium-dextran scintigraphy. Pediatrics 2003;112(3 Pt 1):e242-7.
32. John AS, Phillips SD, Driscoll DJ, Warnes CA, Cetta F. The use of octreotide to successfully treat protein-losing enteropathy following the Fontan operation. Congenit Heart Dis 2011;6(6):653-6.
33. Uzun O, Wong JK, Bhole V, Stumper O. Resolution of protein-losing enteropathy and normalization of mesenteric Doppler flow with sildenafil after Fontan. Ann Thorac Surg 2006;82(6):e39-40.
34. Maeda J, Yasuda M, Oyanagi T, Shibata A, Kono K, Kodo K, et al. Effectiveness of sildenafil on protein-losing enteropathy after Fontan operation. Pediatr Cardiol Cardiac Surg 2012;28(3):186–91.
35. John AS, Driscoll DJ, Warnes CA, Phillips SD, Cetta F. The use of oral budesonide in adolescents and adults with protein-losing enteropathy after the Fontan operation. Ann Thorac Surg. 2011;92(4):1451-6.
36. Vyas H, Driscoll DJ, Cabalka AK, Cetta F, Hagler DJ. Results of transcatheter Fontan fenestration to treat protein losing enteropathy. Catheter Cardiovasc Interv 2007;69(4):584-9.
37. Sheikh AM, Tang AT, Roman K, Baig K, Mehta R, Morgan J, et al. The failing Fontan circulation: successful conversion of atriopulmonary connections. J Thorac Cardiovasc Surg 2004;128(1):60-6.
38. Peršić M, Browse NL, Prpić I. Intestinal lymphangiectasia and protein losing enteropathy responding to small bowel restriction. Arch Dis Child 1998;78(2):194.
39. Jayakumar KA, Addonizio LJ, Kichuk-Chrisant MR, Galantowicz ME, Lamour JM, Quaegebeur JM, et al. Cardiac transplantation after the Fontan or Glenn procedure. J Am Coll Cardiol 2004;44(10):2065-72.
40. Feldt RH, Driscoll DJ, Offord KP, Cha RH, Perrault J, Schaff HV, et al. Protein-losing enteropathy after the Fontan operation. J Thorac Cardiovasc Surg 1996;112(3):672-80.

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Review

Clinical applications of *Gymnema sylvestre* against type 2 diabetes mellitus and its associated abnormalities

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Summary. Diabetes mellitus (Madhumeha) is one of the leading metabolic disorder prevalent in the developing countries which is characterized by high blood sugar level and is associated with macrovascular and microvascular complications. The Indian Ayurveda describes several herbs for the management and treatment of diabetes mellitus among which *Gymnema sylvestre* (Asclepiadaceae) is revered as a potential antidiabetic herbal drug which has the capability of simultaneously regenerating β-cell and stimulating insulin secretion. *Gymnema sylvestre* also possesses anti-obesity, anti-hyperlipidemic, anti-inflammatory, and anti-cancerous activities. This review updates the recent developments in the experimental studies conducted on the *Gymnema sylvestre* as an effective remedy for diabetes mellitus evidenced by both animals and human studies. Moreover, this study also discussed the toxicity of *Gymnema sylvestre* and future challenges in the roadmap of formulation for prevention and control of diabetes.

Key words: Diabetes Mellitus, *Gymnema sylvestre*, Anti-diabetic, Anti-hyperlipidemic, Anti-inflammatory, Anti-Cancerous

Introduction

Plants have been used practically in all civilizations as a source of medicine. The World Health Organization (WHO) projects the rampant use of plants based preparations as medicines by almost 80% populace inhabiting the developing nations (1). Countries in Asia are considered to provide crucial information in using herbal species for the treatment of various metabolic conditions (2).The modern pharmacopoeia contains at least 25% of plant-derived drugs. Ayurveda of the Atharva Veda enlists the usage and efficacy of herbal formulations in the healing of diabetes. Diabetes is a cluster of inter-related metabolic anarchy symbolized by hyperglycemia and carbohydrate, lipid and protein metabolic disorders which ultimately results in discrepancies either in the secretion of insulin or its efficacy or both (3). According to WHO, approximately 171 million people were worldwide suffering from diabetes in 2000 and the prevalence is projected to 366 million by 2030 (4).

Gymnema sylvestre-A Potential Herbal Cure

Gymnema sylvestre (Gurmar/Madhunashini) is one of the natural herbals that has been extensively used in traditional medicine for almost two thousand years. It is a woody, plant species native to India, particularly in South Indian forests. It is also found in tropical Africa and in Australia as well as in Asia, Malaysia, Japan, Vietnam and Sri Lanka (5). The main plant parts of *Gymnema sylvestre* used for herbal preparations are its leaves and roots (6). The leaf powder is yellow in

color and soft in nature having poor flow ability and compatibility (7). G*ymnema* possesses hepatoprotective and sugar suppressing potential (8, 9). The age-old use of G*ymnema* includes the usage of its dried leaf and root, for ailments such as cough, leprosy, skin diseases and wounds. Liquid extract procured from the roots of *Gymnema* has been used to cure nausea, vomiting, and dysentery and while the paste formed from the plant when mixed with mother's milk is effective enough to cure mouth ulcer (10, 11).

This present review aims at providing the latest developments on the pharmacological and clinical research conducted to establish the hypoglycemic effect of the plant, unraveling its active hypoglycemic constituents in the treatment of diabetes. Moreover, the study discussed other activities of *Gymnema sylvestre* such as anti-microbial, anti-cancerous and anti-arthritic activity.

Various applications of *Gymnema sylvestre*

Gymnema sylvestre is highly preferred by Ayurvedic physicians to dexterously combat and manage diabetes. This figure explains the antidiabetic, antihyperlipidemic, anti-Obese, anti-oxidant, immunomodulatory and wound healing activities *Gymnema sylvestre* (Fig.1).

Antidiabetic activity of Gymnema sylvestre based on animal studies

The antidiabetic activity of *Gymnema sylvestre* has been studied in diabetic rats by administering them with the alcoholic extract of leaves (100 mg/kg/day) for a period of one month (20). The average blood glucose level was significantly lowered in the animals treated with *Gymnema sylvestre* extract from the second week of therapy. As no effect of *Gymnema sylvestre* was noted on the level of thyroxine even in corticosteroid-induced diabetes mellitus rat, hence it was concluded that possibly *Gymnema sylvestre* does not affect thyroid hormone-mediated type 2 diabetes mellitus.

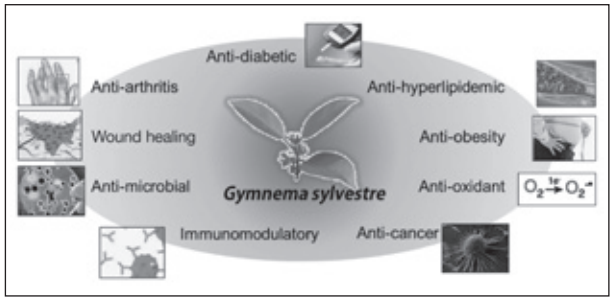

Figure 1. Various roles of *Gymnema sylvestre* in health protection. Anti-diabetic (9), anti-hyperlipidemic (12), anti-obesity (13), anti-oxidant (14), anti-cancer (15), Immunomodulatory (16), anti-microbial (17), wound healing (18), anti-arthritis (19).

While in the diabetic rabbits experiment, *Gymnema sylvestre* demonstrated improvements in glycogen synthesis, glycolysis, gluconeogenesis, and hepatic and muscle glucose uptake (21) (22) and facilitated the hitch of hemoglobin and protein glycosylation (23). Studies carried out by Srivastava and co-workers (1985) have illustrated the anti-hyperglycemic and life-protracting upshot of aqueous extract of the dried leaves of *Gymnema sylvestre* that had been given in four different single doses (of amounts 0.2 g, 0.4 g, 0.6 g, and 0.8 g respectively) in alloxan-induced diabetic rats with various ranges of blood glucose levels. The highest reduction in the level of blood glucose was obtained in the moderately diabetic rats treated with *Gymnema sylvestre* at a dose of 0.6 g. The same group also exhibited highest life-expectancy. While the administration of over 0.6 g of *Gymnema* extract showed no further amelioration in the management of blood glucose (24).

Gymnema sylvestre also possesses insulinotropic activity, the administration of *Gymnema sylvestre* has reduced the fasting glucose levels (at significant variation, $P < 0.001$) together with a considerable lowering of serum lipid levels while concomitantly ameliorating serum protein levels (25). The administration of the ethanolic excerpt (50%) of the leaves of *Gymnema sylvestre* (GS3, 20 mg/day/rat) and the processed residue of GS3 (GS4, 20 mg/day/rat) presented a 30% boost in the total β-cells mass and also in the numbers of the islets ($p < 0.001$) (26). The regeneration of pancreatic tissue also caused the success in the absolute management of the fasting sugar levels within 60 days in GS3 and 20 days in the GS4 group.

The alcoholic extract of *Gymnema sylvestre* stimulates the release of insulin from the HIT-T15, MIN-6, and RINm5F β-cells by increasing membrane permeability (27, 28). Trypan blue exclusion test indicated that extract increased the permeability of cells for dye due to high saponin in glycoside content. Ca^{++} sensitive component leads to the release of insulin through channel in-

dependent influx of Ca^{++} into the β-cells. Similar effects were reported by Liu et al. (29) where aqueous alcoholic extract (0.06-0.25 mg/ml conc.) secreted insulin from MIN6 β-cell line. Higher concentration (> 0.5 mg/ml) causes increased trypan blue uptake and increases β-cell Ca^{++} levels. The methanolic extract of *Gymnema sylvestre* leaf and callus also exhibits the antidiabetic activities through regeneration of β-cells (30).The green compact callus obtained through *in-vitro* culture exposed to stressful circumstances of exposure to blue light with 2, 4-D (1.5 mg/L) and KN (0.5 mg/L) also showed a significant β-cell regeneration. Sujin et al. in 2008 reported that administration of higher doses (5, 10, 15, 20/g/25 days) of *Gymnema sylvestre* did not show significant mortality but behavioral changes were observed *viz*. lethargic movements and suppression of appetite were observed upon 5 g and 20 g dose (31). *Gymnema sylvestre* is known to suppress the taste stimulus in taste buds. A compound isolated from the searing aqueous excerpt of the *Gymnema sylvestre* leaves was seen to condense the neural response of the murine chorda tympani towards sucrose and this matter was confirmed to be the peptide named Gurmarin which comprised of 35 amino acids of 4,000 Dalton molecular mass. The forestalling potential of the peptide on the sweet perception was established by exposing the tongue with above $1x10^{-6}$M of the peptide (32). The inhibitory effect of gurmarin differs among tongue regions and mouse strains (33). A study conducted on the taste perception of rats towards gurmarin revealed that the apex of the tongue is activated upon consumption mostly due to the binding of gurmarin to the receptor protein responsible for the sweet taste perception (34).

The pharmacokinetic and pharmacodynamic interaction was studied by Kamble's colleagues in diabetic rats after concomitant treatment with 400 mg/kg of *Gymnema sylvestre* extract and 0.8 mg/kg of glimepiride drug for four weeks (35). The results revealed a beneficial pharmacodynamic interaction with a significant increase in anti-hyperglycemic activities with no major alterations in the pharmacokinetic parameters. The chitosan nanoparticle with *Gymnema sylvestre* extract was also evaluated against rats induced with streptozotocin and was found to decrease the fasting glucose level and glycosylated hemoglobin at the dose of 100 mg/kg body weight of the rats (36).

Phytochemistry and Bioactive components having anti-diabetic property

The principle component of *Gymnema sylvestre* is gymnemic acid, a complex mixture of at least 17 different saponins (37) mostly oleanane (38) and dammarene classes (39). The longispinogenin 3-O-β-D-glucuronopyranoside, 3-O-β-D-glucopyranosyl (1-6)-β-D-glucopyranosyloleanolic acid 28-O- β-D-glucopyranosyl ester, 21 β-benzoylsitakisogenin 3-O-β-D-glucuronopyranoside 3-O-β-D-xylopyranosyl (1-6)-β-D-glucopyranosyl (1-6)-β-D-glucopyranosyl oleanolic acid 28-O-β -D-glucopyranosyl ester, oleanolic acid 3-O-β-D-xylopyranosyl(1-6)-β-D-glucopyranosyl(1-6)-β-D-glucopyranoside and 3-O-β-D-glucopyranosyl (1-6)-β-D-glucopyranosyl oleanolic acid 28-β -D-glucopyranosyl (1-6)-β-D-glucopyranosyl ester are the oleanane-triterpene glycosides. These have been unraveled by hydrolysis followed by spectrophotometric analysis. Correspondingly, 7 other novel dammarane-style saponins, extracted from the leaf excerpt of *Gymnema sylvestre*, these are the gymnemasides I-VII. The same category saponins that were known earlier are gypenoside XXVIII, XXXVII, LV, LX11 and LXIII.

The saponin components responsible for the anti-hyperglycemic effect of *Gymnema sylvestre* are gymnemosides and gymnemic acid (40). The consumption of glucose by muscles is prevented by the triterpene glycosides fraction of the plant (41, 42). The inhibitory effects of triterpene glycosides and various gymnemosides from *Gymnema sylvestre* were found to inhibit glucose uptake in rat (43). A novel compound named dihydroxy gymnemic triacetate isolated from acetone extract reduced the blood sugar level by 65% and glycosylated hemoglobin by 39.56% and an increased in plasma insulin level by 63% at 20 mg/kg dose (44). The administration of gymnemic acid IV lowered the sugar level (13.5 mg/kg body weight) to 60%, which was similar to the efficiency of glibenclamide (14.8 mg/kg body weight) (45). Recently, the crystallographic analysis of gymnemagenin indicated its good gelling with the target protein's crystallographic constitution (dipeptidyl peptidases, aldose reductase, glucokinase, fructose 1,6-bisphosphate, cytochrome 450, 11β-hydroxysteroid dehydrogenase, tyrosine

phosphatases, protein kinase B, glutamine fructose-6-phosphate amidotransferase, Insulin receptor substrate, Glucose transporter, AMP-activated protein kinase, cholesteryl ester transfer protein) having a key responsibility in the carbohydrate regulation (46).

Antihyperlipidemic activity

Diabetes mellitus is often associated with disturbances in lipid metabolism regulating in levels of lipoproteins (47). The lipoprotein abnormalities cause insulin resistance through different factors which may alleviate lipoprotein lipase (LPL) and peroxisome-proliferator activated receptor (PPAR) gamma, on contrary by elevating acyl-CoA synthetase and transporter of microsomal triglyceride. The administration of 25-100 mg/kg dose of the leaf extract of *Gymnema sylvestre* showed a tectonic decline in the lipid profile in a dose reliant approach when administered orally for two weeks in experimental rats. The extract (at 100 mg/kg) of *Gymnema sylvestre* lowers the serum triglyceride, total cholesterol and atherosclerotic property which was almost comparable to a standard lipid-lowering agent (48).

The hypolipidemic activity of higher dose of aqueous leaf extract (up to 800 mg/kg body weight for 30 days) was examined by Mall et al. in alloxan induced diabetic rats (49). The reduction was examined at all doses (400,600 and 800 mg/kg body weight) but the highest concentration was substantial compared to all observed days. While the administration of the excerpt indicated a consequential diminution in the serum lipids and the fasting blood glucose level, it has also shown proof of desirable increase in the serum high-density lipoprotein (HDL)-cholesterol. Similar considerable dwindle in the other lipid parameters and a significant increase (p <0.05) in HDL level was obtained by Rachh et al. in 2010 when hydroalcoholic leaf extract (200 mg/kg body weight) of the plant was administered in the diet of high cholesterol-fed (2% cholesterol + 1 % sodium cholate + 2% coconut oil) rats (12).

Gymnema sylvestre when mixed with chitosan and ascorbic acid (1:10:2), possesses protective effects against hypercholesterolemia. Significant decrease in serum triglyceride (35.87%), total cholesterol (43.89%), LDL (54.00%), and atherogenic index (AI) (41.47%), was observed at dose of 4.68 g/kg diet (50). Leaf extract consumption in rats was also seen to reduce the noticeable digestion of fat and kick off the emission of neutral sterols and acidic steroids (51). A novel dihydroxy gymnemic triacetate caused a reduction in total cholesterol, triglyceride, LDL by 54%, 55%, and 40% respectively and simultaneously increase in HDL level was 38% observed (44).

Anti-Obese Activity

Abdominal fat deposition is yet another pivotal criterion branded as the harbinger of diabetes. The increase in adipocytes lessens the quantity of insulin receptors on cells that are targets of insulin in our body. This substantially diminishes the requisite amount of insulin to be present in the circulation and potentially reduces its metabolic functions. An alarming 40-80 percentile of diabetics have been reported to be obese. The adipocytes secrete resistin hormone, a 12.5 kDa cysteine-rich protein (52). Studies in murine models have implicated that the prevalence of resistin in the blood circulation accentuates insulin resistance development. Recent *in vivo* and *in vitro* investigations have shown that resistin sways glucose metabolism. In murine models, the inclusion of resistin patently amplifies the creation of glucose from the liver and thus drops the hepatic insulin exploit (53). Pravenec and co-workers (2003) have shown that genetically modified rats which secrete more resistin than required have glucose intolerance and disrupted skeletal muscle glucose metabolism (54).

Gymnema sylvestre is shown to have very useful properties for the management of both obesity and diabetes. Administration of a supplement comprising of *Gymnema sylvestre* with glucomannan, fenugreek, vitamin C and chitosan to a patient with body mass index 30 kg/m^2 or more resulted in significant reduction in their weight and also the overall fat percentage.

Administration of gymnemic acid increased the fecal excretion of steroids and cholesterol (55). Weight gain was eventfully curbed in rats treated with the extract (56). The hexane extract of *Gymnema sylvestre* leaves (150 mg/kg and 250 mg/kg body weight) significantly (p<0.001) reduced increased body weight in Sprague dawley rats (13). A separate study was undertaken to analyze the effect of a new extract of an immensely bio-available, calcium-potassium salt of (–)-hydroxycitric acid for weight loss. The same ex-

cerpt was also used along with a niacin-bound chromium compound and with *Gymnema sylvestre* extract achieved weight loss in mildly fat individuals. Weight loss was analyzed by evaluating body weight fluctuations, body mass index, appetite, serum triglyceride, total cholesterol, HDL, LDL, leptin and serotonin concentrations, the elimination of urinary fat metabolites was also noted (57). *Gymnema sylvestre* extract endorses weight loss as it trims down sweet cravings and thus manages the blood sugar concentrations.

Anti-Oxidant Activity

Oxidative stress is produced under diabetic conditions which are responsible for the development of secondary complications. Reactive oxygen species (ROS) are produced under such conditions through glycation reaction which occurs in various tissues and plays a deleterious role in the diabetic secondary complications (58). The insinuation of oxidative stress in worsening diabetes has been proved by further generation of free radicals, non-enzymatic protein glycosylation, glucose auto-oxidation, alterations of antioxidant enzymes and increase in lipid peroxidation (59).

Antioxidants are effective in reducing diabetic complications. Numerous researchers have professed the healing of insulin resistance in type 2 diabetics and cardiovascular disease patients after the administration of antioxidants such as vitamin C, glutathione and vitamin E. The alcoholic extract of *Gymnema sylvestre* has been seen to restrict the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) and clear up superoxide and hydrogen peroxide. This ability to reduce radicals has also been exhibited in the ferric reducing prototype wherein the antioxidant capability was 17.54 mg/g expressed in terms of ascorbic acid (14). A noteworthy decline (19.27%) (p<0.05) in the plasma alanine aminotransferase potentiality was obtained by administration of a mixture (4.68 g/kg diet) of chitosan, vitamin C and *Gymnema sylvestre* (10:2:1) (50).

Immunomodulatory Activities

Several components of the immune system face discrepancies when inflicted with type 2 diabetes which lead to inflammation and glucose abnormalities. The cytokines are produced when the macrophages enter the adipocytes; these cytokines specifically cause the neighboring liver, muscle or fat cells to become insulin resistant. Several markers of inflammation such as C-reactive protein, fibrinogen, the interleukins and tumor necrosis factor-α (60) get elevated in diabetes.

The extracts of *Gymnema sylvestre* restricted the histamine release *in vitro* (21). The leaf extract had significantly elevated the neutrophil chemotaxis that consequentially elevated neutrophils reduction of Nitro Blue Tetrazolium dye to form formazan thus ascertaining intracellular carnage aspect and a total increase in metabolic activity of phagocytosing neutrophils. This is perhaps due to the presence of tannins present in *Gymenma* leaves which have anti-inflammatory and immunomodulatory attributes (16). The methanolic extract of *Gymnema sylvestre* leaves showed a potential effect at 100μg/ml in nitric oxide and ROS generation in macrophage and 20μg/ml in lymphocyte proliferation leading to stimulation of myeloid and lymphoid elements of the immune system and thereby restoring the innate immunity (61).

Wound Healing Activity

Diabetes exponentially reduces the wound healing capacity of the body, hence the recurrence of a severe, never-healing infection from a simple wound is always a major threat (18). In one of the recent studies, Carbopol gel was prepared from the hydroalcoholic extracts of *Gymnema sylvestre* and *Tageteserecta Linn.* to determine its wound healing activity in albino mice (62). In both, the models, a prominent decrease in the time required for the occurrence of epithelial tissues was evidently noticed and the combined gel exhibited hastening of the wound healing process. The hydro alcoholic extracts could possibly accentuate wound healing as they have antioxidant potential and the phytoconstituent (flavonoids) prevalent in it that fastens the process of wound healing.

Clinical Studies and Trials on Humans

Gymnema sylvestre is shown to be an effective antidiabetic agent in the clinical trials. The insulinotropic activity of *Gymnema sylvestre* was observed in adult human subjects (25-40 year age group) by administering the 2 g/day in two doses (25). A water based excerpt

of *Gymnema sylvestre* leaves when administered at a dosage of 2 gm thrice daily to 10 normal individuals over a period of ten days and in 6 diabetics for fifteen days potentially lowered the fasting and oral glucose tolerance test (OGTT) glucose intensity barring the OGTT in the normal cluster (63). Quotidian administration of 400 mg leaf excerpt of *Gymnema sylvestre* twice has been observed to diminish the glycosylated hemoglobin (HbA1C) concentration in diabetics (64).

The universal applicability of the *Gymnema sylvestre* in diabetes with either type 1 or type 2 has been established by various studies. In one of the studies, the efficacy of quotidian consumption of 400 mg GS4 was examined on 22 type 2 diabetes patients (65). *Gymnema sylvestre* was seen to radically shrank plasma glucose (p<0.001), HbA1c (p<0.001), and glycosylated plasma protein (GPP) levels during the 18–20-month of valuation time. In another individual study, the efficacy of the *Gymnema sylvestre* excerpt on 27 type 1 diabetics was investigated for 6–30 months (66). It was observed that *Gymnema sylvestre* drastically diminished the GPP levels in the initial six to eight months, and then reduced serum amylase (p<0.001) within 16–18 months. As compared with insulin therapy (n=37), *Gymnema sylvestre* appreciably boosted serum C-peptide concentration in 16–18 months' time (p<0.001). The outcome of the administration of the leaf powder of *Gymnema sylvestre* on plasma glucose concentration of 20 type 2 diabetic women aged between 40-60 years living in Udaipur, Rajasthan was studied by Paliwal et al. in 2009 (67). Everyday 6 gm of *Gymnema sylvestre* leaf powder was given to the subjects in three divided doses and dietary survey using 24 hours recall method was also adopted. Results of intervention revealed that the powder effectively worked on reducing sugar levels with no undesirable side effects, hence it is an effective remedy and a therapeutic agent in lowering sugar level.

The polyherbal formulation made from *Gymnema sylvestre* (GSPF kwath; mixture of 10 herbs) exerted a significant curtailment of blood glucose (23.5 % and 26.7% for fasting and postprandial glucose level, respectively) and glycosylated hemoglobin (11.7%). 6 months of GSPF therapy reduce the serum cholesterol (14.4%), triglycerides (21.7%), LDL (26.8%) and VLDL (21.7%) levels. A marked increase was also recorded in the biochemical marker for oxidative stress (68). In another study, the short-term supplementation of G-400, a polyherbal formulation (1000mg/day for 8 weeks) was used to attenuate the hyperglycemia and hyperlipidemia in the patients (69). Yadav et al. reviewed the preventive and therapeutic aspects of *Gymnema sylvestre* as a potential herbal drug in type 2 diabetes (70).

Other Therapeutic Activates of *Gymnema sylvestre*

The *Gymnema sylvestre* has proved to possess several other therapeutic activities. Various parts of the plant though pungent act as a tonic for liver, vomiting causative, promote the production of urine, refrigerant, astringent, heal, treatment of anomalies in liver and spleen, gas, acidity, constipation, jaundice, helminthiasis and abnormal menstruation.

Antimicrobial Activity

In vitro, antibiotic activity of *Gymnema sylvestre* extracted with petroleum ether then with chloroform and lastly with a mixture of water: Ethanol (1:1) was effective to inhibit *Bacillus subtilis*, *Staphylococcus aureus* but not *Escherichia coli* (71). The hexane extract of *Gymnema sylvestre* also showed maximum inhibition against *Serratia marcescens* (MTCC 86) (72). The ethanol-based excerpt of the leaves of *Gymnema sylvestre* have exhibited microbicidal potential against *Bacillus pumilis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and but was ineffective in case of *Proteus vulgaris* and *Escherichia coli* (17). The bioactive molecule, Gymnemic acid is also used as an antifungal agent as it inhibits the *Candida albican* from yeast to hyphal transition which is a key virulence factor (73).

Anticancer Activity

The efficacy of chloroform, ethyl acetate and alcoholic extracts of *Gymnema sylvestre* were tested on A549 (human lung cancer) cell lines and MCF7 (human breast cancer) cell lines *in vitro* by MTT Assay (74). At 50 and 100 µg/ml concentration, the extracts positively affect the MCF 7 cell lines while the activity was dose-dependent with similar IC_{50} values their efficacy on the A549 cells was trivial. However, the effectiveness of chloroform and ethyl acetate excerpts was better when compared to that of the alcoholic extract

on the A549 cell lines. The anticancer activity may be due to the gymnemic acids. The cytotoxic activity of isolated saponin, gymnemagol from leaves was tested on HeLa cells and reported to be a good cytotoxic agent (63%) (75). The green synthesis of silver and gold nanoparticle from *Gymnema sylvestre* showed cytotoxic activity against Hep2 cells with the IC_{50} value of 121 μg/ml for sliver nanoparticle and 38% inhibition for gold nanoparticle at a concentration of 250 μg/ml. However, silver nanoparticles of *Gymnema sylvestre* proved much better for their antiproliferative effects in Hep2 cells which were mediated through induction of apoptosis (76). The gold nanoparticle of *Gymnema sylvestre* was also synthesized by Arunchalam et al. (15) evaluated against HT29 and Vero cell lines at different concentration. It was observed that the gold nanoparticles were more sensitive toward human cancer cell line (HT29) as compared to Vero cell line.

Anti-Arthritic Activity

The leaf extracts of *Gymnema sylvestre* is has been utilized for demonstrating the anti-arthritic activity in albino rats. The anti-arthritic action of petroleum ether extract and aqueous extract was studied in Freund's adjuvant-induced arthritis in rats (19). The presence of steroids, triterpenoids and saponin glycosides in the leaves *Gymnema sylvestre* leads to the potential anti-arthritic activity. The petroleum ether extract treated groups exhibited significantly reduced paw swelling by blocking the inflammatory cells (77).

Toxicological Evaluation of *Gymnema sylvestre*

Even in the long-term study, there was no report of any undesirable effect. However, hypoglycemia is one of the possibilities of its administration.

High doses of *Gymnema sylvestre* leaves did not exhibit any adverse effect on the gastrointestinal mucosa; hence, it poses to be a harmless gastrotoxic anti-inflammatory response compared with other anti-inflammatory agents (78). While in an acute toxicity investigation in mice a negative behavioral changes, neurologic and autonomic upshots were evident. Safety ratio (LD50/ED50) in the diabetics was sixteen while in the healthy rats it was eleven (79).

Evidence regarding the toxicity of the plant or its parts on the human has been documented rather in patients with quotidian consumption of *Gymnema sylvestre* the serum urea, uric acid, and hemoglobin levels remain normal. However, it is suggested to avoid the administration of *Gymnema sylvestre* during pregnancy. Thus, *Gymnema sylvestre* is generally safe and devoid of side effect. The administration is recommended under the clinical supervision of the healthcare professional.

Future Perspectives of Herbal Drug and its Challenges

The use of plants and their parts for the effective healing of any ailment has been the fulcrum of the investigation. It is estimated that nearly 70% of the drugs used in India are derived from plants. Its efficacy has mounted its marketability with a twelve-monthly growth of 5% and 15%. The gross global herbal market of US $ 62 billion approximately may exponentially sprout to US $ 5 trillion by 2050. Ayurveda contributes Rs. 3500 crores (US $ 813 million) annually to international market (80).

Gymnema sylvestre is a magical herb effective for combating a wide range of health conditions. Every part of the plant has been attributed with medicinal values which have been immensely exploited for the same. Various herbal products based on *Gymnema sylvestre* are formulated and sold in the market. Several brands of extracts, tablets and herbal tea are based on this herb. There are various products of *Gymnema*, *viz. Gymnema* 4G (M1320/M1325), *Gymnema sylvestre* 75, *Gymnema* tea are now regularly used in the day today life.

As with any type of herbal supplement, safety and strength of the formulation are not clinically proven. Thus, taking a note of the intake dose is imperative to minimize the risk of *Gymnema sylvestre* side effects. No established and safe methodology is available for the product eminence and effectiveness. The improvement and materialistic functionality of herbal medicine industries are largely reliant upon its accessibility to amenities and in a row concerning the isolation, refining, and advertisement the industrial potential of plants. Therefore, a systematic investigation is mandated to develop drugs based on the components of *Gymnema sylvestre*. An ef-

Illustration of the bioactive compounds isolated from different parts of *Gymnema sylvestre*

Bioactive compounds	Part used	Extraction	Study results	Model used to induce diabetes	Beneficial role	References
Conduritol	Stem	Ethanol	Exhibited antidiabetic activity by elevating thymus, pancreas, splencia index or inhibiting the atrophy of thymus, pancreas, splencias of diabetic rat	Diabetic rats induced by alloxan	Antidiabetic activity	(81)
Dihydroxy gymnemic triacetate	Leaves	Acetone	Acquire the hypoglycemic and antihyperlipidemic activity in streptozotocin-induced diabetic rats	Streptozotocin-induced diabetic rats	Hypoglycemic and antihyperlipidemic	(44)
Gymnemic acids I-IV and gymnemasaponin V	Leaves	Methanol extract	Gymnemic acid IV treatment decrease glucose uptake and blood glucose level, Increase plasma insulin	Streptozotocin-induced diabetic mice.	Anti-obese and antihyperglycemic pro-drug.	(82)
Deacyl gymnemic acid	Leaves		Administration of deacyl gymnemic acid reduced the blood pressure and improved fasting sugar level. A decrease in HOMA-IR with fair improvement in lipid profile was observed.	High fructose diet for 20 days to induce metabolic syndrome in a rat model	Deacyl gymnemic acid alleviates the insulin resistance a rat model of metabolic syndrome.	(83)
Gymnemate (GA), a mixture of triterpene of glucuronides	Leaves	Water extract	Gymnemate treatment improved the lipid profiles, total cholesterol was decreased about 1/3, LDL, VLDL decreased about 1/2. The ratio of HDL-C to the total cholesterol was increased. The serum triglyceride was decreased to the 1/4 of OLETF control.	Genetic multifactor syndrome animal (OLETF rat)	Administration with gymnemate promoted weight loss was due to the anti-lipidemic action	(84)
Saponin rich gymnema. sylvestre	Leaves	Water extract	Lowers body weight and organ weights. Moreover, Plasma TC, TG, VLDL, LDL-C were also reduced.	High fat diet induced wistar rats	Saponin rich *Gymnema* sylvestre R.Br aqueous leaf extract can be used for obesity treatment.	(85)
Gymnemagenin and gymnemic acids	Leaves	Ethanol extract	A decrease in the blood glucose, serum TG, LDL, TC. Increase in serum insulin and antioxidant enzymes such as glutathione, catalase, and reduced glutathione was reported.	Sprague-Dawley rats	Increase in the antioxidant level and lowering lipid peroxidation	(86)
GS3, and GS4	Leaves	Alcoholic extract	Raise the serum insulin to levels closer to normal fasting levels. diabetic rat pancreas, Treatment with both GS_3, and GS_4, compound were able to double the islet number and β cell number	Streptozotocin treated rats	Probably due to repair/regeneration of the endocrine pancreas	(87)
Triterpene glycoside Glycoside	Leaves	90 % alcohol	Decrease the blood glucose ($p < 0.05$) at 2 and 4 hr after glucose load in the glucose tolerance test.	Streptozotocin induced diabetic rats	Hypoglycemic activity in control and streptozotocin induced diabetic rats.	(88)
Gymnemoside b	Leaves	Methanol extract	Gymnemoside b produced some inhibitory activity on glucose absorption after oral glucose loading in rats.	Male Wister rat	Inhibitory activity on glucose absorption	(89)

Abbreviation: HOMA-IR, (homeostatis model assessment- insulin resistance; OLETF rat, Otsuka Long Evans Tokushima Fatty; LDL, low density lipoprotein VLDL, very low density lipoprotein; HDL-C, high density lipoprotein; GS, Gymnema sylvestre

fective and safe drug can be flourished after an extensive clinical investigation which would be effective, natural, pharmacologically non-toxic and pristine.

Conclusions

Diabetes mellitus is the most common metabolic disorder affecting human beings and is characterized by chronic hyperglycemia. The prevalence of diabetes is rising and aggravating all over the world is being associated with an increase in financial burden, a decrease in quality of life, morbidity and mortality. In the past few years, *Gymnema sylvestre* has emerged as a cost-effective and potential intervention by targeting the etiological factors connected with diabetes. It functions as a blood sugar lowering agent, insulin stimulator, β-cell regenerator, facilitator of anti-obesity and an anti-inflammatory agent. It produces not only blood glucose homeostasis but also showed anti-cancerous, anti-microbial, anti-arthritic activities.

Gymnema sylvestre holds a definite promise in the management of diabetes mellitus. This review has updated the pharmacological, toxicological and clinical evaluation of this plant for treatment of diabetes and its associated abnormalities. The ethnomedical approach for diabetes using *Gymnema sylvestre* is practical, logical and economically worthwhile. But still, it requires scientific and technological validation, standardization for justification of its wide acceptability among a modern system of medicine. One can look toward to an integrated approach to future medicine using this traditional drug.

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References

1. Shelar DB, Shirote PJ. Natural product in drug discovery: back to future. J Pharm Res 2010; 3: 2007-8.
2. Kala CP, Farooquee NA, Dhar U. Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. Biodivers Conserv 2004; 13: 453-69.
3. Shah D, Agarawal V, Parikh R. Noninvasive insulin delivery system: a review. Int J Appl Pharm 2010; 2: 35-40.
4. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047-53.
5. Pham HTT, Hoang MC, Ha TKQ et al. Discrimination of different geographic varieties of Gymnema sylvestre, an anti-sweet plant used for the treatment of type 2 diabetes. Phytochemistry 2018; 150: 12-22.
6. Yadav D, Tiwari A, Mishra M et al. Anti-hyperglycemic and anti-hyperlipidemic potential of a polyherbal preparation "Diabegon" in metabolic syndrome subject with type 2 diabetes. Afr J Tradit Complement Altern Med 2014; 11: 249-56.
7. Singh SP, Patra CN, Dinda SC. A comparative evaluation of the flow and compaction characteristics of Gymnema sylvestre leaf powder. . J Adv Pharm Res 2010; 1: 1-11.
8. Hajare R. Comparing Modified and Relationship Study of Gymnema Sylvestre Against Diabetes. SF J Pub Health 2018; 2.
9. Smruthi G, Mahadevan V, Sahayam S, Rajalakshmi P, Vadivel V, Brindha P. Anti-Diabetic Potential of Selected Indian Traditional Medicinal Plants-An Updated Review. Journal of Pharmaceutical Sciences and Research 2016; 8: 1144.
10. Ekka NR, Dixit VK. Ethno-pharmacognostical studies of medicinal plants of jashpur district (Chhattisgarh). Int J Green Pharm 2007; 1.
11. Meena AK, Bansal P, Kumar S. Plants-herbal wealth as a potential source of ayurvedic drugs. Asian J Tradit Med 2009; 4: 152-70.
12. Rachh PR, Rachh MR, Ghadiya NR et al. Antihyperlipidemic activity of Gymenma sylvestre R. Br. leaf extract on rats fed with high cholesterol diet. Int J Pharmcol 2010; 6: 138-41.
13. Manish K, Aditi K, Renu A, Gajraj S, Poonam M. Antiobesity property of hexane extract from the leaves of Gymnema sylvestre in high fed cafeteria diet induced obesity rats. International Research Journal of Pharmacy 2011; 2: 112-6.
14. Rachh PR, Patel SR, Hirpara HV et al. In vitro evaluation of antioxidant activity of Gymnema sylvestre r. br. leaf extract. Romanian J Biology Plant Biol 2009; 54: 141-8.
15. Arunachalam KD, Arun LB, Annamalai SK, Arunachalam AM. Potential anticancer properties of bioactive compounds of Gymnema sylvestre and its biofunctionalized silver nanoparticles. Int J Nanomedicine 2015; 10: 31.
16. Malik JK, Manvi FV, Nanjwade BK, Alagawadi KR, Sinsh S. Immunomodulatory activity of Gymnema sylvestre R. Br.. leaves on in vitro human neutrophils. J Pharm Res 2009; 2: 1284-6.
17. Satdive RK, Abhilash P, Fulzele DP. Antimicrobial activity of Gymnema sylvestre leaf extract. Fitoterapia 2003; 74: 699-701.
18. Meyer JS. Diabetes and wound healing. Crit Care Nurs Clin North Am 1996; 8: 195-201.

19. Malik JK, Manvi FV, Nanjware BR, Dwivedi DK, Purohit P, Chouhan S. Anti-arthritic activity of leaves of Gymnema sylvestre R. Br. leaves in rats. Pharm Lett 2010; 2: 336-41.
20. Gupta SS, Seth CB. Experimental studies on pituitary diabetes. II. Comparison of blood sugar level in normal and anterior pituitary extract-induced hyperglycaemic rats treated with a few Ayurvedic remedies. Indian J Med Res 1962; 50: 708.
21. Porchezhian E, Dobriyal RM. An overview on the advances of Gymnema sylvestre: chemistry, pharmacology and patents. Pharmazie 2003; 58: 5-12.
22. Shanmugasundaram KR, Panneerselvam C, Samudram P, Shanmugasundaram ERB. Enzyme changes and glucose utilisation in diabetic rabbits: the effect of Gymnema sylvestre, R. Br. J Ethnopharmacol 1983; 7: 205-34.
23. Shanmugasundaram ERB, Venkatasubrahmanyam M, Vijendran N, Shanmugasundaram KR. Effect of an isolate from Gymnema sylvestre, R. Br. in the control of diabetes mellitus and the associated pathological changes. Anc Sci Life 1988; 7: 183.
24. Srivastava Y, Nigam SK, Bhatt HV, Verma Y, Prem AS. Hypoglycemic and life-prolonging properties of Gymnema sylvestre leaf extract in diabetic rats. Isr J Med Sci 1985; 21: 540.
25. Shanmugasundaram KR, Panneerselvam C, Samudram P, Shanmugasundaram E. The insulinotropic activity of Gymnema sylvestre, R. Br. An Indian medical herb used in controlling diabetes mellitus. Pharmacol Res Commun 1981; 13: 475-86.
26. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given Gymnema sylvestre leaf extracts. J Ethnopharmacol 1990; 30: 265-79.
27. Persaud SJ, Al-Majed H, Raman A, Jones PM. Gymnema sylvestre stimulates insulin release in vitro by increased membrane permeability. J Endocrinol 1999; 163: 207-12.
28. Sheoran S, Panda BP, Admane PS, Panda AK, Wajid S. Ultrasound-assisted extraction of gymnemic acids from Gymnema sylvestre leaves and its effect on insulin-producing RINm-5 F β cell lines. Phytochem Anal 2015; 26: 97-104.
29. Liu B, Asare-Anane H, Al-Romaiyan A et al. Characterisation of the insulinotropic activity of an aqueous extract of Gymnema sylvestre in mouse β-cells and human islets of Langerhans. Cell Physiol Biochem 2009; 23: 125-32.
30. Ahmed ABA, Rao AS, Rao MV. In vitro callus and in vivo leaf extract of Gymnema sylvestre stimulate β-cells regeneration and anti-diabetic activity in Wistar rats. Phytomedicine 2010; 17: 1033-9.
31. Sujin RM. Anti-diabetic effect of Gymnema sylvestre (asclepiadaceae) powder in the stomach of rats. Ethnobot Leafl 2008; 2008: 153.
32. Imoto T, Miyasaka A, Ishima R, Akasaka K. A novel peptide isolated from the leaves of Gymnema sylvestre—I. Characterization and its suppressive effect on the neural responses to sweet taste stimuli in the rat. Comp Biochem Physiol A Comp Physiol 1991; 100: 309-14.
33. Shigemura N, Nakao K, Yasuo T et al. Gurmarin sensitivity of sweet taste responses is associated with co-expression patterns of T1r2, T1r3, and gustducin. Biochem Biophys Res Commun 2008; 367: 356-63.
34. Miyasaka A, Imoto T. Electrophysiological characterization of the inhibitory effect of a novel peptide gurmarin on the sweet taste response in rats. Brain Res 1995; 676: 63-8.
35. Kamble B, Gupta A, Moothedath I et al. Effects of Gymnema sylvestre extract on the pharmacokinetics and pharmacodynamics of glimepiride in streptozotocin induced diabetic rats. Chem Biol Interact 2016; 245: 30-8.
36. Venkatachalam P, Thiyagarajan M, Sahi SV. Fabrication of bioactive molecules loaded chitosan nanoparticles using Gymnema sylvestre leaf extracts and its antidiabetic potential in experimental rat model. J Bionanosci 2015; 9: 363-72.
37. Yoshikawa K, Kondo Y, Arihara S, Matsuura K. Antisweet natural products. IX. Structures of gymnemic acids XV-XVIII from Gymnema sylvestre R. Br. V. Chem Pharm Bull (Tokyo) 1993; 41: 1730-2.
38. Ye WC, Zhang QW, Liu X, Che CT, Zhao SX. Oleanane saponins from Gymnema sylvestre. Phytochemistry 2000; 53: 893-9.
39. Yoshikawa K, Arihara S, Matsuura K, Miyaset T. Dammarane saponins fromGymnema sylvestre. Phytochemistry 1992; 31: 237-41.
40. Murakami N, Murakami T, Kadoya M, Matsuda H, Yamahara J, Yoshikawa M. New hypoglycemic constituents in "gymnemic acid" form gymnema sylvestre. Chem Pharm Bull (Tokyo) 1996; 44: 469-71.
41. Shenoy RS, Prashanth KVH, Manonmani HK. In Vitro Antidiabetic Effects of Isolated Triterpene Glycoside Fraction from Gymnema sylvestre. Evid Based Complement Alternat Med 2018; 2018: 7154702.
42. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). Int J Diabetes Metab 2006; 14: 1.
43. Yoshikawa M, Murakami T, Matsuda H. Medicinal foodstuffs. X. Structures of new triterpene glycosides, gymnemosides-c,-d,-e, and-f, from the leaves of Gymnema sylvestre R. Br.: influence of gymnema glycosides on glucose uptake in rat small intestinal fragments. Chem Pharm Bull (Tokyo) 1997; 45: 2034-8.
44. Daisy P, Eliza J, Farook KAMM. A novel dihydroxy gymnemic triacetate isolated from Gymnema sylvestre possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats. J Ethnopharmacol 2009; 126: 339-44.
45. Sugihara Y, Nojima H, Matsuda H, Murakami T, Yoshikawa M, Kimura I. Antihyperglycemic effects of gymnemic acid IV, a compound derived from Gymnema sylvestre leaves in streptozotocin-diabetic mice. J Asian Nat Prod Res 2000; 2: 321-7.
46. Rathore PK, Arathy V, Attimarad VS, Kumar P, Roy S. In-silico analysis of gymnemagenin from Gymnema sylvestre (Retz.) R. Br. with targets related to diabetes. J Theor Biol 2016; 391: 95-101.

47. Omae T, Shimamoto C, Hiraike Y et al. Hyperlipidemia and fat absorption in model rats with type 2 diabetes mellitus. Bull Osaka Med Coll 2006; 52: 45-58.
48. Bishayee A, Chatterjee M. Hypolipidaemic and antiatherosclerotic effects of oral Gymnema sylvestre R. Br. Leaf extract in albino rats fed on a high fat diet. Phytother Res 1994; 8: 118-20.
49. Mall GK, Mishra PK, Prakash V. Antidiabetic and hypolipidemic activity of Gymnema sylvestre in alloxan induced diabetic rats. Global J Biotechnol Biochem 2009; 4: 37-42.
50. Osman M, Fayed SA, Ghada IM, Romeilah RM. Protective effects of chitosan, ascorbic acid and gymnema sylvestre against hypercholesterolemia in male rats. Aust J Basic Appl Sci 2010; 4: 89-98.
51. Shigematsu N, Asano R, Shimosaka M, Okazaki M. Effect of administration with the extract of Gymnema sylvestre R. Br leaves on lipid metabolism in rats. Biol Pharm Bull 2001; 24: 713-7.
52. Steppan CM, Bailey ST, Bhat S et al. The hormone resistin links obesity to diabetes. Nature 2001; 409: 307-12.
53. Rajala MW, Obici S, Scherer PE, Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule–β selectively impair insulin action on glucose production. J Clin Invest 2003; 111: 225-30.
54. Pravenec M, Kazdová L, Landa V et al. Transgenic and recombinant resistin impair skeletal muscle glucose metabolism in the spontaneously hypertensive rat. J Biol Chem 2003; 278: 45209-15.
55. Nakamura Y, Tsumura Y, Tonogai Y, Shibata T. Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in Gymnema sylvestre leaves. J Nutr 1999; 129: 1214-22.
56. Shigematsu N, Asano R, Shimosaka M, Okazaki M. Effect of long term-administration with Gymnema sylvestre R. BR on plasma and liver lipid in rats. Biol Pharm Bull 2001; 24: 643-9.
57. Preuss HG, Bagchi D, Bagchi M, Rao CVS, Satyanarayana S, Dey DK. Efficacy of a novel, natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacin-bound chromium and Gymnema sylvestre extract in weight management in human volunteers: A pilot study. Nutr Res 2004; 24: 45-58.
58. Kaneto H, Kajimoto Y, Miyagawa J et al. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. Diabetes 1999; 48: 2398-406.
59. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J Biophys 2008; 18: 225-36.
60. Rizvi AA. The role of inflammation in diabetes and its complications. South Med J 2006; 99: 8-10.
61. Singh VK, Dwivedi P, Chaudhary BR, Singh R. Immunomodulatory Effect of Gymnema sylvestre (R. Br.) Leaf Extract: An In Vitro Study in Rat Model. PLoS One 2015; 10: e0139631.
62. Kiranmai M, Kazim SM, Ibrahim M. Combined wound healing activity of Gymnema sylvestere and Tagetes erecta Linn. Int J Pharm Appl 2011; 1: 135-40.
63. Khare AK, Tondon RN, Tewari JP. Hypoglycaemic activity of an indigenous drug (Gymnema sylvestre,'Gurmar') in normal and diabetic persons. Indian J Physiol Pharmacol 1983; 27: 257.
64. Joffe DJ, Freed SH. Effect of extended release gymnema sylvestre leaf extract (Beta Fast GXR) alone or in combination with oral hypoglycemics or insulin regimens for type 1 and type 2 diabetes. Diabetes Control Newslett 2001; 76.
65. Baskaran K, Ahamath BK, Shanmugasundaram KR, Shanmugasundaram ERB. Antidiabetic effect of a leaf extract from Gymnema sylvestre in non-insulin-dependent diabetes mellitus patients. J Ethnopharmacol 1990; 30: 295-305.
66. Shanmugasundaram ERB, Rajeswari G, Baskaran K, Kumar BRR, Shanmugasundaram KR, Ahmath BK. Use of Gymnema sylvestre leaf extract in the control of blood glucose in insulin-dependent diabetes mellitus. J Ethnopharmacol 1990; 30: 281-94.
67. Paliwal R, Kathori S, Upadhyay B. Effect of Gurmar (Gymnema sylvestre) powder intervention on the blood glucose levels among diabetics. Stud Ethno-Med 2009; 3: 133-5.
68. Mahajan S, Chauhan P, Subramani SK et al. Evaluation of "GSPF kwath": A Gymnema sylvestre-containing polyherbal formulation for the treatment of human type 2 diabetes mellitus. Eur J Integr Med 2015; 7: 303-11.
69. Kurian GA, Manjusha V, Nair SS, Varghese T, Padikkala J. Short-term effect of G-400, polyherbal formulation in the management of hyperglycemia and hyperlipidemia conditions in patients with type 2 diabetes mellitus. Nutrition 2014; 30: 1158-64.
70. Yadav D, Cho K-H. Preventive and therapeutic aspects of selected herbal medicines in diabetes mellitus. Progress in Nutrition 2017; 19: 117-26.
71. Saumendu DR, Jeet K, Dipankar S, Singh TB, Prabha BA. In vitro antibiotic activity of various extracts of Gymnema sylvestre. Int J Pharma Res Devel 2010; 2.
72. Thalikunnil ST, Sukesh K, Densingh J. Phytochemical investigation and antibacterial activity of Gymnema sylvestre and Andrographis paniculata from western ghats. Phytomedicine 2011; 3: 254.
73. Vediyappan G, Dumontet V, Pelissier F, d'Enfert C. Gymnemic acids inhibit hyphal growth and virulence in Candida albicans. PLoS One 2013; 8: e74189.
74. Jayachitra A, Muniyandi MJ. Pharmacological potential of Gymnema sylvestre: A review. J Pharmacol Toxicol Investig 2015; 1: 54-8.
75. Khanna V, Kannabiran K. Anticancer-cytotoxic activity of saponins isolated from the leaves of Gymnema sylvestre and Eclipta prostrata on HeLa cells. Int J Green Pharm 2009; 3: 227.
76. Nakkala JR, Mata R, Bhagat E, Sadras SR. Green synthesis of silver and gold nanoparticles from Gymnema sylvestre leaf extract: study of antioxidant and anticancer activities. J Nanopart Res 2015; 17: 151.
77. Eric G, Lawrence J. Rheumatoid arthritis and its therapy. The textbook of therapeutics drug and disease management Baltimore: Williams and Wilkins Company 1996: 579-95.

78. Diwan PV, Margaret I, Ramakrishna S. Influence of Gymnema sylvestre on inflammation. Inflammopharmacology 1995; 3: 271-7.
79. Chattopadhyay RR. A comparative evaluation of some blood sugar lowering agents of plant origin. J Ethnopharmacol 1999; 67: 367-72.
80. Joshi K, Chavan P, Warude D, Patwardhan B. Molecular markers in herbal drug technology. Curr Sci 2004; 87: 159-65.
81. Wei J, Zhen H, Qiu Q, Chen J, Zhou F. [Experimental [corrected] study of hypoglycemic activity of conduritol A of stems of Gymnema sylvestre]. Zhongguo Zhong Yao Za Zhi. 2008; 33: 2961-5.
82. Sugihara Y, Nojima H, Matsuda H, Murakami T, Yoshikawa M, Kimura I. Antihyperglycemic effects of gymnemic acid IV, a compound derived from Gymnema sylvestre leaves in streptozotocin-diabetic mice. J Asian Nat Prod Res 2000; 2: 321-7.
83. Bhansali S, Shafiq N, Pandhi P et al. Effect of a deacyl gymnemic acid on glucose homeostasis & metabolic parameters in a rat model of metabolic syndrome. Indian J Med Res 2013; 137: 1174.
84. Luo H, Kashiwagi A, Shibahara T, Yamada K. Decreased bodyweight without rebound and regulated lipoprotein metabolism by gymnemate in genetic multifactor syndrome animal. Mol Cell Biochem 2007; 299: 93-8.
85. Reddy RM. I, Vijaya T, Pushpa Latha B, Dattatreya Rao S. Saponins rich aqueous extract of Gymnema sylvestre R. Br reduces high fat diet induced obesity in wistar rats. J Pharmacy Res 2011; 4: 1237-9.
86. Kang M-H, Lee MS, Choi M-K, Min K-S, Shibamoto T. Hypoglycemic activity of Gymnema sylvestre extracts on oxidative stress and antioxidant status in diabetic rats. J Agric Food Chem 2012; 60: 2517-24.
87. Shanmugasundaram E, Gopinath KL, Shanmugasundaram KR, Rajendran V. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given Gymnema sylvestre leaf extracts. J Ethnopharmacol 1990; 30: 265-79.
88. Verma N, Shakya V, Saxena R. Antidiabetic activity of glycoside isolated from Gymnema sylvestre in streptozotocin induced diabetic rats. Asian J Chem 2008; 20: 5033.
89. Yoshikawa M, Murakami T, Kadoya M et al. Medicinal foodstuffs. IX. The inhibitors of glucose absorption from the leaves of Gymnema sylvestre R. BR. (Asclepiadaceae): structures of gymnemosides a and b. Chem Pharm Bull (Tokyo) 1997; 45: 1671-6.

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Original article

Is there any link between vitamin D and coronary no-reflow phenomenon?

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Summary. Coronary no-reflow phenomenon (CNP) is associated with an increased risk of major cardiovascular adverse events. Vitamin D is closely associated with hypertension, stroke, myocardial infarction, cardiovascular adverse events, and endothelial dysfunction. Considering endothelial dysfunction is one of the main responsible factors of CNP. We aim to evaluate the association between vitamin D and CNP. The study group consisted of 109 patients. Taking into consideration the inclusion criteria, 60 patients with CNP and 49 patients without CNP were included in the study. CNP defined as TIMI grade <3 at the end of the procedure in the absence of any coronary dissection/spasm and/or less than 70% ST resolution at first hour ECG. Prevalence of CNP was found 55% in this study group. On univariate analysis age, balloon predilatation, stent diameter, serum creatinine, vitamin D, CRP level, initial TIMI flow <2, and reperfusion time >3 h were associated with CNP. On multivariate analysis reperfusion time >3 h, initial TIMI flow <2, and serum creatinine level were the independent predictors for CNP (OR 5.182; 95% CI: 3.159-8.327; p<0.001, OR 4.061; 95% CI: 2.729-6.327; p<0.001, OR 3.301; 95% CI: 1.937-4.623; p<0.001; respectively). In our study, we have found that reperfusion time >3 h, initial TIMI flow <2, and serum creatinine level were an independent predictor for CNP. Vitamin D was not found to be an independent predictor of CNP.

Key words: vitamin D, ST-elevation myocardial infarction, no-reflow phenomenon

Background

Despite the medical and technological improvements of the revascularization procedures in coronary artery diseases, ST-elevation myocardial infarction (STEMI) remains a significant health concern. The primary percutaneous coronary intervention (pPCI) is a favourable treatment option for restoring perfusion to the affected area of the myocardium as soon as possible. Coronary no-reflow phenomenon (CNP) was defined as Thrombolysis in myocardial infarction (TIMI) flow grade <3 after vessel recanalisation despite the absence of angiographic stenosis, spasm, dissection, or thrombosis after pPCI. CNP occurs in 11-41% of STEMI patients treated by primary pPCI and is associated with the poor left ventricular function, arrhythmias, poor in-hospital and one-year survival (1).

The mechanism of CNP is complex and not fully understood. Possible mechanisms of CNP were microthromboemboli, irreversible cardiomyocyte and endothelial damage/dysfunction caused by ischemia, activation of reactive oxygen species, endothelial cell necrosis leads to the destruction of tight and adherens junction and loss of vascular integrity (2). Myocardial contrast echocardiography, magnetic resonance imaging (MRI), myocardial blush grade, intracoronary pressure measurements, intracoronary Doppler, Virtual histology intravascular ultrasound were a useful

diagnostic tool for CNP. A rapid resolution of ST-segment elevation is highly suggestive of reperfusion. ST-segment resolution <70% at 60 min is a descriptive marker of CNP (1, 3).

Vitamin D is a fat-soluble steroid responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects. Vitamin D receptors are expressed in a variety of tissues, including cardiomyocytes, vascular smooth muscle cells, and endothelial cells. Vitamin D deficiency contributes to the development of cardiovascular diseases through its association with risk factors, such as diabetes mellitus (DM), hypertension (HT), and atherosclerosis and also through events such as myocardial infarction, stroke, and congestive heart failure. Also, vitamin D has anti-inflammatory effects and prevents cholesterol removal by macrophages and foam cells formation on vessel walls. Vitamin D deficiency causes endothelial dysfunction through its direct or indirect association through the up-regulation of the renin-angiotensin system or via induction of smooth muscle proliferation and a pro-inflammatory state (4-9). Sugden et al. (10) demonstrated that decreased serum vitamin D level was associated with decreased flow-mediated dilatation, and vitamin D supplementation can improve endothelial dysfunction in patients with DM.

In this context, we aim to evaluate the association between serum vitamin D level and CNP in patients underwent pPCI.

Methods

The study group consisted of 124 consecutive patients, who underwent primer PCI due to first acute STEMI were evaluated prospectively. Among them, 15 patients were excluded from the study because of not undergoing pPCI, cardiogenic shock, symptom to balloon time more than 12 hours, history of coronary artery disease (previous coronary artery bypass graft surgery or PCI), and not achieving coronary artery patency.

The diagnosis of acute STEMI was established using The Joint European Society of Cardiology, American College of Cardiology Foundation, the American Heart Association definition of STEMI.(11) All patients received aspirin (300 mg) and clopidogrel (600 mg) prior to their transfer to the catheterisation laboratory. Emergency coronary angiography was performed using the percutaneous femoral or radial approach. Heparin (100 U/kg) was administered in all patients during pPCI procedure. Occlusion of the infarct-related coronary artery was crossed using various guide wires, and predilatation was performed via balloon angioplasty if necessary. Routine stenting was attempted directly or following balloon angioplasty. The drug-eluting stent was implanted at the site of the ruptured atherosclerotic plaque. 12-lead ECG was obtained 60 minutes after successful pPCI. All of the patients were treated according to the recommendations of the latest ESC Guidelines for the Management of Patients with STEMI (12). The usage of thrombus aspiration catheter and administration of glycoprotein IIb/IIIa inhibitors were chosen according to the interventional cardiologist's decision.

Epicardial coronary blood flow was quantified visually using the Thrombolysis in Myocardial infarction (TIMI) flow grade classification (13). Initial TIMI flow was assessed at the beginning of the procedure prior to wire insertion, and final TIMI flow was assessed at the end of the procedure. No-reflow was defined as TIMI grade <3 at the end of the procedure in the absence of any coronary dissection or spasm. The TIMI flow grades were evaluated by 2 blind cardiologists. The frame rate of cine images were 30 frames per seconds.

ST-segment elevation in mm was measured 20 ms after the J point. The sum of ST-segment elevations was calculated for all leads. A percentage ST-segment resolution <70% was accepted as an ECG sign of the No-reflow. Electrocardiograms were evaluated in a blinded manual manner by two experienced cardiologists. CNP was described as TIMI flow <3 after pPCI and/or <70% ST-segment resolution at 60 minute ECG in our study.

Peripheral venous blood samples were obtained from all patients and controls. Each blood sample was left to coagulate for 30 minutes, and then centrifuged at 2000 g. for 15 minutes to separate serum. Serum aliquots were immediately labeled and stored at -20°C until analysis.

Serum 25-OH Vitamin D level was analyzed using commercial reagents by the Chemiluminescent Microparticle Immunoassay method on Abbott

Architect i2000SR (Abbott Laboratory Abott park, Chicago, IL, USA). The method of measurement was carried out according to the manufacturer instructions.

The data of patients were analysed for the demographic features, echocardiographic parameters including ejection fraction, biochemical parameters, and coronary angiography.

Echocardiographic examination

Transthoracic echocardiography was performed within 48 hours of admission using a Vivid S5 (GE healthcare) echocardiography device and Mass S5 probe (2-4 MHz). Standard two-dimensional and colour flow Doppler views were acquired according to the guidelines of American Society of Echocardiography and European Society of Echocardiography (14). The ejection fraction was measured according to the Simpson's method.

Patients with DM were identified on admission as those with documented DM using either oral hypoglycemic agents or insulin treatment. Hyperlipidemia (HL) was defined as total cholesterol at least 200 mg/dL or using antihyperlipidemic therapy on admission. HT was defined as blood pressure above 140/90 mmHg or using antihypertensive therapy on admission.

Statistical analysis

Statistical analysis was performed using the SPSS (version 20.0, SPSS Inc., Chicago, Illinois) software package. Continuous variables were expressed as the mean±standard deviation (mean± SD), and categorical variables were expressed as a percentage (%). The Kolmogorov-Smirnov test was used to evaluate the distribution of variables. Student's t-test was used to evaluate continuous variables showing normal distribution, and Mann-Whitney U-test was used to evaluate variables that did not show normal distribution. A p-value <0.05 was considered statistically significant. To identify predictors of CNP, the following variables were initially assessed in a univariate model: age, reperfusion time >3h, initial TIMI flow <2, stent diameter, balloon predilatation, serum creatinine, vitamin D, and CRP. Significant variables in univariate analysis were then entered into a multivariate logistic-regression analysis using backward stepwise selection.

Results

Prevalance of CNP was found 55% in this study group. The demographic characteristics of both group are summarized in Table 1. There was no significant difference between both groups regarding female gender, HT, current smoking, HL, and Body mass index (69.4% vs. 68.3% p=0.906, 32.6% vs. 33.3% p=0.738, 53.1% vs 58.3% p=0.304, 20.4% vs 25% p=0.322, 28.3 vs 30.1 kg/m^2 p=0.437; respectively) (Table 1). There was significant diffrence between both groups regarding age and DM (59.4±10.7 vs. 64.1±10.1 years p=0,022, 34.6% vs. 48.3% p<0.001; respectively) (Table 1).

There was no significant difference between both groups regarding biochemical parameters such as white blood cell, haemoglobin, and urea (11.5±3.5 vs 12.1±2.8 10^3/µL p=0.291, 13.8±1.9 vs 14.3±1.2 g/dl p=0.065, 39.1±16.2 vs 37.3±7.3 mg/dl p=0.494; respectively) (Table 2). There were significant diffrence between both groups regarding reperfusion time >3h, balloon predilatation, stent diameter, initial TMI flow <2, serum vitamin D, C reactive protein (CRP), creatinine level (16.3% vs 45% p<0.001, 53.1% vs 71.7% p=0.045, 3.05±0.41 vs 2.85±0.34 mm p=0.008, 46.9% vs 71.6% p<0.001, 19.3±6.9 vs 13.2±8.5 ng/ml p<0.001, 1.6±2.1 vs 2.4±2.2 mg/dl p:0.041, 0.81±0.17 vs 0.88±0.12 mg/dl p=0.007; respectively) (Table 2).

On univariate analysis age, serum creatinine, vitamin D, CRP, balloon predilatation, stent diameter,

Table 1. General characteristics of patients

Patient characteristics	Coronary no-reflow phenomenon		p
	- (49)	+ (60)	
Age (years)	59.4±10.7	64.1±10.1	**0.022**
Female Gender, n(%)	34 (69.4)	41 (68.3)	0.906
Hypertension, n(%)	16 (32.6)	20 (33.3)	0.738
Diabetes Mellitus, n(%)	17 (34.6)	29 (48.3)	**0.02**
Current Smoking, n(%)	26 (53.1)	35 (58.3)	0.304
Hyperlipidemia, n(%)	10 (20.4)	15 (25)	0.322
Body Mass Index, (kg/m^2)	28.3 (22.1±35.7)	30.1 (24.6±36.9)	0.437

Table 2. Laboratory, echocardiographic, and angiographic parameters

Variables	Coronary no-reflow phenomenon		p
	- (49)	+ (60)	
White Blood Cell (10^3/μL)	11.5±3.5	12.1±2.8	0.291
Haemoglobin (g/dl)	13.8±1.9	14.3±1.2	0.065
Urea (mg/dl)	39.1±16.2	37.3±7.3	0.494
Creatinine (mg/dl)	0.81±0.17	0.88±0.12	**0.007**
C reactive protein (mg/dl)	1.6±1.1	2.4±2.2	**0.041**
Vitamin D (ng/ml)	19.3±6.9	13.2±8.5	**<0.001**
Ejection Fraction (%)	37.9±10.3	35.4±9.7	0.549
Initial TIMI flow <2 (n,%)	23 (46.9)	43 (71.6)	**<0.001**
Stent Diameter (mm)	3.05±0.41	2.85±0.34	**0.008**
Stent Length (mm)	20.7±7.1	22.7±5.5	0.055
Balloon Predilatation (n,%)	26 (53.1)	43 (71.7)	**0.045**
Reperfusion Time >3 h, (n,%)	8 (16.3)	27 (45)	**<0.001**

Table 3. Univariate analysis of predictors for coronary no-reflow phenomenon

Predictor variables	OR (95% C.I.)	p
Age (years)	2.681 (1.376-4.327)	**<0.001**
Serum Creatinine (mg/dl)	3.153 (1.839-4.638)	**<0.001**
Serum Vitamin D (ng/ml)	2.147 (1.373-3.427)	**<0.001**
Serum C Reactive Protein (mg/dl)	2.549 (1.937-3.581)	**0.01**
Balloon dilatation (n,%)	1.951 (1.327-2.638)	**0.001**
Stent Diameter (mm)	3.926 (2.379-5.734)	**<0.001**
Reperfusion Time >3 h (n,%)	4.269 (2.837-6.823)	**<0.001**
Initial TIMI Flow <2 (n,%)	3.929 (2.837-7.634)	**<0.001**

reperfusion time >3h, and initial TIMI flow <2 were associated with CNP (OR 2.681; 95% CI: 1.376-4.327; p<0.001, OR 3.153; 95% CI: 1.839-4.638; p<0.001, OR 2.147; 95% CI: 1.373-3.427; p<0.001, OR 2.549; 95% CI: 1.937-3.581; p=0.01, OR 1.951; 95% CI: 1.327–2.638; p=0.001, OR 3.926; 95% CI: 2.379-5.734; p<0.001, OR 4.269; 95% CI: 2.837-6.823; p<0.001, OR 3.929; 95% CI: 2.837-7.634; p<0.001; respectively) ((Table 3).

Table 4. Multivariate analysis of predictors for coronary no-reflow phenomenon

Predictor variables	OR (95% C.I.)	p
Serum creatinine (mg/dl)	3.301 (1.937-4.623)	**<0.001**
Reperfusion time >3 h (n,%)	5.182 (3.519-8.359)	**<0.001**
Initial TIMI flow <2 (n,%)	4.061 (2.729-6.327)	**<0.001**

On multivariate analysis serum creatinine level, reperfusion time >3 h, and initial TIMI flow<2 were independent predictors for CNP (OR 3.301; 95% CI: 1.937-4.623; p<0.001, OR 5.182; 95% CI: 3.519-8.359; p<0.001, OR 4.061; 95% CI: 2.729-6.327; p<0.001; respectively) (Table 4).

Discussion

In our study, we have found that serum creatinine level, reperfusion time >3 h, and initial TIMI flow <2 were an independent predictor for CNP. Vitamin D was not found to be an independent predictor of CNP.

In previous studies, CNP occurs in 11-41% of STEMI patients treated by pPCI (1). In our study, the prevalence of CNP was 55%. Our higher prevalence of CNP was associated with our definition of CNP. In our study, CNP was described as <70% ST-segment resolution at 60 minute ECG and/or TIMI flow <3 after pPCI. Although the most of the studies used TIMI flow method for assessing CNP TIMI flow method is less accurate than myocardial blush grade and MRI. Therefore, not only TIMI flow grade but also ECG criteria of CNP (<70% STR) were used to evaluate of CNP in our study. Our higher ratio of CNP might be associated with definition criteria of CNP.

The pathophysiology of CNP is still not fully understood. Possible etiological factors of CNP are pre-existing microvascular dysfunction, distal microthrombo-embolization due to high platelet activity, increased thrombus burden, ischemic injury, reperfusion injury, swelling of myocardial cells compressing microvascular vessels, and individual susceptibility. Endothelial dysfunction is one of the major mechanisms leading to CNP, as we found in our results that high creatinine levels in patients with CNP were signifi-

cantly increased. The reduction in renal function has been proven to cause retention of vasotoxic substances and cause metabolic changes that lead to increase reactive oxygen species. These changes are believed to have an important role in creating an atherogenic milieu. As a result, the plasma concentration of endothelium-derived protein will be increased, and endothelium-dependent vasodilatation will be decreased. The changes of this level are responsible for increasing soluble vascular cell adhesion molecule-1 expression, the earlier step of endothelial dysfunction (15). In our study, we found that serum creatinine level was independently associated with CNP.

It is well known that prolonged ischemia was associated with distal capillary oedema, swelling of myocardial cells, neutrophil plugging and alterations of capillary integrity (16). Additionally, delayed reperfusion can result in more organised intracoronary thrombus which might increase the risk of distal embolisation (17). The thrombus is rich in thrombocytes in the early phase of STEMI, with a longer time to reperfusion, the thrombus takes on more erythrocytes and becomes more firm. Prolonged ischemia may disrupt the microvascular bed, and the degree of this disruption is known to be a key component in the pathogenesis of CNP (18). Delayed reperfusion leads to the greater destruction of the microvasculature, which is why an increased rate of no-reflow is seen in cases of prolonged reperfusion. Animal studies showed that longer duration of occlusion of the coronary artery is associated with CNP after reopening the artery (19). Jawad et al. (20)reported that that delayed presentation >6 h from symptom onset to be independently associated with CNP. In accordance with previous studies (18-20), we found that prolonged revascularisation (reperfusion time >3 h) was an independent predictor of CNP.

Several studies showed that initial TIMI flow was independently associated with CNP (20, 21). Brodie et al. (22) reported that procedural success rate was better in patients with initial TIMI 2-3 flow. Additionally, De Luca et al. (23) showed that initial good TIMI flow was strongly associated with post-procedural TIMI 3 flow and myocardial blush grade 2-3. Good patency of the infarct-related artery prior to PCI associated with lower thrombus burden, spontaneous endogenous lysis of the thrombus, resolution of vasospasm, and smaller infarct size. In accordance with previous studies (20-23), in our study, we found that initial TIMI flow grade was an independent predictor of CNP.

Growing evidence demonstrated that there was a strong association between vitamin D and cardiovascular disease. Vitamin D deficiency causes endothelial dysfunction through its direct or indirect association through the up-regulation of the renin-angiotensin system or via induction of smooth muscle proliferation and a pro-inflammatory state (4-9). Several studies demonstrated that vitamin D deficiency was associated with decreased flow-mediated dilatation, and vitamin D supplementation can improve endothelial dysfunction in various study population such as healthy subjects, DM, stroke, obesity, rheumatoid arthritis, and systemic lupus erythematosus (24, 25). Considering that endothelial dysfinction is one of the possible mechanism of CNP, we conducted this study. In our study, we found that vitamin D was not an independent predictor of CNP.

Our study has some limitations. First, small sample size. Second, we didn't use intravascular USG to quantify evaluate thrombus burden and plaque content. Third, we didn't perform the myocardial blush grade. Fourth, we didn't evaluate serum calcium and parathyroid hormone level.

Conclusion

In our study, we have found that serum creatinine level, reperfusion time >3 h, and initial TIMI flow <2 were an independent predictor for CNP. Vitamin D was not found to be an independent predictor of CNP. Although endothelial dysfunction is one of the possible mechanism of the CNP, other important pathophysiological mechanisms including microthromboemboli, irreversible cardiomyocyte and endothelial damage/dysfunction caused by ischemia, activation of reactive oxygen species, endothelial cell necrosis might outweigh rather than endothelial dysfunction. Further studies are needed to evaluate the association between CNP and vitamin D.

References

1. Harrison RW, Aggarwal A, Ou FS, et al. Incidence and outcomes of no-reflow phenomenon during percutaneous coronary intervention among patients with acute myocardial infarction. Am J Cardiol 2013; 111: 178-84.
2. Bouleti C, Mewton N, Germain S. The no-reflow phenomenon: State of the art. Archives of Cardiovascular Diseases 2015; 108: 661-74.
3. Faruk Akturk I, Arif Yalcin A, Biyik I et al. Effects of verapamil and adenosine in an adjunct to tirofiban on resolution and prognosis of noreflow phenomenon in patients with acute myocardial infarction. Minerva Cardioangiol 2014; 62: 389-97.
4. Siadat ZD, Kiani K, Sadeghi M, et al. Association of vitamin D deficiency and coronary artery disease with cardiovascular risk factors. J Res Med Sci 2012; 17: 1052-5.
5. Cerit L. Vitamin D as a Modifiable Risk Factor for Incident Heart Failure in Atrial Fibrillation. JACC Heart Fail 2018; 6: 85-6.
6. Cerit L. Cardiac Syndrome X, Vitamin D, and Lipoprotein(a). Heart Lung Circ 2017; 26: e104.
7. Cerit L. Vitamin D as a Predictor of Postoperative Atrial Fibrillation. Ann Thorac Surg 2017; 104: 1433.
8. Cerit L, Duygu H. Association of pre-ablation level of vitamin D with atrial fibrillation recurrence after catheter ablation. Europace 2017; 19: 1586.
9. Cerit L, Kemal H, Gulsen K, Ozcem B, Cerit Z, Duygu H. Relationship between Vitamin D and the development of atrial fibrillation after on-pump coronary artery bypass graft surgery. Cardiovasc J Afr 2017; 28: 104-7.
10. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitaminD levels. Diabet Med 2008; 25: 320-5.
11. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Circulation 2012; 126: 2020-35.
12. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2017; 39: 119-77.
13. Gibson MC, Cannon CP, Murphy SA, Marble SJ, Barron HV, Braunwald E; TIMI Study Group. Relation-ship of TIMI myocardial perfusion grades, flow grades, frame count and percuta-neous coronary intervention to long-term outcomes after thrombolytic administration in acute myocardial infarction. Circulation 2002; 105: 1909-13.
14. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification. Eur J Echocardiogr 2006; 7: 79-108.
15. Sabin P, Koshy AG, Gupta PN, et al. Predictors of no- reflow during primary angioplasty for acute myocardial infarction, from MedicalCollege Hospital, Trivandrum. Indian Heart J 2017; 69: 34-45.
16. Kloner RA, Rude RE, Carlson N, Maroko PR, DeBoer LW, Braunwald E. Ultrastructural evidence of microvascular damage and myocardial cell injury after coronary artery occlusion. Circulation 1980; 62: 945-52.
17. Yang Y, Li J, Xu W, et al. Thrombus aspirated from patients with ST-elevation myocardial infarction: Clinical and angiographic outcomes. Kokyu to Jyunkan 2002; 50 :315-320. J Int Med Res 2016; 44: 1514-23.
18. De Luca G, Suryapranata H, Zijlstra F, et al. Symptom-onset-to-balloon time and mortality in patients with acute myocardial infarction treated by primary angioplasty. J Am Coll Cardiol 2003; 42: 991-997.
19. Schwartz BG, Kloner RA. Coronary no reflow. J Mol Cell Cardiol 2012; 52: 873-882.
20. Mazhar J, Mashicharan M, Farshid A. Predictors and outcome of no-reflow post primary percutaneous coronary intervention for ST elevation myocardial infarction. Int J Cardiol Heart Vasc 2015; 10: 8-12.
21. Kirma C, Izgi A, Dundar C, et al. Clinical and procedural predictors of no-reflow phenomenon after primary percutaneous coronary interventions: experience at a single center. Circ J 2008; 72: 716-21.
22. Brodie BR, Stuckey TD, Hansen C, Muncy D. Benefit of coronary reperfusion before intervention on outcomes after primary angioplasty for acute myocardial infarction. Am J Cardiol 2000; 85: 13-8.
23. De Luca G, Ernst N, Zijlstra F, et al. Preprocedural TIMI flow and mortality in patients with acute myocardial infarction treated by primary angioplasty. J Am Coll Cardiol 2004; 43: 1363-7.
24. Al Mheid I, Patel R, Murrow J, et al. Vitamin D status is associated with arterial stifness and vascular dysfunction in healthy humans. J Am Coll Cardiol 2011; 58: 186-92.
25. Karakas Y, Sahin G, Urfali FE, Bal C, Degirmenci NA, Sirmagul B. Efect of vitamin D supplementation on endothelial dysfunction in hemodialysis patients. Hemodial Int 2017; 21: 97-106.

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Original article

Investigation of nutritional status of security officers working in shifts and correlation with obesity

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Summary. Adhering to a healthful diet propensity and way of life, assume a crucial part ofhealthy and agreeable life. Undesirable dietary proclivities are among the significant hazard factors for obesity and related unending ailments, especially if received during early adulthood. Numerous natural elements, way of life, working discipline and financial circumstance influence the nourishing status. This investigation aimed to feature nutritiousconditions and dietary propensities of security officers laboring in shifts and decide the obesity status. Volunteer security officers working from December 2017 to March 2018 in shifts in the city of Istanbul in Turkey were chosen for a cross-sectional investigation. A standard questionnaire was used to collect data through face to face negotiations with security officers regarding socio-statistic characteristics, work status, and dietary patterns in turn of work. Analysis of data was performed using SPSS 25 adaptations. The average age of participants was 34.75±7.12 years. Of the 963 participants, 24 were female and 939 were male. The number of participants who were heavyweight, obese, and morbid obese were 549, 143 and 4 respectively. Nutritional supplements used by participants were vitamin B12, fish oil, vitamin C, Ca, Zinc, vitamin D, iron, probolis, bamboxs, bee pollen. Vitamin C and vitamin D were the most commonly used vitamins with the ratio of 22% and 25.9%, respectively. The obesity rate was higher in those who did not take vitamin and mineral supplements as compared to that in those who took.Participants working in the monthly shift change system were more likely to be overweight, obese and morbid obese than participants who worked in the weekly shift system. The rate of being obese was found to be higher among the participants who had average one time and four times night shifts. Participants preferred mostly fruit-vegetable-type snacks with the rate of 29.7%. Breakfast was the most skipped meal among workers in day and night shifts. Participants consumed eggs every day and in addition, they preferred full-fat cheese mostly with the ratio of 33.4%. The most consumed vegetable by participants during the day was tomato with the rate of 6.3%.Participants mostly preferred to consume white bread and its derivatives with the ratio of 33.7%. Participants drank mostly tea in a day with the maximum rate of 77.6% among regular beverages and among consumption of fat, olive oil was consumed at the highest rate of 46.5% per day. Our study findings indicates that, security officers working in shifts have irregular eating habits.

Key words: eating habits, nutritional status, obesity, shift-workers

Introduction

Regular dietary patterns are the primary need of a healthy way of life. Irregular nutritional habits, for example, skipping breakfast and eating the food rich in carbohydrate and fat, more than need, are the primary elements causing nutrition problem. Irregular feeding may cause or increase the potential risk of ceaseless illnesses including obesity, cardiovascular problems, osteoporosis, and cancer (1). The food product type, the preparation methods, the quantity of meals and the length of the time interval between two meals seems to be very significant regarding a healthy diet.

The basic factors, which may cause energetic imbalance in whole body, contributing to the incidence of overweight and obesity are, increased intake of poor nutrients, nutrient containing high-energy ingredients, high amount of fats, sugar and salt. Also, the other most crucial factor is the lacking or no physical activity (2). The food preference of human is depicted as a complicated process including an assortment of affecting perspectives, for example, the financial and social level and accessibility of food, the educational level and age interval of an individual (3). Undesirable dietary propensities are among the significant hazard factors for obesity and related incessant illnesses, especially if embraced amid early adulthood (4, 5). Investigations on the Middle East reveals that young people and grown-ups eating practices are unfavorably being impacted by the differentiating environmental effects prompting alarming rates of overweight, obesity and higher metabolic hazard factors causing diabetes, hypertension, and other unending illnesses (6, 7). Overweight and obesity are metabolic illnesses common all around the world. Today they are achieving epidemic extents and consequently being the fifth driving factors for worldwide mortality. No less than 2.8 million grown-ups die every year because of being overweight or having an obesity problem (8). Shift working, is the method used to regulate the working hours in full-day period to realize the necessary supply-demand equilibrium in the market (9). Shift system is additionally depicted as antisocial time in light of the fact that the shift system is by and large completed while a critical piece of the general public is resting, along these lines restricting the social existence of shift workers. Thus, shift workers are encountered with numerous variables causing them a problematic life. Nourishment is the most vital of these elements. Food is one of the factors influencing the production rate of workers. At the point when the vitality required for creation is not accessible, laborers restrain the work and produce less. Insufficient and unequal feeding likewise diminishes body resistance, bringing about the more recurrent diseases and more accidents in work, resulting in an expansion in worsening the health regardless of a lessening in the generation rate (10).

A nourishing appraisal is critical regarding the increase in the potential of work, lessening wellbeing uses and diminishing burden of citizens. The study was carried out with the aim of determining the nutritional status of the security guards working in the city of Istanbul and determining the their obesity status.

Materials and Methods

A cross-sectional investigation was performed with volunteer security officers working in shifts from December 2017 to March 2018 from the sixprivate security organizations in Istanbul. Ethics committee approval for the investigation was acquired from the ethics council of Dicle University Medical Faculty. Participation in the survey was on volunteer bases, every member was required to give written informed consent and namelessness was ensured. This investigation was directed to evaluate the nutritional status and relationship of which with obesity among security officers working in shifts in Istanbul from December 2017 to March 2018.The total number of security officers at the time of the study was 1109. The number of participants who agreed to participate in the survey is 963 because of the survey on a volunteer basis. The number of participants comprised of 963 security officers working in shifts (n=939 males, 24 females) with age average of 34.75±7.12 years. A standard questionnaire was used to get information via face to face personal meetings. The survey comprised of inquiries on security officers working in movements' socio-statistic characteristics, shift work status, and dietary patterns.

Anthropometric estimations: The body weights of uniform-wearing security officers without shoes were estimated as the value closest 0.5 kg with a portable scale. Height and abdomen perimeter of each member is estimated to the nearestvalue with the use of 0.1 cm fiber-glass tape. Body Mass Index (BMI: weight/height^2, kg/m^2) was computed for each in agreement with the standards of World Health Organization.

Dietary patterns: Feeding was determined by questionnaire with an inquiry concerning the sum and utilization recurrence of dairy products, meat and meat products, fruit and vegetables, legumes, breakfast and grain products, fat, sugar and sweet, drinking tea, cof-

fee and alcoholic beverages. It was addressed whether they had omitted the meals or not.

Shift working status: Shift working status was surveyed by questions concerning shift change framework, working time in the system, working recurrence of the nightshift, the effect of shift work framework on weight, the effect of the shift work framework on way of life.

Statistical investigation

SPSS 25 was used to analyze all statistical data. Results were evaluated confidence level of 95% and the value $p<0.05$ was decided as a statistically significant outcome. Continuous variables data were expressed as mean±SD in the case of a normal distribution or as median (25-75 percentiles) in that of abnormal distribution. Factors of the categoryappeared as frequency (percentage). Chi-square test was performed to survey the distinctions and connections amongst groups and elements. Keeping in mind the end goal to have the capacity to utilize the chi-square examination, the number of classifications with the standard value under five ought not to surpass 20% of the total number of classifications,and this value must be higher than one in all classes. Toward this path, the quantity of data in the pores was considered before the chi-square estimation was performed.

Accordingly, a few groups with a small number of members were participated in the survey by consolidating with a subgroup or a higher group.

Results

The average age of participants is 34.75±7.12 years. Of the 963 participants, 24 were female and 939 were male. Of the participants, 549 individuals were heavyweight, 143 were obese and 4 of them were morbid obese. Nutritional supplements taken by participants include vitamin B12, fish oil, vitamin C, Ca, Zinc, vitamin D, iron, probolis, bamboxs and bee pollen. The most commonly used vitamins were vitamin C (22.0%) and vitamin D (25.9%). Participants who used vitamin-mineral supplements were found to have a higher obesity rate than participants who did not use vitamin-mineral supplements. There was no statistically significant relationship between the use of vitamin-mineral supplements and BMI ($\chi^2=3.65$; $p>0.05$). There was a significant correlation between the BMI and the type of shift system ($\chi^2=22.82$; $p<0.05$).Participants working in the monthly shift change system were more likely to be obese than participants who worked in the weekly shift system. There was a significant correlation between BMI and average shift time interval ($\chi^2=39,90$; $p<0,05$). Participants who had average one time and four time shifts were found to have higher obesity tendencies. Participants with the rate of 29.7% prefer mostly fruit-vegetable-dried fruits-type snacks. Breakfast is the most skipped meal among workers in day and night shift. 40.7% of daytime workers skip the breakfast more as compared night-time workers with the rate of 38.8%. Participants consume full-fat cheese mostly with the ratio of 33.4%. The most consumed vegetable by participants everyday was tomato with the ratio of 6.3% and mostly preferred to consume white bread and it's derivatives with the ratio of 33.7% and olive oil with a maximum rate of 46.5%.

Table 1 has shown that the number of male participants is 939 (97,5%). A vast majority of participants with the number of 499 (51.8%) were high school graduates. The number of married participants were 331 (34.4%). The number of participants who smoke and drink alcohol were 517(53.7%) and 139 (14.4%), respectively. According to WHO BMI classification the number of obese participants were 139 (14.4%).

Table 2 has shown that nutritional supplements used by participants were vitamin B12, fish oil, vitamin C, Ca, Zinc, vitamin D, iron, probolis, bamboxsand, bee pollen. Six of the 27 participants (22.21%) who used vitamin-mineral supplements indicated that they used vitamin supplements for more than three months. The most commonly used vitamin is vitamin D with a rate of 29.6%.

Table 3 has shown that among participants using vitamin-mineral supplements, obesity rate (11.1%) was lower than in participants who did not use vitamin-mineral supplements (15.0%).There was no statistically significant relationship between the use of vitamin-mineral supplements and BMI ($\chi^2=3.65$; $p>0.05$).

While 27.9% of the participants indicated that the shift work system was effective on weight gain and

Table 1. Demographic characteristics of participants

Demographic characteristics	Group	Number (n)	Percent(%)
Demographic characteristics gender	Female	24	2,5
	Male	939	97,5
Education	Primary School	52	5,4
	Secondary School	314	32,6
	High School	499	51,8
	University	81	8,4
	Postgraduate	17	1,8
Marital Status	Single	632	65,6
	Married	331	34,4
Smoking	Yes	517	53,7
	No	294	30,5
	Stop	152	15,8
Alcohol Use	Yes	139	14,4
	No	698	72,3
	Stop	128	13,3
Vitamin-mineral supplementation	Yes	27	2,8
	No	936	97,2
BMI	Normal (18,5-24,9)	271	28,1
	Overweight (25,0- 29,9)	549	57,0
	Obese (30-39,9)	139	14,4
	Morbid obese (≥40)	4	0,4

Table 2. Vitamin-minerals used by participants and frequency of use

Vitamin-mineral	Number (n)	Percent (%)
Vitamin B12	5	18,5
Fish oil	1	3,7
vitamin C	6	22,2
Ca	1	3,7
Zinc	1	3,7
Vitamin D	8	29,6
Iron	4	14,8
Probolis, Bamboks, Bee polen	1	3,7
Duration of Vitamin-minerals used		
0-1 month	10	37,03
1-3 months	11	37,73
>3 months	6	22,21

they did, 43.9% of them have stated that the shift work system was not effective on weight gain.

Table 4 has shown that there was a significant correlation between the BMI and the type of shift system (χ^2=22.82; p<0.05). Participants working in the monthly shift change system were more likely to be overweight, obese and morbid obese.

Table 5 has shown that there was a significant correlation between BMI and average shift time interval (χ^2=39,90; p<0,05). Participants who had average one time and four times shifts were found to have higher obesity and morbid obese tendencies.

Discussion

With the industrial revolution, the shift work system used intensively in factories and mines, this sys-

Table 3. Relationship between the use of vitamin-mineral and BMI

Use of vitamin-mineral	BMI								χ^2	p
	Normal		Overweight		Obese and morbid obese		Total			
	n	%	n	%	n	%	n	%		
Use of vitamin-mineral										
Yes	12	44,4	12	44,4	3	11,1	27	100,0	3,65	0,16
No	259	27,7	537	57,4	140	15,0	936	100,0		
Total	271	28,1	549	57,0	143	14,8	963	100,0		

Table 4. The relation between shift type and BMI

Shift type	BMI								χ^2	p
	Normal		Overweight		Obese and morbid obese		Total			
	n	%	n	%	n	%	n	%		
Weekly	189	32,9	319	55,6	66	11,5	574	100,0		
Monthly	82	21,1	230	59,1	77	19,8	389	100,0	22,82	0,00
Total	271	28,1	549	57,0	143	14,8	963	100,0		

Table 5. The correlation between average night shift time and BMI

Average night shift time	BMI								χ^2	p
	Normal		Overweight		Obese and morbid obese		Total			
	n	%	n	%	n	%	n	%		
Once	33	20,1	91	55,5	40	24,4	164	100,0		
Twice	105	37,4	151	53,7	25	8,9	281	100,0		
Three times	104	29,0	209	58,2	46	12,8	359	100,0	39,90	0,00
Four times	29	18,2	98	61,6	32	20,1	159	100,0		
Total	271	28,1	549	57,0	143	14,8	963	100,0		

tem is an indispensable part of many industries (11). However, deterioration of worker's body balances due to shifts time intervals leads to social and physical negativities, which is affirmed as a drawback of the system (12). The current literature points out that there is a significant relationship between loss of sleep, nutrition and metabolic changes (13). In his study, Ermis shown that the individuals sleeping less are more likely to be obese (14). This may be attributed to the increased level of ghrelin hormone and decreased the level of leptin hormone (15), but it is also a major risk for obesity, especially in shift workers, that the distortion of dietary habits and pattern of diet and continual meal-skipping are observed continuously (16). This study was conducted to examine the nutritional status of shift workers and to determine the frequency of obesity.

In this study, 53.7% of participants smoke and 14.4% of them use alcohol. In a survey conducted with 2090 shift workers in Korea, use of alcohol ($p<0.015$) and smoke ($p<0.001$) were found to be more frequent as compared to whom work daytime and three shift (6 hours) (17). In a study performed by Esquirol *et al.*, there is no difference between smoking and alcohol use in shift workers as compared to daytime workers. This situation can be explained by the personal habits of smoking and alcohol consumption (18.

While it was observed that 549 (57%) and 143 (14.8%) of participants were overweight, obese and morbid obese, a study reported that 48% of workers, 65 male and 44 female, were overweight and obese (19). Similarly, in a survey conducted in Norway, it was seen that as night work hours increased, BMI was increased (20). In this case, it can be interpreted that the eating habits of individuals working in the shift system and getting food healthfully are affected by irregular working hours, and thus he incidence of obesity is more frequent in parallel with this.

In this study, nutritional supplements used by participants were vitamin B12, fish oil, vitamin C, Ca, Zinc, vitamin D, iron, probolis, bamboxs, bee pollen. Vitamin C (22%) and vitamin D (25.9%) were the ones used mostly. Research conducted by Linseisen *et al*. has shown that intake of zinc, vitamin A and D, as well as dietary fiber, were also below the level recommended (21). A study conducted in Japan showed that vitamin D levels in serum of participants were also in normal range and these are similar in level between shifts period (22). In a study conducted by Romano *et al*. on 196 workers in Italy, serum 25-OH vitamin D levels of workers working at night (13.4±5.3 ng/mL versus 21.9±10.7 ng/mL, p<0.001)was found lower as compared to whom work daytime (23). This result may be due to reduced making use of sunlight by shift workers and the use of vitamin D supplementation. Participants may also need to use food supplements due to inadequate nutritional status.

In this study, obesity-morbid obesity was observed higher in those who did not take vitamins and minerals as compared to whom take, and there was no statistically significant difference. There was no significant correlation between BMI and the use of vitamin-mineral supplements (χ^2=3.65; p>0.05). In a study conducted by Y Li *et al*., a relationship between use of vitamin-mineral supplements and parameters such as BMI, body fat mass were investigated and found that 87 females taking multivitamin-mineral supplements had significantly lower BMI despite placebo (24). Similarly, another randomized, double-blind, placebo-controlled study reveals the significant correlation between use of multivitamin-mineral supplements and the control appetite, increased resting energy consumption, and weight loss (25). This may be related to the energy thermogenesis contribution of vitamins and minerals to improve energy consumption and/or to decrease energy intake.

In this study, 27.9% of participants indicated that the shift work system was effective on weight gain and they did, whereas 43.7% of them stated that the shift work system was not effective on weight gain. Participants who worked in the monthly shift change system, were found to have a higher tendency to be overweight, obese and morbid obese, and thus a significant correlation was found between BMI and the type of shift system worked at (χ^2=22,82; p<0,05). In the study involving male and female nurses in Korea, 0.9 kg weight gain was observed among nurses during the five years period of shift work, and there was no statistically significant correlation found. There are studies in the literature that have no significant difference regarding BMI between workers at daytime and at night-time shift, too (26). This can be explained by the effect of differences in nutritional status of shift workers outside the working life on weight gain, or the dietarydifferences that employees prefer in the working time.

Fast-food (sandwiches, Turkish doner, hamburger, etc.) by 8.7% have the lowest rate and 24.1% of the participants consumed caffeinated beverages, whereacid was observed that nutritional impairment was due to difficulty in reaching healthy foods. Similarly, in the study performed by Zverev and Misiri on shift workers showed that increasing the tendency to have a snack (27). In a study conducted, most of the 109 participants stated that as a result of skipping meals, they consumed more high-energy snacks such as crisps, chocolate, biscuits (28). In the retrospective study using the data of the Cancer and Nutrition Cohort Study in the Netherlands, 7173 daytime workers were compared with 683 night-timeworkersregarding nutrition, and it was observed that night-time workers preferred high-sugar containing snacks (29). This can be explained by the fact that shift workers cannot leave the working environment (to go places such as markets, groceries, etc.), and because there were no places available for food preparation, they prefered to eat foods which wereeasy to access, practical and accessible without wasting time.

In this study, it was observed that the breakfast is the most skipped meal for the day and night shift

workers. Daytime workers (40.7%) are more likely to skip breakfast as compared to those work night-times (38.8%). In a study conducted in Japan, it was observed that night shift workers were delayed during meal times as compared to daytime workers, and breakfast was frequently omitted (30). Similarly, in a study performed in southern France, it was shown that daytime workers consume four or five meals while night shift workers eat more than five meals a day. Shift workers consumed breakfast and lunch 30% and 10% less than it should be, respectively (31). This can be explained by the fact that daytime shift workers wake up in the early hours and do not feel hungry or they do not want to eat something in the morning because they are sleepy, so they skip breakfast, and in the case of night-time, night shift workers skip breakfast and prefer sleeping because they work until very late.

In this study, 33.4% of participants stated that they consumed full-fat cheese among the milk and dairy products, while 97.3% of participants never consumed skimmed milk. In a survey conducted in Kanazawa University on 2254 workers, it was shown that 408 workers, those working at night shifts especially in between 20-29 years of age, had lower consumption of dairy products than daytime workers (32). In a similar study conducted with 41 airport staff, dairy consumption was found to be lower among daytime staff. This result can be explained by the fact that they think light products are harmful to them or tasteless and thus prefer to eat full-fat product (31).

In this study, 26.6% of participants indicated that they consumed eggs every day. In a survey conducted in Kanazawa University on 2254 workers, it was reported that the red meat consumption by night-time workers was lower than that by daytime workers (30). Similarly, in a study conducted with 207 workers working daytime and 210 workers working in three shifts registered to the health system of the Canary Islands, it was reported that shift workers consumed more red meat and eggs (32). In the retrospective study conducted-with the data of Cancer and Nutrition Cohort study in the Netherlands, 7173 daytime workers were compared with the 683 night-time workersregarding nutrition, and it was observed that red meat and fish consumption of night-time workers were higher.Higher consumption of red meat and eggs may indicate that obesity is more prevalent in occupational groups working in shifts, with an increased intake of saturated fat (33). In this study, 77.6% of participants indicated that they consumed tea every day. In a similar survey conducted by Mashadi*et al*. in Iran on airports staff, the consumption of hot drinks such as tea and coffee were found to be higher in daytime workers (31). On the contrary, in a study involving 66 shift workers in Brazil, consumption of alcoholic beverages increased as the number of night-time shifts increased (18). This can be explained by differences in beverage choices of individuals due to cultural differences. Participants were found to consume tomato regularly up to the rate of 6.3%. In a survey conducted in Kanazawa University on 2254 workers, it was reported that fresh vegetable consumption of night-time workers was lower than those of daytime workers (30). In the study done by Knutson *et al.,* workers transferred to shift work, consumption of fresh vegetable-fruit was reported to be low (33). This may be explained by the limited length of cooking time due to the extent of shift duration of workers and the less preference for vegetable dishes, which is referred to as cookware meal, as they prefer practical and less time-consuming dishes and bread, such as macaroni, instead.

Conclusion

Nutrition is one of the most critical factors that affect health as well as constitute one of the basic necessities of a human being. In this study, it was observed that the security guards working in shifts skipped meals, mainly breakfast and had an inadequate and unbalanced nutrition habits due to irregular working hours. It is important to determine the nutritional status of shift workers who continue their lives in indefinite working hours, to increase work efficiency and to reduce the burden of society.

In this context, the policies in the country, where the conditions are challenging, should re-examine the working conditions and hours. Employers or workplaces may be required to provide health refreshment including fruit, nuts, yogurt, etc. to their employees at certain times to overcome the difficulties in accessing healthy food and thus omitting meals because of shift

times. In particular, employers may offer breakfast meals for those who start very early in the morning shift.

References

1. Wahlqvist ML. Nutrition and prevention of chronic diseases: a unifyingeco-nutritionalstrategy. Nutrition, Metabolism and Cardiovascular Diseases 2014; 14: 1-5.
2. Leech RM, McNaughton SA, Timperio A. The clustering of diet, physical activity and sedentary behavior in children and adolescents: a review. International Journal of Behavioral Nutrition and Physical Activity 2014; 11(1): 4.
3. Al-Nakeeb Y, Lyons M, Dodd LJ, Al-Nuaim A. An investigation into the lifestyle, health habits and risk factors of young adults. International Journal of Environmental Research and Public Health 2015; 12(4): 4380-4394.
4. Nasreddine L, Naja F,Tabet M, Habbal MZ, El-Aily A, Haikal C, Sidani S, Adra N, Hwalla N. Obesity is associated with insulin resistance and components of the metabolic syndrome in Lebanes eadolescents. Annals of Human Biology 2012; 39(2): 122-128.
5. Rinaldi AE, de Oliveira EP, Moreto F, Gabriel GF, Corrente JE, Burini RC. Dietary intake and blood lipid profile in overweight and obese school children. BMC Research Notes 2012; 5: 598.
6. Alsheikh-Ali AA, Omar MI, Raal FJ, Rashed W, Hamou O, Kane A, Alami M, Abreu P, Mashhoud WM. Cardiovascular risk factor burden in Africa and the middleeast: the Africa middleeast cardiovascular epidemiological (ACE) study. PLoSOne 2014; 9(8): e102830.
7. Salim EI, Moore MA, Al-Lawati JA, Al-Sayyad J, Bazawir A, Bener A, Corbex M, El-Saghir N, Habib OS, Maziak W, Mokhtar HC, Seif-Eldrin IA, Sobue T. Cancer epidemiology and control in the Arab world–past, present and future. Asian Pacific journal of cancer prevention 2009; 10(1): 3-16.
8. World Health Organization (WHO). October 2017. Obesity and overweight. .Retrieved on May 30, 2018 from WHO Website: http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
9. Letourneux V. Precarious Employment and Working Conditions in Europe. Dublin, Ireland: Wyatt Ville Road Loughlinstown. 1993.
10. Baysal A. Beslenme. 10th ed. Ankara: Hatiboğlu Yayınları. 2004
11. Korkusuz R. Vardiyalı (postalar halinde) çalışma ve Türk iş hukukundaki düzenlemesi. Gazi Üniversitesi Hukuk Fakültesi Dergisi 2005;9(1-2), 1-18.
12. Yücecan S. Optimal beslenme. Hacettepe Üniversitesi Sağlık Bilimleri Fakültesi Beslenme ve Diyetetik Bölümü. Ankara: Klasmat Matbaacılık. 2008.
13. Günaydın N. Bir Devlet hastanesinde çalışan hemşirelerin uyku kalitesi ve genel ruhsal durumlarına etkisi. Journal of Psychiatric Nursing 2014;5(1), 33-40.
14. Ermiş E, Doğan E, Erilli NA, Satıcı A. Üniversite öğrencilerinin beslenme alışkanlıklarının incelenmesi: Ondokuz Mayıs Üniversitesi Örneği. Spor ve Performans Araştırmaları Dergisi 2015; 6(1): 30-40.
15. Manenschijn L, van Kruysbergen RG, de Jong FH, Koper JW, van Rossum EF. Shift work at young age ıs associated with elevated long-term cortisol levels and body mass index. The Journal of Clinical Endocrinology & Metabolism 2011; 96(11): 1862-1865.
16. Zhao I, Turner C. The impact of shiftwork on people›s daily health habits and adverse health outcomes. Australian Journal of Advanced Nursing 2008; 25(3): 8.
17. Oh J, Yim HW. Association between rotating night shift work and metabolic syndrome in Korean workers: differences between 8-hour and 12-hour rotating shift work. Industrial Health 2018; *7*; 56(1): 40-48.
18. Esquirol Y, Bongard V, Mabile L, Jonnier B, Soulat JM, Perret B. Shift work and metabolic syndrome: respective impacts of job strain, physical activity and dietary rhythms. Chronobiology International 2009; 26(3): 544-559.
19. Nea FM, Pourshahidi LK, Kearney JM, Livingstone MBE, Bassul C, Corish CA. A qualitative exploration of the shift work experience: the perceived effect on eating habits, life style behaviours and psychosocial wellbeing. Journal of Public Health 2018; 1-11.
20. Buchvold HV, Pallesen S, Øyane NM, Bjorvatn B. Associations between night work and BMI, alcohol, smoking, caffeine and exercise - a cross-sectional study. BMC Public Health 2015; 15: 1112.
21. Linseisen J, Wolfram G. Nährstoffzufuhr bei Dauernachtschicht-Arbeitern [Nutrient intake in permanent night shift workers]. Zeitschrift für Ernährungswissenschaft 1994; 33(4): 299-309.
22. Itoh H, Weng Z, Saito H, Ogawa Y, Nakayama K, Hasegawa-Ohira M, Morimoto K, Maki S, Takahashi M. Association between Night-shift Work and Serum 25-hydroxyvitamin D Levels in Japanese Male Indoor Workers: A Cross-sectional Study. Industrial Health 2011; 49(5): 658-62.
23. Romano A, Vigna L, Belluigi V, Conti DM, Barberi CE, Tomaino L, Consonni D, Riboldi L, Tirelli AS, Andersen LL. Shift work and serum 25-OH vitamin D status among factory workers in Northern Italy: Cross-sectional study. Chronobiology International 2015; 32(6): 842-847.
24. Li Y, Wang C, Zhu K, Feng RN, Sun CH. Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. International Journal of Obesity 2010; 34: 1070-077.
25. Major GC, Doucet E, Jacqmain M, St-Onge M, Bouchard C, Tremblay A. Multivitamin and dietary supplements, body weight and appetite: results from a cross-sectional and a randomised double-blind placebo-controlled study. British Journal of Nutrition 2008; 99: 1157-1167.
26. Ha M, Park J. Shiftwork and metabolic risk factors of cardiovascular disease. The Journal of Occupational Health 2005; 47: 89-95.

27. Zverev YP, Misiri HE. Perceived effects of rotating shift-work on nurses' sleep quality and duration. Malawi Medical Journal 2009; 21(1): 19-21.
28. Hulsegge G, Boer JM, van der Beek AJ, Verschuren WM, Sluijs I, Vermeulen R, Proper KI. Shift workers have a similar diet quality but higher energy intake than day workers. Scandinavian Journal of Work, Environment & Health 2016; 42(6): 459-468.
29. Yoshizaki T, Kawano Y, Noguchi O, Onishi J, Teramoto R, Sunami A, Yokoyama Y, Tada Y, Hida A, Togo F. Association of eating behaviours with diurnal preference and rotating shift work in Japanese female nurses: a cross-sectional study. BMJ Open 2016; 28; 6(11): e011987.
30. Morikawa Y, Miura K, Sasaki S, Yoshita K, Yoneyama S, Sakurai M, Ishizaki M, Kido T, Naruse Y, Suwazono Y, Higashiyama M, Nakagawa H. Evaluation of the Effects of Shift Work on Nutrient İntake: A Cross-sectioanal Study. Journal of Occupational Health 2008; 50: 270-278.
31. Mashhadi NS, Saadat S, Afsharmanesh MR, Shirali S. Study of association between beverage consumption pattern and lipid profile in shift workers. Diabetes &Metabolic Syndrome: Clinical Research & Reviews 2016; 10(4): 227-229.
32. Fernández Rodríguez MJ, Bautista Castaño I, Bello Luján L, Hernández Bethencourt L, Sánchez Villegas A, Serra Majem L. Nutritional evaluation of health shift workers from the Canary Islands. Nutrición hospitalaria 2004; 19(5): 286-91.
33. Knutson A, Andersson H, Berglund U. Serum lipoproteins in day and shift workers: a prospective study. British Journal of Industrial Medicine 1990; 47(2): 132-134.

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Original article

Contribution of food groups to sodium and potassium intakes by their ratio in Korean adults

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Summary. *Background/Aims:* Koreans have relatively high sodium intakes, and a higher sodium-to-potassium (Na/K) ratio is associated with increased mortality resulting from cardiovascular diseases. The purpose of this study was to analyze the dietary patterns and food groups that contribute to sodium and potassium intakes according to dietary Na/K ratio in Koreans. *Methods:* A 24-hour dietary recall was collected twice from 640 healthy adults (aged 19-69 years) in four Korean provinces. The subjects were divided into groups of Na/K<1 and Na/K≥1, and their dietary pattern focused on sodium and potassium intakes and Na/K ratio of major food groups were analyzed and compared. *Results:* The average Na/K ratio of the subjects was 1.5; the highest ratio was in the twenties. Daily sodium and potassium intakes were significantly high in the Na/K≥1 group and the Na/K<1 group, respectively. Sodium intake from grains, meat, fish, and seasonings of the Na/K≥1 group and potassium intake from potatoes, beans, nuts, and fruits of the Na/K<1 group were significantly higher than the counterpart groups. Food groups with the lowest Na/K (0.1) were fruits, cooked rice, and beans, and a Na/K ratio greater than 10.0 included seasonings and salted seafood. The Na/K<1 group consumed more fruits and potatoes and the Na/K≥1 group consumed more grains, beverages, meats, and seasonings. *Conclusions:* Dietary Na/K ratio of Koreans was significantly higher than Korean or WHO guidelines. Recommendation of reducing sodium intake from grains, meats, and seasonings and increasing potassium intake from fruits, potatoes, and beans may be suggested for lowering the Na/K ratio.

Key words: sodium, potassium, Na/K ratio, food group, 24-hour dietary recall

Introduction

Excessive sodium intake can induce hypertension, stroke, cardiovascular diseases (CVD), and renal diseases, and can also accelerate osteoporosis (1-5). Therefore, to improve public health, sodium intake should be reduced. The Ministry of Health and Welfare in Korea launched Health Plan 2020 in an attempt to reduce 15% of the population's sodium intake to less than 2000 mg/day (6). Additionally, the Ministry of Food and Drug Safety implemented a similar sodium reduction project with the goal of decreasing sodium intake by 20% (3900 mg/day) by 2017. When the goal was achieved by 2014, a second goal was set of reducing sodium intake to 3500 mg/day by 2020.

In the last ten years, the average Korean daily sodium intake was 5256.6 mg in 2005, 4831.1 mg in 2010, 4583.1 mg in 2012, and 3889.9 mg in 2014 (7).

Sodium intake has continuously decreased since 2010 when national initiatives actively promoted sodium reduction. However, the average daily sodium intake of Koreans is still twice the recommended value of the World Health Organization (8). Approximately 80% of the population ingests more than 2000 mg of sodium per day (7).

Appropriate potassium intake along with sodium reduction aids in the excretion of excess sodium (9, 10), promotes bone health by suppressing urine excretion of calcium as a result of excessive sodium intake, and helps prevent hypertension and stroke (11-13). Despite the health benefits of appropriate potassium intake, potassium is the second least ingested nutrient in Korea after calcium. The Korea National Health and Nutrition Examination Survey (KNHANES) reported that the average daily potassium intake level was 2773.2 mg in 2005, 2843.0 mg in 2008, 2998.5 mg in 2010, 2918.0 mg in 2012, and 2983.3 mg in 2014. All levels were lower than 3500 mg, the recommended intake (RI) for adults (14), and the percentage of people who did not meet the RI was about 80%, suggesting a high rate of insufficient potassium intake.

In addition, the sodium-to-potassium (Na/K) ratio can affect the risk of hypertension (15). Cook et al. (16) reported that the Na/K in urine is correlated to blood pressure and serves as a strong predictive risk factor for CVD prediction in comparison to simple sodium or potassium levels. The INTERSALT study, an international study of electrolyte excretion and blood pressure, also reported that the urinary Na/K ratio in female is associated with increased mortality rates resulting from cerebrovascular diseases (17). Therefore, researches are needed to investigate the dietary Na/K ratio and suggest adequate intake level.

However, studies on the dietary Na/K ratio are still insufficient in Korea. The KNHANES investigated sodium and potassium intakes separately, but analysis of their ratio was not conducted. While the survey investigated the sodium and potassium content in each food group and the annual trends in source foods and food groups with high contribution rates, no study has been conducted that investigates the dietary patterns of the Na/K ratio. To solve both problems, high sodium intake and low potassium intake, at the same time, it is necessary to evaluate the dietary Na/K ratio and to suggest effective dietary strategies for simultaneously lowering sodium intake and increasing potassium intake. Therefore, the purpose of this study was to investigate the dietary patterns and food groups that contribute to sodium and potassium intakes according to dietary Na/K ratio in Korean adults with typical common diet.

Materials and Methods

Subjects

Between August and December 2014, 640 healthy adults (320 males, 320 females) were recruited through city halls, health centers, university home pages, and senior welfare facilities in the following four provinces of Korea: Seoul, Chungcheong, Gyeongsang, and Jeolla. Individuals were excluded if they were taking medicine, pregnant or lactating, on dietary control, or diagnosed with hypertension, heart failure, diabetes, renal diseases or cancer. A total of 160 subjects were recruited from each region, and 64 males and 64 females were recruited from each of the following age groups: 19-29 years, 30-39 years, 40-49 years, 50-59 years, and 60-69 years. WHO recommended that the general population consume a sufficient amount of potassium to maintain the molar ratio of Na/K of 1:1 (8). The recommended Na/K ratio in Korea is 0.57 (i.e., 2000 mg sodium per day and 3500 mg potassium per day) as a weight ratio (14). However, most people do not meet this recommended level because they consume too much sodium and not enough potassium. The average Korean Na/K ratio was 1.3 (i.e., 3889.9 mg sodium per day and 2983.3 mg potassium per day) in 2014 (7). Yang et al. (18) reported a beneficial protective effect of usual Na/K<1 on CVD and all-cause mortality. Therefore, the subjects of this study were divided into two groups (<1 and ≥1) depending on the dietary Na/K ratio. The study protocol was approved by the Institutional Review Board of Kyungpook National University (KNU 2014-0053), and all subjects provided their written informed consent to participate in the study.

Analysis of sodium and potassium intakes

A 24-hour dietary recall survey was conducted twice for meals on ordinary days, excluding weekends

and holidays, and the survey was carried out by trained dieticians following the method of the Food Intake Survey in the KNHANES. To ensure efficient dietary recall, subjects directly documented meal information on a provided record sheet. Subjects were pre-trained to take a picture of each meal, and the recall survey was conducted through direct interviews. To increase the accuracy of survey intake reporting, Food Photos for Quantity Estimation: Korean Genome and Epidemiology Study (19) were used in conjunction with comparative measuring tools, such as food models and rulers.

The results of the food intake survey were analyzed using a nutritional assessment program, CAN-Pro 4.0 (Computer Aided Nutritional Analysis Program version 4.0, Korea Nutrition Society, 2010). The main database of the CAN-Pro 4.0 is based on the National Standard Food Composition Table (20, 21). For processed foods and foods not registered in CAN-Pro 4.0, calculations were performed by adding the nutrient contents based on the nutrition facts of the corresponding food.

Analysis of Na/K ratio

Based on the classification standards of the KNHANES and the National Standard Food Composition Table for each food, this study classified a total of 16 food groups: grains, potatoes, sugars, beans, nuts and seeds, vegetables, mushrooms, fruits, meats, eggs, fish and shellfish, seaweeds, milk, oils and fat, beverages, and seasonings. This study also classified a total of 31 food groups for detailed evaluation of sodium and potassium sources. The sodium and potassium intake and their ratio of the listed food or detailed food groups were calculated.

Statistical analysis

All data of this study were statistically analyzed with the SAS 9.3 program (Version 9.3 SAS Institute, Cary, NC, USA). A *t*-test for continuous variables and chi-square test for categorical variables were used to assess differences between the Na/K groups, and ANOVA and Duncan's multiple-range tests were used to assess differences among age groups. The level of significance of all analyses was set to $p<0.05$.

Results

General characteristics

Table 1 shows the general characteristics of the subjects according to Na/K ratio. Na/K<1 subjects had lower proportion of men, higher age and lower height and weight compared with Na/K≥1 subjects ($p<0.05$). The average Na/K ratio of the subjects was 1.5; the highest ratio was in the twenties (Figure 1).

Daily sodium and potassium intakes

Sodium and potassium intakes in the two groups of the Na/K ratio are shown in Table 2. Daily energy intake was significantly lower in the Na/K<1 group than in the Na/K≥1 group (1813.5 kcal vs. 1972.3 kcal, $p<0.01$). Daily total intake or intake per 1000 kcal of the Na/K<1 group was significantly lower than in the Na/K≥1 group for sodium and was higher for potassium ($p<0.001$). The proportion of the subjects who consumed >2000 mg sodium in the Na/K<1 group was significantly lower than that in the Na/K≥1 group (74.8% vs. 95.5%, $p<0.001$). The proportion of subjects who ingested <3500 mg potassium was significantly lower in the Na/K<1 group than in the Na/K≥1 group (64.9% vs. 84.1%, $p<0.001$).

Sodium and potassium intakes from food groups

Table 3 shows daily sodium and potassium intakes from each food group between the subjects according to the Na/K ratio. Sodium intake from grains, meat, fish, and seasonings showed a significant difference between the Na/K groups and was higher in the Na/K≥1 group. Potassium intake from potatoes, beans, nuts, and fruits was significantly higher in the Na/K<1 group, while potassium intake from meat and seasonings was higher in the Na/K≥1 group. Table 4 summarizes the Na/K ratio of detailed food groups. The food groups with the lowest Na/K ratio (0.1) were fruits, cooked rice, and beans/nuts/seeds; seasonings and salted seafood were the food groups with a Na/K ratio greater than 10.0.

Comparison of food intakes between the Na/K groups

Daily intake of each food group between the Na/K groups is shown in Figure 2. Fruits and potatoes intakes of the Na/K<1 group was significantly higher

Table 1. General characteristics of the subjects according to dietary Na/K ratio

	Na/K<1	Na/K≥1	p value
All subjects	n=111	n=529	
Men, %	32.4	53.7	<0.001
Age, y	50.9±12.3[1)]	42.2±14.5	<0.001
Height, cm	162.3±8.5	165.9±8.3	<0.001
Weight, kg	61.6±10.5	64.5±11.6	0.015
BMI, kg/m^2	23.3±2.9	23.3±3.1	0.910
Waist circumference, cm	81.5±9.5	81.4±9.4	0.907
Hip circumference, cm	95.7±6.6	96.5±5.9	0.214
WHR	0.85±0.06	0.84±0.07	0.242
Men	n=36	n=284	
Age, y	52.2±12.9	42.6±14.2	<0.001
Height, cm	171.3±5.9	171.5±5.8	0.881
Weight, kg	71.1±8.9	71.5±9.7	0.845
BMI, kg/m^2	24.3±2.8	24.3±3.0	0.934
Waist circumference, cm	87.7±9.4	85.8±8.0	0.182
Hip circumference, cm	99.1±7.2	98.3±5.8	0.441
WHR	0.88±0.05	0.87±0.05	0.248
Women	n=75	n=245	
Age, y	50.2±12.0	41.7±14.8	<0.001
Height, cm	158.0±5.7	159.4±5.7	0.059
Weight, kg	57.0±7.8	56.4±7.8	0.562
BMI, kg/m^2	22.8±2.9	22.2±2.9	0.104
Waist circumference, cm	78.5±8.0	76.3±8.3	0.040
Hip circumference, cm	94.0±5.6	94.3±5.3	0.674
WHR	0.83±0.06	0.81±0.06	0.001

[1)] *Mean±standard deviation*

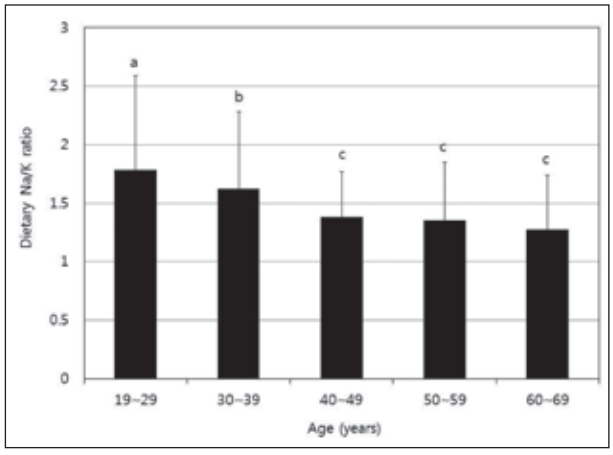

Figure 1. Dietary Na/K ratio of the subjects according to age groups (each group: n=128). Different letters indicate significant difference among age groups by Duncan's multiple range test at p<0.05. Different letters (a, b, c) over the graph indicate significantly difference among age groups at p<0.05 by Duncan's multiple-range test

than the Na/K≥1 group. However, the Na/K≥1 group significantly and highly consumed grains, beverages, meats, and seasonings compared to the Na/K<1 group (p<0.001).

Discussion

In this study, the dietary pattern and contributing food groups of sodium and potassium according to Na/K levels of Korean adults were analyzed. As main results, sodium intake from grains, meat, fish, and seasonings of the Na/K≥1 group and potassium intake from potatoes, beans, nuts, and fruits of the Na/K<1 group were significantly higher than the counterpart groups. Also, the Na/K<1 group consumed more fruits and potatoes and the Na/K≥1 group consumed more grains, beverages, meats, and seasonings.

Table 2. Daily intakes of Na and K of the subjects according to dietary Na/K ratio

	Na/K<1 (n=111)	Na/K≥1 (n=529)	p value
Energy, kcal/d	1813.5±456.4[1)]	1972.3±543.5	0.002
Na			
mg/d	2673.3±1026.3	3982.5±1508.3	<0.001
mg/1000 kcal·d	1464.6±399.4	2056.4±665.9	<0.001
>2000 mg/d, %	74.8	95.5	<0.001
K			
mg/d	3320.0±1320.7	2615.1±891.5	<0.001
mg/1000 kcal·d	1812.2±483.8	1356.5±389.7	<0.001
<3500 mg/d, %	64.9	84.1	<0.001
Na/K	0.8±0.1	1.6±0.6	<0.001

[1)]Mean±standard deviation

Table 3. Daily intakes of Na and K from food groups of the subjects according to dietary Na/K ratio

Food groups	Na		K	
	Na/K<1 (n=111)	Na/K≥1 (n=529)	Na/K<1 (n=111)	Na/K≥1 (n=529)
Grains	267.3±297.5[1)]	572.9±651.3[***2)]	341.1±209.7	333.9±165.4
Potatoes	8.1±20.4	11.1±40.3	443.1±544.1	178.5±266.2[***]
Sugars	1.1±3.4	1.7±7.1	2.4±15.0	3.0±13.2
Beans	19.8±56.6	16.4±58.1	169.9±261.7	85.4±123.6[**]
Nuts and seeds	6.0±12.9	4.9±15.7	58.7±104.6	33.7±69.5[*]
Vegetables	342.9±242.1	375.2±333.3	800.2±414.9	723.3±409.3
Mushrooms	0.4±0.7	0.4±0.8	30.5±56.3	28.4±57.6
Fruits	19.3±19.1	28.5±171.3	462.4±501.0	200.0±229.9[***]
Meats	89.0±145.0	187.4±270.4[***]	134.5±135.0	216.2±201.0[***]
Eggs	38.8±37.3	46.3±56.9	41.0±38.1	47.1±45.7
Fish and shellfish	225.9±213.9	363.1±351.9[***]	200.2±253.2	225.6±200.8
Seaweeds	84.7±188.2	91.4±207.0	146.0±289.7	128.1±242.0
Milk	52.4±73.7	53.0±77.0	119.5±161.8	96.7±149.6
Oils and fat	2.3±9.0	1.1±6.0	2.0±11.0	0.4±2.5
Beverages	19.3±44.4	24.4±49.7	210.2±661.5	113.6±215.2
Seasonings	1496.0±700.5	2204.8±1107.6[***]	158.5±108.6	208.3±187.2[***]
Total	2673.3±1026.3	3982.5±1508.3[***]	3320.0±1320.7	2615.1±891.5[***]

[1)]Mean±standard deviation.
[2)]Significantly different between two groups of Na/K by unpaired t-test. [*]p<0.05, [**]p<0.01, [***]p<0.001

Na/K groups of this study were classified on the basis of previous study that Na/K<1 is associated with a lower risk of mortality resulting from CVD and various diseases (21). Recently, the sodium intake of Koreans by KNHANES was 3889.9 mg/d, exceeding the recommendation of 2000 mg, and the potassium intake was 2983.3 mg/d, which was below the 3500 mg recommendation level. As a result, the dietary Na/K ratio highly exceeded the recommendation of <0.57 (i.e., 2000 mg/d and 3500 mg/d) as well as <1. In a study using NHANES data, it was reported that only <0.015% of the population met the recommenda-

Table 4. Dietary Na/K ratio from detailed food groups of the subjects

Food groups	Na (mg/d)	K (mg/d)	Na/K
Fruits	10.1±15.3[1)]	205.4±305.1	0.1±0.1
Cooked rice	13.7±23.3	215.0±162.1	0.1±0.1
Beans, nuts and seeds	3.7±15.1	34.5±75.6	0.1±0.3
Grains and potatoes	9.0±79.1	131.7±328.7	0.3±1.2
Alcoholic beverages	4.6±13.1	16.3±39.7	0.3±0.3
Vegetables and seaweeds	39.9±176.4	77.6±157.8	0.4±1.0
Beverages and tea	44.4±85.4	175.9±367.2	0.5±1.3
Others	6.5±47.4	17.1±131.9	0.6±1.4
Milk and dairy products	40.2±62.2	88.2±147.3	1.0±2.3
Fats and oils	0.3±3.5	0.3±4.6	1.0±0.4
Meat, fish and eggs	15.2±56.0	23.4±105.9	1.0±1.6
One-dish meals	207.9±456.4	144.1±206.4	1.5±1.1
Kimchi	253.1±282.8	155.1±139.8	1.6±1.2
Stews	253.4±363.2	152.3±215.6	1.8±0.9
Roasted foods	183.9±574.6	112.3±209.8	1.9±2.6
Seasoned vegetables	314.4±372.5	192.6±236.5	2.0±2.6
Stir-fried foods	411.0±431.4	219.2±237.1	2.2±1.5
Pan-fried foods	97.0±187.1	49.7±93.5	2.2±2.7
Sugars	8.4±46.0	4.1±18.3	2.3±4.1
Soups	481.5±499.3	239.8± 257.3	2.3±1.6
Fried foods	125.2±248.0	74.9±150.6	2.4±2.2
Steamed foods	137.0±267.0	65.8±147.3	2.9±2.0
Meat and fish products	45.1±128.6	15.7±41.6	3.1±2.0
Bread and snacks	182.5±287.3	69.5±114.8	3.3±11.9
Braised foods	158.9±252.0	64.8±108.8	3.3±1.8
Porridge	28.8±113.1	27.4±122.6	3.6±6.8
Noodles	431.4±676.4	116.8±164.3	3.9±3.1
Rice cake	44.0±118.5	14.0±43.6	5.1±3.0
Pickled and salted foods	98.9±301.6	17.2±38.6	8.0±5.9
Seasonings	71.6±169.9	11.0±24.9	12.6±46.2
Salted seafoods	33.5±122.9	5.9±30.5	14.6±13.1

[1)] *Mean±standard deviation*

tion of Na/K<0.83 (22). Baily et al. (23) also reported that about a quarter of US adults meet the Na/K<1. In the present study, sodium intake of the subjects was 3755.4 mg/d and the potassium intake was 2737.3 mg/d thus, the average Na/K ratio was 1.5. About 17% of the subjects were Na/K<1, similar to previous study (23). These results show the necessity of research to suggest both dietary guidance and a strategy to lower Na/K intake.

Dietary Na/K ratio decreased with age, and subjects in their 20s had the most undesirable level. This finding is believed to be attributed to the trend of individuals in their twenties having a high consumption rate of processed foods such as noodles and bread/snacks, which have high sodium and low potassium contents (24). Further, this age group showed a lower intake of vegetables and fruits, which have high potassium content. The subjects with Na/K<1 had a

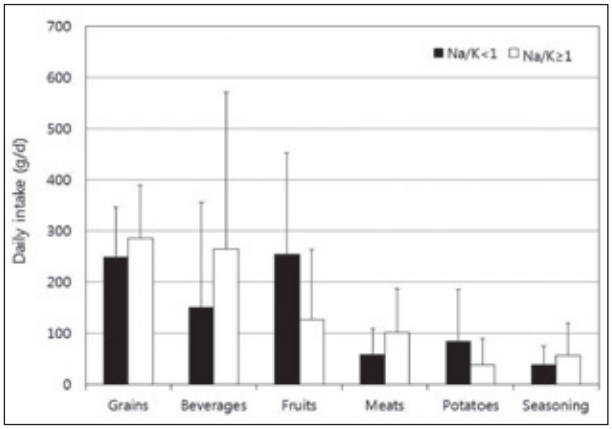

Figure 2. Daily food intakes of the subjects according to dietary Na/K ratio (Na/K<1: n=111; Na/K≥1: n=529). Six food groups in the figure show significant difference between two groups of Na/K<1 and Na/K≥1 by unpaired Student's *t*-test at p<0.001

lower number of males than subjects with Na/K≥1. This shows that men have a higher Na/K intake than women. This observation is believed to be attributed to the notion that females tend to ingest more fruit and grains/potatoes, which have low sodium and high potassium contents. Nagata et al. (25) reported a 2.4-fold increased risk of death from stroke associated with high sodium intake in men but, in women, the association of sodium intake with stroke mortality was weaker. Considering sodium and potassium intakes related to health (25, 26), nutritional education of appropriate sodium and potassium intake is needed, especially in males and individuals in their twenties.

In this study, sodium intake from grains, meat, fish, and seasonings was higher in Na/K≥1 subjects than in Na/K<1 subjects. These results may be related to the eating patterns of Koreans. The major foods contributing to sodium intake in Korea are liquids in soups and stews, fermented foods such as kimchi and salted fish, and noodles such as ramen (7, 27). This study also found that sodium intake from soups was the highest followed by noodles, seasoned vegetables, stews, and kimchi. Ogawa et al. (28) reported that miso soup and Japanese pickles were the top contributors of sodium intake among 197 common Japanese dishes. Na/K≥1 subjects may have a high intake of grains and potatoes because of their high intake of liquid and kimchi. Some evidence exists that sodium intake is related to energy intake and obesity risk (29, 30). This study also showed similar result that daily energy and sodium intake were higher in the Na/K≥1 group.

The main sources of potassium are unprocessed grains, vegetables and fruits, especially tomato, cucumber, zucchini, eggplant and root vegetables such as sweet potato and potato (7, 27). The present study also found that potassium intake from potatoes, beans, nuts, and fruits was higher in Na/K<1 subjects. In the complex aspect of sodium and potassium intakes, Na/K<1 subjects had a low intake of sodium from grains, meat, fish, and seasonings and a high intake of potassium from potatoes, beans, and fruits.

Reports indicate that blood pressure increases as the Na/K ratio increases (31), and the intake of potassium is appropriate when the molar Na/K ratio is close to 1.0 (8). In the present study, food groups with a Na/K ratio less than 1.0 were fruits, beans, grains and potatoes, vegetables and seaweeds, beverages and tea. Rhee (32) reported that foods with a Na/K ratio less than 1.0 were mushrooms, fruit, potatoes, beans, tea, and seaweeds; foods with a Na/K ratio greater than 1.0 were seasonings and spices, fish and shellfish, sugar, and cereal. These findings are similar to the results of the present study. As a geographical and cultural comparison, Meneton et al. (33) examined food intake patterns of adults and children in France, reporting the foods with the highest Na/K ratio to be cheese, pork, pastry and sugar products, cereal, bread, soup, and fast food, and the foods with the lowest Na/K ratio to be fruits, vegetables, and dairy products.

Na/K≥1 subjects consumed more grains, beverages, meats, and seasonings and less fruits and potatoes compared to Na/K≥1 subjects. These results were similar to the sodium and potassium intake patterns from the food group. As expected, Na/K≥1 subjects consumed more sodium sources and less main sources of potassium. However, grains and beverages intakes were high in Na/K≥1 subjects despite their low Na/K ratio, most likely because of the high sodium intake resulting from the high intake of main dishes and beverages. Considering the results of this study, for lower Na/K intake, a nutritional guide is needed to choose more foods with low Na/K ratio such as fruits, beans, potatoes, vegetables and seaweeds.

This study has several limitations. First, food intake status was surveyed using the 24-hour recall

method, which reflects only short-term food intake levels of individuals. Thus, it is difficult to examine ordinary intake levels. Second, the nutrient database used in this study lacks nutrient data on processed foods, fusion foods, and international foods. Therefore, the sodium and potassium content database of processed foods and other foods needs to be established in advance for accurate examination of the sodium and potassium intake status. Nevertheless, this study differs from previous studies that evaluated sodium or potassium intakes separately. Additionally, the strength of this study is the present results can be effectively applied to dietary guidance that recommends lowering Na/K intake by analyzing sodium and potassium intakes in various food groups and the dietary pattern that focuses on the Na/K ratio.

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References

1. Chobanian AV, Hill M. National heart, lung, and blood institute workshop on sodium and blood pressure: A critical review of current scientific evidence. Hypertension 2000; 35: 858-63.
2. De Wardener H, MacGregor G. Harmful effects of dietary salt in addition to hypertension. J Hum Hypertens 2002; 16: 213.
3. Meneton P, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. Physiol Rev 2005; 85: 679-715.
4. Cook NR, Cutler JA, Obarzanek E, et al. Long term effects of dietary sodium reduction on cardiovascular disease outcomes: Observational follow-up of the trials of hypertension prevention (TOHP). BMJ 2007; 334: 885.
5. Bedford JL, Barr SI. Higher urinary sodium, a proxy for intake, is associated with increased calcium excretion and lower hip bone density in healthy young women with lower calcium intakes. Nutrients 2011; 3: 951-61.
6. Ministry of Health & Welfare. The Health Plan 2020 [Internet]. Ministry of Health & Welfare; 2017 [cited 2017 July 1]. Available from: http://www.mohw.go.kr/front_new/jb/sjb030301vw.jsp?PAR_MENU_ID=03&MENU_ID=0319&CONT_SEQ=257824&page=1.
7. Ministry of Health and Welfare, Korea Centers for Disease Control and Prevention. Korea Health Statistics 2015: Korea National Health and Nutrition Examination Survey (KNHANES VI-2). Cheongwon: Korea Centers for Disease Control and Prevention; 2015.
8. World Health Organization, Food and Agriculture Organization of the United Nations. Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation. Geneva: World Health Organization; 2003. WHO Technical Report Series 916.
9. Jackson EK, Herzer WA. Angiotensin II/prostaglandin I2 interactions in spontaneously hypertensive rats. Hypertension 1993; 22: 688-98.
10. Morris RC Jr, Sebastian A, Forman A, Tanaka M, Schmidlin O. Normotensive salt sensitivity: Effects of race and dietary potassium. Hypertension 1999; 33: 18-23.
11. Frassetto LA, Morris RC Jr, Sellmeyer DE, Sebastian A. Adverse effects of sodium chloride on bone in the aging human population resulting from habitual consumption of typical American diets. J Nutr 2008; 138: 419S-22S.
12. Brancati FL, Appel LJ, Seidler AJ, Whelton PK. Effect of potassium supplementation on blood pressure in african americans on a low-potassium diet: A randomized, double-blind, placebo-controlled trial. Arch Intern Med 1996; 156: 61-7.
13. Bazzano LA, He J, Ogden LG, et al. Dietary potassium intake and risk of stroke in US men and women: National health and nutrition examination survey I epidemiologic follow-up study. Stroke 2001; 32: 1473-80.
14. Ministry of Health and Welfare, The Korean Nutrition Society. Dietary Reference Intakes for Koreans 2015. Sejong: Ministry of Health and Welfare; 2015.
15. Kwok TCY, Chan TYK, Woo J. Relationship of urinary sodium/potassium excretion and calcium intake to blood pressure and prevalence of hypertension among older Chinese vegetarians. Eur J Clin Nutr 2003; 57: 299-304.
16. Cook NR, Obarzanek E, Cutler JA, et al. Joint effects of sodium and potassium intake on subsequent cardiovascular disease: The trials of hypertension prevention follow-up study. Arch Intern Med 2009; 169: 32-40.
17. Xie JX, Sasaki S, Joossens JV, Kesteloot H. The relationship between urinary cations obtained from the INTERSALT study and cerebrovascular mortality. J Hum Hypertens 1992; 6: 17-21.
18. Yang Q, Liu T, Kuklina EV, et al. Sodium and potassium intake and mortality among US adults: prospective data from the Third National Health and Nutrition Examination Survey. Arch Intern Med 2011; 171: 1183-91.
19. Ministry of Health and Welfare, Korea Centers for Disease Control and Prevention. Food Photos for Quantity Estimation: Korean Genome and Epidemiology Study. Cheongwon: Korea Centers for Disease Control and Prevention; 2012.
20. Rural Development Administration. Food Composition Table. 7th ed. Suwon: Rural Development Administration; 2007.
21. Rural Development Administration. Food Composition

Table. 8th ed. Suwon: Rural Development Administration; 2011.
22. Drewnowski A, Maillot M, Rehm C. Reducing the sodium-potassium ratio in the US diet: a challenge for public health. Am J Clin Nutr 2012; 96: 439-44.
23. Bailey RL, Parker EA, Rhodes DG, et al. Estimating sodium and potassium intakes and their ratio in the American diet: data from the 2011-2012 NHANES. J Nutr 2016; 146: 745-50.
24. Webster JL, Dunford EK, Neal BC. A systematic survey of the sodium contents of processed foods. Am J Clin Nutr 2010; 91: 413-20.
25. Nagata C, Takatsuka N, Shimizu N, Shimizu H. Sodium intake and risk of death from stroke in Japanese men and women. Stroke 2004; 35: 1543-7.
26. Du S, Neiman A, Batis C, et al. Understanding the patterns and trends of sodium intake, potassium intake, and sodium to potassium ratio and their effect on hypertension in China. Am J Clin Nutr 2014; 99: 334-43.
27. Lee HS, Duffey KJ, Popkin BM. Sodium and potassium intake patterns and trends in South Korea. Hum Hypertens 2013; 27: 298-303.
28. Ogawa K, Tsubono Y, Nishino Y, et al. Dietary sources of nutrient consumption in a rural Japanese population. J Epidemiol 2002; 12: 1-8.
29. Ma Y, He FJ, MacGregor GA. High salt intake: independent risk factor for obesity? Hypertension 2015; 66: 843-9.
30. Nam GE, Kim SM, Choi MK, et al. Association between 24-h urinary sodium excretion and obesity in Korean adults: A multicenter study. Nutrition 2017; 41: 113-9.
31. Dahl LK, Leitl G, Heine M. Influence of dietary potassium and sodium/potassium molar ratios on the development of salt hypertension. J Exp Med 1972; 136: 318.
32. Rhee MY. Study for the Effect of Natrium Intake on the Prevalence of Cardiovascular Disease. Cheongju: National Institute of Food and Drug Safety Evaluation; 2013. Report No. 13162consumer106.
33. Meneton P, Lafay L, Tard A, et al. Dietary sources and correlates of sodium and potassium intakes in the French general population. Eur J Clin Nutr 2009; 63: 1169-75.

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Original article

Large-scale investigation on healthy status of teachers in Jiangsu Province, China

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Summary. *Objective:* We conducted this cross section study to investigate the basic health status of teachers in Jiangsu. *Design:* Healthy data of teachers from Chinese Teacher Cohort were collected by an online questionnaire and scored by a diet behavior system with maximum score of 28. *Setting:* The health of teachers is not only a sociopolitical topic, but also an interdisciplinary challenge, while few researches focused on the basic healthy information of above teacher population. *Subjects:* A total of 22,956 teachers aged 20-65 year registering in Jiangsu Province Education System completed the questionnaire survey. *Results:* The average Dietary habits score of participants was 14.0±3.0 based on a full score of 28, which is the worst. The Dietary habits score increased with age (P<0.001). A worse score was detected in males compared with females, in the subjects with abnormal BMI (<18.5 or >25) compared with the ones with normal BMI (18.5-23.0) (P<0.001), in the subjects with smoking or drinking history compared the ones without such history (both P<0.001), and in the subjects from South of Jiangsu (vs Central region, P<0.05; vs North region, P<0.01). A higher score (21-28 scores vs. 7-13 scores) was significantly negatively associated with high blood pressure, high serum glucose, hyperlipidemia, and sleeplessness (all P were <0.001), after adjusted for sex, age, smoking and drinking status. *Conclusion:* The results indicate baseline healthy data of teachers in Jiangsu, and provide reliable evidences for future feasible strategies making on prevention of occupation related chronic diseases including high blood pressure, high serum glucose, hyperlipidemia, and sleeplessness. Knowledge of healthy habits and nutritional knowledge in the teacher population needs significant improvement. Developing age/gender-specific program for promoting healthy lifestyle among teachers is recommended.

Key words: teacher population, healthy status, Dietary habits, high blood pressure, high serum glucose, hyperlipidemia, sleeplessness

Introduction

Teachers occupy a privileged position in society, playing an important role in human development and the educational process. The health of teachers is not only a sociopolitical topic, but also an interdisciplinary challenge. The current health status of teachers is not optimistic. For example Seibt *et al* reported that 18% of full time teachers had impaired mental health, 53% of full-time teachers suffered from high blood pressure, and low physical fitness was observed in only 6% of full-time teachers in an occupational health screening in German (1).

Jiangsu locates in eastern China, and plays an important role in the education system of China, containing 137 colleges and universities, 2,885 second-

* Chunlin Zheng, Cheng Shi, and Jianlin Bai contributed equally to the present study and should be regarded as joint first authors

ary schools, 4,068 primary schools, and 106 special education schools. Totally 696,600 full-time teachers serve in Jiangsu education system (2). Till now, few researches focused on the basic healthy information of above teacher population, the understanding of healthy status in this population is critical for sustainable development of local education system, corresponding performance of control and prevention strategies of chronic non-infectious diseases. Therefore we conducted this cross section study by online questionnaire to investigate the basic health status of teachers in Jiangsu and its relationship with their dietary habits and knowledge reserve on health.

Materials and Methods

Study Population

Teachers certified in Jiangsu Province register in the Teacher Management Information System and are managed by Jiangsu Provincial Department of Education. We distributed a designed questionnaire to all registered teachers using the online Teacher Training Management System of Jiangsu from July 10 to Aug 20, 2015. A total of 22,956 teachers aged 20-65 year registered and completed the questionnaire survey. After excluding subjects who were pregnancy, aged less than 20 years old, and missing data, the analysis enrolled 22,179 participants, yielding a participation rate of 96.6%. All the participants were from 89 local schools in primary, high school or colleges in Jiangsu province.

Questionnaire survey

In Chinese Teacher Cohort (CTC), the standardized questionnaire was constructed based on the Adult Dietary Behavior Scale (3). The questionnaire contains: 1) basic social information: including age, gender, height, body weight, and region; 2) dietary habit including breakfast, lunch, and supper diets, and snacks, dietary structure of three meals; 3) healthy habit including smoking status, drinking status, sleep quality, exercise situation, and occupational stress; 4) healthy condition including blood pressure, blood lipid, blood glucose, chronic disease history, use habit of health care products.

In our questionnaire instructions, Using BMI scores, this study further classified the participants as underweight (under 18.5 kg/m^2), normal weight (between 18.5 kg/m^2 and 23.0 kg/m^2), overweight (between 23.0 kg/m^2 and 25.0 kg/m^2), obese(>25.0 kg/m^2) based on the World Health Organization's classifications suggested and revised for the Asia-Pacific region (4). Hypertension was defined as blood pressure>140/90 mmHg; Hyperlipidemia was defined as serum triglyceride >1.7mmol/L; Hyperglycemia is defined as blood glucose >6.1; Sleeplessness is defined as sleep time <6 hour. The nighttime snacking was defined as any food consumption after 09:00 PM. The night snack consumption time period examined was at least a week before the subjects filled out the questionnaire.

The study was approved by IRB of local institutes of authors and informed consent was signed by each participant before questionnaire.

Dietary habits score

We analyzed the frequency of 1) breakfast, 2) coarse food grain (corn, wheat, sorghum, buckwheat, oat), 3) bean products, 4) dairy product, 5) vegetables or fruits, 6) midnight snack, and 7) fat meal taken by subjects in one week. The 7 items derived from the questionnaire survey and their respective scoring criteria are presented in Supplemental Table 1. Healthy diet behavior including breakfast, coarse food grain, bean products, dairy product, and vegetables or fruits taken contains four options, 7 times taken per week got 1 score; 4-6 times/week got 2 scores, 1-3 times/week got 3 scores, 0 times/week got 4 scores. Unhealthy diet behavior including midnight snack and fat meal taken contains four options, 7 times taken per week got 4 scores; 4-6 times/week got 3 scores, 1-3 times/week got 2 scores, 0 times/week got 1 score. The dietary habits score was defined as the summary of above 7 diet behaviors.

Statistical analysis

The total dietary habits score was a summation of scores from the 7 items and ranged from 0 to 28. A greater total score represents a dietary pattern reflective of unhealthier dietary habits. We used mean±standard deviation (SD) and frequency (percentage) to describe general characteristic. Chi-square test was analyzed to compare difference between categorical variables. The association between the various variables and total dietary habits score was evaluated through linear regres-

Table1. Characteristics of the subjects

	Participants, n (%)	Dietary habits score	P-values
Total	22,179 (100%)	14.0±3.0	
Gender			<0.001
Male	10,547	14.7±3.0	
Female	11,612	13.5±2.9	
Age (Years)			< 0.001
<30	4,166	13.8±3.0	
30-50	16,539	14.1±3.0	
>50	1,474	13.8±2.8	
BMI (kg/m^2)			<0.001
<18.5	6,601	14.4±2.9	
18.5-24.99	11,896	13.6±3.0	
25-28	3,083	14.7±2.9	
>28	599	15.3±3.2	
Smoking status			<0.001
Never	19,175	13.8±2.9	
Ever	3,004	15.2±3.1	
Drinking status			<0.001
Never	15,624	13.6±3.0	
Ever	6,555	15.0±2.8	
Region			0.087
South of Jiangsu	5,559	14.1±2.8	
Central of Jiangsu	7,604	14.0±3.0	
North of Jiangsu	9,016	14.0±3.1	

sion models. Furthermore multiple logistic regression models adjusted for gender, age, BMI, smoking status, and alcohol use were used to explore the relationship between dietary habits and human health condition. SPSS software version 19.0 (SPSS Inc, Chicago, IL, USA) were performed to analyze all statistical analyses in our study. We regarded *P*-value less than 0.05 as the cut-off for statistical significance.

Results

Study participant characteristics

A total of 22,179 participants comprising 11,763 female teachers (53.03%) and 10,416 male teachers (46.96%) were surveyed in this study. The mean age of participants was 37.68 years, with standard deviation (SD) of 8.49, and 30-50 years old people were major subjects, accounting to 74.57% (Table 1).

Of the participants, body mass index (BMI) of 12,313 subjects (55.51%) was normal (18.5-23.0), 19,847(89.48%) reported no smoke history, and 16,171(72.91%) reported no drink history. Based on a full score of 28, which is the worst score of Dietary habits, the average Dietary habits score was 14.0±3.0.

Dietary habits score analysis of the enrolled population

Table 2 presents factors associated with Dietary habits score. There was significant difference in the mean score according to age, gender, BMI, smoking and drinking status (all *P*<0.001). The score decreased with age (*P*<0.001). The male teachers had a higher score on Dietary habits than females (*P*<0.001). Compared with the subjects with normal BMI (18.5-23.0),

Table 2. Factors associated with Dietary habits score

Parameter	Estimate	SE	t	P value
Intercept*	14.86	0.12	16576.47	<0.001
Age	-0.03	0.003	108.08	<0.001
Gender				
Male	0	0	-	-
Female	-0.67	0.05	185.78	<0.001
BMI (kg/m^2)				
<18.5	0.69	0.04	252.92	<0.001
18.5-23.0	0	0	-	-
23.0-25	0.82	0.06	203.64	<0.001
>25	1.27	0.12	114.77	<0.001
Smoking status				
Never	0	0	-	-
Ever	0.62	0.06	100.45	<0.001
Drinking status				
Never	0	0	-	-
Ever	0.80	0.05	246.05	<0.001
Region				
South of Jiangsu	0	0	-	-
Central of Jiangsu	-0.12	0.05	5.96	0.015
North of Jiangsu	-0.25	0.05	26.53	<0.001

*Never smoking: smoking <100 cigarettes in the past years; ever smoking: smoking ≥20 cigarettes in the last year; Never drinking: no drink in the past years; Ever drinking: at least once drink in the last year. *Adjusted by age, gender, BMI, smoking status, drinking status, region.*

Table 3. Relationship between dietary habits with chronic non-infectious disease

Health condition	Healthy dietary habits (7-13 points)	Average dietary habits (14-20 points)	Unhealthy dietary habits (21-28 points)	P-trend
Blood pressure High blood pressure vs normal	Reference value	1.12 (0.77, 1.61)	3.44 (1.71, 6.92)	0.001
Blood glucose >6.1 mmol vs normal	Reference value	0.88 (0.79, 0.99)	1.00 (0.67, 1.50)	<0.001
Serum lipid Hyperlipidemia vs normal	Reference value	1.26 (1.17, 1.36)	1.65 (1.28, 2.14)	<0.001
Sleeplessness <6 hour *vs* ≥6 hour	Reference value	1.69 (1.55, 1.84)	4.27 (3.35, 5.44)	<0.001

High blood pressure is defined as blood pressure >140/90 mmHg; hyperlipidemia is defined as serum lipid >1.7mmol/L. is defined as blood glucose >6.1. Sleeplessness is defined as sleep time <6 hour. Data were adjusted by gender, age, BMI, smoking and drinking status

the subjects with abnormal BMI (<18.5 or >25) had higher scores on Dietary habits (both P<0.001), which means a worse score on dietary habits. The increase of score is significant (P<0.001). The Dietary habits score of subjects with smoking or drinking was higher than that of the population without such habits (both P<0.001). The subjects from South of Jiangsu had a higher score on Dietary habits than the people from Central of Jiangsu and North of Jiangsu (P<0.05).

We further performed the association analysis between dietary habits score with chronic non-infectious diseases including hypertension, hyperglycemia sleeplessness, and hyperlipidemia after adjustment of gender, age, BMI, smoking and drinking status , and the results showed that above diseases were all significantly related to the increase trend of dietary habits score (all P <0.01), which indicated the important of normal diet habits on chronic non-infectious diseases incidence.

Discussion

The teaching profession is with a high prevalence of work-related stress, which may lead to sustained physical and mental health problems in teachers. Current few studies on the health status of teachers were conducted, and little was known about this baseline of such population, which is the reason why we conducted this investigation.

For all subjects, more than 50% have normal BMI without smoking and drinking history. We found that there is a trend of healthy diet habit with age increase (P<0.001).

The teachers aged from 30-39 had the highest score on diet habit, which means such a group had a most unhealthy diet habit in normal life. The teachers aged from 30-39 are the rock of social, on the one side they are in the critical stage of career, facing heavy tasks in teaching and research, and on the other side they need to support the elderly and raise children. The double pressure from family and career make it difficult for the population to develop a healthy diet habit. The group with age more than 50 years old has the best diet habit compared to other age groups because the subjects have a comparably stable life, and are more concern about health. Meng *et al* investigated the sub-health status of teachers in universities and colleges, and they found that the sub-health status was lowest detected in the sub-group with more than 51 years old, which was consistent with our results (5).

Our results showed that the females had a significant better diet habit compared with that of males, who had a significant worse score on smoking and drinking status, fruits and dairy products intake, whether or not to eat breakfast (data are not shown). And we get similar results on life style between genders compared with the report from Czech (6).

Reng *et al* investigated the health literacy among urban primary school teachers in 6 provinces in China, and the results showed that the level of health living

style and behavior was 44. 94%, but there is no difference between genders, and literacy level was decreased with the age growing (7). The difference may be caused by the various sources of subjects, in our study all investigated teachers were from Jiangsu Province, which is located southeastern coastal area with developed economy and culture, while Reng *et al* investigated six provinces of China covering eastern, central and western sections, including the developed and developing area with different diet habits (7).

Compared with the subjects with normal BMI (18.5-23.0), the subjects with abnormal BMI (<18.5 or >25) had higher scores on Dietary habits (all $P<0.001$), which means a worse score on dietary habits. The increase of score is significant ($P<0.001$). The Dietary habits score of subjects with smoking or drinking was higher than that of the population without such habits (both $P<0.001$).

The subjects from South of Jiangsu had a higher score on Dietary habits than the people from Central of Jiangsu and North of Jiangsu ($P<0.05$). The analysis showed that the difference mainly came from the various dietary models and structure. It is interesting to find that the abnormal rate of fasting blood-glucose was significantly higher in people from South of Jiangsu (7.7%) compared to the people from Central of Jiangsu (3.5%) and North of Jiangsu(3.5%) ($\chi^2=16.03$, $P<0.01$) (8), diabetes incidences are various in different regions in China, which may be related to the dietary structure.

The life style including diet habit plays a critical role in the incidence of chronic non-infectious diseases including hypertension, hyperglycemia, sleeplessness, and hyperlipidemia. The multiple logistic regression results showed that hypertension, hyperglycemia, sleeplessness, and hyperlipidemia were all significantly associated to the dietary habits in the teacher population after adjustment of gender, age, BMI, smoking and drinking status, and the unhealthy trend of diet habits is significantly associated with the increasing incidence of chronic non-infectious diseases.

In conclusion, based on our results, we recommend that the conception of healthy diet should be emphasized for teachers by promotion of healthy education programs for teachers especially for 30-39 age group and males.

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Authors' contributions:

Chunlin Zheng and Cheng Shi: data collection; Jianling Bai, Wentao Shao, Chunlan Zhang: statistical analysis; Jin Bu: study design and manuscript writing

Ethical Standards Disclosure:

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethics Committee of Nanjing Medical University. Written informed consent was obtained from all subjects.

References

1. Seibt R, Matz A, Hegewald J, et al. Working conditions of female part-time and full-time teachers in relation to health status. Int Arch Occup Environ Health 2012; 85: 675-687.
2. Jiangsu statistical yearbook. http://www.jssb.gov.cn/2016nj/nj15/nj1517.htm
3. Giannini SP, Latorre Mdo R, Fischer FM, et al. Teachers' voice disorders and loss of work ability: a case-control study. Journal of Voice 2015; 29(2): 209-217.
4. Wei Sheng. The study of the adult eating behavior evaluation. Wuhan. Huazhong University of Science and Technology. 2004.
5. Meng XP, Liu ZS, Huang XP. The Age distribution of college teachers in sub-health state and countermeasures. Journal of Nursing Science (Surgery Edition) 2007; 22 (10), 64-66 (In Chinese).
6. Zejglicová K1, Kratěnová J, Malý M, et al. The middle-aged urban population showed high incidence of influenceable risk factors for chronic diseases. Men compared to women had higher rates of risk factors and were at higher risk of developing cardiovascular diseases. Cas Lek Cesk 2006; 145(12): 936-42.
7. Reng Z, Li YH, Nie XQ, et al. Status of health literacy among urban primary school teachers in 6 provinces in China. Chinese Journal of Health Education 2016; 32 (4): 301-306 (In Chinese).
8. Investigation of abnormal rate of fasting blood-glucose in Jiangsu residents. Practice Preventive Medicine 2016, 23(6): 728-729,761.

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Original article

Prevalence of vitamin D deficiency and the effect of anthropometric and lifestyle factors on thevitamin D statuses of healthy women residing in Riyadh

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Summary. *Objective:* Hypovitaminosis D is a global health issue, and its increasing prevalence affects people of all ages. This study aimed to determine the vitamin D statuses of healthy women residing in Riyadh, based on measured serum calcidiol ($25(OH)_2D_3$) levels and to assess the effects of demographic, anthropometric, and lifestyle factors on vitamin D status. *Method:* A structured questionnaire was designed and completed after patient consent acquisition to obtain demographic, anthropometric, and lifestyle information. A food frequency questionnaire was used to assess dietary intake, and an immunoassay was used to determine serum vitamin D levels. *Result:* The mean age of the participants was 33.46 years, and approximately 56% of the participants reported no exposure to sunlight. Only 26.98% of the participants had a normal body mass index (BMI), and 42.86% of the participants had a waist circumference <80 cm. Approximately 86% of the participants were identified as vitamin D deficient (mean, 22.73 nmol/L), and only 7.94% had a normal vitamin D concentration (mean, 96.34 nmol/L) with a cutoff value of 50 nmol/L (for deficiency). A highly significant difference ($p \leq 0.05$) was found between the means of various vitamin D status groups. Regarding BMI, the difference in vitamin D levels was highly significant ($p \leq 0.05$). There was a strong, positive, and highly significant ($p \leq 0.05$) correlation between BMI and waist circumference and a negative (or inverse) correlation between BMI and vitamin D and between waist circumference and vitamin D. The participants' diets were found to be deficient in vitamin D-rich foods. *Conclusion*: Education to enhance awareness of the importance of vitamin D is needed, and vitamin D supplementation apart from adequate sun exposure and suitable dietary intake is suggested for individuals with serum calcidiol $25(OH)_2D_3$ levels below 50 nmol/L.

Key words: vitamin D, BMI, sunlight, Riyadh, obesity

Introduction

Vitamin D is a fat-soluble vitamin and prepro-hormone (1) that is known to be found in two forms, vitamin D_2 (also known as ergocalciferol) and vitamin D_3 (also known as cholecalciferol) (2). Vitamin D_2 is mostly obtained from foods, such as fish, egg yolk, and mushrooms, and vitamin D_3 is formed in the skin after exposure to sunlight or ultraviolet light (3-4). Hypovitaminosis D is a worldwide issue, and approximately one billion people are at risk regardless of their ethnicity and age group (5-6). There are several causes of vitamin D deficiency, including decreased bioavailability, decreased synthesis, increased catabolism or metabolic demands, and increased urinary loss (7). Other factors that contribute to a low vitamin D level are cultural norms that lead to a lack of sunlight exposure, such as clothing style, housing designs, and outdoor sun protection (8, 9). Although sun exposure is considered a major source of vitamin D, the prevalence of its defi-

ciency is paradoxically much higher in countries with a sunny climate, such as Saudi Arabia (10), Egypt (11), and the United Arab Emirates (12) than in those with a less sunny climate. The status of vitamin D is of great interest given that vitamin D insufficiency is widespread worldwide (13). Vitamin D is recognized for its physiological role in regulating calcium homeostasis (14) by increasing the efficacy of intestinal calcium and phosphorus absorption to maintain signal transduction, neuromuscular function, and metabolic activities and to promote skeletal mineralization (15). Its receptors are expressed in multiple tissues within the body, including vascular smooth muscle, the endothelium, and cardiomyocytes (14). Inadequate vitamin D levels in the body have been linked to osteomalacia due to a skeletal mineralization defect, and its insufficiency is a risk factor for osteoporosis in adults (16). In children, its deficiency leads to rickets (8).

Increasing evidence from biochemical, cellular, animal, and epidemiological investigations also highlights vitamin D as a pleiotropic regulator of several physiological processes, including the modulation of immune responsiveness, cellular differentiation and proliferation and central nervous system function (17). Epidemiological studies have reported that lower vitamin D levels are associated with diabetes-related microvascular complications (18), retinopathy (19), multiple sclerosis (20) hypertension and cardiovascular disease (21) and that a higher vitamin D level is related to a reduced cancer incidence and decreased cancer-related mortality (22).

Although Saudi Arabia receives a substantial amount of sunlight throughout the year, many Saudi Arabian people have severe vitamin deficiency, particularly Saudi Arabian women (23). The objective of this study was to determine vitamin D status by measuring serum calcidiol ($25(OH)_2D_3$) levels and to assess the effects of demographic, anthropometric, and lifestyle factors on the vitamin D statuses of healthy women (young and old) residing in Riyadh.

Methods

Research design

A descriptive cross-sectional approach was used in the present study. Sixty-three women (age range between 20 and 80 years) were recruited using a random sampling method. A pro forma of inclusion and exclusion criteria for selection of participants was prepared, and a house-to-house survey was then conducted to find participants.

Inclusion criteria

All nonpregnant and nonlactating Saudi women who consented to provide blood samples were included in this study.

Exclusion criteria

Women without Saudi Arabian nationality; women who were pregnant and lactating; women previously diagnosed with metabolic bone diseases, diabetes mellitus, cardiovascular disease, or renal, hepatic endocrine or autoimmune diseases; and women unwilling to provide blood samples were excluded from the study.

Ethical considerations

This study was conducted in accordance with research policies of the King Saud University Research Centre. The purpose of the study was explained to all participants, and written consent was obtained. Participants' blood samples were withdrawn by a qualified nurse, and the participants were assured that the information they provided would be used exclusively for scientific purposes and be kept confidential.

Demographic characteristics

A structured questionnaire was completed after the acquisition of informed consent while interviewing the participants to obtain demographic information. Demographic characteristics included age and occupation. The second section of the questionnaire included risk factors for vitamin D deficiency, such as housing conditions, nonexposure to sunlight, and clothing habits. Participants were questioned about their duration of time spent in sunlight and principal exposure times during the day, their use of sunscreen, and their dietary habits. Anthropometric measurements were undertaken by the study researcher. The comprehensiveness and relevance of the study tools were reviewed by a panel of experts, and a pilot study was undertaken to test the validity and applicability of the measurement tools.

Anthropometric measurements

Anthropometric measurements in this study included body weight, height and waist circumference. All anthropometric measures were assessed by the researcher, and the participants were measured barefoot and wearing light-weight clothing. Height and weight were used to calculate BMI (weight (kg)/height^2 (m)^2). The participants were classified as follows: underweight (BMI <18.5), normal (BMI between 18.5 and 24.9), overweight (BMI between 25 and 29.9), or obese (BMI >30) (24).

Dietary assessment

A food frequency questionnaire was used for dietary assessment. Participants were interviewed by a nutritionist to gather information regarding their consumption frequency of vitamin D-related food items, such as milk, fish, eggs, and mushrooms. The questionnaire included details regarding the exact portion sizes of the food consumed (serving size), the parts consumed (whole, half, quarter), and the fat content of the food (skim, low-fat, full-fat, fortified).

Biochemical tests

All participants were asked to keep a dietary record and were instructed not to take part in any high-intensity physical activity 12 hours prior to providing blood samples. Blood samples were acquired by a specialized nurse at King Khalid University Hospital after the participants had fasted for 8 hours. Samples were collected in sample tubes and centrifuged at 3,000 rpm for 10 minutes to prepare the serum. Vitamin D serum ($25(OH)_2D_3$) levels were assessed with an immunoassay using a radioactive device (Roche Analyzer Cobas e170 Immunoassay, Roche Diagnostic, USA) according to the method proposed by Vieth et al. (25).

A general definition of vitamin D deficiency could not be determined because there is no universally agreed cutoff value or normal range for serum $25(OH)_2D_3$ levels. The cutoff values for vitamin D have long been debated. The US Endocrine Society and the American Geriatric Society consider vitamin D levels inadequate if serum $25(OH)_2D_3$ levels are <50 nmol/L (<20 ng/ml). The US Institute of Medicine and the UK National Osteoporosis Society consider serum $25(OH)_2D_3$ levels <30 nmol/L (<12 ng/ml) to be inadequate, and both the WHO and the German Nutrition Society consider serum $25(OH)_2D_3$ levels <25 nmol/L (<10 ng/ml) to be inadequate (26). For the present study, the participants were grouped according to their vitamin D levels as follows: deficient (<50 nmol/L), relative insufficient (between 50 nmol/L and 72 nmol/L) and normal (>72 nmol/L) (27).

Statistical analysis

Data were analyzed using the SPSS statistical software package (version 22). Frequencies are presented as percentages (%), and continuous variables are presented as the mean ± standard deviation. The participants were stratified into 3 groups according to their serum $25(OH)_2D_3$ concentrations as follows: <50 nmol/L, between 50 nmol/L and 72 nmol/L, and >72 nmol/L. Pearson's chi-square test was performed to compare frequencies and to test differences between group proportions for categorical variables, and one-way ANOVA was used to determine associations between quantitative variables.

Results

Table 1 depicts the demographic characteristics, anthropometric measurements, and lifestyle habits of the participants. In this study, 49.21% of the participants were between 20 and 30 years of age, and 22.22% and 17.46% were between 31 to 40 and 41 to 50 years of age, respectively. Only 11.11% of the participants were greater than 50 years old. Approximately 30% of the participants were students, 27% were currently employed, and 42.86% were housewives. Sixty percent of the participants lived in villas, and the remainder lived in apartments. A total of 79% of the participants reported no vitamin D supplementation consumption of any type, and approximately 56% of the participants reported no exposure to sunlight. Approximately 42% of the participants were obese, and 28.57% were overweight. The percentage of participants with a normal BMI was 26.98%. A total of 42.86% of the participants had a waist circumference <80 cm, while 23.81% of the participants had a waist circumference between 80 cm and 90 cm, and 33.33% of the participants exhibited a waist circumference of more than 90 cm. Ta-

Table 1. Participant demographic characteristics, anthropometric measurement, and lifestyle habits

		Frequency	Percentage (%)
Demographic characteristics and lifestyle habits			
Age (Years)* (33.46±15.26)	20-30	31	49.21
	31-40	14	22.22
	41-50	11	17.46
	51 and over	7	11.11
Occupation	Student	19	30.16
	Job/Working	17	26.98
	Housewife	27	42.86
Housing	Villa	38	60.32
	Apartment	25	39.68
Do you take a vitamin D supplement?	Yes	13	20.63
	No	50	79.37
Do you expose yourself to sunlight?	Yes	28	44.44
	No	35	55.56
How often (days) do you expose yourself to sunlight?	Daily	17	26.98
	Alternate days	4	6.35
	No routine	7	11.11
	No exposure at all	35	55.56
Time of sunlight exposure (n=28)	6 to 9 am	5	17.86
	9 to 12 pm	16	57.14
	12 to 3 pm	7	25
Anthropometric measurements			
BMI* (30.42±9.67)	Underweight	2	3.18
	Normal	17	26.98
	Overweight	18	28.57
	Obese	26	41.27
Waist circumference* (84.43±16.65)	>80	27	42.86
	80-90	15	23.81
	<90	21	33.33

**Data are expressed as the mean ± std dev.*

Table 2. Distribution of serum $25(OH)_2D_3$ status of participants

Serum $25(OH)_2D_3$ status	**Mean $25(OH)_2D_3$**	**Frequency**	**Percentage (%)**	**p value**
Deficiency	22.73±9.42[a]	54	85.72	0.00
Relative insufficiency	60.35±6.25[b]	4	6.34	
Normal	96.34±8.33[c]	5	7.94	

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript abc indicate significant differences among serum $25(OH)_2D_3$ statuses as indicated by ANOVA followed by Duncan's multiple range test.

Table 3. Distribution of serum $25(OH)_2D_3$ levels, based on BMI and age

	N	Deficiency		Relative insufficiency		Normal		Chi squared value	P value
		No:	%	No:	%	No:	%		
Based on BMI								13.124	0.041
Underweight* (48.95±38.25)	2	1	50	0	0	1	50		
Normal* (30.23±22.15)	17	15	88.24	1	5.88	1	5.88		
Overweight* (32.29±25.52)	18	16	88.89	2	11.11	0	0		
Obese* (25.88±14.64)	26	22	84.62	1	3.85	3	11.53		
Based on Age								6.995	0.321
20-30* (26.79±19.48)	31	29	93.55	0	0	2	6.45		
31-40* (34.90±28.72)	14	11	78.57	1	7.14	2	14.29		
41-50* (37.17±28.61)	11	8	72.73	2	18.18	1	9.09		
51 and above* (28.04±17.33)	7	6	85.71	1	14.29	0	0		

** Data are expressed as the mean± standard deviation; p values < 0.05 are significant.*

ble 2 depicts the distribution of the serum $25(OH)_2D_3$ level statuses of the participants, and Table 3 depicts their BMI and age distributions. In the present study, the mean serum $25(OH)_2D_3$ level was 30.96±23.28 nmol/L (range, 5-105 nmol/L). Approximately 86% of the participants were found to be vitamin D deficient, with a mean value of 22.73 nmol/L, while the vitamin D concentrations in 6.34% of the participants were relatively insufficient, with a mean value of 60.35 nmol/L. Only 7.94% of the participants had a normal vitamin D concentration, with a mean value of 96.34 nmol/L, at a cutoff value of 50 nmol/L (for deficiency). A highly significant difference ($p \leq 0.05$) was found between the means of various vitamin D status groups, and the difference in vitamin D levels was also highly significant ($p \leq 0.05$) with regard to BMI. Only 17 women had a normal BMI, whereas 44 women were either overweight or obese. The results show that the highest number of participants (93.55%) deficient in serum $25(OH)_2D_3$ belonged to the age group between 20 and 30 years of age, followed by the older participants classified in the age group 51 years and older (85.71%), with mean serum $25(OH)_2D_3$ levels of 26.79 nmol/L and 28.04 nmol/L, respectively. A strong, positive, and highly significant ($p \leq 0.05$) correlation was found between BMI and waist circumference (i.e., larger waist circumference measurements were correlated with a higher BMI), and a negative (or inverse) correlation was found between BMI and vitamin D (i.e., a higher BMI was correlated with a decreased vitamin D concentration) and between waist circumference and vitamin D (i.e., larger waist circumference

Table 4. Correlation between BMI, waist circumference and serum $25(OH)_2D_3$ concentration in the studied sample

	BMI	Waist	Vitamin D
BMI	1	-0.658**	-0.006*
Waist	0.658**	1	-0.136
Vitamin D	-0.006*	-0.136	1

*** Correlation is significant at the 0.01 level (2-tailed)*

measurements were correlated with a decreased vitamin D concentration (Table 4). Figure 1 gives a brief description of the serum $25(OH)_2D_3$ level distribution based on dietary intake. The percentage of participants consuming fish and mushrooms was very low, although fish is one of the best sources of vitamin D.

Discussion

Addressing health disparities is a major focus of current public health efforts (28), and during the last decade, no other micronutrient has gained as much attention in the health and biomedical research community as vitamin D (29). This study identified a number of demographic, environmental, and lifestyle factors associated with vitamin D levels. Various factors, such as environmental and lifestyle factors as well as demographic factors, are determinants of serum $25(OH)_2D_3$ concentrations. Some studies suggest that vitamin D intake, sex, outdoor activity, latitude, season, age, and body fat levels are major determinants of the serum $25(OH)_2D_3$ concentration (13, 30). Serum $25(OH)_2D_3$ is most reflective of overall vitamin D stores and is used in research and clinical practice to determine vitamin D status.

In this study, only one fourth of the participants were exposed to sunlight on a daily basis, while more than 50% reported never being exposed to sunlight at all, which is consistent with the results of a cross-sectional study performed on females from Riyadh (31). Limited sun exposure is one of the main causes of vitamin D deficiency (14, 32) in addition to having an indoor lifestyle, prolonged breastfeeding, and lack of a

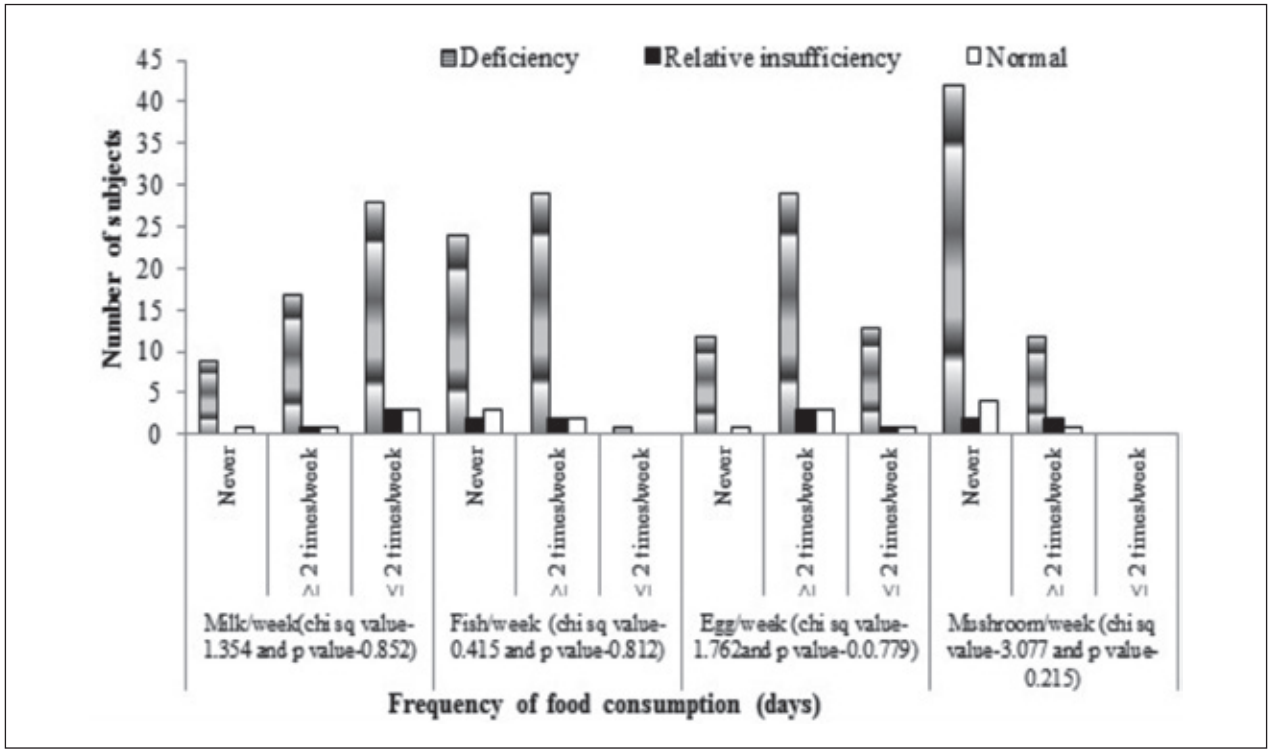

Figure 1. Distribution of serum $25(OH)_2D_3$ levels based on dietary intake.
p values < 0.05 are significant.

balanced diet (33, 34). Moreover, because the body is covered for cultural reasons and because of intense heat and high temperatures, people tend to remain mainly indoors (6). In Muslim countries such as Saudi Arabia, Muslim women wear clothes (the abaya, hijab, and niqab) that completely cover their head and body; they seldom have any chance to expose their bodies to sunlight and are often vitamin D deficient (35). In a previous study, it was reported that covering the skin with clothing prevents the skin from contacting UV-B rays, which are essential for the production of vitamin D (6). The color of the skin of Saudi individuals varies from light brown to dark. Dark pigmentation has been found to decrease skin synthesis of vitamin D because ultraviolet light cannot reach the appropriate layer of the skin (36). In a study on Turkish women who wore veils (only the hijab), the mean serum $25(OH)_2D_3$ level was 32 nmol/L, while it was only 9 nmol/L when they were completely covered (niqab) and 56 nmol/L in those who wore Western-style clothing (37,38). In contrast, another study found no difference in vitamin D levels between veiled and nonveiled Bangladeshi women (39).

In this study, a large percentage (86%) of the participants was identified as vitamin D deficient, which is very similar to the results (83.0%) reported by Yousef et al. (40). Mogbel (41) and Ardawi et al. (42) reported prevalence rates of vitamin D deficiency of 79.1% and 80%, respectively, in their respective studies on Saudi Arabian women. Other reports from various countries show that more than 50% of the global population is at risk of hypovitaminosis (7, 43). One study in the United States revealed that almost 75% of individuals of European ethnic origin and 90% of those of nonEuropean ethnic origin suffered from vitamin D insufficiency (44). The mean $25(OH)_2D_3$ serum levels reported in elderly people were 28 nmol/L in Siberia (45), 30 nmol/L in Japan (46), and 36 nmol/L in India (47).

Similar to this study, Masoompour et al. (48) found that serum 25-hydroxyvitamin D levels did not decline with age, while in contrast to these findings, Smotkin-Tangorra et al. (49) concluded that vitamin D insufficiency was associated with an increase in age. Obese women were more deficient than women with a normal BMI in terms of vitamin D levels. Yousef et al. (40) and Al-Sultan et al. (50) found that vitamin D levels were significantly higher in the lean control participants than in obese subjects in their study and showed a significant decline in relation to categories of obesity. In a bidirectional Mendelian randomization analysis of multiple international adult cohorts, a high BMI was shown to directly lead to lower serum $25(OH)_2D_3$ levels (51). Vitamin D3 supplementation led to a reduction in body fat mass (52), and results from clinical trials in adults have suggested that decreasing vitamin D storage sites by reducing fat mass is the only way to restore normal serum $25(OH)_2D_3$ levels in overweight and obese individuals (53). Reinehr et al. (54) and Arunabh et al. (55) also reported a significant and inverse relationship between BMI and $25(OH)_2D_3$ serum levels. Moreover, several studies have shown no association between serum $25(OH)_2D_3$ levels and BMI, body weight, or fat-free mass (56,57,58). Additionally, an inverse relationship among total serum $25(OH)_2D_3$ concentrations, waist circumference and BMI has been observed (40), which is similar to the result reported in this study. The populations in the abovementioned studies differed to some extent with regard to their age, sex, and pregnancy, and most of these populations were more likely to trend towards being overweight than obese. These factors may explain the differences in the results between the studies. Although the interaction between the mechanism underlying the association between the serum $25(OH)_2D_3$ status and adiposity remains unclear, the most strongly supported elucidation is that increased body fat leads to increased sequestration of vitamin D in the adipose tissue, decreasing the circulating $25(OH)_2D_3$ serum levels (59, 60), or the effect of volume dilution due to the larger body size of obese individuals (61). The effect of BMI on serum $25(OH)_2D_3$ levels may be explained by people with a high BMI usually having a high body fat content and adipose tissue acting as a reservoir for lipid-soluble vitamin D and not releasing vitamin D to compensate for low serum calcium levels (62). It has been reported that reduced vitamin D bioavailability triggers a hypothalamic response that leads to increased hunger and decreased energy expenditure. Additionally, the resulting secondary hyperparathyroidism leads to an upregulation of lipogenesis (60). As the amount of adipose tissue increases, the uptake and clearance of vitamin D are further enhanced (62).

In this study, no significant association was observed between total serum $25(OH)_2D_3$ concentrations and vitamin D intake. Sadat Ali et al. (63) reported that food items consumed in the Kingdom of Saudi Arabia were either not fortified or contained very little vitamin D. In addition, they found no significant association between total serum $25(OH)_2D_3$ concentrations and vitamin D intake, which may have been due to the small sample size, and these results were similar to those in this study. In contrast, Al Mogbel (41) demonstrated a significant relationship between serum $25(OH)_2D_3$ levels and dietary intake. The recommended dietary allowance of vitamin D was found to be 600 IU/d for individuals between 1 and 70 years of age, corresponding on average to a 25-hydroxyvitamin D (25OHD) serum level of at least 50 nmol/L (20 ng/ml), and 800 IU/d for those older than 70 years of age (64).

In this study, most of the participants almost never ate any form of fish. Al Mobgel (41) reported that the Riyadh region is remotely located away from the seacoast; therefore, the people of Riyadh depend mainly on cattle and poultry meat rather than seafood, which is known to be a good source of vitamin D.

Conclusion

In conclusion, our study suggests that low vitamin D levels are prevalent in healthy females in all age groups residing in Riyadh, which may be related to a lack of adequate sunlight, a lack of consumption of vitamin D-rich foods, and a lack of awareness concerning the importance of vitamin D. This study identified a strong association between BMI and serum $25(OH)_2D_3$ levels. As such, obesity is another factor underlying hypovitaminosis D. There is no unified cut-off level for vitamin D deficiency; therefore, the development of local guidelines is needed in addition to providing vitamin D supplementation apart from adequate sun exposure and diet to all individuals whose serum $25(OH)_2D_3$ levels fall below 50 nmol/L. Public health education should be made available, focusing on the importance of consuming fish and dairy products and the health benefits of regular exposure to sunlight and regular physical exercise.

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References

1. Maldonado G, Paredes C, Guerrero R, Rios C. Determination of vitamin D status in a population of Ecuadorian subjects. Sci World J 2017; 2017:1-5.
2. Kulie T, Groff A, Redmer J, Hounshell J, Schrager S. Vitamin D: An Evidence-Based Review. J Am Board Fam Med 2009; 22: 698-706.
3. Moyad MA. Vitamin D: A Rapid Review. Dermatol Nurs 2009; 21:25-30.
4. Lehann B, Meurer M. Vitamin D Metabolism. Dermatol Therapy 2010; 23:2-12.
5. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008; 87: 1080S-1086S.
6. Christie FT, Mason L. Knowledge, attitude and practice regarding vitamin D deficiency among female students in Saudi Arabia: a qualitative exploration. Int J Rheum Dis 2011; 14: 22-29.
7. Sadat Ali N, Al Elq A, Al Farhan M, Sadat NA. Fortification with vitamin D: Comparative study in the Saudi Arabian and US markets. J Family Community Med 2013; 20: 49-52
8. Elshafie DE, Al- Khashan HI, Mishriky AM. Comparison of vitamin D deficiency in Saudi married couples. Eur J Clin Nutr 2012; 66: 742-5.
9. Al Saleh Y, Al Daghri NM, Khan N, et al. Vitamin D status in Saudi school children based on knowledge. BMC Pediatr 2015; 15:53.
10. Al Turki HA, Sadat Ali M, Al Elq AH, Al Mulhim FA, Al Ali AK. 25 hydroxyvitamin D levels among healthy Saudi Arabian women. Saudi Med J 2008; 29:1765-68.
11. Lotfi A, Abdel-Nasser AM, Hamdy A, Omran AA, El-Rehany MA. Hypovitaminosis D in female patients with chronic low back pain. Clin Rheumatol 2007; 26: 1895-1901.
12. Laleye LC, Kerkadi AH, Wasesa AA, Rao MV, Aboubacar A. Assessment of vitamin D and vitamin A intake by female students at the United Arab Emirates University based on self-reported dietary and selected fortified food consumption. Int J Food Sci Nutr 2011; 62: 370-6.
13. Mithal A, Wahl DA, Bonjour JP, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 2009; 20:1807-20.
14. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-81.
15. Babelghaith SD, Wajid S, Al-Zaaqi MA et al. Knowledge and practice of vitamin D deficiency among people lives in

Riyadh, Saudi Arabia-A cross-sectional study. Biomedical Research 2017; 28:3114-18.
16. Eastell R, Riggs BL. Vitamin D and osteoporosis. In: Feldman D, Pike JW, Glorieux FH, Editors.Vitamin D, 2nd ed. MA: Elsevier academic press, Burlington: 2005; 1101-20.
17. Forman JP, Giovannucci E, Holmes MD, et al. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007; 49: 1063-9.
18. Joergensen C, Hovind P, Schmedes A, Parving HH, Rossing P. Vitamin D levels, microvascular complications, and mortality in type 1 diabetes. Diabetes Care 2011; 34: 1081-5.
19. Kaur H, Donaghue KC, Chan AK, et al. Vitamin D deficiency is associated with retinopathy in children and adolescents with type 1diabetes. Diabetes Care 2011; 34: 1400-02.
20. Munger KL, Zhang SM, O' Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology 2004; 62:60-5.
21. Wang TJ, Penciana MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008; 117:503-11.
22. Zhao XY, Feldman D. The role of vitamin D in prostate cancer. Steroids 2001; 66:293-300.
23. Al Faraj S, Al Mutairi K. Vitamin D deficiency and chronic low back pain in Saudi Arabia. Spine 2003; 28: 177-9.
24. WHO. Appropriate body mass index for Asian populations and its implications for policy and intervention strategies. The Lancet 2004; 363:157-63.
25. Vieth R, Bischoff-Ferrari H, Boucher BJ, et al. The urgent need to recommend an intake of vitamin D that is effective. Am J Clin Nutr 2007; 85:649-50.
26. Al DaghriNM, Al-SalehY, Aljohani N, et al. Vitamin D status correction in Saudi Arabia: an experts' consensus under the auspices of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis, and Musculoskeletal Diseases (ESCEO).Arch Osteoporos 2017; 12: 1-8.
27. Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M, Estruch R. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med 2008; 168: 2449-58.
28. Weishaar Tom, Vergili JM. Vitamin D status is a biological determinant of health disparities. J Acad Nutr Diet 2013; 113:643-51.
29. Al Daghri NM, Sabico S, Al Saleh Y, et al. Calculated adiposity and lipid indices in healthy Arab children as influenced by vitamin D status. J Clin Lipidol 2016; 10:775-81.
30. Mai XM, Chen Y, Camargo CA. Jr. Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. Am J Epidemiol 2012; 175:1029-36.
31. Habib FM, Al Motairi WA, Al Mutairi WM. Vitamin D deficiency: knowledge and practice among adult Saudi females. Glo Adv Res J Med Sci 2014; 3:95-101.
32. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. J Intern Med 2000; 247:260-8.
33. Fuleihan G. Vitamin D deficiency in the Middle East and its health consequences for children and adults. Clinic Rev Bone Miner Metab 2009; 7:77-93.
34. Dawodu A, Wagner C. Mother-child vitamin D deficiency: an international perspective. Arch Dis Child 2007; 92:737-40.
35. Sedrani SH, Al-Arabi K, Abanmy A, Elidrissy A. Vitamin D status of Saudis II. Effect of regional and environmental location. Saudi Med J 1992; 13:206-13.
36. Lips P. Vitamin D physiology. Prog Biophys Mol Biol2006; 92:4-8.
37. Atli T, Gullu S, Uysal AR, Erdogan G. The prevalence of vitamin D deficiency and effects of ultraviolet light on vitamin D levels in elderly Turkish population. Arch Gerontol Geriatr 2005; 40:53-60.
38. Alagöl F, Shihadeh Y, Boztepe H et al.Sunlight exposure and vitamin D deficiency in Turkish women. J Endocrinol. Invest 2000; 23: 173-7.
39. Islam MZ, Akhtaruzzaman M. Hypovitaminosis D is common in both veiled and non-veiled Bangladeshi women. Asia Pac Clin Nutr 2006; 15:81-7.
40. Yousef FM, Ola A. Alsayed, Elham A. Al Nagshabandi. The relationship between vitamin d and body mass index among faculty members of King Abdulaziz University. Middle-East J Sci Res 2016; 24: 1628-37.
41. Al Mogbel ES. Vitamin D status among adult Saudi females visiting primary health care clinics. Int J Health Sci 2012; 6:99-107.
42. Ardawi MS, Qari MH, Rouzi AA, Maimani AA, Raddadi RM. Vitamin D status in relation to obesity, bone mineral density, bone turnover markers and vitamin D receptor genotypes in healthy Saudi pre and postmenopausal women. Osteoporos Int 2011; 22:463-75.
43. Looker AC, Johnson CL, Lacher DA,Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS Data Brief 2011; 59:1-8.
44. Adams JS, Hewison M. Update in Vitamin D. J Clin Endocrinol Metab 2010; 95:471–8.
45. Bakhtiyarova S, Lesnyak O, Kyznesova N, Blankenstein MA, Lips P. Vitamin D status among patients with hip fracture and elderly control subjects in Yekaterinburg, Russia. Osteoporos Int 2006; 17:441-6.
46. Nakamura K, Nashimoto M, Matsuyama S, Yamamoto M. Low serum concentrations of 25-hydroxyvitamin D in young adult Japanese women: a cross sectional study. Nutr 2001; 17: 921-5.
47. Harinarayan CV. Prevalence of vitamin D insufficiency in postmenopausal south Indian women Osteoporos Int 2005; 16:397-402.
48. Masoompour SM, Sadegholvaad A, Larijani B, Ranjbar-Omrani G. Effects of age and renal function on vitamin D status in men. Arch Iran Med 2008; 11: 377–81.
49. Smotkin-Tangorra M, Purushothaman R, Gupta A, Nejati G, Anhalt H, Ten S. Prevalence of vitamin D insufficiency

in obese children and adolescents. J Pediatr Endocrinol Metab 2007; 20: 817–23.
50. Al Sultan AI, Amin TT, Abou-Seif MA, Al Naboli MR. Vitamin D, parathyroid hormone levels and insulin sensitivity among obese young adult Saudis. Eur Rev Med Pharmacol Sci 2011; 15: 135-47.
51. Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. Plos Med 2013; 10:e1001383.
52. Salehpour A, Hosseinpanah F, Shidfar F, et al. A 12-week double-blind randomized clinical trial of vitamin D_3 supplementation on body fat mass in healthy overweight and obese women. Nutr J 2012; 11:78.
53. Muscogiuri G, Sorice GP, Prioletta A, et al. 25-Hydroxyvitamin D concentration correlates with insulin-sensitivity and BMI in obesity. Obesity 2010; 18:1906-10.
54. Reinehr T, de Sousa G, Alexy U, Kersting M, Andler W.Vitamin D status and parathyroid hormone in obese children before and after weight loss. Eur J Endocrinol 2007; 157: 225-32.
55. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. J Clin Endocrinol Metab 2003; 88: 157-61.
56. Bolland MJ, Grey AB, Ames RW, et al. Age, gender and weight related effects on levels of 25 hydroxyvitamin D are not mediated by vitamin D binding protein. Clin Endocrinol 2007; 67: 259-64.
57. Winters SJ, Chennubhatla R, Wang C, Miller JJ. Influence of obesity on vitamin D–binding protein and 25-hydroxy vitamin D levels in African American and white women. Metabolism 2009; 58: 438-42.
58. Baradaran A, Behradmanesh S, Nasri H. Association of body mass index and serum vitamin D level in healthy Iranian adolescents. Endokrynol Pol 2012; 63:29-33.
59. Al Daghri NM. Vitamin D in Saudi Arabia: Prevalence, distribution and disease associations. J Steroid Biochem Mol Biol 2018;175:102-7.
60. Cunha KA, Silva Magalhaes EI, Rodrigues Loureiro LM, Rocha Sant'Ana LF, Queiroz Ribeiro A, Novaes JF. Calcium intake, serum vitamin D and obesity in children: is there an association? Rev Paul Pediatr 2015; 33:222-9.
61. Heller JE, Thomas JJ, Hollis BW, Larson Meyer DE. Relation between vitamin D status and body composition in collegiate athletes. Int J Sport Nutr Exerc Metab 2015; 25:128-35.
62. Institute of Medicine. Food and Nutrition Board. Dietary reference intakes for calcium and vitamin D. National Academies Press Washington, D.C. 2011.
63. Sadat Ali M, Al Elq A, Al Turki H, Al Mulhim F, Al Ali A.Vitamin D levels in healthy men in eastern Saudi Arabia. Ann Saudi Med 2009; 29: 378-82.
64. Aloia JF. The 2011 Report on dietary reference intake for vitamin D: Where do we go from here? J Clin Endocrinol Metab 2011; 96:2987-96.

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Original article

Dysphagia awareness among dietitians

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Summary. *Objective:* This study was conducted to determine dieticians' awareness of dysphagia, as they have an important role in the screening and diagnosis of dysphagia. In terms of being the first study about dysphagia awareness of dietitians in Turkey, the study is of great importance. *Methods:* The study was conducted between November-December 2013 including 85 dietitians who volunteered to participate in the research from University Hospitals and Education and Research Hospitals in Ankara, Turkey. Through a face-to-face interview or email to dietitians participating in the study, they completed a questionnaire that included questions about their working time, dysphagia symptoms, diagnostic methods, and dietary practices. *Findings:* Of dietitians, 35.3% said that they frequently encounter patients with dysphagia and 7.1% of them didn't. The awareness scores of dieticians in terms of recognition of dysphagia symptoms vary from 0 to 12, with an average of 5.2+2.7 and a median score of 5. The most common aware of dysphagia symptoms were coughing and suffocation (77.6%) during meals. There was no significant difference between the awareness scores and the study period and the units they worked in ($p>0.05$). 82.4% of the dieticians didn't receive any training about dysphagia after undergraduate and postgraduate education. *Conclusion:* This study shows that dieticians do not have enough knowledge about dysphagia symptoms. Dietitians training in this area may provide an important contribution to reducing the negative consequences of dysphagia.

Key words: deglutition, deglutition disorder, dysphagia, dietitian, awareness

Introduction

Swallowing is a sensorimotor behavior beginning in the central cortex of the central nervous system and created by participation of many levels of structures including the bulbusa, ensuring food passes from within the mouth to the stomach. To complete a normal, safe, aspiration-free swallowing action, there needs to be integrated coordination of many neuromuscular structures, functional anatomy and physiology in the head and neck (1). The first swallowing actions begin in the 12-16th week of pregnancy by swallowing amniotic fluid and reaches adult levels at 6 years of age. However, babies born before 32 weeks may have difficulty sucking-swallowing (2). Dysphagia (difficulty swallowing) is a symptom of mechanical obstruction of the transfer of food from the mouth to the stomach, reduction in the power of muscles ensuring the swallowing motion or disrupted coordination (1).

The incidence of dysphagia increases with age, and affects 40-60% of the geriatric population (3). Dysphagia is observed in nearly 12% of patients admitted to hospital, and in 30-60% of patients requiring home care, with the incidence of dysphagia linked to neurological diseases comprising 75-80% of all swallowing disorders (1). High risk patient groups for dysphagia include diseases like stroke, head-neck cancers, cerebral palsy, Parkinsons, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and dementia (1,4).

As a result of dysphagia, insufficient nutrition and malnutrition, lengthened hospital stays, dehydration, acute and chronic aspiration pneumonia due to food and drink entering the airway, permanent lung damage and death may occur (5). Development of malnutri-

tion due to dysphagia negatively affects the rehabilitation duration and functional amelioration speed. Malnutrition causes complications like reduced immunity, pneumonia and compression wounds, and increases mortality. Additionally, dysphagia increases mean hospital stay by 1.64 days, this delay is 4.6 days if accompanied by dehydration and thus, increases hospital costs. Mortality rates are increased 13.7% compared to patients without dysphagia (6, 7).

In oral or pharyngeal dysphagia, the following symptoms are at the forefront; coughing and feeling of suffocation while swallowing, difficulty initiating swallow, feeling food stuck to the throat, sialorrhea, unexplained weight loss, changes in eating habits, recurrent pneumonia, changes in voice and talking (wet voice) and more common nasal regurgitation. During esophageal dysphagia, there may be feeling of food stuck to the chest or throat, oral or pharyngeal regurgitation, changes in nutritional habits and recurrent pneumonia (8, 9).

A multidisciplinary approach is important to increase the success of dysphagia rehabilitation. Important workers include ENT experts, physiotherapist, gastrologist, speech and language therapists, dietitians, radiologists, pediatrists, psychologists/psychiatrists, gerontology and neurology teams (4, 9). Dietitians are a part of early diagnosis and treatment due to awareness of dysphagic patients, and a correct nutritional plan is important for rehabilitation. As a result, dietitians should be knowledgeable and educated about dysphagia and risk factors (10).

As dietitians monitor the nutritional status of patients admitted to hospital, this study inquired about awareness of dysphagia among hospital dietitians and aimed to research the potential for early identification of dysphagia among inpatients.

It is a primary goal in trying to find the answer of the question whether the dieticians are aware of the dysphagia of the patient rather than the approach to the dysphagia.

Material and Method

Research type, location and timing

This research was a descriptive study with the aim of assessing dietitians' awareness of dysphagia and dysphagia symptoms. The research included 85 dietitians working at University Hospitals and Education and Research Hospitals in Ankara province from total 126 dietitians, who volunteered to participate in the study from November 2013 to December 2013.

A table which included to 12 items that may caused of dysphagia or caused of symptoms by dysphagia was presented to dietitians in the study. It was asked participants to whichone of items make dieticians think of exist to dysphagia among patients. The total "yes" answers were determined as an awareness score (between 0-12 points) (Table 1)

In addition, the relationship with the awareness levels between the graduated university, the lessons they received, the working times and the units they work in were evaluated.

Data collection

Using the face-to-face interview technique or e-mail, dietitians completed a survey including questions related to length of employment, dysphagia symptoms, diagnostic methods and dietary applications.

Statistical analysis of data

When assessing results obtained in the study, IBM SPSS 22 (IBM SPSS, Turkey) was used for statistical analyses. When evaluating study data, normal distribution of parameters was assessed with the Kolmogorov-Smirnov test and it was identified that parameters

Table 1. Whichone of items make dietitians think of exist to dysphagia among patients on the table?

	Yes	**No**
Weight loss		
Not fully finishing meals		
Weak control of saliva by patient		
Coughing, feeling suffocated while eating		
Coughing, feeling suffocated after eating		
Presence of tracheostomy		
Patient's choice of food consistency		
Lengthened duration of eating		
Presence of wet, hoarse, breathy voice while talking		
Disrupted posture of patient		

did not have normal distribution. Descriptive statistical methods (mean, standard deviation, frequency), in addition to the Kruskal Wallis test for comparison between the groups for parameters with quantitative data, were used when assessing study data. Spearman's rho correlation analysis was used to investigate the relationships between parameters. Significance was assessed at $p<0.05$.

Limitations of the Research

The research was only completed in University and Education and Research Hospitals located in Ankara.

Results

When unit of employment was examined, 45.9% of dietitians worked in the internal medicine ward, 17.6% in surgical wards, 15.3% on rotation, 9.4% on the nutrition team, 7.1% in food services and 4.7% in clinics. The working durations in their organization varied from 6 months to 26 years, with mean of 4.99±5.81 and median duration of 3 years. Of dietitians, 57.6% stated they sometimes encountered patients with swallowing complaints, with 35.3% encountering them frequently, with 7.1% stated they had never encountered these patients.

While 38.8% of dietitians felt they had sufficient knowledge about dysphagia, 14.1% felt they were very knowledgeable, with 47.1% feeling insufficient. In terms of training/course/symposiums related to dysphagia, 17.6% had participated during undergraduate education, while 17.6% participated after undergraduate education. Of dietitians, 77.6% stated they referred patients with dysphagia symptoms to other experts for assessment.

When dietitians were questioned about symptoms leading to consideration of dysphagia, 63.5% said weight loss, 45.9% said not fully finishing meals, 43.5% said weak control of saliva in patients, 77.6% said coughing or feeling of suffocation during eating, 25.9% said coughing or feeling of suffocation after eating, 40% said tracheostomy, 71.8% said patients choice of consistency in food, 54.1% said longer eating times, 27.1% said wet, hoarse or breathy voice when talking, 14.1% said bad posture of the patient, 22.4% said orientation/cooperation disorder, and 34.1% said recurrent pneumonia/respiratory tract infections (Table 2).

Awareness scores given to dietitians according to knowing dysphagia symptoms varied from 0 to 12, with mean of 5.2+2.7 and median of 5 (Table 3). It means dietitians recognize that average 5 of the 12 symptoms as dysphagia.

There was no statistically significant difference found between awareness scores and graduated university, working duration or clinic of hospital ($p>0.05$).

Dieticians who have enough information about dysphagia (sufficient and very sufficient) score of awareness 5.91±3.13 and 4.58±2.23, respectively (Table 4).

When dietitians were asked about approaches to dysphagia patients, 95.3% stated changes in consistency were made, and 54.1% stated they kept nutritional consumption records to enable assessment of the patient's nutritional status. Of dietitians, 60% recommended oral enteral nutritional products for patients with dysphagia, 29.4% recommended nutrition through a nasogastric tube, 14.1% recommended nutrition with gastrostomy and 11.8% recommended parenteral nutrition.

Table 2. Distribution of dietitians according to their recognition of dysphagia symptoms

	N	%
Weight loss	54	63.5
Not fully finishing meals	39	45.9
Weak control of saliva by patient	37	43.5
Coughing, feeling suffocated while eating	66	77.6
Coughing, feeling suffocated after eating	22	25.9
Presence of tracheostomy	34	40.0
Patient's choice of food consistency	61	71.8
Lengthened duration of eating	46	54.1
Presence of wet, hoarse, breathy voice while talking	23	27.1
Disrupted posture of patient	12	14.1
Disruption of orientation/cooperation	19	22.4
Recurring pneumonia/respiratory tract infections	29	34.1
Other	2	2.4

Table 3. The minimum, maximum, mean, standard deviation, and median values of the awareness score

	N	Minimum	Maximum	Mean±SD	Medyan
Awareness score	85	0,00	12,00	5,2±2,7	5

Table 4. Assessment of awareness score according to levels of feeling informed about dysphagia

Level of feeling informed about dysphagia	Awareness Score	
	Mean±SD	Median
Sufficient	5.91±3.13	6
Very	4.58±2.23	4
Insufficient	4.80±2.35	4
P	0.215	
Kruskal Wallis Test		

Table 5. Distributions of consistency often applied in consistency change practise

	N	%
None	1	1,2
Clear liquid	15	17,6
Fluid liquid	20	23,5
Honey consistency	30	35,3
Soft, smooth (yoghurt, custard ..)	82	96,5
Soft, bite sized	56	65,9

Of dietitians stating they would make consistency changes, 96.5% chose smooth/soft, 65.9% said shredded solids, 35.3% said consistency of honey, 23.5% said fluids and 17.6% said clear liquids. Additionally, 77.6% of dietitians encountering dysphagia referred the patient to another expert for assessment (Table 5).

When knowledge of diagnostic and treatment methods for dysphagia was questioned among dietitians, 62.4% said bedside assessment, 28.2% said MBSS and 21.2% said they knew no methods.

Of dietitians, 98.8% stated they thought dietitians should have an active role in dysphagia rehabilitation; however, of these 76.5% wished to be included in a training process related to dysphagia or were interested in dysphagia rehabilitation.

Discussion

Dysphagia is not rare among hospital patients and is a cause of significant morbidity and morbidity. The main diseases where dysphagia is observed include neurological diseases like stroke, Parkinsons, muscular dystrophy, amyotrophic lateral sclerosis, and multiple sclerosis and it is commonly observed in patients undergoing heart-lung surgery, with head-neck region radiotherapy, with head-neck surgery or with tracheostomy (8, 11). Untreated or neglected dysphagia may cause disrupted quality of life, dehydration, weight loss, aspiration pneumonia and even result in death of the individual (12). Additionally, it negatively affects the general improvement after disease, and increases hospital stays and long-term care requirements (13). However, even with very significant clinical results dysphagia is not sufficiently noticed by health professionals. Additionally, due to an absence of screening protocols and expert personnel related to the topic, dysphagia diagnosis and treatment is delayed which increases hospital malnutrition (14). In Turkey, while the NRS-2002 screening for malnutrition, EAT-10 applications for dysphagia screening test are not yet routine practice.

The role of dietitians in dysphagia diagnosis and treatment encompasses a broad range from traditional nutritional management, to consistency changes within the framework of dynamic nutrition, dysphagia assessment and organization. A patient-centered approach does not only maintain integrity of nutrition and hydration but at the same time is very important to increase the quality of life of the individual (15). In monitoring dysphagic patients, the dietitian tracks the patient's nutritional intake for 3 or 7 days, and calculates levels of consistency selection of foods and energy nutritional element requirements (16). Of dietitians participating in our research, 54.1% reported they would make a nutritional consumption record.

Nutritional consumption records identify whether the patient is receiving sufficient energy and nutritional elements. Thus, necessary interventions are provided in timely manner reducing the risk of malnutrition (17).

There are many methods used to assess dysphagic patients. The most practical and rapidly applied among these is bedside evaluation (18). The presence of symptoms like patient posture, integrity of anatomic structures, weight loss, eating duration, coughing or feeling suffocated during/after eating, wet voice, and fever may lead to consideration of dysphagia and aspiration (19). In this study, 21.2% of dietitians reported they have never heard of bedside assessment. This situation may be due to differences in the employment durations and units of dietitians.

In our research, the majority of dietitians assessed coughing and feeling suffocated during eating (77.6%), weight loss (63.5%) and lengthened eating duration (54.1%) as dysphagia symptoms. Fewer dietitians associated dysphagia with disrupted posture (14.1%), disrupted orientation (22.4%), coughing after eating (25.9%) and wet, hoarse voice (27.4%) (Table 2). Dietitians were given awareness points from 0 to 12 in terms of knowing dysphagia symptoms and mean points were 5.2±2.7. The fact that dieticians know average 5 of the 12 dysphagia symptoms means that patients with other symptoms cannot be considered dysphagic. This is an important problem in terms of causing malnutrition of these patients and delay in recovery processes.

Additionally, the mean awareness points of those dietitians reporting they felt they had sufficient and very sufficient knowledge about dysphagia were 5.91±3.13 and 4.58±2.23, respectively (Table 4). Unfortunately, dietitians, who thought that they had enough knowledge, did not recognize much of the dysphagia symptoms. This situation may be due to the insufficient number of dietitians working in hospitals not allowing sufficient time to assess patients. Because, in hospitals, even a dietitian per 100 beds is not allowed. Of dietitians participating in the study, 82.2% had not received any training on this topic in undergraduate or after undergraduate education. With no speech and language therapists or insufficient dietitians in hospitals, providing basic training about this topic may increase the efficacy of dysphagia rehabilitation.

One study about dysphagia risk screening for stroke patients separately investigated bedside evaluation and diet recommendations by dietitians and speech-language therapists and dietitians identified 40% risk for patients, while speech-language therapists identified 31.5% risk. They concluded that the two study areas had perfect compliance in terms of dysphagia risk and oral/nonoral nutritional recommendations, fluid intake and diet consistency recommendations (20). Diets with changed consistency form the basis of dysphagia management. Modifications may increase quality of life, improve well-being, reduce the risk of malnutrition and dehydration, reduce the risk of aspiration pneumonia and ensure continued oral feeding (21-23). The aspiration risk of fluids and clear liquids are high; however, they may be chosen in patients with bolus conduction problems (22). As a result, it is necessary to choose the consistency according to dysphagia type and level. Of dietitians participating in the research, 95.3% reported they first made consistency changes for dysphagic patients. When making these changes, the most commonly chosen were smooth, pureed food (96.5%) and shredded, blended food (55.9%) (Table 5). Consistency of honey, fluids and clear liquids were chosen less often. A study by Steele et al. showed that nectar-like liquids were primarily recommended for patients with oral insufficiency and laryngeal penetration (24). The fact that all dysphagia symptoms are not recognized by dietitians, will cause not recognise the patients who need to change their consistency. It will also cause mistake in selecting the correct consistency.

Teamwork is important for diagnosis and treatment of dysphagia. It is recommended that dietitians and swallow therapists work together, consistency standards be developed and common and sufficient time be allotted to patient observation (10,5). A study in Spain assessed the food consistency and risk analyses recommended for patients by 30 dietitians and 30 speech and language therapists working in 14 separate hospitals and determined that some differences may lead to significant results in terms of missing aspiration pneumonia, especially, and emphasized that working together would contribute to patient healing (25). In a similar study. Dietitians first assessed patients in the neurology ward in terms of dysphagia over 2 months

and dietitians observed mealtimes and prepared a scale related to dysphagia symptoms. After diet recommendations and regulation was performed, speech-language therapists blind to the study assessed the patients. Of 35 patients with dysphagia identified, 4 were recommended to cease oral nutrition by both dietitians and speech-language therapists. Neither group recommended thin fluids like water or milk, etc. for any patient. It was concluded that dietitians and therapists played a reliable and primary role in oral-nonoral nutrition decisions due to the compatibility between decisions by dietitians and therapists (10).

Conclusion

While the research has been done only 4 university were giving the nutrition and dietetic education in Turkey and the profession of dieticians is obtained by a 4 year undergraduate education. There is no additional qualification exam after graduation. During their training, dietitians receive education about malnutrition and participate in activities aiming to reduce malnutrition. However, one of the significant causes of hospital and geriatric malnutrition, especially, of dysphagia is not sufficiently or carefully screened. Dysphagia rehabilitation, requiring coordinated work by multiple units, is a very specific application area. Dietitians may undertake a key role in these teams with a significant and effective duty in the diagnosis and treatment of dysphagia. Thus, in addition to increasing the quality of life of the patients, it will be possible to reduce the formation of additional diseases, shorten healing times and reduce malnutrition.

This research also carries the character of being a stimulant for dietitians in the hospitals where the data are collected. They will also notice the importance of their role in dysphagia rehabilitation.

References

1. Özdemir A, Çekin AH. Disfajiye Genel Yaklaşım. Güncel Gastroenteroloji 2011; 15(4): 227-234.
2. Logeman J. Evaluation and treatment of swallowing disorders. Austin, TX. Pro-ed Publisher 1999; ss: 24-26, 139-156.
3. Cassel SG. Case Reports: Trial Dysphagia Interventions Conducted via Telehealth. Int J Telerehabil 2016; 5: 8(2): 71-76.
4. Çiyiltepe M. Yutma Bozuklukları Tanı ve Rehabilitasyonu. Ankara, Türkiye: GATA Basımevi. 2005.
5. Heis CJ, Goldberg L, Dzarnoski M. Registered Dietitians and Speech-Language Pathologists: An Important Partnership in Dysphagia Management. Journal of the American Dietetic Association 2010; s: 1290-1293.
6. Leonard R. Integrated Management of Dysphagia and Malnutrition. Satellite Symposium Proceedings. 7th EUGMS Congress. Málaga, Spain. 2011.
7. Umay EK, Gürçay E, Ünlü E, Ekşioğlu E, Çakcı A. Erken Dönem İnmeli Hastalarda Disfajinin Fonksiyonel ve Nütrisyonel Etkisi. J Med Sci 2010; 30(3): 925-931.
8. Aksoy EA, Öz F. Yutma Bozukluklarında Tanı. Acıbadem Üniversitesi Sağlık Bilimleri Dergisi 2012; 3(1): 1-6.
9. Wieseke A, Bantz D, Siktberg L, Dillard N. Assessment and Early Diagnosis of Dysphagia. Geriatr Nurs 2008; 29: 376-383.
10. Broady R, Decker RT, Vonhagen S, Maillet JO. Journal of the American Dietetic Assosiation 2000; 100 (10): 1029-1037.
11. Wirth R, Dziewas R. Neurogenic dysphagia. Internist (Berl) 2017; 58(2): 132-140 doi: 10.1007/s00108-016-0178-8.
12. Harvey S, Gordon L. Recognısıng Dysphagıa Rısk And Compromısed Nutrıtıon In Patıents Wıth Advanced Chronıc Obstructıve Pulmonary Dısease (Copd): Explorıng The Role Of Speech And Language Therapy And Dıetetıcs. BMJ Support Palliat Care 2013; 3: A7.
13. Shelat VG, Garvi JP. Seminars in Dysphagia "Nutritional Support in Dysphagia." http://dx.doi.org/10.5772/61243. 2015; s:121-136.
14. Tuncay F, Taşbaş Ö, Borman P, Geçene M, Coşkun Ö. The Bedside Clinical Evaluation of Swallowing Function of Stroke Patients in Acute Stage. J PMR Sci 2011; 14: 33-8.
15. Macleod M, O'shea S. The Dietitian's Role in Diagnosis and Treatment of Dysphagia. Medical Radiology. Springer, Berlin, Heidelberg. 2017. https://doi.org/10.1007/174_2017_124.
16. Cichero J. Science Supporting Better Nutrition. Clinical Nutrition Highlight 2012; 8(3): 1-8.
17. Kraaijenga SAC, Y, Stuiver MM, P, Al-Mamgani A, Brekel MWMVD, Hilgers FJM Efficacy Of A Novel Swallowing Exercise Program For Chronic Dysphagia In Long-Term Head And Neck Cancer Survivors. Head Neck 2. 2017. Doi: 10.1002/Hed.24710.
18. Trapl M, Enderle P, Nowotny M, Teuschl Y, Matz K, Dachenhausen A, Brainin M. Dysphagia Bedside Screening for Acute-Stroke Patients The Gugging Swallowing Screen. Stroke 2007; 38(11): 2948-52.
19. Selçuk B. Dysphagia in Stroke. Turk J Phys Med Rehab 2006; 52(Suppl B): 38-44.
20. Castellanos VH, Butler E, Glucgh L, Burke B. Use of Thickened Liquids in Skilled Nursing Facilities. Journal of the American Dietetic Association 2004; 104(8): 1222-1226.

21. Macleod M. Dietary Treatment in Dysphagia, 33rd Congress of Clinical Nutrition & Metabolism, ESPEN. 2011.
22. Garcia JM, Chambers E, Matta Z, Clark M Serving Temperature Viscosity Measurements of Nectar- and Honey-Thick Liquids. Dysphagia 2008; 23(1): 65-75.
23. Leonard R, Kendall K. Dysphagia Assesment and Treatment Planning. San Diego, California. Singular Publishing Group 2008; ss: 139-156.
24. Steele C, Van Lieshout PH, Goff HD. The rheology of liquids: a comparison of clinicians' subjective impressions and objective measurements. Dysphagia 2003; 18(3): 182-195.
25. Amaral ACF, Rodrigues LA, Furlan RMMM, Vicente LCC, Motta A. Speech-Language and Nutritional Sciences in hospital environment: analysis of terminology of food consistencies classification. CoDAS 2015; 27(6): 541-9.

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Original article

The implementation and evaluation of a nutrition education programme about Mediterranean diet for adolescents

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Summary. The purpose of this study is to assess the compliance of the dietary habits in adolescents with the Mediterranean diet by detecting their nutrition knowledge levels and to educate the adolescents on the Mediterranean diet. The research was conducted with adolescents aged between 11 and 16 years in Eskişehir Province. The experimental group (n=76) and the control group (n=84) were applied pretest and posttest. The experimental group was educated on the Mediterranean diet by using different teaching methods for a total of 18 hours (8 weeks). The compliance of the nutrition status of adolescents with the Mediterranean diet was assessed using the Mediterranean Diet Quality Index (KIDMED). Before the education, the KIDMED scores of 25.0% of adolescents had been poor (≤3 points), 56.6% of them had been medium (4-7 points) and 18.4% of them had been high (≥8 points) whereas after the education, the KIDMED score of all the adolescents (100 %) were high (≥8 points). In consequence of the education provided to the experimental group, the nutrition knowledge and KIDMED scores of the adolescents increased. It is important to provide individuals with proper nutrition knowledge and habits beginning from their childhood to prevent health problems at later ages.

Key words: Mediterranean Diet Quality Index (KIDMED), Mediterranean diet education, adolescent nutrition

Introduction

Child nutrition takes form according to the social dietary habits. Healthy eating habits adopted since early ages have influence on a healthier lifetime for individuals in the later years (1-4). The fact that the diseases such as cardiovascular diseases, diabetes, colon cancer and even Alzheimer are less common in the countries with coasts to the Mediterranean Sea, has aroused researchers' interests in the dietary habits in these countries. In the studies conducted in recent years, it has been detected that various tumors (prostate, colon cancer etc.) as well as chronic diseases such as diabetes, obesity, childhood asthma and rhinitis are also less common in the regions where this diet is applied and that there is a connection between individuals' eating habits and frequency of these diseases (5-11). A study conducted in order to determine the impact of olive oil consumption on lipidosis in patients with non-alcoholic fatty liver disease revealed that olive oil may decrease lipidosis (12).

The Mediterranean diet contains plenty of fresh natural foods, especially fruits and vegetables as well as olive oil, fish, hazelnut, peanut, walnut. The basis of the Mediterranean diet, the definition of which was primarily developed by Keys, is composed of vegetable variety and whole-grain foods (13). The essence of this diet includes regular daily activities, consumption of natural and fresh foods as well as avoidance of too much processed food. The consumption of olive oil is particularly the most important element of the diet (14-15). By means of this study, the compliance of the

adolescents' nutrition status with the Mediterranean diet had been assessed according to their nutrition knowledge and then the adolescents were provided with education on nutrition.

Materials and Methods

Objective and procedure

It was examined in what proportion the adolescents' daily nutrition status complies with the Mediterranean diet and whether or not their nutrition knowledge levels and the compliance of their diets with the Mediterranean diet are correlated. The adolescents were provided with an education on the Mediterranean diet and their nutrition knowledge scores and KIDMED scores were assessed before and after the education.

The study was conducted between 9 February 2015 and 18 May 2015 with adolescents aged between 11 and 16, living in Sivrihisar district of Eskişehir province. The target population of the study was the students receiving education in the middle schools in Sivrihisar district (n=1067). The sample included 160 students: 76 students from Ertuğrul Yavuz Gülerce Middle School (experimental group) and 84 students from Dümrek Middle School (control group). The sample number was determined by means of the Sample versus Target Population Table (16). The schools in the sample were determined via the stratified teleological sampling method (17) and assigned as experimental and control groups. The schools of experimental and control groups were chosen from the regions that have the same demographic characteristics. Before it was launched, the study had been approved by the Ministry of National Education. With the decision of Eskisehir provincial National Education Directorate, research approval was obtained (Date: 19 November 2014, No: 5439716). In addition, parents were informed and approved for the research.

Instruments and data collection

In the study, "Two Group Pretest and Posttest Experiment" adaptation of the "Classic Experimental Design" was used. Two different data collection instruments were used in the research. The first one of the data collection instruments was the information form developed by the researcher. During the preparation of the information form, the relevant resources and questionnaire forms of the research previously done about this subject were referred to. In the form, there are 8 questions for determining the demographic characteristics of the students, 7 questions for their dietary habits.

The other data collection instrument was the Mediterranean Diet Quality Index (KIDMED) which had been developed in order to assess the compliance of kids' daily nutrition status with the Mediterranean diet. The development of the KIDMED index is based on the principles of Mediterranean dietary patterns as well as the factors that undermine it. The index ranged from 0 to 12, and was based on a 16 questions test that could be self-administered or conducted via an interview (pediatrician, dietitian, etc.). Questions denoting a negative connotation with respect to the Mediterranean diet were assigned a value of -1, and those with a positive aspect were scored +1 (18).

According to the KIDMED index (16 questions):

≤8 points shows "optimal" diet quality.

4-7 points "average" (improvement needed).

≥3 points "very low" (diet quality).

Developed by Stefan et al., validity and reliability of KIDMED index was initially used by Köksal, Tek and Pekcan. In addition to the KIDMED index, a nutritional knowledge test developed by the researchers was used to determine the nutritional knowledge of participants. The nutritional knowledge test included 20 questions. Writers were asked to submit a questionnaire form with questions on nutritional knowledge. These questions were scored according to a 5-point Likert-type scale: very important=5 points; considerably important=4 points; important to some extent=3; minimal importance=2 points; not important=1 point. Potential scores ranged from 20 to 100 points. The validity of the nutritional knowledge questions was tested, and found to have a Cronbach's alpha score of 0.844. These forms were also used as pretest and posttest. Knowledge points of the students were evaluated.

In the research, a planned education was implemented and the experimental group was provided with nutrition education. The education, which took place twice a week during the school hours, was completed in

8 weeks. One month after the completion of the education program, the students were given a consolidation education with the same subjects for two hours. The period of education took 18 hours in total. The education program, which had been prepared by the researcher in form of a presentation, was given to adolescents with the assistance of blackboard via computer and projector. The subjects related to the adequate and balanced nutrition, the importance of nutrition in the adolescence period, the healthy and balanced nutrition suggestions of the World Health Organization, the basic rules of a healthy nutrition, the characteristics of the Mediterranean diet and its effects on the health were explained in these slides. The researcher particularly aimed to make the students comprehend the importance of the Mediterranean diet. They emphasized the importance of the Mediterranean dietary pattern in detail.

Of all the teaching methods; discussion, question and answer, and brainstorming methods were administered. Audio-visual materials were used during the education. The students prepared some documents such as banners, leaflets etc. Posters prepared by children were hung on the wall and not pulled down therefom until the end of research. Moreover, the adolescents organized activities such as writing drama, poems, songs, plays about the Mediterranean diet and during these activities, they consolidated their knowledge.

Data analysis

The data collected as a result of pretest and posttest was evaluated by using the SPSS 21.00 (Statistical Package of the Social Sciences) program and studied with 95% reliance. The relation between the experimental and control groups and their dietary habits was examined with X^2 relation test. The distribution of nutrition and KIDMED scores in the experimental and control groups before and after the education was determined through independent sample t test and their differentiation according to the gender, which is one of the demographic variables, was evaluated.

Results

The demographic characteristics of the participants of the research are shown in Table 1.

Amongst the adolescents who participated in the research; 36.8 % were 8th grade and 60.5% were boys, 22.4% were 11 years old in the experimental group. With regard to the educational level of participants' mothers, the results showed that 68.4 % were elemantery school in experimental group. For participants' fathers educational levels were similar to mothers educational level and 61.8% of fathers were primary school graduates.

The table shows that 25.0 % of the adolescents had a very poor diet, 56.6 % had a diet that needed improvement and 18.4.9% followed a diet of optimal quality before education. The KIDMED scores (≥8) of all students increased after the education (Table 2).

While, before the education, the number of both boys and girls who received high KIDMED scores (≥8) had been low in the experimental group, all adolescents' scores scaled up to high (≥8) after the education. This result shows that the students understood the significance of the Mediterranean diet and its effects on health (Table 3). Moreover, there is no difference between KIDMED scores of adolescents according to the educational status of their parents ($p<0.05$).

There are significant differences between the scores of male students before and after the education for all of the items about nutrition knowledge. While the score obtained after the education for the items "consumption of only the favorite foods, generally eating veal and generally eating lamb" was lower than the one before the education, the score obtained after the education was higher for other items. This result especially shows that the adolescents realized that the red meat consumption should be limited in the Mediterranean diet. There were no statistically significant differences between the scores of female students before and after the education for the items "abundant consumption of fruit, conformity of dietary habits to the age and health and generally eating veal" ($p>0.05$). While the score obtained after the education for the items "consumption of only favorite foods, generally eating veal and lamb", excluding the items above, was lower than the score before the education, this score was higher for the other items after the education (Table 4).

While there was no statistically significant difference between the nutrition knowledge pretest results

Table 1. Demographic Information

		Experimental Group (n=76)		Control Group (n=84)	
		n	%	n	%
Grade	5	17	22.4	22	26.2
	6	17	22.4	15	17.9
	7	14	18.4	17	20.2
	8	28	36.8	30	35.7
Gender	Boy	46	60.5	48	57.1
	Girl	30	39.5	36	42.9
Age	11	17	22.4	25	29.8
	12	16	21.1	12	14.3
	13	19	25.0	18	21.4
	14	20	26.3	23	27.4
	15-16	4	5.2	6	7.1
Father's educational level	Illiterate/just literate	5	6.6	5	5.9
	Elementary School	47	61.8	26	31.0
	Middle School	18	23.7	25	29.8
	High School or College	6	7.9	28	33.3
Mother's educational level	Illiterate	7	9.2	4	4.8
	Literate	6	7.9	7	8.3
	Elementary School	52	68.4	44	52.4
	Middle School	6	7.9	26	30.9
	High School	5	6.6	3	3.6
Father's occupation	Farmer	58	76.3	52	61.9
	Self-employed	15	19.7	14	16.7
	Civil servant	3	4.0	18	21.4
Mother's occupation	Housewife	76	100.0	84	100.0

Table 2. KIDMED Scores of Adolescents in the Experimental Group (n=76)

KIDMED Score	Before Education		After Education	
	n	%	n	%
Very poor (≤3)	19	25.0	-	-
Diet needs improvement (4-7)	43	56.6	-	-
Optimal Mediterranean diet (≥8)	14	18.4	76	100.0

of the students in the experimental group and those of the control group, there was a significant difference between the posttest results. The average of nutrition knowledge score posttest results of the experimental group was determined as $\bar{X}$=86.42 while that of the control group was determined as $\bar{X}$=74.12. There is a significant difference between the KIDMED pretest results of the students in the experimental group and those of the students in the control group. The average of KIDMED pretest results of the experimental group was 5.20 while that of the control group was 6.06. There is a significant difference between the KIDMED posttest results of the students in the experimental group and those of the students in the control group. The average of KIDMED posttest results of the experimental group was 11.84 while that of the control group was 5.26 ($p<0.05$). It was observed that

Table 3. Distribution of KIDMED Scores of Adolescents by Gender (n=76)

KIDMED Score	Boy				Girl			
	Before Education		After Education		Before Education		After Education	
	n	%	n	%	n	%	n	%
Very poor (≤3)	15	32.6	-	-	4	13.3	-	-
Diet needs improvement (4-7)	20	43.5	-	-	23	76.7	-	-
Optimal Mediterranean diet (≥8)	11	23.9	46	100.0	3	10.0	30	100.0

Table 4. Distribution of nutrition knowledge scores of adolescents in the experimental group before and after education according to gender

Nutrition Knowledge	Boy		t	P	Girl		t	P
	Before Education $\bar{X}$=SD	After Education $\bar{X}$=SD			Before Education $\bar{X}$=SD	After Education $\bar{X}$=SD		
Should have adequate nutrition	4.57±0.93	4.98±.15	-2.929	.005*	4.57±.73	4.97±.18	-2.845	.008*
Should have balanced nutrition	4.24±0.97	4.96±.21	-4.959	.000*	4.23±0.86	4.90±.31	-4.325	.000*
Consumption of only the favorite foods	3.17±1.32	1.65±.97	6.282	.000*	3.60±1.19	1.53±.86	7.511	.000*
Abundant consumption of fruit	4.50±0.96	4.80±.40	-2.197	.033*	4.57±0.68	4.60±.50	-.226	.823
Abundant consumption of vegetable	3.93±1.34	4.72±.46	-3.936	.000*	3.80±1.21	4.57±.50	-3.096	.004*
No consumption in late hours at night	2.46±1.41	4.65±.48	-10.236	.000*	2.97±1.63	4.80±.48	-5.633	.000*
Should consume low-fat food	3.54±1.43	4.65±.53	-5.190	.000*	3.73±1.08	4.47±.73	-3.194	.003*
Chewing the food sufficiently	4.41±0.93	4.76±.43	-2.185	.034*	4.27±0.87	4.73±.52	-2.379	.024*
Should adopt nutrition habits according to age and health status	4.15±1.15	4.70±.47	-3.012	.004*	4.40±1.04	4.73±.45	-1.624	.115
Avoiding processed foods containing additives	3.59±1.41	4.78±.42	-5.759	.000*	3.80±1.32	4.80±.41	-3.746	.001*
Avoiding too much calorie intake	3.26±1.44	4.65±.53	-6.496	.000*	3.90±1.16	4.73±.52	-3.785	.001*
Should maintain ideal weigh	3.57±1.52	4.76±.48	-5.205	.000*	3.93±1.62	4.77±.43	-2.816	.009*
Avoiding too much salty foods	3.91±1.33	4.65±.48	-3.417	.001*	4.10±1.16	4.80±.41	-3.034	.005*
Avoiding high-sugar foods	3.76±1.51	4.72±.46	-4.311	.000*	3.83±1.42	4.80±.41	-3.713	.001*
Should have three main meals a day	4.00±1.35	4.85±.36	-4.161	.000*	4.07±1.01	4.83±.38	-4.173	.000*
It is beneficial to consume fish	3.48±1.44	4.63±.53	-5.467	.000*	3.23±1.33	4.53±.73	-4.782	.000*
It is beneficial to consume chicken	3.52±1.35	4.65±.53	-5.507	.000*	3.43±1.38	4.53±.51	-4.557	.000*
It is beneficial to consume veal	3.30±1.41	2.04±.63	5.218	.000*	2.50±1.48	2.23±.63	0.869	.392
It is beneficial to consume mutton	3.87±1.07	2.15±.67	9.523	.000*	3.43±1.45	2.33±.61	3.485	.002*
Should consume pulpy fibrous products	3.35±1.35	4.72±.50	-7.023	.000*	3.47±1.20	4.67±.48	-5.288	.000

***p<0.05*

Table 5. Comparison of Nutrition Knowledge Scores and KIDMED Scores of adolescents

	Group	n	$\bar{X}$± SD	t	p
Nutrition Knowledge Score (Pretest)	Experimental	76	75.08±8.93	-1.487	.139
	Control	84	77.24±9.39		
Nutrition Knowledge Score (Posttest)	Experimental	76	86.42±3.70	8.660	.000*
	Control	84	74.12±11.87		
KIDMED Score (Pretest)	Experimental	76	5.20±2.45	-2.366	.019*
	Control	84	6.06±2.16		
KIDMED Score (Posttest)	Experimental	76	11.84±0.52	22.304	.000*
	Control	84	5.26±2.65		

**P<0.05*

Table 6. Comparison of Experimental and Control Groups Nutrition Knowledge Scores and KIDMED Scores

Groups		n	$\bar{X}$± SD	t	p
Experimental Group	Nutrition Knowledge Score (Pretest)	76	75.08±8.93	-11.579	.000*
	Nutrition Knowledge Score (Posttest)	76	86.42±3.70		
	KIDMED Score (Pretest)	76	5.20±2.45	-23.204	.000*
	KIDMED Score (Posttest)	76	11.84±0.52		
Control Group	Nutrition Knowledge Score (Pretest)	84	77.24±9.39	2.181	.032*
	Nutrition Knowledge Score (Posttest)	84	74.12±11.87		
	KIDMED Score (Pretest)	84	6.06±2.16	2.578	.012*
	KIDMED Score (Posttest)	84	5.26±2.65		

**P<0.05*

both the nutrition knowledge scores and KIDMED scores of the experimental group increased following the education and that the education was beneficial to the students (Table 5).

Whereas the nutrition knowledge score of the experimental group was $\bar{X}$=75.08 in the pretest, it was determined as $\bar{X}$=86.42 after the posttest. This difference between the pretest and the posttest is statistically significant ($p<0.05$). While the KIDMED score of the experimental group was $\bar{X}$=5.20 in the pretest, it increased to $\bar{X}$=11.84 in the posttest and this difference is significant as well ($p<0.05$). There is also a significant difference between the KIDMED pretest score and posttest score of the control group ($p<0.05$) (Table 6).

Discussion

Since the 60s', Mediterranean diet has been applied, especially in the olive growing areas of the Mediterranean region. This diet includes consumption of plenty of olive oil, a large quantity of fruits and vegetables, dairy products, especially cheese, meat and meat products in small quantities (21). Turkey has a coast on the Mediterranean Sea and in these regions, this diet is only partially applied. The dietary habits of individuals differ towards central and eastern regions.

Different researches have been conducted on the Mediterranean diet. Among these researchs, Sahingoz and Sanlier (22) detected that out of 890 adolescents, 464 of whom were boys and 426 of whom were

girls, 17.9% had poor (≤3), 59.2% had a mid-quality (4-7) and 22.9% had optimal quality (≥8) KIDMED scores. In the study conducted by Philippou et al (23) 23 male and 11 female swimmers ranging from 13 to 19 and their parents were provided with nutrition education. They assessed the influence of education on the nutrition knowledge and its compliance with the Mediterranean diet. Following the study, they observed an increase in the adherence to the Mediterranean diet of the adolescents, whose parents also received the education. The increase in the KIDMED score and the compliance with the Mediterranean diet that had firstly been % 21 (≥8) rose to 47 % (≥8) after the education, which was stated to be a positive development ($p<0.01$). In the study conducted by Serra-Majem etc. (18) (2004) in Spain, it was detected that out of the children and adolescents ranging from 2 to 24, 2.9% had (≤3), 48.6% had (4-7) and 48.5% had (≥8) KIDMED scores. It was detected in our study that the KIDMED scores of 25.0% of the students was very poor (≤3), 56.6% was a mid-quality (4-7) and 18.4% was optimal quality (≥8). After the education, the KIDMED scores of all the students increased to ≥8 (Table 2). Within the scope of our study, it was identified that KIDMED scores of adolescents do not show any differences according to the educational status of their parents ($p<0.05$). The study carried out by Bawaked etc (24) revealed that there is a significant association between adherence to the Mediterranean diet, gender and parental education status ($p<0.01$). It was determined that adherence to Mediterranean diet was higher among children of well-educated parents compared to children of parents with elementary school level of education. As pursuant to our study, there is no difference between KIDMED scores of adolescents according to their parents' level of education. This may result from the fact that parents entail a relative low degree of education.

In the study conducted by Serra-Majem etc. (18) (2004) in Spain, out of the boys among the children and adolescents aged between 2 and 24 years, 3.2% had poor (≤3), 47.8% whose diets needed to be improved had a mid-quality (4-7) and 49.0% had high (≥8) KIDMED scores. However, it was detected that, out of the girls, 2.5% had poor (≤3), 49.5% whose diets need to be improved had a mid-quality (4-7) and 47.9% had optimal quality (≥8) KIDMED scores. In the study conducted by Kabaran and Gezer (25) in the Turkish Republic of Northern Cyprus, it was determined according to the gender that out of the children and adolescents ranging from 9 to 13, 7.9% of boys and 4.7% of girls had poor (≤3), 22.9% of boys and 28.7% of girls had a mid-quality (4-7), 10.0% of boys and 10.0% of girls had high (≥8) KIDMED index. The researchers observed that there is no statistically significant difference between the average KIDMED index according to gender and age groups ($p>0,05$). In the research by Farajian et al., (26) it was detected that out of Greek male kids aged between 10 and 12, 48.4% had poor (≤3), 49.3% had a mid-quality (4-7), 51.7% had high (≥8) KIDMED scores. They also determined the scores according to the gender as 3.64±2.29 in boys and 3.66±2.24 in girls; therefore, it was observed that there are no significant differences in KIDMED scores according to the gender ($p>0.05$). In the study in which they examined the compliance of nutrition status of the adolescents in the Southern and Northern Italy with the Mediterranean diet, Noale et al., (27) detected that out of 565 adolescents aged between 12 and 19 years, 38.6% had poor (≤3), 47.4% had a mid-quality (4-7) and 14% had optimal quality (≥8) KIDMED scores. Moreover, they observed that the adolescents in the Southern Italy have more adherence to the Mediterranean diet. In our study, we assessed that 32.6% of the boys and 13.3% of the girls had very poor (≤3) KIDMED scores before the education. It was determined that 43.5% of the boys and 76.7% of the girls should develop their compliance of nutrition status with the Mediterranean diet, which is described as a mid-quality score (4-7). Those who had high (≥8) KIDMED scores were determined as 23.9% of the boys and 10.0% of the girls (Table 3). Other studies show resemblance to the KIDMED scores before the education of our study. This result indicates that adolescents need to improve their diet quality. After the education, the KIDMED scores of both girls and boys were observed to be ≥8.

Providing with nutrition education during childhood is important regarding the development of a healthy eating habit in the youngsters (23). A nutrition education was provided by Demirözü (28) in order to increase the nutrition knowledge level of 78 girls doing

sports aged between 8 and 12 and to create permanent behavioral changes. The researcher determined that the average of the points the experimental group had acquired from nutrition knowledge questions in the pretest was 83.5±10.3 % and that of the control group was 81.8±12.9 %. The difference between the average of groups was not considered as statistically significant ($p>0.05$). However, in the posttest, the average of the experimental group (98.7±2.9%) was found significantly higher than that of the control group (78.8±14.4 %) ($p<0.001$). In the study in which the nutrition education provided, the elementary school students in Özkonak town of Nevsehir province by Kaplan (29) were analyzed, and it was observed that the average of the students' knowledge scores, which had been 11.532±1.560 before the education, increased to 13.337±0.882 following the education. A statistically significant difference was detected between the education and the knowledge scores averages ($p<0.001$). According to the study that examined the influence of the nutrition education provided the students in the regional primary boarding school by Keskin (30) on the food consumption, while the average of the nutrition knowledge test score of the experimental group had been 3.10 in the pretest, it reached to 9.05 in the posttest, increasing 5.94 points ($p<0.001$). In the study conducted with Spanish school children, Serra-Majem et al. (31) assessed that the boys had higher consumptions of milk and dairy products, grain, pastry, legume, fish, red meat, poultry, egg and fruit than girls; that the consumption of vegetable was higher in girls and the consumptions of sugar, dried fruits and nuts were at the same level in boys and girls. Proper nutrition habits may be developed in children by popularizing the sales of fruits, vegetables, dried nuts, milk and dairy products instead of packaged processed foods in school canteens.

The common result of our study and of the other studies is that the nutrition educations provided to the children are effective. The nutrition knowledge scores and KIDMED scores increased significantly after the education with respect to those before the education (Tables 4, 5, 6). All adolescents obtained a KIDMED score of ≥8 following the education. However, the difference between girls'nutrition knowledge scores in pre- and after education period is statistically meaningless in three questions ($p>0.05$). This case gives rise to the thought that girls may have failed to adequately comprehend some points. In the societies lacking adequate and balanced diet, the nutrition problems are inevitable. One of the most important reasons of the inadequate and unbalanced diet in Turkey is considered to be the fact that the individuals are not sufficiently educated on nutrition and that they lack nutrition knowledge (32). There is not any independent course addressing the importance of healthy diet in school programs in Turkey. Currently in Turkish primary schools (1st–8th grade), nutritional programs are still implemented with contributions from and the support of the students' families, without any government support. Food-hour is the time when students eat grab-a-bite food (fruit, fruit juice, milk, ayran, sandwich, etc.) during the 15-min break. While students are eating, they are questioned about food. Other countries that do not possess nutrition courses in their school programs may add a "nutrition" course in their programs and to systematically educate children on healthy and proper nutrition. Because, today, children and adolescents tend to consume fast food, not paying attention to the importance of healthy nutrition. Development of healthy nutrition knowledge and habits will increase the quality of life in individuals and offer them a quality aging.

The studies have shown that the Mediterranean diet has an influence on prevention of many diseases. The adherence to this diet which is based on the dietary habits of the peoples living in the West Anatolian coasts, Prince Islands, Crete and Greece, Southern Spain, France and Italy is asserted to decrease the risks of neurodegenerative diseases (Parkinson and Alzheimer diseases), cardiovascular diseases, metabolic syndrome, cancer (especially colon cancer and breast cancer), type 2 diabetes, obesity, stroke, inflammation, hypertension, intellectual disabilities and depression (27, 33-43). A study conducted with coeliac disease patients revealed that the daily diet of these people had a low adherence to the Mediterranean diet and that it could be beneficial to guide them to follow the Mediterranean diet (44). Liese etc (45) conducted a research in order to identify the impact of dietary quality and way of nutrition on inflammation that can be observed in youth with Type 1 Dia-

betes. KIDMED scores of youth were also evaluated within the scope of this study. Findings indicated that teens had a KIDMED score between 3-12 and that a healthy way of nutrition leads to the avoidance of inflammation in youngs, but has not the same impact on youngs suffering from Type 1 Diabetes. Hernandes etc (46) have examined the impact of children's adherence to Mediterranean diet on bone mineral content and bone density. Children's physical activity status was also evalued within this process. Research results showed that 82.4% of obese children had a low adherence to Mediterranean diet. It has been concluded that a good bone mass can be created through enagaging in high or medium level of physical acitivities and leading a life in adherence to the Mediterranean diet. Another study examining the relationship between Spanish adolescents' adherence to the Mediterranen diet and their academic achievement showed that the Mediterranen diet along with a good quality of sleep have a positive impact on school performance and academic achievement (47).

Comparable to the rest of the world, quality of life in Turkey is affected by factors like obese families, obese children, cardiovas-cular diseases, Type-2 diabetes, hypertension, psycho-social problems and cancer. The entire population can benefit from a Mediterranean diet and information and education about this diet should be distributed and publicized. For instance, the curriculum's of educational institutions, including kindergartens, should highlight the importance of consuming olive oil, fruits, vegetables and wholegrain cereals; balanced and sufficient nutrition; and of physical activity. The health benefits of a Mediterranean diet are profound enough to warrant inclusion and promotion, for the adoption of healthy eating habits by children. With respect to its contribution to the protection of human health, starting from the children in elementary schools, this diet should be taught to adolescents as well as their parents, and be included in their curriculum so that their awareness can be increased.

Governments at the regional, national and European level should take prioritize raising, producing, transporting and commercializing the foods that constitute the Mediterranean diet: olive oil, fruits and vegetables, fish, cheese and yoghurt, nuts, cereals. Families, also, should take responsibility for making the healthiest choices when purchasing food for the home, or at a restaurant (17).

Conclusion

Nutrition has an important influence on healthy growth and development as of infancy and on prevention of health problems. Nutrition and dietary habits in children are essential to plan strategies aiming at the improvement of health in long term in the adult population (48-49). If an individual uses his/her knowledge to make behavioral changes, it means that such knowledge is promoting positive behaviors and habits. This study determined the level of nutritional knowledge amongst adolescents, and the extent to which their nutritional habits are in accordance with the Mediterranean Diet Quality Index. In our study, it was observed that the nutrition knowledge of the adolescents increased and that they comprehended the characteristics of the Mediterranean diet following the education on the Mediterranean diet and healthy nutrition. The Mediterranean diet is also considered to set a good example regarding adequate and balanced nutrition. With respect to its contribution to the protection of human health, starting from the children in elementary schools, this diet should be taught to adolescents as well as their parents, and be included in their curriculum so that their awareness can be increased. Proper nutrition habits may be developed in children by popularizing the sales of fruits, vegetables, dried nuts, milk and dairy products instead of packaged processed foods in school canteens. The results of this study have revealed that a systematical nutrition education is efficient and beneficial for the individuals. In order to reform the wrong nutrition habits and increase the knowledge level, it is essential to provide children and adolescents with applied nutrition education at every stage of education starting from preschool institutions and to sustain this education.

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References

1. Fuhr, J. E., Barclay K. H. The importance of appropriate nutrition and nutrition education. Young Child 1998; 53(1): 74-80.
2. Pirouznia, M. The influence of nutrition knowledge on eating behavior-the role of grade level. Nutr & Food Sci. 2001; 31(2): 62-67.
3. Powers, A. R., Struempler B. J., Guarino A., Parmer. S. M. Effects of a nutrition education program on the dietary behavior and nutrition knowledge of second-grade and third-grade students. J Sch Health 2007; 75(4): 129-133.
4. Sahingoz, S. A. 2015. The Mediterranean diet quality ındex (KIDMED) and nutrition knowledge. In The Mediterranean diet: An evidence-based approach, ed. V. R. Preedy and R. R. Watson, 115-122. USA: Academic.
5. Musaiger, A.O. Nutritional status ans diets habits of adolescent girls in Oman. Ecol Food Nutr 1994; 31(3-4): 227-237.
6. Serra Majem L.¿Másbeneficios de la dietamediterránea?. Nutrición y Obesidad 2001; 4: 43-46.
7. Serra-Majem, L., Roman B., Estruch. R. Scientific evidence of interventions using the Mediterranean diet: A systematic review. Nutr Reviews 2006; 64(1): 27-47.
8. Cawley, J. H. Markets and childhood obesity. Future Child 2006; 16(1): 69-88.
9. Benetou, V., Trichopoulou, A., Orfanos P., Naska, A., Lagiou, P., Boffetta P., Trichopoulos, D. Conformity to traditional Mediterranean diet and cancer incidence: the Greek EPIC cohort. BJC 2008; 99(1): 191-195.
10. Muñoz, M. A., Fíto, M., Marrugat, J., Covas M.I., Schröder H. Adrehence to the Mediterranean diet is associated with better mental and physical health. Br J Nutr 2008; 101(12): 1821-1827.
11. Panagiotakos, D.B., Dimakopoulou, K. Katsouyanni, K. Bellander T., Grau M., Koenig W., Peters. A. Mediterranean diet and inflammatory response in myocardial infarction survivors. Int J Epidemiol 2009; 38(3): 856-866.
12. Rezaei S., Akhlaghi, M., Sasani M.R., Boldaji, R.B. Olive oil lessened fatty liver severity independent of cardiometabolic correction in patients with non-alcoholic fatty liver disease: A randomized clinical trial. Nutrition 2019; 57:154-161.
13. Keys, A. Mediterranean diet and public health: Personal reflections. Am J Clin Nutr 1995; 61(6): 1321-1323.
14. Köksal, O. 2001. Gıda ve beslenme. Kayseri: Erciyes Üniversitesi.
15. Demirci, M. 2011. Beslenme. Istanbul: Onur.
16. Ural, A., Kılıç. I. 2005. Bilimsel arastırma süreci ve SPSS ile veri analizi. Detay Yayıncılık: Ankara.
17. Büyüköztürk, S., Çakmak, E. K., Akgün, O. E., Karadeniz S. Demirel. F. 2008. Bilimsel arastırma yöntemleri. Ankara: Pegem.
18. Serra-Majem, L., Ribas, L., Ngo J., Ortega, R.M., García A., Pérez-Rodrigo C., Aranceta, J. Food, youth and the Mediterranean diet in Spain. Development of KIDMED, Mediterranean Diet Quality Index in children and adolescents. Public Health Nutr; 2004; 7(7): 931-935.
19. Štefan, L., Prosoli, R., Juranko, D., Čule, M., Milinović, I., Novak, D., Sporiš, G. The Reliability of the Mediterranean Diet Quality Index (KIDMED) Questionnaire. Nutrients 2017; 9(4), doi: 10.3390/nu9040419.
20. Köksal, E., Tek, N., Pekcan G. 12-18 yaş grubu adölesanlarda KIDMED (Sağlıklı Beslenme) İndeksi ve 24 saatlik besin tüketim yöntemi ile beslenme alışkanlıklarının saptanması, VI. Uluslararası Beslenme ve Diyetetik Kongresi, 2-6 Nisan 208, Antalya.
21. Ermis, C., Demir, I., Semiz, E., Yalçınkaya, S., Sancaktar O., Tüzüner F. E. Antalya Bölgesindeki koroner arter hastalarının demografik ve klinik özellikleri ve bu özelliklerinin Türkiye ortalamaları ile karsılastırılması. Ana Kar Der 2001; 1: 10-13.
22. Sahingoz, S. A., Sanlier, N. Compliance with Mediterranean Diet Quality Index (KIDMED) and nutrition knowledge levels in adolescents. A case study from Turkey. Appetite 2011; 57(1): 272-277.
23. Philippou, E., Middleton, N., Pistos, C., Andreou E., Petrou M. 2016. The impact of nutrition education on nutrition knowledge and adherence to the Mediterranean Diet in adolescent competitive swimmers. J Sci Med Sport 2017; 20(4): 328-332.
24. Bawaked, R.A. ,Gomez, S. F., Homs, C., Esteve, R.C., Cardenas, G., Fíto, M. and Schröder, H. Association of eating behaviors, lifestyle, and maternal education with adherence to the Mediterranean diet in Spanish children. Appetite 2018; 130(1): 279-285.
25. Kabaran, S., Gezer. C. Determination of the Mediterranean Diet and the Obesity Status of Children and Adolescents in Turkish Republic of Northern Cyprus. Turkish J Pediatric Dis 2013; 7(1): 11-20.
26. Farajian, P., Risvas, G., Karasouli, K., Pounis G. D., Kastorini, C. M., Panagiotakos, D. B., Zampelas A.. Very high childhood obesity prevalence and low adherence rates to the Mediterranean diet in Greek children: the GRECO study. Atherosclerosis, 2011; 217(2): 525-530.
27. Noale, M., Nardi, M. Limongi, F. Siviero, P. Caregaro, L. Crepaldi, G., Mediterranean Diet Foundation Study Group. Adolescents in southern regions of Italy adhere to the Mediterranean diet more than those in the northern regions. Nutr Res, 2014; 34(9): 771-779.
28. Demirözü, B. E. 2011. Spor okullarına devam eden 8-12 yas grubu çocuklara verilen beslenme eğitiminin çocukların beslenme bilgi ve alıskanlıklarına etkisi. Yüksek Lisans Tezi, Marmara Üniversitesi Sağlık Bilimleri Enstitüsü, Istanbul.
29. Kaplan, B. 2011. Nevsehir ili Özkonak kasabasında bulunan Cumhuriyet Ilköğretim Okulu öğrencilerine verilen beslenme eğitiminin etkinliğinin incelenmesi. Yüksek Lisans Tezi, Çukurova Üniversitesi Sağlık Bilimleri Enstitüsü, Adana.
30. Keskin, M. 2009. Yatılı Ilköğretim Bölge Okullarında öğrencilere verilen beslenme eğitiminin besin tüketimlerine

etkisinin arastırılması. Yüksek Lisans Tezi, Gazi Üniversitesi Eğitim Bilimleri Enstitüsü, Ankara.
31. Serra-Majem, L., García-Closas, R., Ribas, L., Pérez- Rodrigo C., Aranceta. , J. Food patterns of Spanish schoolchildren and adolescents: The enKid Study. Public Health Nutr 2001; 4(6a): 1433-1438.
32. Avan, M. 2006. Nutritional behaviours of 6th, 7th and 8th grade primary school students of kızıltepe in mardin MA diss., Selcuk University Social Sciences Institute.
33. Demirci, M. Bölükbaşı. B. 2003. Akdeniz Beslenme Tarzında Zeytinyağının Önemi. Türkiye 1. Zeytinyağı ve Sofralık Zeytin Sempozyumu, İzmir, 41-48.
34. Schröder, H. Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. J. Nutr. Biochem 2007; 18(3): 149-160.
35. Kastorini, C. M., Milionis H.J., Goudevenos J.A., Panagiotakos, D. B. Mediterranean diet and coronary heart disease: Is obesity a link?–A systematic review. Nutr Metab Cardiovasc Dis 2010; 20(7): 536-551.
36. Tognon, G., Hebestreit A., Lanfer A., Moreno L. A., Pala V., Siani A., Lissner L. Mediterranean diet, overweight and body composition in children from eight European countries: Cross-sectional and prospective results from the IDEFICS study. Nutr Metab Cardiovasc Dis 2013; 24(2): 1-9.
37. Azzini, E., Maiani, G. 2014. Mediterranean diet: Antioxidant nutritional status. In The Mediterranean diet: An evidence-based approach, ed. V. R. Preedy, R., and R., Watson, 249-257. USA: Academic.
38. Barbaros, B., Kabaran, S. Mediterranean diet and health protective properties. Nutr Diet J 2014; 42(2): 140-147.
39. Buckland, G., Agudo, A. 2014. The Mediterranean diet and mortality. In The Mediterranean diet: An evidence-based approach, ed. V. R. Preedy, R., and R., Watson, 47-60. USA: Academic.
40. McEvoy, C. T., J. V. Woodside. 2014. Mediterranean diet for prevention of cardiovascular disease and type 2 diabetes. In Glucose Intake and Utilization in Pre-diabetes and Diabetes: Implications for Cardiovascular Disease, ed. R. R. Watson & B. B. Dokken, 327-339. USA: Elsevier.
41. Castro-Quezada, I., B., Román-Viñas, Serra-Majem L. 2015. Nutritional adequacy of the Mediterranean diet. In The Mediterranean diet: An evidence-based approach, ed. V. R. Preedy, R., and R., Watson, 13-21. USA: Academic.
42. Bendall, C. L., Mayr, H. L., Opie, R.S., Bes-Rastrollo, M., Itsiopoulos C., Thomas. C.J. Central obesity and the Mediterranean diet: A systematic review of intervention trials, Crit Rev Food Sci Nutr 2017; 6: 1-5.
43. Giuseppe G., A., Mistretta, A. Frigiola, Bes-Rastrollo M., Itsiopoulos C., Thomas C.J. Mediterranean Diet and cardiovascular risk factors: a systematic review, Crit Rev Food Sci Nutr 2014; 54(5): 593-610.
44. Morreale, F., Agnoli, C., Roncoroni, L., Sieri, S., Lombardo, V., Mazzeo, T, Elli, L. Bardella, M.T., Agostoni, C, Doneda, L., Scricciolo, A., Brighenti, F., Pellegrini, N. Are the dietary habits of treated individuals with celiac disease adherent to a Mediterraneandiet? Nutr Metab Cardiovasc Dis 2018; https://doi.org/10.1016/j.numecd. 2018.06.021
45. Liese, A.D., Ma, X., Ma,X., Mittleman, M.A., The, N.S., Standiford, D.A., Lawrence, J.M., Pihoker, C., Marcovina, S.M., Mayer-Davis, E.J., Puett, R.C. Dietary quality and markers of inflammation: No association in youth with type 1 diabetes. J Diabetes Complications 2018; 32(2): 179-184.
46. Hernandez, M. V., Arenaza, L., Gracia-Marco, L., Medrano, M., Merchan Ramirez, E.,D Martinez Avila D.W., Oses, M., Ruiz, R. J., Ortega, B.F. , Labayen, I. Influence of physical activity on bone mineral content and density in overweight and obese children with low adherence to the Mediterranean Dietary Pattern. Nutrients 2018 Aug 12; doi: 10.3390/nu10081075.
47. Adelantado-Renaul, Mireia., Beltran-Valls, M.R., Esteban-Cornejo, I., Martinez-Vizcaino, V., Santaliestra-Pasias, A.M., Moliner-Urdiales, D. The influence of adherence to the Mediterranean diet on academic performance is mediated by sleep quality in adolescents Acta Pædiatricai 2018; doi:10.1111/apa.14472.
48. Baysal, A. 2008. Diyet el kitabı. Ankara: Hatiboğlu.
49. Piscopo, S. Beyond health: Extending teaching about sustainability of the mediterranean diet in Malta, J Nutr Educ Behav 2017; 49(7): s8.

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Original article

Vitamin D status among female university students in Pakistan

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Summary. Increased prevalence of hypovitaminosis D and the genesis of a myriad associated maladies among a variety of population fractions have been a focus of the scientific community and health professionals. Present cross-sectional study aimed at assessing the levels of serum 25- hydroxyvitamin D [25(OH)D] among female university students (FUSs) in Pakistan. Students were recruited and evaluated for various socio-demographic parameters including skin color, veil status, previous disease history, sun exposure and dietary condition. Serum levels of 25(OH)D were determined and information on lifestyle variables were gathered to ascertain vitamin D deficiency (VDD) and its correlates. Significant difference in serum levels of 25(OH) D among females with dark skin (22 ng/ml) as compared to white skinned (35 ng/ml) subjects was noted suggesting skin pigmentation as a contributory determinant for vitamin D deficiency. Similarly, the results indicated low vitamin D levels among veil observing FUSs. Though, VDD has not been found to be severe as 25(OH)D levels remained >18.0 ng/ml in all groups, however extreme caution is needed to improve these concentrations to optimal levels. Speculation can be drawn from the results that VDD might be much higher among illiterate population groups suggesting further research to ascertain prevalence of VDD among populations from varied socio-economic tiers in Pakistan.

Key words: vitamin D, South Asia, women, socio-economic, osteoporosis, serum 25-hydroxyvitamin D

Introduction

Significance of 25(OH)D, the circulating form of vitamin D, has been well recognized as a fundamental factor for maintaining musculoskeletal health of people of all ages (1). Evidence is available to suggest vitamin D status of the populations to be linked with several chronic disorders such as diabetes and various types of cancers (2,3). Rickets and osteomalacia among children and adults, respectively, are manifested in severe VDD whereas increase bone loss and fracture risk have been widely reported among individuals with less severe deficiency (4).

Prevalence of VDD has been common among people beyond ethnicity, age, gender, race, geographical boundaries signifying approximately one billion people with low vitamin D worldwide (3,5,6). Levels of serum 25(OH)D have been regarded as the most reliable measure to gauge vitamin D status among all population groups. Several studies substantiated a variability in setting the optimal levels of serum 25(OH)D globally on account of ecological and socio-economic variability, however; consensus of international experts has been arrived at by pronouncing severe deficiency, <12.5nmol/L: moderate deficiency, 12.5-25nmol/L: mild deficiency, 25-50 nmol/L: insufficiency >50nmol/L-75nmol/L and sufficiency >75nmol/L (7,8).

Ecological studies have corroborated the increased prevalence of VDD among females of varied age groups in Middle East and North African region

(9). A recent study from Egypt reported 72% of the females in childbearing age as vitamin D deficient (10). The researchers established observing veil and lack of sun exposure to be the underlying causes for heightened VDD among females. Despite the availability of ample resources and plenty of sunshine, many European populations are at the risk of VDD. In an analogue study, researchers examined 824 elderly people from 11 European countries and found 47% of women having 25(OH)D levels <30 nmol/L (11). South East Asian countries such as China, Japan, Hong Kong, North and South Korea and Taiwan are not the exception with respect to vitamin D deficiency. Populations from Nanjing and Beijing in China were shown to suffer from VDD [25(OH)D <25 nmol/L) in winter and fall, respectively (12).

Food Sources of Vitamin D are not abundant thereby necessitating the consumption of vitamin D fortified foods and supplements (12, 13). Fortification of foods with Vitamin D and supplementation have been widely recognized as means to override vitamin deficiency especially in developing economies (12). Evidence is available to confirm supplementation of vitamin D to be extensively contributing in improved bone mineral density leading to reduced risk of falls and fractures among elderly (14, 15). Available information on the extent of the prevalence of VDD amongst pregnant women in Pakistan signified supplementation of vitamin D, as a viable strategy during pregnancy. Researchers have recommended 400 IU to 600 IU as a reasonable dose during pregnancy (12).

High prevalence rates of VDD have been reported in Pakistani women, but there is no reliable data from Southern Punjab to rule out inadequacy of vitamin D among female literate students. Earlier studies carried out suggested VDD to be pandemic in Pakistan, however the researchers did not find any study typically focusing literate university female students' community. The primary objective of the study in question was to test the hypothesis that VDD {(25(OH)D level, ≤15 ng/mL)} is prevalent among the literate female fraction of Southern Punjab, and to identify correlation with a range of VDD determinants such as skin color, veil status, disease condition, sun exposure and dietary condition.

Methods

Study population and design

This prospective observational study was conducted at Bahauddin Zakariya University, Multan, Pakistan. The study was approved by the ethical committee for research on human and animal subjects, Bahauddin Zakariya University, Multan, Pakistan. Apparently healthy female students (n=60) aged between 20-25 years were selected after preliminary screening following predefined inclusion/exclusion criteria. The criteria for inclusion in this study were non-smoker and healthy university graduating female students. Informed consent was solicited from the selected participants and a detailed interview was conducted on skin color, veil status, sun exposure, dietary habits and previous disease history. Questions regarding skin color include dark or white skin. Veil status was inquired for complete body cover including face and unveiled as face and forearm exposed. Information on sun exposure was comprising length of time exposed to sun during peak hours i.e., 11:00 to 13:30 hr with open face and forearm. Dietary habits were evaluated for consumption frequency of dairy products, fish, calcium / vitamin D fortified foods, fast foods, tea, coffee and carbonated beverages.

Serum 25-hydroxy vitamin D analysis

Fasting blood samples (5cc) were drawn from the selected participants by professional nursing staff. Samples were taken in vacutainer coated with clotting activator and stored in refrigerator (4°C). Serum was isolated from the stored samples by centrifugation within an hour of sample collection. Serum 25-hydroxy vitamin D concentration was measured using Enzyme-Linked Immunosorbent Assay (ELISA) technique. Ten microlitre 25(OH)D was pipetted in designated wells of 96-well ELISA plate. Control and serum samples (10 µl) were added to the well plate according to the sampling plan. Freshly prepared working solution i.e., Biotinylated 25 (OH)D reagent was added to each well. The contents of the well plate were manually mixed by gentle shaking for 30 sec and the plate was incubated in dark chamber for 90 min. Washing of the plate contents was performed twice with wash buffer (300 µl). Two hundred microlitre conjugate was pipet-

ted in each well subsequent to washing. Contents of the well-plate were incubated for 30 min at 25°C. Second washing was performed with washing buffer (300 µl). Two hundred microlitre TMB substrate was added in each well and the plate was again incubated for 30 min at 25°C. Stop solution (50 µl) was added to the wells to stop enzymatic reaction. Absorbance was measured on ELISA reader at 450 nm within 10 min of adding stop solution to the well. Concentration of 25(OH)D in each sample was calculated by plotting absorbance values in standard curve. Cut-off values of 25 (OH) D were established as severe deficiency, <12.5 nmol/L: moderate deficiency, 12.5-25 nmol/L: mild deficiency, 25-50 nmol/L: insufficiency >50 nmol/L-75 nmol/L and sufficiency >75 nmol/L (7,8).

Statistical analysis

The Statistical Package for Social Sciences, SPSS (release 25.0, standard version, copyright© SPSS), was used for data analysis. Descriptive analysis was done for demographic variables and proportions were determined. Univariate analyses and ANOVA tests, were performed among subjects for skin color, veil wearing, sun exposure, dietary conditions and pathological conditions. The association of serum 25(OH)D concentrations between these 5 groups were studied by linear regression and correlation. *p*-value less than 0.05 (2-sided test) was considered statistically significant.

Results and Discussion

Data presented in Table 1 depict a mean concentration of 25(OH) D level ranging from 25 to 27 ng/ml among five groups (Table 1). Skin pigmentation has been shown to play a seminal role in raising serum 25(OH)D among population belonging to non-western regions. Evidence is available to suggest higher prevalence of VDD among dark skinned populations especially from South Asian and African regions (16-18).

Our study confirmed the results of foregoing studies reporting a significant difference in serum 25(OH)D levels among female university students i.e., 22 and 35 ng/ml among dark skinned and white skinned female students, respectively (Table 2). These finding are a proxy of skin pigmentation to be one of the contributory factors for increased 25(OH)D levels among adolescents and girls of child bearing age and are supported by evidence from literature (19).

Non-observance of veil in most of the Muslim societies is generally grinned and grimaced at as a non-religious practice. Resultantly, Muslim females have to observe veil either by covering the whole body including face or sometimes with only face, arms and feet exposed. This practice generally leads to reduced or sub optimal levels of serum 25(OH)D among females of all age groups in Pakistan. Current study confirms reduced levels of 25(OH)D i.e., 22 ng/ml among FUSs observing veil by covering whole body including face as compared to 25(OH)D (35 ng/ml) among those having some parts of the body exposed to sun (Table 2). Upshots of the present study are in line with the findings of several researchers in terms of veil (*Purdah*) being corollary of increased prevalence of VDD among South Asian populations (20, 21).

Vitamin D serum concentrations have been found to be drastically varying under various disease conditions. Amital et al. (22) demonstrated a significant

Table 1. Descriptive statistics for serum 25(OH)D levels (ng/ml) among female university students aged 20-25 years

	N*	Min.	Max.	Mean	Std. Dev
Skin Color	60	18.00	45.00	26.12	6.66
Veil Status	60	18.00	45.00	26.79	6.93
Disease Status	60	18.00	41.00	25.92	6.38
Sun Exposure	60	17.90	40.00	25.72	6.22
Dietary Condition	60	18.00	39.00	25.00	6.02

** Number of participants*

Table 2. Descriptive statistics for serum 25(OH)D levels (ng/ml) among female university students aged 20-25 years (sub-groups)

		N	Mean	SD	Min.	Max.	Total (%)	p	C I (95%)	
									Upper	Lower
Skin Color	DS	42	22.4	2.73	18.0	29.1	70.0	>0.0001	21.35	23.44
	WS	18	34.8	4.60	29.0	45.0	30.0		33.23	36.43
Veil Status	WV	38	22.1	2.70	18.0	29.3	63.3	>0.0001	21.10	23.17
	NV	22	34.8	3.91	29.8	45.0	36.7		33.47	36.19
Disease Status	WD	39	21.9	2.54	18.0	26.8	65.0	>0.0001	20.94	22.86
	ND	21	34.4	2.80	30.6	39.0	35.0		32.65	35.99
Sun Exposure	Mi.E	42	22.2	2.75	17.9	27.2	70.0	>0.0001	21.26	23.20
	Mx.E	18	33.9	3.97	28.0	40.0	30.0		32.37	35.34
Dietary Condition	PVD	40	22.1	3.05	18.0	28.0	66.7	>0.0001	21.04	23.21
	RVD	20	33.5	4.10	28.4	41.0	33.3		31.98	35.05

** Number of participants: DS=Dark Skin: WS=White Skin: WV=With Veil: NV=No Veil: WD=With Disease: ND=No Disease: MiE=Min. Exposure: MxE=Max. Exposure: PVD=Poor Vit.D: RVD=Rich Vit.D: SD=Standard Deviation: CI=Confidence Interval*

negative correlation between the serum concentration of vitamin D and the standardized values (z-scores) of disease activity scores. Likewise, varying level of vitamin D have been implicated with a variety of diseased conditions and are considered to be a causal factor for the development of CVD (23), statin-associated myalgia (24), breast cancer (25), tuberculosis (26) and asthma (27). Visible difference in serum 25(OH)D levels were noticed among FUSs with diseased and non-diseased heath status (Table 2). Significantly higher concentration of vitamin D levels i.e., 34ng/ml among healthy subjects were observed while vitamin D concentration remained much lower i.e., 22 ng/ml in female subjects who had experienced some disease conditions suggesting the impact of optimal levels of 25(OH)D in maintaining good health.

Several studies demonstrated the role of sun exposure and diet as substantial contributory factor to maintain optimal levels of 25(OH)D, however these studies were not directed to find out vitamin status of the educated females studying at University level where awareness on the role of vitamin D in health were supposed to be higher as compared to the normal population with reduced literacy rates. We concluded that dietary sources of vitamin D and sun exposure have positive impact on avoiding vitamin D deficiency. Present results corroborated the longstanding believe in sun exposure as a fundamental component for acquiring elevated vitamin D levels. Diets rich in vitamin D have also been regarded a good approach to attain sufficient amount of vitamin, however food alone does not meet the increased needs of vitamin D. Therefore, fortification of food has been widely recommended and accepted approach to combat vitamin deficiency in areas and situations where sun exposure does not suffice the needs for vitamin such as genetic factors, age, latitude of the country of residence, life styles and chronic diseases.

Viewing at the extent of sunshine and weather conditions in Pakistan with latitude 24° 35' North and longitude 61° East to 78° East, Southern Punjab has a tropical climate with low humidity, in summer its temperature rises up to 45-48°C and in winter it has minimum temperature 4-5°C. So, intensity of ultra violet B radiations (UVB) radiations is very high in comparison with the European countries. Despite these favorable weather conditions, high prevalence of vitamin D deficiency has been reported in Pakistan (28). Inadequate exposure of sun (UVB) and or vitamin D supplements may affect vitamin D status (29). Pakistan is among the tropical countries where sun is drenched and possibility for the production of vitamin D through UVB throughout the year is higher. Nevertheless, National Nutritional Survey of Pakistan (30) shows that Pakistan is also a victim of high prevalence of vitamin D deficiency. Sunlight exposure provides 90-95% of vitamin D, but depends on latitude, altitude

and pollution. Our finding confirmed that University students aged 20-25 years who were in habit of exposing their body to sun were able to experience optimum levels of 25(OH)D (34 ng/ml) as compared to those observing veil with little or no exposure to sun having 25(OH)D up to 22 ng/ml (Table 2).

The correlation was tested among the parameters to evaluate the influence of one parameter on the other one. The skin color showed negative correlation with the veil (p<0.123, r=–0.201), dietary conditions (p<0.508, r=–0.087) and sun exposure (p<0.082, r=–0.226) but weak correlation was found with disease conditions (p<0.029, r=0.334). Other groups i.e., veil, dietary and sun exposure have highly significant (p<0.001) positive correlation with each other but disease condition showed the negative correlation behavior with veil (-0.186), dietary habits (-0.337) and sun exposure (-0.256) (Table 3).

Conclusions

VDD remains to be a global issue of public health significance. Resource constrained economies are more inflicted with vitamin deficiency owing to a variety of reasons important being poverty, lack of awareness and poor health facilities. Literature confirm high prevalence of VDD in Pakistan that distresses all population segments. Reports are available to demonstrate VDD to be extremely pervasive among various population groups representing skin pigmentation, sex, age, socioeconomic status, urban and rural folk, veil and non-veil observing females and those consuming vitamin D deficient diets. However, little was known about the magnitude of the prevalence of vitamin D among educated young females in Pakistan. Our study on FUS's validated that awareness might have some role to overpower vitamin deficiency as tested groups for vitamin D status with limiting factors like dark skin, veil observance, diseased conditions, restricted sun exposure and consumption of diet poor in vitamin have insufficient levels of 25(OH)D suggesting that education and awareness might be an instrumental determinant for maintaining optimal vitamin D levels. It seems imperative that public is to be educated on the role of vitamin D in health to avoid the complication of vitamin D insufficiency and deficiency by exploiting optimum sunlight exposure and following life style changes to avert nutritional deficiencies.

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Table 3. Correlation among groups female university students aged 20-25 years based on various parameters to assess serum 25(OH) D levels (ng/ml)

		Skin Color	Veil Status	Dietary Status	Sun Exposure	Disease Status
Skin Color	Pearson Correlation	1	-0.201	-0.087	-0.226	0.290
	Sig. (2-tailed)		0.123	.508	.082	0.069
Veil Status	Pearson Correlation	-0.201	1	0.787**	0.679**	-0.186
	Sig. (2-tailed)	0.123		0.0001	0.0001	0.252
Dietary Status	Pearson Correlation	-0.087	.787**	1	0.712**	-0.337*
	Sig. (2-tailed)	0.508	0.0001		0.0001	.034
Sun Exposure	Pearson Correlation	-0.226	0.679**	0.712**	1	-0.256
	Sig. (2-tailed)	0.082	0.0001	0.0001		0.111
Disease Status	Pearson Correlation	. 0.290	-0.186	-0.337*	-0.256	1
	Sig. (2-tailed)	0.069	0.252	0.034	0.111	

*** Correlation is significant at the 0.01 level (2-tailed).*

** Correlation is significant at the 0.05 level (2-tailed).*

References

1. Whiting SJ, Calvo MS. Dietary recommendations for vitamin D: a critical need for functional end points to establish an estimated average requirement. J Nutr 2005; 135: 304-309.
2. Heaney RP. Long latency deficiency disease: insights from calcium and vitamin D. Am J Clin Nutr 2003; 78: 912-919.
3. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr 2008; 87: 1080S-1086S.
4. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001; 22: 477-501.
5. Van der Meer IM, Middelkoop BJC, Boeke AJP, Lips PTAM. Prevalence of vitamin D deficiency among Turkish, Moroccan, Indian and sub-Sahara African populations in Europe and their countries of origin: an overview. Osteoporos Int 2011; 22: 1009-1021.
6. Lips P, van Schoor N. Worldwide vitamin D status. In Vitamin D (Third Edition). 2011; 947-963.
7. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? J Steroid Biochem Mol Biol 2004; 89: 611-614.
8. Holick MF. Sunlight, UV-radiation, vitamin D and skin cancer: how much sunlight do we need?. Ad Exp Med Biol 2008; 624: 1-15.
9. Lamberg-Allardt CJ, Outila TA, Kärkkäinen MU, Rita HJ, Valsta LM. Vitamin D deficiency and bone health in healthy adults in Finland: could this be a concern in other parts of Europe?. J Bone Miner Res 2001; 16: 2066-2073.
10. Cashman KD, Dowling KG, Škrabáková Z et al. Vitamin D deficiency in Europe: pandemic?, 2. Am J Clin Nutr 2016; 103: 1033-1044.
11. Zhang W, Stoecklin E, Eggersdorfer M. Vitamin D status in Mainland China. Nutr 2013; 29: 953-957.
12. Akhtar S, Ismail T, Atukorala S, Arlappa N. Micronutrient deficiencies in South Asia–Current status and strategies. Trends Food Sci Technol 2013; 31: 55-62.
13. Shrapnel W, Truswell S. Vitamin D deficiency in Australia and New Zealand: What are the dietary options? Nutr Diet 2006; 63: 206-212.
14. Ascherio A, Munger KL, Simon KC. Vitamin D and multiple sclerosis. Lancet Neurol 2010; 9: 599-612.
15. Pierrot-Deseilligny C, Souberbielle JC. Is hypovitaminosis D one of the environmental risk factors for multiple sclerosis? Brain 2010; 133: 1869-1888.
16. Zaman S, Hawlader MDH, Biswas A, Hasan M, Jahan M, Ahsan GU. High Prevalence of Vitamin D Deficiency among Bangladeshi Children: An Emerging Public Health Problem. Health 2017; 9: 1680.
17. Lips P, de Jongh RT. Vitamin D deficiency in immigrants. J Bone Rep 2018; 9: 37-41
18. Martin CA, Gowda U, Renzaho AM. The prevalence of vitamin D deficiency among dark-skinned populations according to their stage of migration and region of birth: A meta-analysis. Nutrition 2016; 32: 21-32.
19. Akhtar S. Prevalence and correlates of vitamin D deficiency-perspectives from Pakistan. Pak J Pharm Sci 2016; 29.
20. Akhtar S. Vitamin D status in South Asian populations–risks and opportunities. Crit Rev Food Sci Nutr 2016; 56: 1925-1940.
21. Islam MZ, Akhtaruzzaman M, Lamberg- Allardt C. Hypovitaminosis D is common in both veiled and non-veiled Bangladeshi women. Asia Pac J Clin Nutr 2006; 15: 81-87.
22. Amital H, Szekanecz Z, Szücs G et al. Serum concentrations of 25-OH vitamin D in patients with systemic lupus erythematosus (SLE) are inversely related to disease activity: is it time to routinely supplement patients with SLE with vitamin D?. Ann Rheum Dis 2010; 69: 1155-1157.
23. Anderson JL, May HT, Horne BD et al. Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am J Cardiol 2010; 106: 963-968.
24. Michalska-Kasiczak M, Sahebkar A, Mikhailidi DP et al. Analysis of vitamin D levels in patients with and without statin-associated myalgia—a systematic review and meta-analysis of 7 studies with 2420 patients. Int J Cardiol 2015; 178: 111-116.
25. Sofi NY, Jain M, Kapil U, Seenu V, Kamal VK, Pandey RM. Nutritional risk factors and status of serum 25 (OH) D levels in patients with breast cancer: A case control study in India. J Steroid Biochem Mol Biol 2018; 175: 55-59
26. Saleem S, Khan WA, Ali B et al. Association of vitamin D with tuberculosis patients of Sargodha, Pakistan. PAB 2018; 7: 236-242.
27. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. J Allergy Clin Immunol 2010; 125: 995-1000.
28. Hossain N, Khanani R, Hussain-Kanani F, Shah T, Arif S, Pal L. High prevalence of vitamin D deficiency in Pakistani mothers and their newborns. Int J Obstet Gynaecol 2011; 112: 229-33.
29. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 1995; 61: 638S-645S.
30. Bhutta ZA, Soofi SB, Zaidi SSH, Habib A. Pakistan National Nutrition Survey 2011. Pakistan Medical Research Council, Nutrition Wing, Cabinet Division, Government of Pakistan.

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Original article

Reliability and validity of the Turkish version of the short food literacy questionnaire among university students

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Summary. The aim of this study was translating and adapting the SFLQ to Turkish and evaluating the validity and reliability for adults in Turkey. In accordance with the purpose of the study, a 2-part questionnaire was prepared. The first part focused on the sociodemographic characteristics, including age, sex, and questions that determine food label reading habits. The second part consisted of the Newest Vital Sign (NVS) test, Turkey Health Literacy SCALE-32 (TSOY-32) and Short Food Literacy Questionnaire (SFLQ). The construct validity of the SLFQ was assessed using factor analysis. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.811. Bartlett's test of sphericity was significant (X2=841.958, df=66; p<0.001). A Scree plot and eigenvalues determined that one factor should be retained, which accounted for 32.01% of the variance. The questionnaire factor loadings varied between 0.43 and 0.64. Internal consistency was assessed by calculating Cronbach alfa, and the value was 0.803. There was a positive relationship between SLFQ, TSOY-32 and NVS (r=0.531, p<0.001; r=0.294, p<0.001). As a results of this study, it can be said that SLFQ is a valid and reliable measurement tool, therefore it can be used to describing food literacy among Turkish adults.

Key words: food literacy, validation, health literacy, nutrition

Introduction

Nutrition knowledge is one of the factors, which affects food behaviors and healthy lifestyle (1). However, the food literacy includes skills we have about food choice, food consumption, food preparation and understanding of food effects on human body. It is defined by the experts "the relative ability to basically understand the nature of food and how it is important to you and how able you are to gain information about food, process it, analyze it and act upon it"(2). Food literacy has the advantage of improving community health such as health literacy, therefore understanding, describing and measuring adults' food literacy level may help for intervention to population (3-5). It is new trend and there is not enough study on food literacy effect of human health, even so it is shown that food literacy correlated with healthy food consumption (6).

Non-communicable disease are the most common health problems such as obesity, heart disease are related with diet (7). Enhancing lifestyle is depend on physical activity and what we consuming and during life. It has shown that nutrition education has positive effects of consuming healthy food and well-being (8, 9). Food literacy is a new concept to describe food well-being and a way to make healthy community. Despite there is not enough study focus on adult food literacy, adults' food behaviors has major effects on their children's choices and education and how they will act in the future, because of this it is important to target parents (10). However, there are limited studies on this

topic, most of the researches focus on adolescent's food literacy and most of the intervention studies focus on school children (9, 11-13).

"A short food literacy questionnaire (SFLQ) for adults" is first the instrument to measure food literacy among adults (14). This form developed by Krause et al. in Switzerland. It is short, practical and useful for public health, SFLQ is developed for Swiss population, although it could be used for other countries with adaptation to country profile. Food literacy have complex components, even though this questionnaire focuses on the skills and the abilities for healthy food choices (2, 14, 15). This might be practice to measure adults' food literacy and rapid intervention to improve nutrition abilities.

The objective of this study was to translate and to adapt the SFLQ to Turkish and to evaluate the validity and reliability for adults in Turkey.

Methods

Study Group and Procedure

The methodological study was carried out in Harran University School of Health Vocational School between December 2017 and January 2018 in Şanlıurfa. The study was reviewed and approved by the Harran University Medical Faculty Ethics Committee and Health Vocational School administration. In terms of academic use of the scale, required permission was obtained for the responsible researcher.

The criteria for inclusion in the study were to be between age 18-30, to accept participation in the study, to answer more than 90% of the questionnaire form. The sample size was calculated as 300 people based on the statement "sample size should be 10 to 20 times the number of items in the study questionnaire" (16). All the students who participated in the study were informed about the study and their written approval were obtained. 148 people who don't accepting to participate in the study, being absent when the survey was practiced, and not answering 90% of the questions in the survey were removed from the study group. Finally, the study was conducted with 308 (67,5%) university students.

Due to the adaptation of the scale from different languages and cultures, the validity and reliability study has been carried out in two stages. In the first stage, the validity of language and coverage, in the second stage construct validity, concurrent criterion validity, internal consistency and test-retest reliability were evaluated.

Stage 1: In accordance with the translation-back translation method, the GSOY Scale were translated into Turkish by two different language experts. Then the Turkish form, which was formed by the co-decision of the two experts, was translated into English by another language expert. For the content validity of the Turkish form, it was presented to 10 experts (an academician doctor, 7 doctors, 2 nutritionist). They were asked to assess the items in four groups: "essential"," somewhat convenient - the revision of the item is required", " It is quite appropriate - but minor changes are necessary,", or "unnecessary". The content validity index of items in the questionnaire was 0.67.

Stage 2: Afterwards, a group of 10 students were tested for clarity by applying GSOY Scale and feedback was obtained. The items were understood by the students and no change was requested. After the pilot study, scale was reapplied to 40 students selected from 308 students with an interval of three weeks to evaluate the test-retest reliability

Data Collection Tools

In accordance with the purpose of the study, a 2-part questionnaire was prepared. The first part focused on the sociodemographic characteristics, including age, sex, and questions that determine food label reading habits. The second part was consisted of the Newest Vital Sign (NVS) test, Turkey Health Literacy SCALE-32 (TSOY-32) and Short Food Literacy Questionnaire (SFLQ) (14, 17, 18).

SFLQ was developed by Krause et al (14). This scale which covered crucial elements of nutrition literacy and food literacy definitions has 12-item questionnaire of four- or five-point Likert type. For evaluation, a summary score was calculated that ranged from 7 to 52; the higher score shows the better food literacy. Cronbach's alpha value of SFLQ was 0.82.

TSOY-32 has been developed by Okyay et al. on the basis of the conceptual framework of the European Health literacy Scale study (17). This self-reporting scale was developed to assess people's health

literacy over fifteen years of age with the composition; four-point Likert-type of 32 items. According to score, TSOY-32 was categorized in four categories; inadequate health literacy (0-25), problematic-limited health literacy (>25-33), adequate health literacy (>33-42), excellent health literacy (>42-50).

The NVS is an evidence-based health literacy screening tool that includes a standardized Nutrition Facts label and six accompanying questions, requires basic reading and numeracy skills (18). The ability of a person to read and analyze a nutrition label has been noted to parallel the conceptual and analytic skills, which are needed to understand the majority of health-related instructions. NVS is suitable to be used as a quick screening test for limited literacy in primary health care.

Analysis

Factor Analysis

Exploratory factor analysis was calculated by using a principal factor method with varimax rotation to evaluate the scale's construct validity. Factor analysis adequacy was assessed by applying the Kaiser-Meyer-Olkin (KMO). The KMO result was >0.50, and factor analysis was performed. All the items exhibited factor loadings of >0.40 in the analysis, so there was no need to remove items (19). According to the factor loadings obtained from the factor analysis, items pertained to a subdimension according to their maximum factor weight. One dimension was identified by the factor analysis.

Internal Consistency

Cronbach a coefficient was calculated to evaluate the scale's internal consistency, and coefficients were also calculated for the item-total correlation and for the item elimination. Items which were greater than 0.30 of the total item correlations were considered reliable. None of the items gave values less than 0.30 (20).

Test-retest reliability

Test-retest reliability coefficient was calculated to evaluate the scale's stability over time. The level of agreement between responses at test and retest was measured by using Spearman rank correlation coefficient.

Statistical Analysis

SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for data analysis. The demographic characteristics of the study group were reported using descriptive statistics (frequencies, proportions, mean±SD, Min-Max, median and interquartile range 25%-75% (IQR 25-75)). Initially, the normality of the total scores was tested using the Kolmogorov-Smirnov normality test and graphs. Therefore, the median scores were compared using Kruskal Wallis and Mann-Whitney U tests. The level of agreement between responses at test and retest was measured by using Spearman rank correlation coefficient.

Results

A total of 308 student were included in this study. The mean age was 19,94±2,42 years old and 28.2% of the participants were male. Students' age (r=0,027; p=0,642) and gender (Z=0,003; p=0,998) were not significantly associated with SFLQ score. 85 students (27.6%) reported that they rarely read food labels. In this study, the mean SFLQ score increased with the increase in food label reading habit (p<0,001). The distribution of the SFLQ scores of the study group according to the food label reading habits is given in Table 1.

Table 1. The distribution of the SFLQ scores of the study group according to the food label-reading habits

	N (%)	Mean±SD	Min.-Max	Median	IQR 25-75	Test KW	p
Food Label Reading habit presence							
Rarely-non	85 (27,6)	26,32±8,34	9,0-50,0	27,00	19,4-32,0		
Sometimes	115 (37,3)	27,78±7,85	9,0-46,4	27,40	22,0-33,0	22,109	<0,001
Often-very	108 (35,1)	31,83±7,74	14,0-51,0	31,20,00	26,0-37,5		

Factor Analysis

The construct validity of the SLFQ was assessed by using factor analysis. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.811. Bartlett's test of sphericity was significant (X2=841.958, df=66; p<0.001). A Scree plot and eigenvalues determined that the factor accounted for 32.01% of the variance should be retained. The questionnaire factor loadings varied between 0.43 and 0.64.

Internal Consistency

Internal consistency was assessed by calculating Cronbach α and the value was 0.803. The deletion of any item from the scale produced Cronbach α values that ranged between 0.77 and 0.79 The corrected item total correlation coefficient ranged between 0.32 and 0.52. The Results of Reliability Analysis, and Factor Loading of the SLFQ Items are given in Table in Table 2.

Test-Retest Reliability

Three weeks later, the questionnaire was conducted again to 48 student. A high positive correlation was observed between the total scores of the 2 applications using Spearman rank correlation analysis (r: 0.808, P<0.001). The average score on the first evaluations was 40,89±6,86 while the average score on the second evaluations was 42,61±7,56.

Hypothesis-testing validity

The hypothesis was established that students with higher TSOY-32 and NVS scores would get higher SLFQ scores. 42 students (13,6%) had inadequate health literacy. As the level of health literacy increased, SFLQ total score increased (<0,001). There was a positive relationship between SLFQ, TSOY-32 and NVS (r=0.531, p<0.001; r=0.294, p<0.001). The distribution of SLFQ scores obtained from the students' health lit-

Table 2. The results of reliability analysis, and factor loading of the SLFQ Items

	SLFQ Items	**1**	**2**	**3**
1)	When I have questions on healthy nutrition, I know where I can find information on this issue	0,563	0,436	0,779
2)	In general, how well do you understand the following types of nutritional information? (A) Nutrition information leaflets (B) Food label information (C) TV or radio program on nutrition (D) Oral recommendations regarding nutrition from professionals. (E) Nutrition advice from family members or friends	0,633	0,517	0,780
3)	How familiar are you with the Turkey Ministry of Health Food Pyramid?	0,445	0,347	0,787
4)	I know the official Turkey Ministry of Health recommendations about fruit and vegetable consumption	0,441	0,348	0,790
5)	I know the official Turkey Ministry of Health recommendations about salt intake	0,529	0,427	0,781
6)	Think about a usual day: how easy or difficult is it for you to compose a balanced meal at home?	0,430	0,321	0,789
7)	In the past, how often were you able to help your family members or a friend if they had questions concerning nutritional issues?	0,575	0,464	0,778
8)	There is a lot of information available on healthy nutrition today. How well do you manage to choose the information relevant to you?	0,597	0,468	0,776
9	How easy is it for you to judge if media information on nutritional issues can be trusted?	0,625	0,503	0,772
10)	Commercials often relate foods with health. How easy is it for you to judge if the presented associations are appropriate or not?	0,625	0,504	0,772
11)	How easy is it for you to evaluate if a specific food is relevant for a healthy diet?	0,642	0,515	0,771
12)	How easy is it for you to evaluate the longer-term impact of your dietary habits on your health?	0,622	0,500	0,772

Cronbach α: 0.801

1: Factor Loading, 2: Corrected Item Total Correlation, 3: If Item Deleted Cronbach α

eracy levels and correlation values of the SLFQ with the NVS are given in Table 3, 4.

Discussion

Construct validity refers to whether a scale or test measures the construct adequately. Factor analysis is a method used commonly for evaluating construct validity (20). In this study, the KMO test value was found 0,811. The KMO test result indicated that the SLFQ was reliable, and the Bartlett test result was found statistically significant, which means that the structure of the SLFQ was suitable for factor analysis (21).

Factor loadings of 0.10 were accepted as low, 0.30 as moderate, and values of 0.59 or above are considered high. A high factor loading showed that the item was a valid indicator of the related factor (21). In the current study, it was observed that the factor loading was 0.441 for one item and 0.445 for another item. The rest of the items were greater than 0.5. As a result, the scale could not be separated into components and had a single dimension. These results showed that construct validity of the questionnaire was sufficient. Our results similar with the SLFQ Swiss version, they also had minimum factor loading 0.40 and contribution of factor loading was similar in both study (13). The SLFQ is currently developed and there is no other study on this scale. Although our findings show that scale has similar results with Turkish version, in time new studies will show us factor loading in different populations.

Cronbach's α coefficient, which represents internal consistency reliability, should be higher than 0.70 (22). Cronbach's α coefficients for the SLFQ were 0.803 for the entire questionnaire and greater than 0.771 for if item deleted Cronbach's α (Table 2), which implies that the questionnaire exhibited considerable reliability. This result indicates that the items in the questionnaire are consistent with each other and the items questionnaire contained measured the same characteristic.

Test-retest reliability refers to the correlation coefficient obtained for any variable under similar conditions and after a certain time interval. The test-retest scores less than 0.80 indicated that the participants did not answer the items when they were retested (23). In our study, results of the SLFQ supported the literature and showed that the scale items did not change over time.

Congruent validity was also confirmed via its significant correlation with the SLFQ and NVS. The NVS was one of the instrument to measure the health literacy in adults, which was quickly feasible and acceptable (24, 25). Understanding of healthy food and nutrition literacy associated with the NVS, therefore we used to compare between NVS and SLFQ score (26). 35.1% of participants had adequate literacy and significantly highest SLFQ score. In addition, this showed that SFLQ was associated with the abilities for healthy food choice as it claimed (13).

One of the hypotheses tested in the study was the level of general health literacy correlated with SLFQ scores. In support of the hypothesis, it was found that

Table 3. The distribution of SLFQ scores obtained from students according to health literacy level.

	N (%)	Mean±SD	Min.-Max	Median	IQR 25-75	Test KW	p
TSOY-32							
Inadequate Health Literacy	42 (13,6)	23,98±6,44	14,0-44,20	23,90	18,2-27,4	78,265	<0,001
Limited Health Literacy	96 (31,2)	25,46±6,83	9,20-45,6	25,00	23,3-30,4		
Adequate Health Literacy	113 (36,7)	29,76±7,83	9,0-50,0	30,40	25,4-35,8		
Excellent Health Literacy	57 (18,5)	36,07±7,00	19,8-51,0	36,40	31,4-41,4		

Table 4. Correlation values of the SLFQ with the NVS

					Correlation values with SLFQ score	
	Mean±SD	Min.-Max	Median	IQR 25-75	r	p
NVS	2,53±1,41	0,0-6,0	2,00	2,0-3,5	0,294	<0,001

general health literacy level was positively correlated with SLFQ scores (r=0.531, p<0.001). Similarly, positive correlation was found in the original scale study (r=0.294, p<0.001).

Consequently, it can be said that SLFQ is a valid and reliable measurement tool as a result of the conducted analyzes. However, it should be considered that the study group was consist of university students. Therefore, it is important to conduct studies on different samples for the validity and reliability of the scale.

References

1. Worsley A. Nutrition knowledge and food consumption: can nutrition knowledge change food behaviour? Asia Pacific journal of clinical nutrition. 2002;11:579-85.
2. Vidgen HA, Gallegos D. Defining food literacy and its components. Appetite. 2014;76:50-9.
3. Cullen T, Hatch J, Martin W, Higgins JW, Sheppard R. Food Literacy: Definition and Framework for Action. Canadian Journal of Dietetic Practice and Research. 2015;76:140-5.
4. Bublitz MG, Peracchio LA, Andreasen AR, et al. The quest for eating right: Advancing food well-being. Journal of Research for Consumers. 2011;19:1-12.
5. Beatrice AB, Elizabeth M, Meaghan RB, Lynn R, Rebecca T. The Ontario Food and Nutrition Strategy: identifying indicators of food access and food literacy for early monitoring of the food environment. Health Promotion and Chronic Disease Prevention in Canada : Research, Policy and Practice. 2017;37:313-9.
6. Poelman MP, Dijkstra SC, Sponselee H, et al. Towards the measurement of food literacy with respect to healthy eating: the development and validation of the self perceived food literacy scale among an adult sample in the Netherlands. International Journal of Behavioral Nutrition and Physical Activity. 2018;15(1):54.
7. Pendergast D, Garvis S, Kanasa H. Insight from the public on home economics and formal food literacy. Family and Consumer Sciences Research Journal. 2011;39:415-30.
8. Colatruglio S, Slater J. Food literacy: Bridging the gap between food, nutrition and wellbeing. Sustainable well-being: Concepts, issues, and educational practices. 2014:37-55.
9. Brooks N, Begley A. Adolescent food literacy programmes: A review of the literature. Nutrition & dietetics. 2014; 71: 158-71.
10. National Academies of Sciences E, Medicine. Food Literacy: How Do Communications and Marketing Impact Consumer Knowledge, Skills, and Behavior? Workshop Summary: National Academies Press; 2016.
11. Ronto R, Ball L, Pendergast D, Harris N. Adolescents' perspectives on food literacy and its impact on their dietary behaviours. Appetite. 2016;107:549-57.
12. Nowak AJ, Kolouch G, Schneyer L, Roberts KH. Building food literacy and positive relationships with healthy food in children through school gardens. Childhood Obesity (Formerly Obesity and Weight Management). 2012;8:392-5.
13. Vaitkeviciute R, Ball LE, Harris N. The relationship between food literacy and dietary intake in adolescents: a systematic review. Public health nutrition. 2015;18:649-58.
14. Gréa Krause C, Beer-Borst S, Sommerhalder K, Hayoz S, Abel T. A short food literacy questionnaire (SFLQ) for adults: Findings from a Swiss validation study. Appetite. 2018;120:275-80.
15. Velardo S. The Nuances of Health Literacy, Nutrition Literacy, and Food Literacy. Journal of Nutrition Education and Behavior. 2015;47:385-89.
16. Erefe I. Nursing Research Principles, Process and Methods. 4th ed. Ankara: Odak Ofset; 2004.
17. Okyay P, Abacıgil F. Türkiye Sağlık Okuryazarlığı Ölçekleri Güvenilirlik ve Geçerlilik Çalışması. 1. Baskı. Ankara: Anıl Matbaa; 2016. pp. 43-60.
18. Weiss BD, Mays MZ, Martz W, et al. Quick assessment of literacy in primary care: the newest vital sign. Ann Fam Med. 2005;3:514-22.
19. Field A. Discovering statistics using SPSS. 3th ed. London, England: Sage; 2009.
20. Buyukozturk S. Veri analizi el kitabı. 4. Baskı. Ankara, Turkey: Pagem A Yayıncılık; 2004.
21. Gözüm S, Aksayan S. A guide for trans-cultural scale adaptation II. Psychometric characteristics and cross-cultural comparisons. Turk J Nurs Res 2003;5:3-14.
22. Gunay EY, Gul A. Reliability and validity of the Cambridge Worry Scale in pregnant Turkish women. Midwifery. 2015;31:359-64.
23. Cam MO, Baysan Arabaci L. Qualitative and quantitative steps on attitude scale construction. Turk J Res Dev Nurs. 2010;2:59-71.
24. Ferris L, Norton P. Basic concepts in reliability and validity. Tools for Primary Care Research. Newbury Park, CA: Sage; 1992:64-76.
25. Shah LC, West P, Bremmeyr K, Savoy-Moore RT. Health literacy instrument in family medicine: the "newest vital sign" ease of use and correlates. The Journal of the American Board of Family Medicine. 2010;23:195-203.
26. Rowlands G, Khazaezadeh N, Oteng-Ntim E, Seed P, Barr S, Weiss BD. Development and validation of a measure of health literacy in the UK: the newest vital sign. BMC Public Health. 2013;13:116.
27. Carbone ET, Zoellner JM. Nutrition and health literacy: a systematic review to inform nutrition research and practice. Journal of the Academy of Nutrition and Dietetics. 2012;112:254-65.

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Original article

Evaluation of obesity in university students with neck circumference and determination of emotional appetite

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Summary. *Objective:* In this study, it was aimed to evaluate obesity in university students with neck circumference and other anthropometric measurements and to determine their emotional appetite. *Method:* The study was conducted within the scope of Scientific Research Projects Coordination Unit of Kırıkkale University in June-December 2017 in June-December 2017 term and 4873 students were reached. A questionnaire consisting of descriptive information and emotional appetite scale was applied to students and their obesity status were determined by taking anthropometric measurements. *Results:* Students consist of 56.3% females and 43.7% males. Mean body mass index (BMI) is 23.62±3.03 kg/m^2 in males and 21.66±3.08 kg/m^2 in females ($p<0.001$). Mean neck circumference is 37.50±2.49 cm in males and 32.18±3.51 cm in females ($p<0.001$). According to students' BMI, 18.5% are overweight and obese, to neck circumference 36.6% are obese, and to waist circumference 22.4% are in the risk and high-risk group. 65.8% of males and 19.4% of females are obese according to neck circumference ($p<0.001$). Waist circumference/height ratio of males is 0.478±0.054 while that of females is 0.452±0.053. Central obesity was observed in 30.2% of males and 16.3% of females ($p<0.001$). A positive correlation was found between body weight, BMI, waist circumference, wrist circumference, waist/height ratio and neck circumference. Emotional appetite status of students and values of total positive scores are different from others in at least one of BMI groups ($\chi^2=14.503$; $p=0.002$). Total positive scores of thin students are higher than those who are overweight and obese ($p<0.001$). Total score in negative emotions/conditions is high in students who are obese according to neck circumference ($Z=4.539$; $p<0.001$). In negative and positive emotions/conditions, median score of total emotional appetite scale of males is higher than that of females ($p<0.001$). *Conclusion:* According to neck circumference, it is determined that obesity more common in males than females, males' appetites increase more than females' in positive and negative situations, and overweight and obese students exhibit more eating behaviour when they experience negative emotions.

Key words: neck circumference, obesity, emotional appetite, university students

Introduction

The university period is a period in which young individuals have intense positive and negative feelings due to various environmental and social factors and examination anxiety (1). In this period, nutritional preferences may change with the orientation towards family-independent eating habits, risky behaviours related to nutrition, decrease in physical activity and insufficient and unbalanced nutritional states can be seen,

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symptoms of eating disorders can occur in students, due to emotional eating behaviour and incorrect body perception (2-5). It is stated that the emotional eating behaviour, carried out by various researchers, in order to cope with negative emotions has been examined in individuals with different body weights and that food consumption of individuals is affected by positive and negative emotions and conditions (6-7).

In determining obesity, many simple and practical methods such as height, body weight, BMI, waist circumference, waist circumference/hip circumference ratio are used (8). Among these methods, BMI is the most frequently used method to distinguish underweight, normal, overweight and obese individuals (9). In addition to determining overweight and obesity, the measurement of neck circumference shows a strong correlation with visceral obesity and today, determining abdominal obesity is seen as an optimal measurement instrument in the diagnosis of metabolic diseases and other chronic diseases associated with obesity (10, 11).

The aim of this study was to evaluate obesity with neck circumference and other anthropometric measurements in students of Kırıkkale University and to determine the emotional appetite of the students.

Materials and Methods

The research was conducted at Kırıkkale University in the 2016-2017 spring /2017-2018 fall semesters with the support of the Scientific Research Project Coordination Unit (BAP:2017/45). The sampling size of the study was calculated as at least 2976 students and 4873 students were reached at the end of the study. Foreign students (having different eating habits) and students of the Department of Nutrition and Dietetics (because they have knowledge of nutrition) are not included in the study. The number of sampling was determined by stratified cluster sampling method and the students were taken into the study based on volunteerism. The students who participated in the study were informed about the study and their written consent was obtained. Students were asked questions about their own identifying information about general characteristics and eating habits with a questionnaire developed by Nolan et al. (7) consisted of an emotional appetite scale was applied. The emotional appetite scale (Emotional Appetite Questionnaire (EMAQ)) is a 22-item likert-type 9-point scoring system designed to evaluate emotional eating. The validity and reliability of the scale of Turkish version were conducted by Demirel et al. (12), and it was shown to be a reliable measurement instrument. It is based on Likert type 9 point scoring system. Participants score less (1-4), the same (5) and more (6-9) as the effect of the expressions on their appetite in each item. The presence of emotional eating is evaluated in negative/positive emotions (14 items) and negative/positive conditions (8 items). The sum of negative emotions and negative conditions scores state total negative EMAQ score and the sum of positive emotions and positive conditions scores state total positive EMAQ score. There are no cut-off point related to emotional eating. The scale assesses the presence of emotional eating negative and positive emotions and conditions.

Anthropometric measurements of students (height, body weight, waist circumference, neck circumference, wrist circumference) were determined. Waist circumference (WC), neck circumference (NC) and waist-to-height ratio (WHtR) were used in determining abdominal obesity. BMI was evaluated as follows; <18.5 kg/m^2 underweight, 18.5-24.9 kg/m^2 normal, 25.0-29.9 kg/m^2 overweight, ≥30.0 kg/m^2 obese (13). Waist circumference was considered as risk when ≥80 cm in females and ≥94 cm in males; as high risk when ≥88 cm in females and ≥102cm in males (14). The neck circumference was evaluated as obesity when ≥37 cm in males and ≥34 cm in females (15). Height to wrist circumference ratio (H/WrC) was evaluated to body frame as small when <10.9 in females, <10.4 in males, as medium when 10.9-9.9 in females, 10.4-9.6 in males, as large when <9.9 in females, <9.6 in males (16). For waist-to-height ratio, 0.5 was considered as threshold value, >0.5 was considered as central obesity (17). All measurements were made in accordance with the technique (18).

For statistical analysis and calculations, the IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used. Statistical significance level was accepted as $p<0.05$. The distribution of continuous variables in the study such as age,

BMI, EMAQ scores etc. were examined by normality graphs and Shapiro Wilk test. These variables are expressed as mean ± standard deviation (mean ± sd) and median (minimum-maximum). Categorical variables such as gender and parents' educational status are shown in numbers and percentage (%). In the analyses, Mann Whitney U test was used to compare gender and neck circumference according to the number of groups while Kruskal-Wallis analysis was applied in comparison of EMAQ scores according to BMI groups and breakfast frequency. After Kruskal-Wallis test, Bonferroni-Dunn correction was applied as post-hoc test. The correlation between categorical variables was analysed by chi-square tests. The relation between neck circumference and some anthropometric measurements in gender subgroups was evaluated by Spearman rho coefficient.

Results

In this study, 56.3% (n=2742) of the students were female, 43.7% (n=2131) were male, 50.1% stayed at dormitories and 48.4% allocated a monthly budget of <200 Turkish liras (TL) for nutrition. It was determined that 31.9% of the students exercise and 6.0% did not have breakfast (Table 1).

The mean age of the students was 20.58±1.86 years, the mean BMI was 23.62±3.03 kg/m^2 for males and 21.66±3.08 kg/m^2 for females ($p<0.001$). The mean NC is 37.50±2.49 cm in males, 32.18±3.51 cm in females and the NC of males is higher than females ($p<0.001$). 65.8% of males and 19.4% of females are obese according to their NC ($p<0.001$). According to WC measurements, while 10.3% of male students are at risk and 4.6% are at high risk, 12.3% of female students are at risk and 7.0% are at high risk ($p<0.001$). According to the H/WrC, 43.8% of the students have medium body frame and 30.2% of the males and 16.3% of the females have central obesity ($p<0.001$) (Table 2).

In male and female students, there was a positive correlation between neck circumference measurement and body weight, BMI, waist circumference, wrist circumference, waist/height (Table 3).

In both negative and positive emotions and conditions, total emotional appetite median score of

Table 1. Descriptive characteristics of students

Characteristics	n (%)
Gender	
Female	2742 (56.3)
Male	2131 (43.7)
Accommodation	
With family	883 (18.0)
In dormitory	2445 (50.1)
In a house with friends	1137 (23.2)
With relatives /acquaintances	51 (1.0)
In a house alone	223 (4.6)
Other	154 (3.1)
Monthly total income (TL)	
<299	256 (5.2)
300-499	1508 (30.8)
500-999	2179 (44.5)
1000-2000	789 (16.1)
>2000	164 (3.3)
Money spared to nutrition (TL)	
<200	2366 (48.4)
201-400	1756 (35.9)
401-600	557 (11.4)
601-800	136 (2.8)
>800	78 (1.5)
Status of exercise	
Does exercise	1553 (31.9)
Does not exercise	3322 (68.1)
Frequency of exercise	
Everyday	447 (28.8)
Once in a week	220 (14.2)
Several times a week	802 (51.6)
Several times a month	84 (5.4)
Faculty	
Medical/Dental/Health Sciences /Veterinary/ Sports Sciences	922 (18.9)
Law/Economics and Administrative Sciences	1568 (32.1)
Educational Sciences/ Letters/ Islamic Sciences/Fine Arts	1487 (30.5)
Engineering	904 (18.5)
Class	
1	1169 (23.9)
2	1198 (24.5)
3	1173 (24.0)
4	1353 (27.6)
Having breakfast (day/week)	
Never	293 (6.0)
1-2	691 (14.1)
3-4	1138 (23.2)
5-6	851 (17.4)
Everyday	1924 (39.3)

Table 2. Anthropometric measurement of students

Characteristics	Male Mean±SD Median (min-max)	Female Mean±SD Median (min-max)	Total Mean±SD Median (min-max)	Z	p
Age (year)	20.79±1.99 21 (17-37)	20.42±1.74 20 (17-34)	20.58±1.86 20 (17-37)	6.415	**<0.001**
BMI (kg/m²)	23.62±3.03 23.41 (14.51-37.55)	21.66±3.08 21.30 (14.34-40.40)	22.51±3.21 22.27 (14.34-40.40)	23.641	**<0.001**
Neck circumference (cm)	37.50±2.49 37.7 (28-50)	32.18±2.16 32.0 (26-45)	34.51±3.51 34.0 (26-50)	52.540	**<0.001**
Waist circumference / height	0.478±0.054 0.474 (0.232-0.733)	0.452±0.053 0.444 (0.347-0.718)	0.463±0.055 0.463 (0.232-0.733)	18.442	**<0.001**
	n (%)	**n (%)**	**n (%)**	χ^2	**p**
BMI				290.666	**<0.001***
Underweight	64 (3.0)	340 (12.6)	406 (8.4)		
Normal	1465 (69.7)	2046 (75.6)	3527 (73.1)		
Overweight	512 (24.4)	267 (9.9)	780 (16.2)		
Obese	61 (2.9)	54 (2.0)	115 (2.3)		
Neck circumference				1069.515	**<0.001**
Normal	726 (34.2)	2194 (80.6)	2931 (60.4)		
Obese	1395 (65.8)	529 (19.4)	1924 (39.6)		
Waist circumference				18.784	**<0.001***
Normal	1802 (85.1)	2202 (80.7)	4019 (82.7)		
Risk	218 (10.3)	336 (12.3)	554 (11.4)		
High risk	97 (4.6)	191 (7.0)	288 (5.9)		
Height / wrist circumference				32.326	**<0.001**
Small	874 (41.9)	1089 (40.3)	1963 (41.0)		
Medium	837 (40.1)[1]	1262 (46.7)[1]	2099 (43.8)		
Large	376 (18.0)[2]	350 (13.0)[2]	729 (15.2)		
Waist-to-height ratio				130.726	**<0.001**
Have central obesity	632 (30.2)	442 (16.3)	1074 (22.4)		
Doesn't have central obesity	1463 (69.8)	2268 (83.7)	3731 (77.6)		

For all binary comparison results: p<0.05 1.2 p<0.05

male students is higher than that of female students (p<0.001) (Table 4).

The values of students' total positive scores are different from those of at least one of BMI groups (χ^2=14.503; p=0.002). Total positive scores of underweight students are higher than those of overweight and obese (p<0.001). Positive conditions scores are similar in BMI groups (χ^2=6.902; p=0.075) (Table 5).

Students who are obese according to their neck circumference have a high total scores of negative emotions and conditions (Z=4.539; p<0.001) (Table 6).

Discussion

The university period affects the emotional appetite status of students, changes their food preferences, and causes obesity and eating disorders (19). In our country, research conducted on the nutrition habits of university students has reported that students have serious problems with nutrition in this period, they usually do not pay attention to meals, avoid meals, breakfast in particular, and do not have breakfast every day (19-21). In this study, in accordance with the literature,

Table 3. Correlation between neck circumference and other anthropometric measurements of students

	Neck circumference (cm)					
	Male			Female		
	n	rho	p	n	rho	p
Weight (kg)	2113	0.586	**<0.001**	2704	0.545	**<0.001**
BMI (kg/m²)	2096	0.529	**<0.001**	2690	0.509	**<0.001**
Waist circumference (cm)	2109	0.573	**<0.001**	2715	0.533	**<0.001**
Wrist circumference (cm)	2100	0.421	**<0.001**	2706	0.502	**<0.001**
Waist-to-height ratio	2089	0.507	**<0.001**	2696	0.468	**<0.001**

Table 4. Emotional appetite scores of students by gender

Emotional appetite status	Gender		Z	P
	Male Median (min-max)	Female Median (min-max)		
Negative emotion	33 (9-81)	32 (9-81)	2.749	**0.006**
Positive emotion	30 (5-45)	29 (5-45)	5.847	**<0.001**
Negative conditions	15 (5-45)	13 (5-45)	7.579	**<0.001**
Positive conditions	16 (3-27)	16 (3-27)	3.851	**<0.001**
Total negative	48 (14-126)	45 (14-126)	4.743	**<0.001**
Total positive	46 (8-72)	45 (8-72)	5.528	**<0.001**

Table 5. Students' emotional appetite scores according to BMI groups

Emotional appetite status	BMI groups				χ^2	p
	Underweight Median (min-max)	Normal Median (min-max)	Overweight Median (min-max)	Obese Median (min-max)		
Negative emotion	29 (9-81)^{a,b,c}	33 (9-81)^{a,d}	35 (9-81)^{b,d}	33 (9-81)^{c}	57.118	**<0.001**
Positive emotion	30 (5-45) ^{a,b,c}	29 (5-45)^{a,d}	29 (5-45)^{b}	26 (5-45)^{c,d}	24.630	**<0.001**
Negative conditions	13 (5-41)^{a,b}	13 (5-45)^{c}	15 (5-45)^{a,c}	15 (5-45)^{b}	25.327	**<0.001**
Positive conditions	17 (3-27)	16 (3-27)	16 (3-27)	15 (3-27)	6.902	0.075
Total negative	42 (14-115)^{a,b,c}	46 (14-126)^{a,d}	50 (14-126)^{b,d}	51.5 (15-118)^{c}	42.067	**<0.001**
Total positive	47 (8-72)^{a,b}	46 (8-72)	45 (8-72)^{a}	42 (8-72)^{b}	14.503	**0.002**

** The groups indicated by the same letters are different.*

Table 6. Students' emotional appetite scores according to neck circumference classification

Emotional appetite status	Neck circumference classification		Z	P
	Normal Median (min-max)	Obese Median (min-max)		
Negative emotion	32 (9-81)	34 (9-81)	4.539	**<0.001**
Positive emotion	29 (5-45)	29 (5-45)	0.138	0.890
Negative conditions	13 (5-45)	14.5 (5-45)	5.139	**<0.001**
Positive conditions	16 (3-27)	16 (3-27)	0.831	0.406
Total negative	45 (14-126)	49 (14-126)	4.827	**<0.001**
Total positive	46 (8-72)	45 (8-72)	0.693	0.488

it was determined that the students skipped breakfast and only 39.3% of the students ate breakfast every day.

Various anthropometric measurements are used to determine obesity. In a study conducted by Şanlıer, when the students' obesity status were evaluated according to BMI, it was found that 14.0% in males and 6.4% in females have obesity, and the waist circumference, hip circumference and wrist circumference of male students were higher than that of females (22). In a study conducted by Işık et al., it was found according to the BMI values of university students that 24.8% of females and 33.5% of males were overweight and obese, 40.6% of females and 20.8% of males were in risk and high-risk groups (23). In this study, on the other hand, this study shows that 24.4% of males and 9.9% of females are overweight according to BMI, 10.3% of males are at risk and 4.6% are at high risk group while 12.3% of females are at risk and 7.0% are at high risk group according to waist circumference measurement. In addition, waist circumference, wrist circumference and neck circumference measurements were found to be higher in males than in females ($p<0.001$).

As is known, waist-to-height ratio is an indicator of central obesity, one of the cardiovascular risk factors (24). In the study conducted by Ashwell and Gibson, it was found that while the mean WHtR was 0.55±0.07 in males and 0.51±0.8 in females, the mean WHtR of males were higher than females (24). In a study involving 389 students at Venda University, 102 students (19.2%) were reported to have a WHtR of ≥0.5; in other words, central obesity was reported to be higher in males (11.1%) than females (8.1%). In this study, similarly, WHtR mean of males was higher than females, and central obesity was determined in 30.2% of males and 16.3% of females. Since this study consists of young adult university students, it is thought that the incidence of central obesity was found to be lower.

Studies have shown that NC is associated with BMI, body weight, waist and hip circumference measurements (26,27). Similarly, in this study, a moderate correlation was observed between NC and body weight, BMI, WC, WrC, WHtR values. In addition, according to a study conducted by Saka et al., while 85.1% of males and 38.8% of females were obese compared to the NC (26), in this study it was observed that 65.8% of males and 19.4% of females were obese compared to the NC.

In the studies conducted, it is emphasized that "emotional eating" is one of the factors that cause obesity (28,29). Geliebter and Aversa, in their study conducted with the aim of demonstrating that not only overweight and normal individuals, but also underweight individuals also showed positive and negative emotions found that underweight individuals exhibited less eating behaviour in negative situations, and more in positive situations (6). Nolan et al., in their study to determine the structural validity of EMAQ and its relationship with BMI, reported that overweight and obese individuals exhibited negative emotions, while underweight individuals exhibited positive emotions, and showed more eating behaviour (7). In this study, similarly, it was observed that total positive scores of

underweight students were found to be higher than those who are overweight and obese ($p<0.001$), and overweight and obese students exhibited more eating behaviour in negative situations. Also, in this study, it was determined that the median score of males was higher than the females in total scores of both negative and positive emotions and conditions ($p<0.001$). In other words, both positive and negative cases have been seen to increase the appetite of males compared to females. The results showing that overweight and obese individuals exhibit more eating behaviours when they experience a negative emotion are explained by emotion regulation and that when they experience a negative feeling, they exhibit eating behaviour to reduce this feeling (6, 7). For this reason, it is important to understand the mechanisms of controlling emotional appetite to protect young people from obesity (30).

Conclusion

As a result of this study, it was determined that there was a moderate correlation in the positive direction between the neck circumference of university students and the other anthropometric measurements. Because of its practicality, it was found that BMI and waist circumference, neck circumference, waist circumference/height measurements as well as neck circumference measurements can be used in determining obesity in individuals with this age group. In addition, in negative state and conditions, it is seen that students' appetite can be affected, and excessive eating behaviour caused by emotional appetite may result in overweight and obesity. It is thought that training regularly provides healthy eating and it is beneficial to prevent emotional eating behaviour and obesity, and to cope with stress and negative situations.

References

1. Dyson R, Renk K. Freshmen adaptation to university life: depressive symptoms, stress, and coping. J Clin Psychol. 2006; 62 (10): 1231-44.
2. Korinth A, Schiess S, Westenhoefer J. Eating behaviour and eating disorders in students of nutrition sciences. Public Health Nutr. 2010; 13 (1): 32–37.
3. Ferrara C, Cheryl N, Faina D. Obesity, diet and physical activity behaviors of students in health-related professions. College Student Journal. 2013; 47 (3): 560-565.
4. Bennett J, Greene G, Schwartz-Barcott D. Perceptions of emotional eating behavior. A qualitative study of college students. Appetite. 2013; 60 (1): 187–192.
5. Dingman DA, Schulz MR, Wyrick DL, Bibeau DL, Gupta SN. Factors related to the number of fast food meals obtained by college meal plan students. J Am Coll Health. 2014; 62 (8): 562-9.
6. Geliebter A, Aversa A. Emotional eating in overweight, normal weight, and underweight individuals. Eating Behaviours 3 (2003): 341-347.
7. Nolan LJ, Halperin LB, Geliebter A. Emotional appetite questionnaire. Construct validity and relationship with BMI. Appetite, 2010; 54:314-319.
8. Liubov (Louba) Ben-Noun, Ezra Sohar, Arie Laor. Neck circumference as a simple screening measure for identifying overweight and obese patients. Obesıty Research 2001,9 (8):470-477.
9. Rao G. A New Tool Needed for Identifying and Characterizing Obesity Current Cardiovascular Risk Reports 2013; 7(2) : 85–87
10. Sarah Rosner Preis, Joseph M. Massaro, Udo Hoffmann, et al. Neck Circumference as a Novel Measure of Cardiometabolic Risk: The Framingham Heart Study, J Clin Endocrinol Metab, 2010; 95(8): 3701–3710.
11. Jing-ya Zhou, Hui Ge, Ming-fan Zhuet al Neck circumference as an independent predictive contributor to cardiometabolic syndrome, Cardiovascular Diabetology, 2013; 12:76
12. Demirel B, Yavuz K.F, Karadere E.M, afak Y, Türkçapar M.H. Duygusal iştah anketinin Türkçe geçerlik ve güvenilirliği, beden kitle indeksi ve duygusal şemalarla ilişkisi. Bilişsel Davranışçı Psikoterapi ve Araştırmaları Dergisi 2014;3:171-181. (in Turkish)
13. World Health Organisation (WHO). Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. Geneva: World Health Organization, 2004.
14. World Health Organisation (WHO). Waist circumference and waist-hip ratio: Report of a WHO expert consultation, Geneva, 8-11 December 2008. 2011.
15. Ben-Noun LL, Sohar E, Laor A. Neck circumference as a simple screening measure for identifying overweight and obese patients. Obesity. 2001;9(8):470-7.
16. Pekcan G. Beslenme Durumunun Saptanması. Diyet El Kitabı. S103. 2014. (In Turkish)
17. Ashwell M, Gibson S. Waist-to-height ratio as an indicator of 'early health risk': simpler and more predictive than using a 'matrix' based on BMI and waist circumference. BMJ open. 2016;6(3):e010159.
18. WHO. Measuring obesity. Classification and Description of Anthropometric Data. Report on a WHO Consultation on the Epidemiology of Obesity, Warsaw, 21-23 October 1987.
19. Mazıcıoğlu M, Öztürk A. Üniversite 3 ve 4. sınıf

öğrencilerinde beslenme alışkanlıkları ve bunu etkileyen faktörler. Erciyes Tıp Dergisi 2003;25(4): 172-178. (In Turkish)
20. Güleç M, Yabancı N, Göçgeldi E, Bakır B. Ankara'da iki kız öğrenci yurdunda kalan öğrencilerin beslenme alışkanlıkları. Karadeniz Uluslararası Bilimsel Dergi, 2014;(15):139-149. (In Turkish)
21. Özdoğan Y, Özçelik AÖ, Sürücüoğlu MS. The breakfast habits of female university students. Pakistan Journal of Nutrition; 2010; 9 (9):882-886. ISSN 1680-5194.
22. anlıer N. Gençlerde biyokimyasal bulgular, antropometrik ölçümler, vücut bileşimi, beslenme ve fiziksel aktivite durumlarının değerlendirilmesi. GÜ, Gazi Eğitim Fakültesi Dergisi, 2005; 25 (3): 47-73. (In Turkish)
23. Işık Ö, Yıldırım İ, Yıldırım Y, Karagöz Ş, Ersöz Y, Doğan İ. Üniversite öğrencilerinde farklı ölçüm yöntemlerine göre obezite prevalansı. İnönü Üniversitesi, Beden Eğitimi ve Spor Bilimleri Dergisi, 2017, 4(2):20-33. (In Turkish)
24. Ashwell M, Gibson S. Waist to height ratio is a simple and effective obesity screening tool for CVD Risk factors. Obes Facts. 2009;2(2):97-103. doi: 10.1159/000203363. Epub 2009 Mar 31.
25. Ter Goon D, Libalela M, Lateef O. Amusa and Takalanı C. Muluvhu. Screening for total and abdominal obesity among University of Venda students African Journal for Physical, Health Education, Recreation and Dance (AJPHERD) 2013;19(4:2): 1014-1024.
26. Saka M, Türker P, Ercan A, Kızıltan G, Baş M. Is neck circumference measurement an indicator for abdominal obesity? A pilot study on Turkish Adults. African Health Sciences 2014; 14 (3)
27. Özkaya İ, Tunçkale A. Neck circumference positively related with central obesity and overweight in Turkish university students: A Preliminary Study Cent Eur J Public Health. 2016 Jun; 24(2):91-4. doi: 10.21101/cejph.a4555. Epub 2016 Apr 27.
28. Bongers P, Jansen A, Havermans R, Roefs A, Nedercoorn C. Happy eating. The underestimated role of overeating in a positive mood. Appetite, 74-80. (67) 2013.
29. Klatzkina Rebecca R. Gaffney S, Cyrus K, Bigus E, Brownley Kimberly A. Stress-induced eating in women with binge-eating disorder and obesity. Biological Psychology 131 (2018) 96–106
30. Greene, G. W, Schembre, S. M, White, A. A, Hoerr, S. L, Lohse, B, Shoff, S, Horacek, W, Riebe, D, Patterson, J, Phillips, B. W, Kattelmann, K. K, & Blissmer, B. Identifying clusters of college students at elevated health risk based on eating and exercise behaviors and psychosocial determinants of body weight. Journal American Dietetic Association, 2011;111(3): 394–400.

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Original article

Comparison of dietary quality (Healthy Eating Index-2010) according to metabolic health status in obesity: a cross-sectional study

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Summary. *Objectives:* The aim of the study is to compare dietary quality between metabolically healthy obese (MHO) and unhealthy obese (MUO) individuals. *Methods:* This study was conducted with 67 MHO and 70 MUO participants who consulted the obesity clinic of Adıyaman's Community Health Centre. Data for the study were collected via questionnaire forms by face-to-face interview. The questionnaire form includes socio-demographic characteristics, anthropometric measurements, certain biochemical findings, and dietary intake record with the 24-hour recall method.*Results:* The mean age of the MHO and MUO participants are 39.2±8.7 and 46.5±10.1, respectively. The mean healthy eating index-2010 (HEI-2010) scores of the MHO and MUO participants are 49.0±10.4 and 47.3±8.4, respectively ($p>0.05$). In addition, the scores on "dairy", "empty calories" and "refined grains" were found to be higher in the MHO participants when compared to their counterparts and the difference between the scores is statistically significant ($p<0.05$). Although higher score of HEI-2010 was not associated with metabolic health among the obese subjects (OR 1.55, 95% CI 0.68-3.53, p=0.204), high dairy and low refined grains intake was associated with metabolic health (OR 2.98, 95% CI 0.57-3.06, p=0.035; OR 3.23, 95% CI 1.27-8.21, p=0.014, respectively).*Conclusions:* It is considered that increasing consumption of dairy products and reducing the intakes of refined grains may provide a protective effect in terms of cardiometabolic risk factors. However, there needs to conduct longitudinal follow-up cohort and clinical studies to investigate the efficacy of nutrients and food groups on cardiometabolic health despite increased fat mass.

Key words: cardiometabolic risk, healthy eating index-2010 (HEI-2010), metabolically healthy obese (MHO), obesity

Introduction

Obesity rates have been doubled worldwide since 1980 and it is estimated that more than 650 million people are obese according to WHO's (World Health Organization) 2016 data. It is also predicted that while the burden of diabetes is 44% higher in mildly obese and obese individuals, the burden of ischemic heart diseases is 23% higher in these people. Nevertheless, each obese individual experiences the development of any metabolic disorder in a different way (1). While most obese individuals have complications such as diabetes, hypertension, or dyslipidaemia, others do not have such complications despite similar fat mass and age. Metabolically healthy and unhealthy obese phenotypes have been defined since 1982 according to cardiometabolic status (2).

It is critical to consider cut-off values of each parameter used in the health status-oriented obesity classification to make it clear whether metabolically healthy obese (MHO) individuals are indeed healthy. However, these parameters and cut-off values may dif-

fer in studies (3). It can be stated that MHO individuals are less likely to have atherosclerotic lesion formation (4), cardiovascular diseases (5), and Type II DM incidence (6) when compared to MUO counterparts. Visceral adiposity, inflammation activity, liver steatosis, and macrophage-specific T-cells are lower in the healthy obese than the unhealthy obese (7). Moreover, adiponectin level, mitochondrial function, aerobic fitness, and the incretin response to nutrients are better in healthy obese subjects (8-10).

It is first necessary to lose weight by changing one's lifestyle in the treatment of obesity. This causes an increase in insulin sensitivity thanks to a decrease in adipose tissue and affects the metabolic healthy positively (11-13). A short-term intervention study with energy restriction revealed that the unhealthy obese could switch to the healthy phenotype. However, there is still no consensus on the long-term intervention (7). In recent years, obesity has been considered as a new concept or strategy that can prevent many metabolic disorders and related mortalities thanks to the dietary quality emerged from the principle of adequate and balanced nutrition (14, 15). Yet, studies on the efficacy of dietary composition on metabolic health status despite increased fat accumulation are both limited and inconsistent (8, 16, 17). The aim of this study is to compare the dietary quality of MHO and MUO individuals obtained with the calculation of the components of the healthy eating index-2010 (HEI-2010) and total score.

Methods

Ethical approval and samples size calculation

This study was carried out in the obesity clinic of between February 27 and April 21, 2017. This study was conducted on a voluntary basis in accordance with the regulations set out in the Declaration of Helsinki (as revised in Brazil 2013) and all the procedures were agreed by the participants. In addition, an approval was obtained from Human Ethics Committee for Non-Clinical Research for the present study (Approved no. 85434274-050.04.04/2152). The reporting of this work is compliant with STROBE guidelines. The power analysis for this study was performed using the results of the average HEI-2005 scores of each group in the study on MHO and MUO individuals (16). The minimum sample size for the statistical evaluation of the data obtained from the study; it was determined that 67 individuals for each group was necessary according to G*Power 3.1.9.2 package program with 0.80 power, α=0.05 error level and effect size d=0.43.

Study design

The study was conducted with the individuals who consulted the clinic during the morning hours, met the inclusion criteria, and signed the voluntary consent form. In the preliminary interview, it was regarded that the participants were present in the clinic for the first time and did not lose more than 5% of their body weight over the last 6 months. In addition, it was regarded that participants were between 20-64 years and had at least 30.0 kg/m^2 body mass index (BMI) (BMI=body weight (kg)/height x height (m^2)), It was also considered that the participants did not have any psychiatric disorders, neurological diseases, other diseases causing obesity such as diabetic diseases, hypothalamus diseases, adrenal gland diseases, and genetic diseases such as Prader Willi, Leprechanism, and Rabson-Mendenhall syndrome. Individuals who do not fulfil these criteria were excluded from the study.

Venous blood samples were taken from voluntary participants after an 8-12 hour fasting and their analysis was conducted at the same centre. Blood pressure measurement was performed three times by taking systolic (SBP) and diastolic (DBP) blood pressure. In addition, participants' socio-demographic data and dietary intake record were obtained with a questionnaire form and the face-to-face interview technique.

Body composition measurements

Body weights of the participants were measured with a portable Tanita BC 545 N sensitive to 0.05 kg. For the measurement, the participants had to avoid heavy physical activity 24-48 hours prior to the measurement and alcohol 24 hours before. Moreover, they had to eat at least two hours before and avoid drinking too much water and consumption of other beverages 4 hours before the test. They also had to avoid wearing metal jewellery and cardiac pacemaker. Their body weights were measured with light clothes and with-

out shoes. The obtained data was recorded as a whole number (18).

Body height was measured using a stadiometer. This measurement was done in centimetres from the highest point of the head to the floor in a standing upright position with heels, back, shoulders and back of the head all touching the wall. The participants had to take off their shoes for this measurement (18).

Blood pressure measurement and biochemical analysis

Participants' blood pressure measurements were made by the researcher 3 times at 20-minute intervals with a Medisana MTC 51134 digital blood pressure monitor, and the final 2 values were averaged to determine the values of SBP and DBP (19).

Blood samples from participants were incubated for approximately 25-30 minutes at room temperature in biochemical tubes, then cooled and centrifuged at 4000 rpm at + 4°C for 10 minutes. Serum samples obtained after centrifugation were taken in microcentrifuge tubes for biochemical analyses, incubated at -20°C, and then stored at -20°C until the measurement. Fasting blood glucose (FBG), triglyceride (TG) and high-density lipoprotein (HDL) -cholesterol levels were measured from the blood samples.

Dietary intake assessment

Dietary intakes of the participants were recorded in a weekday on a 1-day retrospective basis with the 24-hour recall method. A catalogue of dishes and foods were utilized to determine measures and amounts. The participants were asked the amount of food they consume in a serving to make calculations. Non-domestic foods were calculated using their standard recipes (20). Average energy and macro- and micronutrient values of the foods were calculated by the Nutrition Information System (BEBIS 7.1).

Calculation of Healthy Eating Index-2010

The Healthy Eating Index is a tool that assesses diet quality in terms of adherence to the Dietary Guidelines for Americans which is a basis for the US nutrition policy. HEI-2010 consists of 12 components. These components are based on daily recommended intake amounts per 1000 calories. While those who consume total fruit (daily intake ≥0.8 cup, maximum score=5), whole fruit (daily intake ≥0.4 cup, maximum score=5), total vegetables (daily intake ≥1.1 cup, maximum score=5), greens and beans (daily intake ≥0.2 cup, maximum score=5), total protein foods (daily intake ≥2.5 oz, maximum score=5), seafood and plant proteins (daily intake ≥0.8 oz, maximum score= 5), dairy products (daily intake ≥1.3 cup, maximum score=10), whole grains (daily intake ≥1.5 oz, maximum score=10), and fatty acids (PUFAs + MUFAs)/SFAs>2.5, maximum score=10) in daily recommended amounts or above get the maximum score, those who remain under the recommended amounts are scored in a proportionally declining manner based on the maximum score. On the other hand, An inverse proportion scoring is made between the threshold values given for maximum and minimum scores to calculate the score on refined grains (daily intake ≤1.8 oz maximum score=10, daily intake >4.3 oz minimum score=0), sodium (daily intake ≤1.1 g maximum score=10, daily intake >2.0 g minimum score=0) and empty calories from solid fats, alcoholic beverages and added sugar (calories ≤19% of total calories, maximum score=20; calories ≥50% of total calories, minimum score=0) (21).

Determination of metabolic health status

MUO was defined as 2 or more cardiometabolic risk factors: triglycerides ≥150 mg/dL or on cholesterol medication, HDL-C <40 mg/dL for men, <50 mg/dL for women or on cholesterol medication, blood pressure ≥130/85mmHg or on blood pressure medication, and fasting glucose ≥100mg/dL or glucose/insulin medication. MHO was defined as having 0 or 1 abnormal cardiometabolic risk factors (9).

Statistical analysis

Statistical analysis was conducted using SPSS 21.0 version (SPSS, Chicago, II, USA). The Kolmogrov-Smirnov test was performed to determine whether continuous variables displayed a normal distribution. If the values did not display a normal distribution: the results were shown as mean±sd, median and 25-75th. Mann-Whitney U test was used to assess the mean between MHO and MUO groups which did not display a normal distribution. If continuous variables were normally distributed, values were expressed as mean±sd and median and independent-t test was

used to assess statistically significance between MHO and MUO groups. Categorical variables were expressed as percentages and differences in categorical variables between MHO and MUO groups were compared using chi-square analyses. Multivariate logistic regression analysis was performed including age, gender, BMI and physical activity level as confounding factors. While total HEI-2010 score and the scores on "dairy" were taken into 3rd quartile, the scores on "refined grains" were taken into 2nd quartile since approximately half of the participants received zero in this component. Most participants got the maximum score on "empty calories" and the minimum score on the component of "seafood and plant protein". Therefore, the multivariate logistic regression analysis was not able to be performed. The results were evaluated at a 95% confidence interval and the values with p <0.05 were accepted as significant (22).

Results

There were 107 (78.1%) female and 30 (21.9%) male participants in the study. It was found out that 5 of the MHO participants (7.5%) and 14 of the MUO participants (20.0%) are illiterate. It was determined that MHO participants have better educational status than their MUO counterparts, and the difference is statistically significant (p=0.004). While 43 (64.2%) of the MHO participants do not have any disorders, 47 (67.1%) of the MUO participants have one or more disorders. 10 (14.9%) MHO and 12 (17.1%) MUO participants were found to smoke (p>0.05). Mean BMI values of the MHO participants were found be statistically lower than that of the MUO participants (p<0.001). While 43 (64.2%) of the MHO individuals had minimal level of physical activity, 41 (58.6%) of the MUO individuals were found to be sedentary and MHO group had better physical activity than the MUO group (p=0.008). Biochemical parameters and blood pressure values of MHO group were found to be better than MUO group and the difference was statistically significant (p<0.05). Daily average energy intakes of the MHO and MUO participants were 1847.5±522.8 and 2084.3±727.6 kcal, respectively, but the difference was not statistically significant (p> 0.05). The percentage of energy intake from protein was statistically higher in the MHO participants (14.8±3.1) than in the MUO participants (13.4±2.5) (p=0.009) (Table 1).

Participants' mean scores on "total fruit", "whole fruit", "total vegetables", "greens and beans", "whole grains", "total protein foods", and "fatty acids" are similar between MHO and MUO groups (p>0.05). It was found that the MHO participants got higher scores (5.0±3.1) on "dairy products" than the MUO ones (4.3±2.7) (p<0.05). The mean scores on "empty calories" were statistically lower in the MUO participants (p<0.05). In terms of the scores on "refined grains", it was found that the MHO and MUO participants got 2.6±3.6 and 1.1±2.6, respectively, and this difference is significant (p=0.004) (Table 2).

Crude and adjusted odds ratio of having a metabolically healthy profile based on total HEI-2010 score and the scores on "dairy" and "refined grains" were given in Table 3. Among the obese subjects, a higher score of a HEI-2010 was not associated with metabolic health (OR 1.55, 95% CI 0.68-3.53, *p*=0.204). In the crude analyses dairy intake was positively associated with metabolic health (OR 2.98, 95% CI 0.57-3.06, p=0.035), but not adjusted model (OR 3.07, 95% CI 1.09-8.58, *p*=0.055). Low refined grains consumption was associated with metabolic health in the adjusted analyses (OR 3.23, 95% CI 1.27-8.21, *p*=0.014).

Discussion

Similar strategies are implemented in the prevention of the metabolic syndrome (MetS) and obesity, and in the medical nutrition treatment. It is thought that an increase in energy intake escalates the risk of obesity. In addition, the macro- and micronutrient content may have an impact on the risk factors of MetS.

137 adult individuals satisfying the criteria were included in the study after signing the informed consent form. It was found that the MHO participants have better educational status than their MUO counterparts. The proportion of smokers was found to be similar in both groups and was consistent with similar studies (8, 17, 23). However, smoking is one of the major risk factors for MetS and CVD. Smoking can reduce insulin sensitivity by increasing the circulation

Table 1. Comparison of socio-demographic and anthropometric data of the MHO and MUO participants (Numbers and percentages; mean and standard deviation)

	MHO (n=67)		MUO (n=70)		Total (n=137)		p[(a)]
	n	%	n	%	n	%	
Gender (F)	52	48.6	55	51.4	107	78.1	0.892
Age (years)	39.2±8.7		46.5±10.1		42.9±10.1		**<0.001****
Marital status (Married)	56	83.5	60	85.7	116	84.6	0.729
Level of education							
Illiterate	5	7.5	14	20.0	19	13.9	
Literate	1	1.5	6	8.6	7	5.1	
Primary School	25	37.3	32	45.7	57	41.6	**0.004****
High School	15	22.4	12	17.1	27	19.7	
Undergraduate and above	21	31.3	6	8.6	27	19.7	
Disorder (None)	43	64.2	23	22.9	66	48.2	**<0.001****
Smoking (Yes)	10	14.9	12	17.1	22	16.1	0.724
Physical activity							
Sedentary	24	35.8	41	58.6	65	47.4	**0.008****
Minimal active	43	64.2	29	41.4	72	52.6	
Anthropometric measurements (Mean±SD)							
BMI (kg/m^2)	33.2±3.5		36.1±4.2		34.7±4.1		**<0.001****
WC (cm)	104.8±8.8		113.6±11.6		109.3±11.2		**<0.001****
Waist/height	0.64±0.06		0.71±0.07		0.67±0.07		**<0.001****
Body fat percentage	38.0±6.8		41.7±5.7		39.9±6.5		**0.001*****
Biochemical parameters and blood pressure (Mean±SD)							
FPG (mg/dL)	90.5±8.5		116.1±33.5		103.6±27.8		**<0.001**
HDL-cholesterol (mg/dL)	62.8±11.0		50.1±14.1		56.3±14.1		**<0.001**
Triglycerides (mg/dL)	117.9±45.2		183.0±77.6		151.2±71.5		**<0.001**
SBP (mmHg)	123.3±12.7		139.2±16.1		131.4±16.5		**<0.001**
DBP (mmHg)	80.5±9.1		86.6±10.3		83.6±10.2		**<0.001**
Dietary intake							
Energy (kcal/d)	1847.5±522.8		2084.3±727.6		1968.7±644.5		0.065
Carbohydrate (%)	49.2±9.9		52.5±10.2		50.9±10.1		0.056
Protein (%)	14.8±3.1		13.4±2.5		14.1±2.9		**0.009*****
Fat (%)	35.9±8.5		33.9±9.6		34.9±9.1		0.214

BMI, body mass index F, Female WC, waist circumference

[(a)] Student's t-test was used to determine the statistical value of continuous variables of the MHO and MUO participants, and χ^2 test was used to determine the statistical value of categorical data.

***p<0.01, ***p<0.001*

of insulin-antagonist hormones such as cortisol and catecholamine (24). In addition, nicotine and carbon monoxide play a role in the development of insulin resistance and dyslipidemia (25). MHO participants were found to have better physical activity levels than MUO ones. Besides components of metabolic syndrome and insulin resistance, physical fitness is an alternative means to define metabolically healthy obesity. Physical activity is the main nongenetic determinant of fitness, and also has beneficial effects on body fat distribution, insulin sensitivity, and other characteristics of the metabolic syndrome (7). Moreover, a more favourable fat distribution, with less visceral fat, was associated with a long-term metabolically healthy profile in obese adults over a period of 10 year, and no excess risk of Type II DM and CVD (26). In recent

Table 2. MHO and MUO participants' mean scores on HEI-2010 components
(Mean and standard deviation; median and 25th and 75th quartiles)

HEI-2010 Components	MHO (n=67)			MUO (n=70)			P[(a)]
	Mean ± SD	Median	25th and 75th quartiles	Mean ± SD	Median	25th and 75th quartiles	
Total fruit	2.4±2.1	2.5	0.04-5.0	2.9±1.9	2.8	0.96-5.0	0.118
Whole fruit	2.8±2.3	4.9	0.09-5.0	3.2±2.1	4.8	0.33-5.0	0.267
Total vegetables	2.7±1.4	2.7	1.6-4.0	2.2±1.1	2.0	1.4-2.0	0.068
Green and beans	2.0±2.0	1.2	0.15-5.0	2.0±1.7	1.6	0.3-3.3	0.726
Whole grains	3.1±3.6	1.6	0.00-5.2	2.9±3.2	1.7	0.00-5.0	0.823
Dairy	5.0±3.1	4.9	2.4-7.7	4.3±2.7	3.9	2.1-6.3	**0.01***
Total protein foods	2.8±2.0	2.9	0.39-5.0	3.3±1.6	3.7	1.9-5.0	0.423
Seafood and plant proteins	0.11±0.69	0.00	0.00-0.00	0.51±2.0	0.00	0.00-0.00	**0.034***
Fatty acids	3.8±3.1	3.1	1.1-6.0	3.5±3.1	2.5	1.0-5.2	0.668
Refined grains	2.6±3.6	0.00	0.00-5.3	1.1±2.6	0.00	0.00-0.00	**0.004****
Sodium	0.98±2.0	0.00	0.00-0.63	2.2±3.3	0.00	0.00-4.1	0.068
Empty calories	19.7±0.97	20.0	20.0-20.0	18.8±7.1	20.0	19.5-20.0	**0.006****
Total HEI-2010 score	49.0±10.4	49.0	41.0-54.0	47.3±8.4	46.2	40.8-53.1	0.278

[(a)] Mann Whitney U test
**P<0.05, **P<0.01*

Table 3. Crude and multivariable-adjusted ratios of the metabolically healthy phenotype according to quartile of total HEI-2010, dairy and refined grains

	Crude OR (%95 CI)	p[(a)]	Adjusted OR (%95 Cl)[(b)]	p[(a)]
Total HEI				
Quartile 1(Minimum)	1 (Reference)		1 (Reference)	
Quartile 2	0.72 (0.31-1.66)	0.204	0.64 (0.24-1.71)	0.195
Quartile 3 (Maximum)	1.55 (0.68-3.53)		1.60 (0.59-4.32)	
Dairy				
Quartile 1 (Minimum)	1 (Reference)		1 (Reference)	
Quartile 2	1.33 (0.57-3.06)	**0.035***	1.06 (0.39-2.88)	0.055
Quartile 3 (Maximum)	2.98 (1.26-7.03)		3.07 (1.09-8.58)	
Refined Grains				
Quartile 1 (Minimum)	1 (Reference)	**0.004****	1 (Reference)	**0.014***
Quartile 2 (Maximum)	2.97 (1.40-6.27)		3.23 (1.27-8.21)	

[(a)] Multivariate logistic regression analysis. Figures are expressed as OR (95% CI)
[(b)] Adjusted according to age, gender, educational status, body mass index (BMI), and physical activity
**p<0.05, **p<0.01*

other studies, it was found that moderate physical activity was higher in MHO participants than in MUO (17, 27).

It was discovered that the MHO participants had a higher percentage of energy from protein but a lower percentage of carbohydrate when compared to

the MUO ones. In some cross-sectional studies, the percentage of macronutrients from energy was found to be similar between the MHO and MUO groups (8, 28). However, in a short-term study on rats, it was observed that high carbohydrate and fat intake and low protein intake escalated the level of FBG by 22 mg/dL and such a result caused dyslipidaemia in the rats. There was also an increase in abdominal fat although there was no significant difference in the weight gain. Therefore, it can be said that high carbohydrate and low protein intakes have a critical role in the MetS since affecting the metabolic health adversely (29).

In the study, there is no significant difference between the MHO (49.0±10.4) and MUO (47.3±8.4) participants in terms of the HEI-2010 scores. Although similar results were found between the groups in dietary composition and total dietary score, it was determined that the MHO participants have a higher consumption of dairy and lower consumption of grains, alcoholic beverages, solid fats, and added sugar. There are also studies revealing that the MHO and MUO individuals have a similar dietary quality (8, 28). In prospective cohort studies rather than cross-sectional ones, it was observed that those who have better dietary quality have low SBP, fasting plasma insulin, FBG, total cholesterol and high insulin sensitivity (30), reduced inflammatory cytokines (31) and low abdominal adiposity (32).

The score on "milk and dairy products" was found to be higher in the MHO participants (5.0±3.1) than in the MUO ones (4.3±2.7) ($p<0.05$). In the study conducted by Camhi et al.(2015), the score on "milk and dairy products" was higher in the male MHO participants (3.9±0.6) than in the MUO counterparts (2.3±0.7) ($p<0.05$). Such a finding is consistent with the present study. It is known that lipogenesis is reduced, and lipolysis is increased in adipose tissue thanks to regular consumption of milk and dairy products. Milk and dairy products with rich calcium and vitamin D content contribute to the reduction of plasma fasting insulin in the obese by increasing lean tissue mass and decreasing total body fat. It was also found that consumption of 3-4 servings of milk and dairy products per day reduces low-grade inflammatory cytokine levels (33) and visceral adiposity (34) within the aetiology of cardiometabolic risk factors. In a cross-sectional study, those who consume yoghurt were found to receive 47% less vitamin B_2, 55% less vitamin B_{12}, 48% less calcium, 38% less magnesium, and 34% less zinc when compared to those who consume milk and dairy products. Moreover, those who consume yoghurt have less likely FBG, blood pressure, TG levels and insulin when compared to the those who do not consume yoghurt. Yoghurt consumption is a good source of certain micronutrients and is proven to play an important role in improving dietary quality (35). As a result, it was shown that consumption of milk and dairy products despite increased fat tissue may have positive effects on metabolic health.

In this study, it was found that the mean score of MHO participants from refined grains was higher than that of MUO ones. In some studies, it was found that the consumption of processed grains was similar between the MHO and MUO groups (8, 16, 28). Processed grains with high glycaemic index and load are critical risk factors for obesity, MetS, CVD, and diabetes (36). The consumption of processed grains is known to escalate the levels of FBG and TG as well as the risk of MetS but decrease HDL-cholesterol (37). Moreover, in a study, it was determined that the high consumption of processed grains has a greater effect on visceral adiposity than on subcutaneous adiposity (38).

The MHO and MUO participants' scores on "sources of empty calories" are 19.7±0.97 and 18.8±7.1, respectively ($p<0.01$). However, such a result is not clinically significant because the mean scores of both groups are close to the maximum score. Simple sugar plays a dramatic role in blood glucose and the increase in insulin secretion and this plays a role in metabolically unhealthy phenotype formation in obesity by leading to the development of abdominal obesity and insulin resistance (39). In addition, simple sugars are rapidly absorbed in the body and converted to lipids in the liver, thus they lead to an escalation in triglyceride levels. The escalation in triglyceride levels is also associated with obesity, dyslipidaemia and insulin resistance (40). In clinical studies, it was found that saturated fat intake is associated with the insulin resistance and coronary artery disease. Especially, saturated fat intake, having an adverse effect on insulin sensitivity, is known to be an important cardiometabolic risk factor.

Even though the MUO participants got higher scores on "seafood and plant proteins" than the MHO

counterparts, the intake of seafood and herbal protein is quite low in both groups. The main reason for this is thought to be the fact that the cuisine culture of the city is mostly based on red meat and milk and dairy products (41).

Strengths and Limitation

This study has some strengths and weaknesses. The strengths of this study include the use of power analysis in determining the sample, the inclusion of those who have never taken a weight-loss diet before and consulted the obesity clinic for the first time, and the use of the most updated version of HEI. The 24-hour recall method was used to obtain dietary composition and quality. For the weaknesses of the study, it can be said that the use of a dietary intake record covering three or more days can yield more effective results. The present study allowed to assess the overall situation as a cross-sectional study. However, it lacks cause and effect relationships.

Conclusion

In conclusion, although both groups have similar a dietary composition and quality, it can be asserted that the high consumption of milk and dairy products and the low consumption of processed grains, saturated fat, added sugar, and alcoholic beverages can result in a protective effect on cardiometabolic risk factors in the obese. Nevertheless, there needs to conduct longitudinal follow-up cohort and clinical studies to investigate the efficacy of micro and macro nutrients and food groups on cardiometabolic health despite increased fat mass. Such studies will contribute significantly to the question of whether healthy obesity is a temporary phenotype depending on the age factor.

Authorship: MA analysed the data and wrote the manuscript. FPA made general checks of the work and adapted it to the format of the journal.

Place of Study: Obesity Clinic of Adıyaman's (Turkey's Province) Community Health Centre

Ethics Approval: Ankara University, Human Ethics Committee for Non-Clinical Research for the present study

References

1. WHO I. Obesity and overweight. Fact sheet WHO http://www mclveganway org uk/publications/who_obesity_and_overweight pdf Zugegriffen. 2014; 26.
2. Samocha-Bonet D, Dixit V, Kahn C, Leibel R, Lin X, Nieuwdorp M, et al. Metabolically healthy and unhealthy obese–the 2013 Stock Conference report. Obesity reviews 2014; 15(9): 697-708.
3. Blüher M. Mechanisms in endocrinology: Are metabolically healthy obese individuals really healthy? European journal of endocrinology 2014; 171(6): R209-R19.
4. Khan UI, Wang D, Thurston RC, Sowers M, Sutton-Tyrrell K, Matthews KA, et al. Burden of subclinical cardiovascular disease in "metabolically benign" and "at-risk" overweight and obese women: the Study of Women's Health Across the Nation (SWAN). Atherosclerosis 2011; 217(1): 179-86.
5. Mørkedal B, Vatten LJ, Romundstad PR, Laugsand LE, Janszky I. Risk of myocardial infarction and heart failure among metabolically healthy but obese individuals: HUNT (Nord-Trøndelag Health Study), Norway. Journal of the American College of Cardiology 2014; 63(11): 1071-8.
6. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. The Journal of Clinical Endocrinology & Metabolism 2012; 97(7): 2482-8.
7. Stefan N, Häring H-U, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. The lancet Diabetes & endocrinology 2013; 1(2): 152-62.
8. Phillips CM, Dillon C, Harrington JM, McCarthy VJ, Kearney PM, Fitzgerald AP, et al. Defining metabolically healthy obesity: role of dietary and lifestyle factors. PloS one 2013; 8(10): e76188.
9. Camhi SM, Waring ME, Sisson SB, Hayman LL, Must A. Physical activity and screen time in metabolically healthy obese phenotypes in adolescents and adults. Journal of obesity 2013; 2013.
10. Schwenk RW, Vogel H, Schürmann A. Genetic and epigenetic control of metabolic health. Molecular metabolism 2013; 2(4): 337-47.
11. Kantartzis K, Machann J, Schick F, Rittig K, Machicao F, Fritsche A, et al. Effects of a lifestyle intervention in metabolically benign and malign obesity. Diabetologia 2011; 54(4): 864-8.
12. Karelis A, Messier V, Brochu M, Rabasa-Lhoret R. Metabolically healthy but obese women: effect of an energy-restricted diet. Diabetologia 2008; 51(9): 1752-4.
13. Miller CT, Fraser SF, Levinger I, Straznicky NE, Dixon JB, Reynolds J, et al. The effects of exercise training in addition to energy restriction on functional capacities and body composition in obese adults during weight loss: a systematic review 2013; 8(11): e81692.
14. Chiuve SE, Sampson L, Willett WC. The association between a nutritional quality index and risk of chronic disease. American journal of preventive medicine 2011; 40(5): 505-13.

15. Tande DL, Magel R, Strand BN. Healthy Eating Index and abdominal obesity. Public health nutrition 2010; 13(2): 208-14.
16. Camhi SM, Evans EW, Hayman LL, Lichtenstein AH, Must A. Healthy eating index and metabolically healthy obesity in US adolescents and adults. Preventive medicine 2015; 77: 23-7.
17. Slagter SN, Corpeleijn E, Van Der Klauw MM, Sijtsma A, Swart-Busscher LG, Perenboom CW, et al. Dietary patterns and physical activity in the metabolically (un) healthy obese: the Dutch Lifelines cohort study 2018; 17(1): 18.
18. Pekcan G. Beslenme durumunun saptanmasi. Diyet El Kitabi Hatipoglu Yayinevi Ankara 2008: 67-141.
19. Casiglia E, Tikhonoff V, Albertini F, Palatini P. Blood Pressure Measurement 2016.
20. Merdol T. Standart Yemek Tarifeleri (3. bs.). Ankara: Hatipoğlu Yayınevi. 2003.
21. Guenther PM, Casavale KO, Reedy J, Kirkpatrick SI, Hiza HA, Kuczynski KJ, et al. Update of the healthy eating index: HEI-2010. Journal of the Academy of Nutrition and Dietetics 2013; 113(4): 569-80.
22. Sümbüloğlu K, Sümbüloğlu V. Biyoistatistik: Hatiboğlu; 2010.
23. Pajunen P, Kotronen A, Korpi-Hyövälti E, Keinänen-Kiukaanniemi S, Oksa H, Niskanen L, et al. Metabolically healthy and unhealthy obesity phenotypes in the general population: the FIN-D2D Survey 2011; 11(1): 754.
24. Artese A, Stamford BA, Moffatt RJJAJoLM. Cigarette Smoking: An Accessory to the Development of Insulin Resistance 2017: 1559827617726516.
25. Calo WA, Ortiz AP, Suárez E, Guzmán M, Pérez CMJIjopm. Association of cigarette smoking and metabolic syndrome in a Puerto Rican adult population 2014; 5(7): 849.
26. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. 2013:DC_121971.
27. Ortega FB, Cadenas-Sanchez C, Migueles JH, Labayen I, Ruiz JR, Sui X, et al. Role of physical activity and fitness in the characterization and prognosis of the metabolically healthy obesity phenotype: a systematic review and meta-analysis. 2018.
28. Hankinson AL, Daviglus ML, Horn LV, Chan Q, Brown I, Holmes E, et al. Diet composition and activity level of at risk and metabolically healthy obese American adults. Obesity 2013; 21(3): 637-43.
29. Wong SK, Chin K-Y, Suhaimi FH, Ahmad F, Ima-Nirwana S. The effects of a modified high-carbohydrate high-fat diet on metabolic syndrome parameters in male rats. Experimental and Clinical Endocrinology & Diabetes 2017.
30. McNaughton SA, Dunstan DW, Ball K, Shaw J, Crawford D. Dietary quality is associated with diabetes and cardio-metabolic risk factors. The Journal of nutrition 2009; 139(4): 734-42.
31. Kastorini C-M, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. Journal of the American College of Cardiology 2011; 57(11): 1299-313.
32. Azadbakht L, Esmaillzadeh A. Dietary diversity score is related to obesity and abdominal adiposity among Iranian female youth. Public health nutrition 2011; 14(1): 62-9.
33. Qureshi MM, Singer MR, Moore LL. A cross-sectional study of food group intake and C-reactive protein among children. Nutrition & metabolism 2009; 6(1): 40.
34. Bradlee ML, Singer MR, Qureshi MM, Moore LL. Food group intake and central obesity among children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES III). Public health nutrition 2010; 13(6): 797-805.
35. Wang H, Livingston KA, Fox CS, Meigs JB, Jacques PF. Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. Nutrition Research 2013; 33(1): 18-26.
36. Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, et al. Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. The American journal of clinical nutrition 2008; 87(3): 627-37.
37. Song S, Lee JE, Song WO, Paik H-Y, Song Y. Carbohydrate intake and refined-grain consumption are associated with metabolic syndrome in the Korean adult population. Journal of the Academy of Nutrition and Dietetics 2014; 114(1): 54-62.
38. McKeown NM, Troy LM, Jacques PF, Hoffmann U, O'Donnell CJ, Fox CS. Whole-and refined-grain intakes are differentially associated with abdominal visceral and subcutaneous adiposity in healthy adults: the Framingham Heart Study. The American journal of clinical nutrition 2010; 92(5): 1165-71.
39. Dhingra R, Sullivan L, Jacques PF, Wang TJ, Fox CS, Meigs JB, et al. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation 2007; 116(5): 480-8.
40. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutrition reviews 2005; 63(5): 133-57.
41. Aksoy M, Sezgi G. Gastronomi turizmi ve Güneydoğu Anadolu bölgesi gastronomik unsurları. Journal of Tourism and Gastronomy Studies 2015; 3(3): 79-89.

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Original article

Nutrition perspective from the view of pregnant women: their understanding of fetal well-being relative to their diet

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Summary. *Introduction*: Nutritionally balanced nutrition assessment, mothers' nutrition motivation, and healthy nutrition information are among the factors that affect infants. Thus, it is possible to shed light on the attempts to reach information in healthy nutrition guides among pregnant women. Aim: The aim of our study was to evaluate the nutritional habits and healthy nutrition knowledge levels of pregnant women. It was aimed to evaluate whether pregnant women should be included in high quality nutrition programs. *Methods*: Our study was performed with volunteer 338 pregnant women who presented to Trakya University obstetrics clinic in March 2018- April 2019. Sociodemographic characteristics were recorded. The General Knowledge Nutrition Questionnaire (GNKQ) was used in our study. *Results*: The differences in GKNQ scores for the pregnant women in our study were evaluated for the four sections and total scores. For the first section, dietary recommendations, the mean was score was 9.66. For the second section (sources of nutrients), the mean score was 32.92. For the third section (choosing everyday foods), the mean sore was 6.23, and for the fourth section (diet-disease relationships), the mean score was 10.24. The mean total score was 59.10. It was seen that most of the pregnant women had not received adequate nutrition education before or during pregnancy. It was observed that some of the pregnant women participated in our study because of the threat of preterm labor and / or premature membrane rupture. 3.84% were found to have preterm history. When evaluated with the GNKQ scale, those with a history of EMR were under investigation and treatment due to the threat of preterm labor. In our study, it was seen that patients who were interned in the ward because of gestational hypertension, preeclampsia, and eclampsia GNKQ scores were lower than in the other patients. This result suggests that nutrition may have an effect on the complications of pregnancy. Unfortunately, although women reported positive changes in lifestyles during pregnancy, it was shown that their dietary intake and knowledge did not meet the recommended nutrient intake for pregnancy. *Discussion*: Considering that diet behavior is very complex, attempts to understand this in terms of nutritional knowledge in pregnant women should start with a clear understanding of awareness. Furthermore, they had a wide range of information, but the correct information was not on a systematic basis. Pregnancy is a life event that triggers a long-term review of nutritional problems. It is important for health professionals to realize that pregnancy is one of the unique opportunities for women to be informed about nutrition. There is a need for greater emphasis on nutritional counseling and education in order to optimize the quality of nutritional habits of pregnant women. Training and forms should be put into practical use for pregnant women. *Conclusion*: Our findings show that evaluating pregnant women with the GNKQ and providing nutritional education will be beneficial on pregnancy outcomes. This study showed that pregnant women had limited knowledge about balanced nutrition rules. It's necessary to increase effective nutrition programs and campaigns for pregnant women. Babies should be provided with a healthy start to life and routine nutrition counseling should be promoted as part of pregnancy care.

Key words: nutritional knowledge, pregnant women, fetal well-being, dietary recommendation

Introduction

Nutritionally balanced nutrition assessment, mothers' nutrition motivation, and healthy nutrition information are among the factors that affect infants. Increased weight gain during pregnancy, macrosomia, leads to an increase in the risk of glucose intolerance and cesarean section (1). Insufficient folic acid and

iodine intake cause preventable birth defects and fetal cognitive problems. Low quality of maternal diet increases the risk of premature birth. Poor maternal nutrition in pregnancy, impaired glucose tolerance, and dyslipidemia may cause increased systemic arterial pressure (2). Fetus exposure to maternal obesity, diabetes, and systemic hypertension may increase the risk of developing obesity and chronic diseases in later ages. Knowledge of the benefits of some basic nutrients such as omega 3 fatty acids and nutrient sources, and deficiencies of nutrients and vitamins and mineral (e.g. iodine, iron) in pregnant women needs to be increased (3, 4).

Dietary behaviors during pregnancy are influenced by a complex set of factors related to the individual and their environment, including physiologic developments. These sociodemographic features, prepregnancy body mass index (BMI), nausea and vomiting during pregnancy, and the effects of the community, especially the family elders, shape women's knowledge and attitudes during pregnancy. Pregnancy gestational diabetes, gestational hypertension, hyperemesis gravidarum, and gastro-esophageal reflux can affect the nutritional profiles of pregnant women (1). There are differences in the motivation of pregnant women and their attitudes towards healthy nutrition. Some women see pregnancy as a turning point towards a healthy diet, while others regard it as a break for healthy nutrition (5).

The level of relationship between the knowledge and awareness level of pregnant women and their compliance with dietary rules should be determined. Thus, it is possible to shed light on the attempts to reach information in healthy nutrition guides among pregnant women and to improve the nutrition quality in their daily lives. In order to support the safe, healthy, and balanced nutrition of women during pregnancy, it is important to have an idea about factors that affect their dietary behaviors.

The aim of our study was to evaluate the nutritional habits and healthy nutrition knowledge levels of pregnant women. It was aimed to evaluate whether pregnant women should be included in high quality nutrition programs.

Methods

Our study was performed with volunteer pregnant women who presented to Trakya University obstetrics clinic for routine pregnancy checks. Written informed consent was obtained from each pregnant woman. A number of specific questions were included in the first part of the questionnaire to characterize the participants.

The study was conducted between March 2018 and May 2019 in Trakya University Women's Diseases and Obstetrics Clinic.

Additional disease information, gestational week, sociodemographic characteristics, and some information about pregnancies (how many weeks of gestation, how many pregnancies) were recorded.

The General Knowledge Nutrition Questionnaire (GNKQ) was used in our study, whose validity and reliability have been proven in the literature (6). The GNKQ represents a more comprehensive assessment of nutritional information than is generally achieved. It is a reliable and valid survey to provide a quality measurement of nutritional information. This tool helps to identify the weak areas of people in the understanding of healthy eating. It also provides useful data to examine the relationship between nutritional information and diet behavior. The scale is useful for individuals in food selection research, providing a clear understanding of the relationship between knowledge and behavior. The GNKQ is also a useful tool for identifying gaps in nutritional knowledge of societies and for evaluating the success of health education campaigns thanks to the wide scope of the scale. The fact that this valuable test is based on a large number of scientific studies makes it more powerful (7,8).

The GNKQ was administered to pregnant women under the guidance of the responsible researcher. In the study, the GNKQ evaluates nutritional profiles and nutritional knowledge levels and includes 135 items covering four nutritional information areas; (i) awareness of current dietary recommendations (11 items), (ii) food sources and nutrient information (73 items), (iii) ability to select daily foods (7 items), and (iv) dietary-disease relationships knowledge level assessment (44 items) (6,7). These four areas underlie the basic considerations of acquisition behavior.

Using the GNKQ, "Do people know what the ex-

isting expert dietary recommendations are?", "Do they know which foods provide the nutrients specified in the experts' recommendations?", Are they aware of the health effects of eating or not eating certain foods?" were examined in the pregnant women.

For the 4 main areas, knowledge subscales and general nutritional knowledge scores were calculated. The raw data obtained from the responses of each participant were coded numerically. The answers were also translated to 1 and 0 for correct and incorrect answers, respectively.

Pregnant women with hyperemesis gravidarum, ectopic pregnancy, missed abortion, gestational trophoblastic diseases, and incomplete and complete abortion were excluded from the study. Pregnant women with special diets (patients with diabetes or those with special diet-regulated chronic diseases) were excluded from the study.

In order to protect and improve the health of both the mother and the baby, we tried to determine deficiencies in order to increase the awareness of balanced nutrition.

Ethical consideration

Ethics committee approval for this study was obtained from Trakya University Ethical Committee of Scientific Research (Decision number: 2019.02.26-21). Written informed consent was obtained from each participant of the study.

Data analysis

All statistical analyses were performed using the IBM SPSS 21.0 package program. The non-parametric Mann-Whitney U test was used for group comparisons. The data were evaluated using appropriate descriptive statistics. Median, minimum, and maximum were calculated as descriptive statistics. Mean and standard deviation are used for descriptive statistics of quantitative variables and percentage and frequency are used for qualitative variables. Significance value (p) for all statistical analyses was defined as 0.05.

Results

Three hundred sixty-nine volunteer pregnant women participated in our study. However, 31 pregnant women were excluded from the evaluated group because they did not fully answer the questions. The study was completed with 338 pregnant women. The ages of the women ranged between 18 and 41 years.

The differences in GKNQ scores for the pregnant women in our study were evaluated for the four sections and total scores. For the first section, dietary recommendations, the mean was score was 9.66±1.60 (mean±standart deviation). For the second section (sources of nutrients), the mean score was 32.92±8.03). For the third section (choosing everyday foods), the mean sore was 6.23±4.51, and for the fourth section (diet-disease relationships), the mean score was 10.24±4.51. The mean total score was 59.10±10.77.

Fifty-four (15.97%) of the pregnant women in our study stated that they received diet training from family physicians during pregnancy checks. There was no statistically significant difference between GNKQ scale total score and sub-scores for 284 (84.02%) preg-

Table 1. GNKQ scores for all pregnant women and evaluation of nutritional education

GNKQ scores	All pregnant women N= 338		According to taken nutritional education (NE) (at least 3 hours/ One month)				
			NE - (n= 284)		NE + (n=54)		*P*
Knowledge section (max score)	Mean±SD		Mean±SD		Mean±SD		
1. Section: Dietary recommendations	9.66	1.60	9.65	1.62	9.74	1.50	0.719
2. Section: Sources of nutrients	32.92	8.03	33.24	7.87	31.25	8.69	0.096
3. Section: Choosing everyday foods	6.23	2.09	6.22	2.15	6.25	1.75	0.922
4. Section: Diet-disease relationships	10.24	4.51	10.32	4.46	9.83	4.77	0.465
Total	59.10	10.77	59.45	10.76	57.27	10.74	0.174

**SD: Standart Deviation*

Table 2. GNKQ scores according to trimesters and number of pregnancies

Parameters	Trimester							Number of pregnancies						
	1 n=57 (16.86%)		2 n=65 (19.23%)		3 n=216 (63.90%)		*p*	1 n=113		2 n=109		3 n=116		*p*
Knowledge section (max score)	Mean±SD		Mean±SD		Mean±SD			Mean±SD		Mean±SD		Mean±SD		
1. Section: Dietary recommendations	9.28	2.10	9.75	1.71	9.74	14.02	*0.135*	9.53	1.73	9.49	1.75	9.96	1.26	0.048*
2. Section: Sources of nutrients	33.61	8.52	33.06	8.54	32.70	7.76	*0.744*	34.15	7.97	31.01	7.78	33.52	8.05	0.008*
3. Section: Choosing everyday foods	5.63	2.24	6.33	1.77	6.36	2.12	*0.059*	6.20	2.08	6.26	2.06	6.23	2.15	0.976
4. Section: Diet-disease relationships	10.87	4.46	10.29	4.33	10.29	4.53	*0.255*	10.56	4.55	9.77	4.41	10.37	4.56	0.391
Total	59.57	11.57	58.69	10.72	59.10	10.61	*0.903*	60.46	10.80	56.64	10.02	60.10	11.10	0.014*

nant women who had not diet education, and among the pregnant women who had diet education (*p=0.174*) (Table 1). It was thought that the pregnant women who were given diet education during pregnancy did not benefit from it enough. It was seen that most of the pregnant women had not received adequate nutrition education before or during pregnancy.

Of the pregnant women, 57 (16.86%) were in the first trimester, 65 (19.23%) were in the second, and 216 (63.90%) were in the third trimester. There was no significant difference between the scores obtained from the GNKQ according to the number of pregnant women. One hundred thirteen (33.43%) pregnant women were primiparous, and 225 (66.56%) were multiparous. There were differences between those with a 2nd pregnancy and those with 3 or more pregnancies. The GNKQ total score *(p=0.014),* sub-scores, section 1 (dietary recommendations) *(p=0.048),* section 2 (sources of nutrients) *(p=0.008)* were statistically different for the second and third trimester. As the pregnancy experience increased, it was observed that the women took more care of their diet (Table 2).

Considering the number of living children, 143 (42.30%) pregnant women had no children, 120 (35.50%) were pregnant women with 1 child, and 75 (22.8%) participants had more than 2 children. As the number of surviving children increased, the scores of mothers who were thought to have increased motherhood experience tended to increase in the GNKQ, but the difference was not statistically significant *(p=0.066)* (Table 3).

It was observed that some of the pregnant women participated in our study because of the threat of preterm labor and / or premature membrane rupture. The difference between pregnant women without these symptoms in our study was examined. Thirteen (3.84%) were found to have early membrane rupture (EMR) and preterm history. When evaluated with the GNKQ scale, those with a history of EMR were under investigation and treatment due to the threat of preterm labor. Although it was observed that the GNKQ total score and the sources of nutrients, choosing everyday foods, and diet-disease relationships tended to get lower scores, there was not statistical significance between them (Table 4). In our study, it was seen that patients who were interned in the ward because of gestational hyperten-

Table 3. GNKQ scores according to the number of children

Parameters	Number of living children n (%)								
	0 n=143 (42.30%)		1 n=120 (35.50%)		2 n=59 (17.45%)		≥3 n=16 (4.73%)		*p*
Knowledge section (max score)	Mean±SD		Mean±SD		Mean±SD		Mean±SD		
1. Section: Dietary recommendations	9.54	1.70	9.57	1.75	10.11	1.03	9.81	0.98	0.111
2. Section: Sources of nutrients	33.53	8.10	31.73	8.10	33.79	8.28	33.25	4.85	0.242
3. Section: Choosing everyday foods	6.27	1.92	6.10	2.24	6.32	2.20	6.50	2.16	0.841
4. Section: Diet-disease relationships	10.57	4.52	10.98	4.26	10.98	4.26	9.25	4.21	0.145
Total	59.93	10.25	57.12	11.56	61.22	10.58	10.24	4.51	0.066

Table 4. GNKQ scores according to risk of preterm labor / premature membrane rupture / Preeclampsia / Gestational Hypertension

Parameters	Risk of preterm labor (TPL)/ Premature membrane rupture (PMR)							Preeclampsia /or Eclampsia / or Gestational hypertension (PEG)				
	TPL (-) PMR (-) n=246		TPL (+) PMR (-) n=77		TPL (+) PMR (+) n=13		*p*	PEG (-) n=281		PEG (+) n=57		*p*
Knowledge section (max score)	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
1. Section: Dietary recommendations	9.71	1.61	9.36	1.64	10.38	0.86	*0.061*	9.72	1.56	9.38	1.78	0.145
2. Section: Sources of nutrients	33.31	8.01	32.05	7.99	29.92	8.19	*0.193*	33.65	8.21	29.33	5.95	*<0.001**
3. Section: Choosing everyday foods	6.32	1.99	5.96	2.47	6.07	1.55	*0.410*	6.45	2.02	5.14	2.13	*<0.001**
4. Section: Diet-disease relationships	10.34	4.35	9.84	4.93	9.84	4.91	*0.662*	10.69	4.48	8.03	3.95	*<0.001**
Total	59.74	10.39	57.22	11.91	56.23	8.81	*0.125*	60.56	10.45	51.89	9.37	*<0.001**

**Risk of preterm labor (TPL)/ Premature membrane rupture (PMR)*
** Preeclampsia /or Eclampsia / or Gestational hypertension (PEG)*

Table 5. GNKQ scores and pregnancy outcomes and folate intakes

Parameters	Hospitalization time					Folate Intake (FI) (at least 400 mcg/ a day)				
	> 3 day (n=132)		≤ 3 day (n=206)		*p*	FI - (n=108)		FI + (n=230)		*p*
Knowledge section (max score)	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
1. Section: Dietary recommendations	9.14	1.83	10.00	1.34	*P<0.001**	9.05	1.73	9.95	1.45	*<0.001**
2. Section: Sources of nutrients	30.25	6.21	34.64	8.59	*P<0.001**	28.3	5.43	35.06	8.17	*<0.001**
3. Section: Choosing everyday foods	6.01	2.18	6.37	2.03	*P=0.125*	5.77	2.14	6.44	2.04	*0.006**
4. Section: Diet-disease relationships	8.47	4.07	11.37	4.41	*P<0.001**	7.90	3.93	11.34	4.34	*<0.001**
Total	53.96	8.24	62.40	10.92	*P<0.001**	51.22	6.79	62.80	10.30	*<0.001**

**Statistical significance*

sion, preeclampsia, and eclampsia GNKQ scores were lower than in the other patients *(p<0.001)*.

When the pregnant women were asked as to whether they received regular ≥400 mcg / day folate from the first month of pregnancy, it was observed that those with regular folate intake had higher GNKQ scores *(p<0.001)*. As the level of knowledge in the direction of healthy nutrition increased, the use of folic acid for the protection of fetal health was increased. When pregnant women are evaluated according to the duration of hospitalization, there was a significant difference between GNKQ the total scores of patients who were hospitalized for more than 3 days and those hospitalized for 3 days or less *(p<0.001)* (Table 5).

Some parameters of the sociodemographic characteristics of the pregnant women in our study were exam-

Table 6. GNKQ scores and pregnant women in sociodemographic parameters

Parameters	Profession / Job							Graduation degree						
	Housewife (n=217)		Employee (n=67)		White-collar (n=54)		*p*	Elementary education (n=132)		Secondary education (n=137)		Undergraduate / Graduate (n= 69)		*p*
Knowledge section (max score)	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	
1. Section: Dietary recommendations	9.83	1.40	9.20	2.14	9.57	1.49	*0.018**	9.78	1.33	9.48	1.92	9.81	1.35	0.235
2. Section: Sources of nutrients	32.99	8.01	32.16	7.80	33.61	8.43	*0.604*	30.71	6.77	34.00	8.45	35.02	8.49	*<0.001**
3. Section: Choosing everyday foods	6.17	2.13	6.41	1.93	6.25	2.14	*0.698*	6.19	2.21	6.21	2.07	6.34	1.94	0.879
4. Section: Diet-disease relationships	10.29	4.36	9.13	4.43	11.42	4.91	*0.020**	9.99	4.47	9.67	4.37	11.85	4.52	0.030*
Total	59.29	10.62	57.07	10.18	60.87	11.83	*0.142*	56.68	8.83	59.45	11.69	63.04	11.12	*<0.001**

**Statistical significance*

ined. The number of pregnant women with an undergraduate or above degree was 69 (20.41%); the number of pregnant women in the secondary education range was 137 (40.53%); the number of pregnant women in the primer education was 132 (39.05%). GNKQ total score *(p<0.001)*, diet of disease (section 2) *(p<0.001)* and diet-disease relationships (section 4) *(p=0.030)* subscale scores increased as the range increased. Simply having more education was an important factor regarding high diet knowledge. Having well job was also important factor for well being in dietary recommendations *(p=0.018)* and diet-disease relationships *(p=0.020)* (Table 6).

The pregnant women were divided into three groups according to their education level. As the level of education increased, it was observed that GNKQ scores increased. In this study, it was observed that, especially the third sub-section (diet foods) and fourth sub-section (diet-disease relationships) affected the feeding habits of pregnant women. When the family structure was questioned, pregnant women living in the nuclear family; GNKQ total scores were higher in the extended family (Table 7).

The women were asked about where they learned the information about their nutrition. Only 104 (30.76%) of the pregnant women had received information from health personnel. Most of the media reported that they received information from the media (46.15%). Only the difference between the scores ob-

Table 7. GNKQ scores and husband's profession and family structure for the pregnant women

Parameters	Husbands profession							Family structure for pregnant women				
	Self-employed (n=125)		Employee (n=154)		White-collar (n=59)		*p*	Nuclear family (n=295)		Extended family (n= 43)		*p*
Knowledge section (max score)	Mean±SD		Mean±SD		Mean±SD			Mean±SD		Mean±SD		
1. Section: Dietary recommendations	9.64	1.61	9.57	1.73	9.96	1.14	*0.272*	9.66	1.60	9.69	1.61	0.899
2. Section: Sources of nutrients	32.27	7.54	32.72	8.18	34.84	8.46	*0.116*	33.36	8.17	29.93	6.24	0.009*
3. Section: Choosing everyday foods	5.63	2.11	6.65	1.96	6.40	2.12	*<0.001**	6.20	2.03	6.46	2.49	0.440
4. Section: Diet-disease relationships	9.40	4.30	10.62	4.81	11.05	3.86	*0.025**	10.34	4.52	9.58	4.40	0.302
Total	56.95	10.34	59.64	10.92	62.27	10.46	*0.005**	59.60	11.01	55.67	8.21	0.025*

Table 8. Where the pregnant women heard their diet knowledge before today

Parameters	TV. Radio (n=156)		Health professionals (n=104)		Web pages (n=52)		Book. Journal (n=26)		*p*
Knowledge section (max score)	Mean±SD		Mean±SD		Mean±SD		Mean±SD		
1. Section: Dietary recommendations	9.60	1.55	9.97	1.38	9.57	1.90	9.00	1.89	0.035*
2. Section: Sources of nutrients	33.15	7.74	32.93	8.54	33.00	7.87	*31.42*	8.23	0.793
3. Section: Choosing everyday foods	6.24	2.20	6.20	1.91	6.19	2.21	6.38	2.02	0.981
4. Section: Diet-disease relationships	9.93	4.43	10.74	4.71	10.28	4.46	10.03	4.24	0.565
Total 338	9.66	1.60	32.92	8.03	6.23	2.09	*10.24*	4.51	0.615

**Statical significance*

Table 9. Pregnant women's age, Body Mass Index (BMI), and p scores

Parameters	Mean	Median	SD	Min	Max	*P*				
						1. Section: Dietary recommendations	2. Section: Sources of nutrients	3. Section: Choosing everyday foods	4. Section: Diet-disease relationship	Total
Age	30.09	30.50	5.95	18	41	*0.028*	*0.155*	*0.992*	*<0.001**	*0.001**
BMI	25.77	27.16	8.64	18.24	45.52	*0.661*	*0.141*	*0.926*	*0.955*	*0.235*

**Statical significance*

tained from the dietary recommendations was significant *(p=0.035)* (Table 8).

In our study, the difference between age and GNKQ scores was significant, and as the age increased, the scores of the age group increased and the knowledge and awareness levels of the young women started to reach better levels *(p=0.001)*. When pre-pregnancy BMIs (before pregnancy) were questioned, no significant difference was found between the GNKQ scores, suggesting that women of conception age do not have adequate nutritional education and are unable to regulate their lives (Table 9).

Discussion

Considering that diet behavior is very complex, attempts to understand this in terms of nutritional knowledge in pregnant women should start with a clear understanding of awareness. Pregnant women tended to focus on a particular area of knowledge, such as fat or cholesterol. Furthermore, they had a wide range of information, but the correct information was not on a systematic basis. These criteria provide a different perspective to research results in pregnant women by looking at the information-behavior relationship in the field of nutrition.

Studies have shown that having good nutrition knowledge and showing a positive attitude with a healthy diet does not always result in high diet quality (8-10). Our study also found that pregnant women were well fed and received nutritional education. However, when evaluated using the GNKQ, they were not able to achieve high scores. In order for future generations to be healthy, systematic training should be planned to increase the knowledge and awareness levels of pregnant women about diet. Due to potential health risks, it is important that women comply with the principles of healthy nutrition during pregnancy. Unfortunately, although women reported positive changes in lifestyles during pregnancy, it was shown that their dietary intake and knowledge did not meet the recommended food and nutrient intake for pregnancy.

The highest scores were achieved in the first section of the GNKQ (dietary recommendations) in previous studies (9,10). In our study, it was also observed that the pregnant women obtained the highest score in the first part. They answered most commonly; 'Which of these breads contain the most vitamins and minerals? The women in our study results most commonly answered: 'wholegrain.'

In the literature, studies on the intake of fruits, vegetables, iron, and sodium found that the participants had sufficient knowledge (11, 12). In our study, pregnant women stated that they had increased the intake of fruits, vegetables, and iron for healthy eating habits, and that they had reduced the intake of fat and salt. However, their knowledge of meat consumption, vitamin D intake, and glucose consumption was not sufficient. The findings suggest that these nutrients should be targeted in future interventions in this population. Nutritional information is among the determinants that increase the quality of diet (12-14). Positive attitudes towards healthy nutrition are constantly associated with high dietary quality. In our study, diet quality was higher in pregnant women with good nutritional information. The family may have a positive effect on eating habits with regard to developing healthy dietary habits among women in societies.

Pregnancy is a life event that triggers a long-term review of nutritional problems. Factors that can follow the trimesters in pregnancy and affect the nutritional awareness among these passages were evaluated in previous studies. However, the practical or clinical effects of differences in nutritional awareness have not been established (15-17). In addition, a recent Australian study showed that diet quality decreased as pregnancy progressed (18). In our study, the nutrition information level did not increase with the pregnancy trimester. The GNKQ scores tended to decrease as the pregnancy progressed. Besides, in our study, it was evaluated according to the number of pregnancies between pregnant women. Although there was a statistically significant difference between the GNKQ total scores *(p=0.014)*, it has not been determined that there was a practical and clinical effect.

It is important for health professionals to realize that pregnancy is one of the unique opportunities for women to be informed about nutrition. This increased awareness can benefit maternal, fetal, and baby health. It can also have positive consequences for postnatal

nutrition because the gains acquired during pregnancy are of great importance for families and society.

Vitamins and minerals are essential for healthy functioning of the body, fetal growth, and development (19). Preterm action, small for gestational age (SGA), low birth weight (LBW), premature rupture of membranes, and risk of preeclampsia increase in nutritional insufficiencies. Insufficient nutritional knowledge, low zinc intake, iron deficiency, and LBW are associated with premature rupture of membranes (20, 21). Especially in middle- and low-income countries, women have poor nutritional knowledge, and lack of nutritional deficiencies for ongoing health during pregnancy. However, multiple-micronutritient supplementation (MMN) is not required, and nutritional information should be encouraged to increase the nutritional level of pregnant women (21-23). In our study, GNKQ scores were lower among pregnant women at risk for preterm labor and those with EMR than in those without these problems. Preeclampsia, gestational hypertension, followed by eclampsia and those who continued treatments had lower GNKQ scores for nutritional knowledge than healthy pregnant women In order to be able to correct prenatal outcomes, adequate training should be provided and diet regulation should be implemented.

Studies have shown that folic acid information is limited in pregnant women. Most women in previous studies were aware of the need to take a folic acid supplements to prevent fetal spina bifida. However, less than half knew what dose and when to take folic acid (24, 25). In our study, there was a lack of awareness of the need to take folic acid and information about how often the women would be checked. In addition, folic acid replacement was also shown to be negligible even in those with high knowledge levels in the GNKQ. One benefit of raising nutritional awareness is the increased use of folic acid to prevent fetal neural tube defects in pregnant women.

In many studies, it is stated that the education level of participants has a significant effect on GNKQ scores (26, 27). Our study showed that women with high education levels had more nutritional information and healthy nutrition efforts *($p<0.001$)*. At the same time, the scores of working women tended to be higher than those of non-working women. This seems to be both a logical and consistent finding.

In the literature, it was found that the level of quality nutrition of the pregnant women with low socioeconomic level was low (27, 28). In our study, GNKQ scores of women with high family income were higher *($p=0.005$)*. The family structure of a husband, wife, and children living in a nuclear family achieved higher scores *($p=0.025$)*.

Recent studies showed that nutrition education was insufficient in pregnancy. Therefore, increasing awareness and understanding of dietary rules can be an important step in increasing women's ability to assess diet quality against a 'healthy, balanced diet' as defined by the Australian Dietary Guidelies (ADG). This can then force women to make positive nutritional changes. Previous research suggests that primary healthcare providers during pregnancy may be the most appropriate to provide this information (28-30). However, it may require additional resources such as time and training. The effectiveness of such strategies among women who believe that their diet quality is low but their diet is healthy can be affected by their desire to increase nutritional information and a healthy diet. Our study shows that diet changes are minimal. In general, although almost two-thirds of women are reported to make dietary changes especially for pregnancy, the degree to which diet quality changes before pregnancy cannot be determined from the available data. Furthermore, in our study, the number of women whose knowledge came from media such as television and radio was high, and the scores were moving dramatically towards better levels.

The findings of this study show that there is a need for greater emphasis on nutritional counseling and education in order to optimize the quality of nutritional habits of pregnant women. It was observed that pregnant women had limited nutritional knowledge, which could lead to less nutritional intake. Primary healthcare providers, family physicians, general practitioners, obstetricians, nurses, and midwives are in a position to reach the community to provide nutritional information. Diet quality was poor in the pregnant women in our study.

Nutritional knowledge and attitudes were found to be important determinants of diet quality. It has been shown that nutrition interventions that encour-

age healthy eating habits in pregnant women should focus on developing the determinants of diet behavior. Training and forms should be prepared and put into practical use for pregnant women. In order to contribute to the improvement of fetal and maternal health, it is seen that nutrition education should be given importance in pregnant women.

Limitations

Nutritional education should be given to women during pregnancy and in the preconception period. However, in our study, it was not possible to provide this training. Furthermore, more studies are needed to facilitate access to healthy food sources and to increase their use. Finally, the majority of women in this study were aware of some areas of dietary recommendations; however, the responses of pregnant women to the scale showed that they had limited knowledge about the rules of diet for healthy nutrition during pregnancy. Although the women reported positive changes in their eating habits, they did not have enough diet information to meet their nutritional requirements during pregnancy.

Conclusion

Our findings show that evaluating pregnant women with the GNKQ and providing nutritional education will be beneficial on pregnancy outcomes. The use of iron, folic acid, and nutritional education in low- and middle-income populations should be encouraged to improve preterm labor, low birth weight, systemic arterial hypertension, and possibly reduce premature births.

This study showed that pregnant women had limited knowledge about balanced nutrition rules. Nutritional guidelines and nutritional counseling provided in practice were not clear enough. Objective-oriented strategies should be developed in order to increase the awareness about the rules of quality nutrition in pregnancy. The findings also emphasize that a small number of women receive nutritional advice as part of their pregnancy care. It is necessary to increase effective nutrition programs and campaigns for pregnant women. Babies should be provided with a healthy start to life and routine nutrition counseling should be promoted as part of pregnancy care.

Implications for research

Based on the results from our subgroup analysis, further research can be conducted. Studies should be performed to better understand the basic nutritional status of pregnant women and how they can affect pregnancy and birth outcomes after increasing nutritional knowledge. In addition, determination of the most appropriate formulation for vitamin and mineral supplements such as folic acid, iron, and zinc may have practical implications. Vitamin, mineral supplementation, and nutritional education supplementation will be useful to better understand maternal and fetal health.

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References

1. Langley-Evans S. Nutrition in early life and the programming of adult disease: a review. Journal of Human Nutrition and Dietetics. 2015;28:1-14.
2. Englund-Ögge L, Brantsæter AL, Sengpiel V, Haugen M, Birgisdottir BE, Myhre R, et al. Maternal dietary patterns and preterm delivery: results from large prospective cohort study. Bmj. 2014;348:g1446.
3. Martin JC, Savige GS, Mitchell EK. Health knowledge and iodine intake in pregnancy. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2014;54(4):312-6.
4. De-Regil LM, Peña-Rosas JP, Fernández-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database of Systematic Reviews. 2015(12).
5. Hamilton EAA, Nowell AK, Harden A, Thangaratinam S. Conduct and reporting of acceptability, attitudes, beliefs and experiences of pregnant women in randomised trials on diet and lifestyle interventions: A systematic review. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2018;225:243-54.
6. Parmenter K, Wardle J. Development of a general nutrition knowledge questionnaire for adults. European journal of clinical nutrition. 1999;53(4):298.
7. Trakman GL, Forsyth A, Hoye R, Belski R. Developing and validating a nutrition knowledge questionnaire: Key methods and considerations. Public health nutrition. 2017;20(15):2670-9.

8. Jones AM, Lamp C, Neelon M, Nicholson Y, Schneider C, Swanson PW, et al. Reliability and validity of nutrition knowledge questionnaire for adults. Journal of nutrition education and behavior. 2015;47(1):69-74.
9. Spronk I, Kullen C, Burdon C, O'Connor H. Relationship between nutrition knowledge and dietary intake. British Journal of Nutrition. 2014;111(10):1713-26.
10. Cooke R, Papadaki A. Nutrition label use mediates the positive relationship between nutrition knowledge and attitudes towards healthy eating with dietary quality among university students in the UK. Appetite. 2014;83:297-303.
11. Variyam JN. Do nutrition labels improve dietary outcomes? Health Economics. 2008;17(6):695-708.
12. Kim S, Douthitt RA. The role of dietary information in women's whole milk and low-fat milk intakes. International Journal of Consumer Studies. 2004;28(3):245-54.
13. Biltoft-Jensen A, Groth MV, Matthiessen J, Wachmann H, Christensen T, Fagt S. Diet quality: associations with health messages included in the Danish Dietary Guidelines 2005, personal attitudes and social factors. Public health nutrition. 2009;12(8):1165-73.
14. Graham DJ, Laska MN. Nutrition label use partially mediates the relationship between attitude toward healthy eating and overall dietary quality among college students. Journal of the Academy of Nutrition and Dietetics. 2012;112(3):414-8.
15. Vahidinia A, Hazavehei SMM, Karimi-Shahanjarini A, Poorolajal J, Erfani H, Entezari MH, et al. Nutrient intake and unhealthy dietary pattern of Iranian women: a cross sectional study. Progress in Nutrition. 2018;20(2-S):106-18.
16. Szwajcer E, Hiddink G, Maas L, Koelen M, van Woerkum C. Nutrition awareness before and throughout different trimesters in pregnancy: a quantitative study among Dutch women. Family practice. 2012;29(suppl_1):i82-i8.
17. Szwajcer EM, Hiddink GJ, Koelen M, Van Woerkum C. Nutrition-related information-seeking behaviours before and throughout the course of pregnancy: consequences for nutrition communication. European journal of clinical nutrition. 2005;59(S1):S57.
18. Moran L, Sui Z, Cramp C, Dodd J. A decrease in diet quality occurs during pregnancy in overweight and obese women which is maintained post-partum. International journal of obesity. 2013;37(5):704.
19. Haider BA, Bhutta ZA. Multiple-micronutrient supplementation for women during pregnancy. Cochrane Database of Systematic Reviews. 2017(4).
20. Terrin G, Berni Canani R, Di Chiara M, Pietravalle A, Aleandri V, Conte F, et al. Zinc in early life: a key element in the fetus and preterm neonate. Nutrients. 2015;7(12):10427-46.
21. Zhou C, Zhang R, Cai X, Xiao R, Yu H. Trace elements profiles of maternal blood, umbilical cord blood, and placenta in Beijing, China. The Journal of Maternal-Fetal & Neonatal Medicine. 2017:1-7.
22. Imdad A, Bhutta ZA. Routine iron/folate supplementation during pregnancy: effect on maternal anaemia and birth outcomes. Paediatric and perinatal epidemiology. 2012;26:168-77.
23. Smith LK, Blondel B, Van Reempts P, Draper ES, Manktelow BN, Barros H, et al. Variability in the management and outcomes of extremely preterm births across five European countries: a population-based cohort study. Archives of Disease in Childhood-Fetal and Neonatal Edition. 2017;102(5):F400-F8.
24. Lee A, Belski R, Radcliffe J, Newton M. What do pregnant women know about the healthy eating guidelines for pregnancy? A web-based questionnaire. Maternal and child health journal. 2016;20(10):2179-88.
25. Harrison A, Pentieva K, Ozaki M, McNulty H, Parle-McDermott A. Assessment of candidate folate sensitive-differentially methylated regions in a randomised controlled trial of continued folic acid supplementation during the second and third trimesters of pregnancy. Annals of human genetics. 2019;83(1):23-33.
26. Kullen CJ, Iredale L, Prvan T, O'Connor HT. Evaluation of general nutrition knowledge in Australian military personnel. Journal of the Academy of Nutrition and Dietetics. 2016;116(2):251-8.
27. Obayashi S, Bianchi LJ, Song WO. Reliability and validity of nutrition knowledge, social-psychological factors, and food label use scales from the 1995 Diet and Health Knowledge Survey. Journal of nutrition education and behavior. 2003;35(2):83-92.
28. Ledoux T, Van Den Berg P, Leung P, Berens PD. Factors associated with knowledge of personal gestational weight gain recommendations. BMC research notes. 2015;8(1):349.
29. Lucas C, Charlton KE, Yeatman H. Nutrition advice during pregnancy: do women receive it and can health professionals provide it? Maternal and child health journal. 2014;18(10):2465-78.
30. Malek L, Umberger W, Makrides M, Zhou SJ. Adherence to the Australian dietary guidelines during pregnancy: evidence from a national study. Public health nutrition. 2016;19(7):1155-63.

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Original article

Evaluation of Gastroesophageal Reflux Disease and variables related with its severity in adults

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Summary. Gastroesophageal Reflux Disease is a chronic gastrointestinal disease that significantly reduces the quality of life and causes serious complications such as esophageal stricture, gastrointestinal bleeding or Barrett's esophagus in some patients. This study was undertaken to evaluate some factors that are considered to be related to Gastroesophageal Reflux Disease and severity among individuals who are 40 years of age and over. This study is a cross-sectional survey conducted with individuals who were at the ages of 40 years and over who applied to 6 Family Health Centers and Family Health Centers serving in the districts of Alpu and Mahmudiye located in 3 settlements with different socioeconomic levels in Eskişehir City Center between 01 November and 30 December 2016. During the study, Individuals with symptoms of retrosternal burning and regurgitation at least 1 day a week was accepted as Gastroesophageal reflux disease in the study and the severity of the disease was assessed with the National Institutes of Health Promis Gerd Scale. Stop Bang Scale was used in assessing the risk level of obstructive sleep apnea syndrome. Among the study sample; 920 individuals were (46.8%) male and 1046 (53.2%) were female. Their ages ranged from 40 to 80 years with a mean of 56.8 ± 10.6 years. Gastroesophageal Reflux Disease was found in 18.3% (n = 360) of the study group. In our study, female gender, moderate family income status, non-steroidal antiinflammatory drug use, family history of gastroesophageal reflux disease, previous gastroesophageal reflux disease, consumption of carbonated beverage, consumption of fatty food, consumption of fermented food, presence of chronic obstructive pulmonary disease story, asthma story presence and having high risk in terms of obstructive sleep apnea syndrome were found to be important risk factors for gastroesophageal reflux disease(each; $p \leq 0.05$). Drug therapy was the most common treatment method among individuals who previously had Gastroesophageal Reflux Disease diagnosis (76.6%). In the study group; the most common Gastroesophageal Reflux Disease severity was "most symptomatic" in individuals with high risk of Obstructive Sleep Apnea Syndrome (60.6%). In this study, it was determined that Gastroesophageal Reflux Disease is an important health problem in adults. In terms of preventing gastroesophageal reflux disease and risk factors; screening should be made and health education and information services should be provided to increase the level of awareness.

Key words: Gastroesophageal Reflux Disease, over 40 years of age, Eskişehir

Introduction

Gastroesophageal Reflux Disease (GERD) is defined as the escape of stomach contents to the esophagus, causing symptoms and/or complications that disturb the person (1). Although the cause of GERD is not fully understood, genetic factors are thought to be more forward than environmental factors (2). Important risk factors for GERD include lifestyle factors such as smoking, chocolate, consumption of spicy

foods, consumption of cigarettes and alcohol, obesity, and the use of drugs such as aspirin and nonsteroidal antiinflammatory drugs (3-6).

In the physiopathology of GERD, a variety of theories such as transient lower esophageal sphincter loosening, reduced resting lower esophageal sphincter pressure, impaired esophageal acid clearance, delayed gastric emptying, and defects in esophageal epithelial preservation are suggested and the most important one among these is transient lower esophageal sphincter loosening (7, 8). Typical symptoms of GERD include pyrosis (retrosternal burning, heartburn) and regurgitation (9, 10). However, dysphagia, odynophagia, angina-like chest pain, globus sensation, belching and chronic cough can also be seen (4).

GERD diagnosis is based on anamnesis, and invasive techniques are used in the presence of atypical symptoms or for the assessment of complications (7). Clinical and laboratory methods or some scales are used to assess the severity of GERD, including the National Institutes of Health (NIH) PROMIS GERD scale (4, 11). It has been reported that the frequency of GERD is 6.2-31.3% in some studies among adults in various countries (12-14). In Turkey, this frequency ranges from 19.3% to 33.9% (15-17).

GERD and non-malignant complications such as esophageal erosion, ulcer, bleeding, perforation, stricture rarely cause death (18). Barrett's esophagus, a complication of GERD, causes adenocancer to cause death but this condition is not very often (19). GERD leads to significant economic losses as it affects health-related quality of life in a negative way, resulting in symptoms and inability to go to work, decreased productivity and reduced daily activities (20-23). In a study conducted by Zhao and colleagues in the USA, the total national hospital costs for hospitalization of individuals with GERD were reported to have risen from $ 509 million in 1998 to $ 622 million in 2005 (24).

One of the accompanying diseases of GERD is Obstructive Sleep Apnea Syndrome (OSAS); which is a disease characterized by intermittent partial or complete occlusion of the upper respiratory tract during sleep that causes decreased blood oxygen saturation (25, 26). It is suggested that in patients with OSAS, the increase in negative intrathoracic pressure during the apneic episode caused GERD by creating a vacuum effect on the gastric content (27).

This study aimed to evaluate some factors that are considered to be related to GERD presence and severity among individuals who were 40 years of age and over.

Materials and Methods

This study is a cross-sectional survey conducted with individuals who were at the ages of 40 years and over who applied to 6 Family Health Centers and Family Health Centers serving in the districts of Alpu and Mahmudiye located in 3 settlements with different socioeconomic levels in Eskişehir City Center between 01 November and 30 December 2016.

Eskisehir province is in the 7th place in terms of socio-economic development order. Livelihood resources are based on industry in the province center and agriculture in the districts (28). According to the data of the Turkish Statistical Institute (TUIK) 2015; the total population of Eskişehir is 826,716 and the population over age 40 is 351,532 (29). Eskişehir Osmangazi University Non-Interventional Clinical Research Ethics Board approved this study with decision numbered as 80558721 / G-268 and dated as 18 October 2016. In order to collect data, necessary permissions were obtained from Eskişehir Provincial Public Health Directorate and Family Health Centers.

There are 52 Family Health Centers (FHC) in the center of Eskişehir.A total of 8 FHC including 6 FHC in 3 settlements with different socioeconomic levels and 1 FHC in Alpu and 1 FHC in Mahmudiye were included in the study according to Eskisehir Provincial Public Health Directorate's opinion. The prevalence of gastroesophageal reflux disease was estimated to be 20%, the error margin was 5%, the confidence interval was 95%, the minimum number of people to be reached for each FHC was 245 and the total number of people was calculated as 1960 so that the reliability of the results could be accepted.

A questionnaire form was prepared using the appropriate literature in accordance with the purpose of the study, (30-34). The questionnaire contains some sociodemographic characteristics of the individuals,

some factors that are thought to be related to GERD, questions about the NIH PROMIS GERD Scale and STOP-BANG Scale.

After informing individuals aged 40 years and over who applied to FHC during the study period about the subject and the purpose of study; 1966 people who agreed to participate in the study formed the working group. Pre-prepared questionnaires were filled in by face-to-face interviewers. This process took approximately 20-25 minutes.

Patients with burning symptoms in the retrosternal region for at least 1 day in the last week were identified as retrosternal burning positive and patients with regurtitation of foods and drinks without vomiting were defined as regurtitation positive. Subjects with symptoms of retrosternal burning and regurgitation at least 1 week in the study were accepted as GERD (34, 35) and the NIH Promis Gerd Scale was used to assess severity. This scale was developed by the National Institutes of Health (NIH) in 2014 (30, 36) and the validity and reliability study in Turkey was conducted by Özşeker et al.. The scale consists of 13 questions with 5 likert types and the scores from each question range from 0-4. Scores to be taken from this scale ranged from 0 to 52, with 16 and over points were the most symptomatic, between 8-15 points were moderate symptomatic, between 4-7 points were mild symptomatic, between 1-3 points were the least symptomatic and 0 point was considered as asymptomatic (31).

Stop Bang scale was used to assess OSAS risk level. The Stop-bang scale was developed by Chung et al. in 2008 and the validity and reliability study in Turkey was conducted by Acar et al. in 2013. The scale consists of a total of eight questions whose answers were yes-no. Those who gave "yes" to three more questions were considered as high risky for OSAS and those who gave two or less "yes" responses were considered as low risky for OSAS (32, 37).

In this study, family income status was evaluated as "poor, moderate and good" according to their own perceptions.

The data were evaluated in the SPSS (version 15.0) Statistical Package Program. Chi-square test and Logistic Regression Analysis (Stepwise Backward Wald Regression) were used for the analyzes. Statistical significance was accepted as $p \leq 0.05$.

Results

Among the study sample; 920 individuals were (46.8%) male and 1046 (53.2%) were female. Their ages ranged from 40 to 80 years with a mean of 56.8±10.6 years. Gastroesophageal Reflux Disease was found in 18.3% (n=360) of the study group. The distribution of the study group members according to some sociodemographic characteristics is given in Table 1.

Results of Logistic Regression Analysis; which were created with variables that were detected to be associated with GERD in Chi-Square analysis such as place of residence, gender, education status, family income status, regular exercise, non steroidal anti-inflammatory drug (NSAID) use, presence of a disease that requires continuous drug use, GERD diagnosis in

Table 1. Distribution of the study group according to sociodemographic characteristics

Sociodemographic characteristics	Number (n)	Percentage (%)
Place of residence		
Provincial center	1334	67.9
District	632	32.1
Age group		
40-49	559	28.4
50-64	904	46.0
Over 65 years	503	25.6
Gender		
Male	920	46.8
Female	1046	53.2
Education status		
Under primary education	1182	60.1
Primary school graduate	231	11.8
High school and above	553	28.1
Personality type		
A	950	48.3
B	1016	51.7
Family type		
Nuclear family	1548	78.7
Extended family	365	18.6
Fragmented family	53	2.7
Family income		
Poor	231	11.7
Moderate	1391	70.8
Good	344	17.5
Total	1966	100.0

family, previous GERD diagnosis, obesity, eating how many hours before bed time, spicy, fatty, salty, fermented food consumption, consumption of carbonated beverages, Diabetes Mellitus, Hypertension, Chronic Obstructive Pulmonary Disease (COPD), Asthma, OSAS risk status; were shown in Table 2.

Female gender, moderate family income status, NSAID use, family history of GERD, previous GERD, consuming carbonated beverages, consumption of fatty foods, consumption of fermented foods, presence of COPD history, presence of asthma, and having high risk in terms of OSAS; were all found as important risk factors for GERD in our study according to Logistic Regression Analysis result (each; $p \leq 0.05$).

Of the study group, 474 (24.1%) individuals had previously diagnosed Gastroesophageal Reflux Disease and 417 (88%) of them had received any treatment due to Gastroesophageal Reflux Disease. Drug therapy was the most common treatment method among individuals who previously had Gastroesophageal Reflux Disease diagnosis (76.6%). The distribution of the individuals with GERD diagnosis in study group according to treatment methods was given in Table 3.

Table 2. Results of the Logistic Regression Model, which were formed by variables determined to be related to GERD in the study group (final step)

Variables	ß	SE[a]	p	OR[b]	95% CI[c]
Gender (reference: Male)					
Female	0.463	0.144	0.001	1.589	1.199-2.105
Family income (reference: good)					
Moderate	0.450	0.195	0.021	1.568	1.071-2.295
Poor	0.291	0.258	0.260	1.338	0.806-2.221
Use of NSAID (reference: No)					
Yes	0.404	0.134	0.002	1.498	1.153-1.946
Family history of GERD (reference: No)					
Yes	0.489	0.137	0.000	1.631	1.246-2.135
Previous GERD diagnosis (reference: No)					
Yes	1.747	0.136	0.000	5.735	4.396-7.483
Consumption of carbonated beverages (reference: No)					
Yes	0.401	0.153	0.009	1.494	1.106-2.018
Consumption of fatty foods (reference: No)					
Yes	0.714	0.137	0.000	2.043	1.562-2.673
Consumption of fermented foods (reference: No)					
Yes	0.294	0.149	0.048	1.342	1.002-1.796
Diabetes Mellitus (reference: No)					
Yes	0.285	0.156	0.068	1.330	0.979-1.807
COPD (reference: No)					
Yes	0.707	0.329	0.032	2.028	1.064-3.865
Asthma (reference: No)					
Yes	0.589	0.211	0.005	1.802	1.191-2.724
OSAS risk status (reference: Low)					
High	0.545	0.173	0.002	1.725	1.228-2.422
Constant	-4.302	0.297	0.000	-	-

SE[a]: Standard error, OR[b]: Odd's ratio, CI: Confidence interval

Table 3. Treatment methods applied by individuals who already had GERD diagnosis in the study group

Treatment methods	Number (Percentage)
No treatment	57 (12.0)
Diet treatment	69 (14.6)
Medication	363 (76.6)
Other	4 (0.8)
Total	493 (100.0)

**Numbers were evaluated through treatment methods.*

Table 4. Distribution of GERD according to severity in individuals with high OSAS risk in the study group

GERD severity in individuals with high OSAS risk	Number (Percentage)
Mild symptomatic	14 (4.8)
Moderate symptomatic	101 (34.6)
Most symptomatic	177 (60.6)
Total	292 (100.0)

In the study group; OSAS was found to be high risky in 73% (n = 1440) and low risky in 27% (n = 526). Among the study group; the most common GERD severity was "most symptomatic" in individuals with high risk of OSAS (60.6%). Distribution of GERD according to severity in individuals with high OSAS risk in the study group was given in Table 4.

Discussion

GERD is a chronic gastrointestinal disease that significantly reduces the quality of life and causes serious complications such as esophageal stricture, gastrointestinal bleeding or Barrett's esophagus in some patients (38). The prevalence of GERD in this study was detected as 18.3%. It has been reported in some studies among adults in various countries that the frequency of GERD varies between 6.2-31.3% (12-14). In Turkey, this frequency varies between 19.3% - 33.9% (15-17). The lack of standardization of the methods used to diagnose GERD in these studies can be shown among the reasons for the different results reported.

In our study, the frequency of GERD in women was 1.589 times higher than that of men. Similar results have been reported in a study conducted by Shaha and his colleagues in Bangladesh (39). Whereas Kay and his colleagues reported that the frequency of GERD in men was higher than in women (40) and on the other handin Locke et al.'s study in Minnesota, there is no difference in the frequency of GERD between men and women (41).

In our study, the GERD frequency was higher in families with moderate income when compared with families that had good income (OR=1.568; p≤0.05). In a study conducted in Israel, it was reported that the frequency of GERD was high in families with low income (42). In a study conducted by Yönem and colleagues, it was reported that there was no relationship between family income status and GERD (15). Such an outcome may have occurred because individuals in the study assessed family income according to their own perceptions.

NSAID use is an important risk factor for GERD symptoms because it increases the duration of acid reflux (43). In our study, GERD frequency was found to be higher in people using NSAID (OR=1.498; p≤0.05). Similar results were reported in a study conducted by Martín-de-Argila et al. (44). However, in a study conducted by Bor et al. in Moscow, it was reported that there was no difference between GERD with use of NSAID (13).

It is known that genetic contribution is important in the etiology of GERD. The presence of upper gastrointestinal disease in the family is a risk factor for GERD (45). In the study group, GERD was found to be 1.631 times higher in those who had a diagnosis family history of GERD than those without family history of GERD. Similar results have been reported in a study by Rabieeve et al., in Iran (46).

Several factors play a role in the pathogenesis of GERD. According to recent studies, genetic polymorphism in genes affecting the host's inflammatory response, drug metabolism, cell cycle regulation, xenobiotic pathways, DNA repair, mutagenesis, esophageal sensory function and gene silencing are associated with GERD risk (47). In the logistic regression analysis; It

was found that the frequency of GERD was found to be 5,735 times higher in those who previously had GERD diagnosis history. In a multi-centered work by Kulig and his colleagues; duration of GERD was reported among the main factors related to the occurence of GERD (48). The most important reason for the high rate in our study can be uneffective and unsufficient treatment of GERD.

Carbonated drinks; increases gastric acid load and increases GERD probability (49). In this study, consumption of carbonated drinks was found to be a risk factor for GERD (OR=1.494; p≤0.05). Similar results have been reported in a study by Fass et al. (50). In a study conducted by Darvishmoghadam and his colleagues in Iran, it was reported that there was no difference in the frequency of GERD among those who consumed and did not consume carbonated beverages (51).

Fatty food consumption reduces the lower esophageal sphincter pressure, increases the frequency of GERD by delaying gastric emptying and extending esophageal acid exposure duration (52). Frequency of GERD in those who consume fatty foods were 2.043 times higher than those who did not consume. Similar results have been reported in the study of El-Serag et al. (53). In a study conducted by Mansour-Ghanaei et al., no relation was found between fatty food consumption and GERD prevalence (54).

Fermented foods (sour products) and drinks (alcohol) cause reflux by reducing the lower esophageal sphincter pressure, delaying gastric emptying, stimulating the sensory receptors in the esophagus and increasing gastric acid secretion (38). In the study group, consumption of fermented food was found to be an important risk factor for GERD (OR=1.342; p≤0.05). In a study conducted by Song and colleagues in Korea, it was reported that the frequency of GERD was higher in those who consume fermented food (breads) (55). However, Mansour-Ghanaei and colleagues reported that there was no difference in the frequency of GERD among those who consumed and did not consume fermented drink (drinking dough-yogurt with water) (54).

In patients with COPD, diaphragm flattening with increased respiratory distress, intra-abdominal pressure increase, and negative intrathoracic pressure may facilitate reflux of gastric contents (56). In our study, the frequency of GERD in patients with COPD was found to be 2.028 times higher. A similar result was reported in a study by Mokhlesi et al. (57). In a study by Çil and colleagues, GERD was reported to be not a risk factor for patients with COPD in the acute exacerbation and respiratory insufficiency situation (58).

In asthmatic patients; the reduction of the diaphragm in pulmonary hyperinflation may cause the lower esophageal sphincter to hernify into the chest by increasing the pressure difference between the abdomen and chest. For this reason, it is expected that the frequency of GERD in asthmatic patients is higher (59). It was found that GERD frequency was higher in asthmatic patients in the study group (OR=1.802; p≤0.05). A similar result was reported in a study conducted by Yönem et al. (15). In a study conducted by Almadi and colleagues, it was reported that there was no difference in the frequency of GERD between those with asthma stories and those without asthma (60).

It is possible that the GERD frequency is higher due to the vacuum-like effect of the intrathoracic negative pressure increasing in the upper airway obstruction in OSAS and the tension of the phrenoesophageal ligament (61). We found that GERD frequency was higher in those who were at high OSAS risk status when compared with those who were not at high OSAS risk status (OR=1.725; p≤0.05). The results of some studies in the literature support our work (62, 63). However, a study by Kim and his colleagues found no relation between GERD symptoms and OSAS (64).

Conclusions

In this study, it was determined that Gastroesophageal Reflux Disease is an important health problem in adults. In terms of preventing gastroesophageal reflux disease and risk factors; screening should be made and health education and information services should be provided to increase the level of awareness. There is a need for more extensive work in order to establish the relationship between Gastroesophageal Reflux Disease and Obstructive Sleep Apnea Syndrome.

References

1. Vakil N, Van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. The American journal of gastroenterology 2006; 101(8): 1900-20.
2. Cameron AJ, Lagergren J, Henriksson C, Nyren O, Locke GR, Pedersen NL. Gastroesophageal reflux disease in monozygotic and dizygotic twins. Gastroenterology 2002; 122(1): 55-9.
3. Scott V. Gastroesophageal reflux disease: diagnosis and management. Journal of the Association for Academic Minority Physicians: the official publication of the Association for Academic Minority Physicians 1999; 11(1): 12-4.
4. Hawkey C, Bosch J, Richter JE, Garcia-Tsao G. Textbook of clinical gastroenterology and hepatology: John Wiley & Sons; 2012.
5. Vossoughinia H, Salari M, Amirmajdi EM, Saadatnia H, Abedini S, Shariati A, et al. An Epidemiological Study of Gastroesophageal Reflux Disease and Related Risk Factors in Urban Population of Mashhad, Iran. Iranian Red Crescent Medical Journal 2014; 16(12).
6. Asl SF, Mansour-Ghanaei F, Samadi H, Joukar F. Evaluations of life style factors and the severity of Gastroesophageal reflux disease; a case-control study. International journal of molecular epidemiology and genetics 2015; 6(1): 27.
7. Scott L, Friedman KRM, James H. Grendell. Lange Current Gastroenteroloji Tanı ve Tedavi2007.
8. Dodds WJ, Dent J, Hogan WJ, Helm JF, Hauser R, Patel GK, et al. Mechanisms of gastroesophageal reflux in patients with reflux esophagitis. New England Journal of Medicine 1982; 307(25): 1547-52.
9. Locke Gr, Talley NJ, Fett SL, Zinsmeister AR, Melton Lr. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. Gastroenterology 1997; 112(5): 1448-56.
10. Klauser A, Schindlbeck N, Müller-Lissner S. Symptoms in gastro-oesophageal reflux disease. The Lancet 1990; 335(8683): 205-8.
11. Özseker B, Yasar NF, Bilgin M, Kurt Y, Balcioglu H, Bilge U. Turkish validation of National Institutes of Health (NIH) patient-reported outcomes measurement information system (PROMIS®) Gastroesophageal Reflux Disease (GERD) scale. Biomedical Research. 2016.
12. Wang R, Yan X, Ma X-Q, Cao Y, Wallander M-A, Johansson S, et al. Burden of gastroesophageal reflux disease in Shanghai, China. Digestive and Liver Disease 2009; 41(2): 110-5.
13. Bor S, Lazebnik L, Kitapcioglu G, Manannikof I, Vasiliev Y. Prevalence of gastroesophageal reflux disease in Moscow. Diseases of the Esophagus 2016; 29(2): 159-65.
14. Bretagne J-F, Richard-Molard B, Honnorat C, Caekaert A, Barthélemy P. Le reflux gastro-œsophagien dans la population générale française: Résultats d'une enquête sur 8 000 sujets adultes. La presse médicale 2006; 35(1): 23-31.
15. Yönem Ö, Sivri B, Özdemir L, Nadir I, Yüksel S, Uygun Y. Gastroesophageal reflux disease prevalence in the city of Sivas. The Turkish journal of gastroenterology: the official journal of Turkish Society of Gastroenterology 2012; 24(4): 303-10.
16. Bor S, Mandiracioglu A, Kitapcioglu G, Caymaz-Bor C, Gilbert RJ. Gastroesophageal reflux disease in a low-income region in Turkey. The American journal of gastroenterology 2005; 100(4): 759-65.
17. Köksal AŞ, Dilek O, Özden A. Türkiye'de birinci basamak sağlık kurumlarına başvuran hastalarda dispepsi görülme sıklığı. Akademik Gastroenteroloji Dergisi 2008; 7(1).
18. Becher A, El-Serag HB. Mortality associated with gastroesophageal reflux disease and its non-malignant complications: a systematic review. Scandinavian journal of gastroenterology 2008; 43(6): 645-53.
19. Van der Burgh A, Dees J, Hop W, Van Blankenstein M. Oesophageal cancer is an uncommon cause of death in patients with Barrett's oesophagus. Gut 1996; 39(1): 5-8.
20. Eloubeidi MA, Provenzale D. Health-related quality of life and severity of symptoms in patients with Barrett's esophagus and gastroesophageal reflux disease patients without Barrett's esophagus. The American journal of gastroenterology 2000; 95(8): 1881-7.
21. Yürekli ÖT, Ersoy O. Gastroözofageal Reflü Hastalığı; Tanım, Klinik Özellikler, Epidemiyoloji ve Doğal Seyir. Turkiye Klinikleri Journal of Gastroenterohepatology Special Topics 2012; 5(2): 1-9.
22. Revicki DA, Wood M, Maton PN, Sorensen S. The impact of gastroesophageal reflux disease on health-related quality of life. The American journal of medicine 1998; 104(3): 252-8.
23. Wahlqvist P. Symptoms of gastroesophageal reflux disease, perceived productivity, and health-related quality of life. The American journal of gastroenterology 2001; 96(8): S57-S61.
24. Zhao Y, Encinosa W. Gastroesophageal Reflux Disease (GERD) Hospitalizations in 1998 and 2005. 2008.
25. Ing AJ, Ngu MC, Breslin AB. Obstructive sleep apnea and gastroesophageal reflux. The American journal of medicine 2000; 108(4): 120-5.
26. Senior BA, Khan M, Schwimmer C, Rosenthal L, Benninger M. Gastroesophageal reflux and obstructive sleep apnea. The Laryngoscope 2001; 111(12): 2144-6.
27. Kerr P, Shoenut JP, Millar T, Buckle P, Kryger MH. Nasal CPAP reduces gastroesophageal reflux in obstructive sleep apnea syndrome. Chest 1992; 101(6): 1539-44.
28. T.C.Eski□şehi□rVali□l2016[Availablefrom:http://www.eskisehir.gov.tr/genel-bilgiler.
29. Turkey Statistical Institute Data 2015 [Available from: https://biruni.tuik.gov.tr/medas/?kn=95&locale=tr.
30. Cohen E, Bolus R, Khanna D, Hays RD, Chang L, Melmed GY, et al. GERD symptoms in the general population: prevalence and severity versus care-seeking patients. Dig Dis Sci 2014; 59(10): 2488-96.
31. Özseker B, Yasar NF, Bilgin M, Kurt Y, Balcioglu H, Bilge U. Turkish validation of National Institutes of Health (NIH) patient-reported outcomes measurement informa-

tion system (PROMISÂŪ) Gastroesophageal Reflux Disease (GERD) scale. Biomedical Research 2016; 27(2).
32. Acar HV, Kaya A, Yücel F, Erdem M, Günal SE, Özgen F, et al. Obstrüktif Uyku Apnesi Tarama Testi Olarak Kullanılan STOP-Bang Testinin Türk Popülasyonunda Geçerliliğinin Saptanması. Turkish Journal of Anesthesia & Reanimation 2013; 41(4).
33. Kumar S, Sharma S, Norboo T, Dolma D, Norboo A, Stobdan T, et al. Population based study to assess prevalence and risk factors of gastroesophageal reflux disease in a high altitude area. Indian Journal of Gastroenterology 2011; 30(3): 135-43.
34. Dent J, El-Serag H, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2005; 54(5): 710-7.
35. Ip S, Bonis P, Tatsioni A, Raman G, Chew P, Kupelnick B, et al. Comparative effectiveness of management strategies for gastroesophageal reflux disease. 2005.
36. Spiegel BM, Hays RD, Bolus R, Melmed GY, Chang L, Whitman C, et al. Development of the NIH patient-reported outcomes measurement information system (PROMIS) gastrointestinal symptom scales. The American journal of gastroenterology 2014; 109(11): 1804-14.
37. Chung F, Yegneswaran B, Liao P, Chung SA, Vairavanathan S, Islam S, et al. STOP QuestionnaireA Tool to Screen Patients for Obstructive Sleep Apnea. The Journal of the American Society of Anesthesiologists 2008; 108(5): 812-21.
38. Jarosz M, Taraszewska A. Risk factors for gastroesophageal reflux disease: the role of diet. Prz Gastroenterol 2014; 9(5): 297-301.
39. Shaha M, Perveen I, Alamgir M, Masud M, Rahman M. Prevalence and risk factors for gastro-esophageal reflux disease in the North-Eastern part of Bangladesh. Bangladesh Medical Research Council Bulletin 2013; 38(3): 108-13.
40. Kay L, Jørgensen T, Jensen KH. Epidemiology of abdominal symptoms in a random population: prevalence, incidence, and natural history. European journal of epidemiology 1994; 10(5): 559-66.
41. Locke GR, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Risk factors associated with symptoms of gastroesophageal reflux. The American journal of medicine 1999; 106(6): 642-9.
42. Moshkowitz M, Horowitz N, Halpern Z, Santo E. Gastroesophageal reflux disease symptoms: prevalence, sociodemographics and treatment patterns in the adult Israeli population. World journal of gastroenterology: WJG. 2011;17(10):1332.
43. Ruszniewski P, Soufflet C, Barthélémy P. Nonsteroidal anti-inflammatory drug use as a risk factor for gastro-oesophageal reflux disease: an observational study. Alimentary pharmacology & therapeutics 2008; 28(9): 1134-9.
44. Martín-de-Argila C, Martínez-Jiménez P. Epidemiological study on the incidence of gastroesophageal reflux disease symptoms in patients in acute treatment with NSAIDs. Expert review of gastroenterology & hepatology 2013; 7(1): 27-33.
45. Mohammed I, Cherkas L, Riley S, Spector T, Trudgill N. Genetic influences in gastro-oesophageal reflux disease: a twin study. Gut 2003; 52(8): 1085-9.
46. Rabiee B, Motamed N, Hosseini V, Hemasi GR, Maadi M, Zamani F. Gastro esophageal reflux disease (GERD) prevalence and related risk factors in north of Iran. Esophagus 2016: 1-7.
47. Ghoshal UC, Chourasia D. Genetic factors in the pathogenesis of gastroesophageal reflux disease. Indian Journal of Gastroenterology 2011; 30(2): 55.
48. Kulig M, Nocon M, Vieth M, Leodolter A, Jaspersen D, Labenz J, et al. Risk factors of gastroesophageal reflux disease: methodology and first epidemiological results of the ProGERD study. Journal of clinical epidemiology 2004; 57(6): 580-9.
49. Johnson T, Gerson L, Hershcovici T, Stave C, Fass R. Systematic review: the effects of carbonated beverages on gastro-oesophageal reflux disease. Alimentary pharmacology & therapeutics 2010; 31(6): 607-14.
50. Fass R, Quan SF, O'Connor GT, Ervin A, Iber C. Predictors of heartburn during sleep in a large prospective cohort study. CHEST Journal 2005; 127(5): 1658-66.
51. Darvishmoghadam S, Zahedi M-J, Abbasi M-MH, Haghdoost A-A, Khalilyzade M, Goughari EK. Review of Clinical Spectrum of Gastroesophageal Reflux Disease in a General Population; A Study from South-East Iran. Middle East Journal of Digestive Diseases (MEJDD). 2016; 8(4): 310-7.
52. Kürklü Ns, Kaner G. Gastroözofageal Reflü Hastalığında Beslenmeye Güncel Bakış.
53. El-Serag H, Satia J, Rabeneck L. Dietary intake and the risk of gastro-oesophageal reflux disease: a cross sectional study in volunteers. Gut 2005; 54(1): 11-7.
54. Mansour-Ghanaei F, Joukar F, Atshani SM, Chagharvand S, Souti F. The epidemiology of gastroesophageal reflux disease: a survey on the prevalence and the associated factors in a random sample of the general population in the Northern part of Iran. International journal of molecular epidemiology and genetics 2013; 4(3): 175.
55. Song JH, Chung SJ, Lee JH, Kim Y-H, Chang DK, Son HJ, et al. Relationship between gastroesophageal reflux symptoms and dietary factors in Korea. 2011.
56. Pacheco-Galván A, Hart SP, Morice AH. Relationship between gastro-oesophageal reflux and airway diseases: the airway reflux paradigm. Archivos de Bronconeumología ((English Edition)). 2011; 47(4): 195-203.
57. Mokhlesi B, Morris AL, Huang C-F, Curcio AJ, Barrett TA, Kamp DW. Increased prevalence of gastroesophageal reflux symptoms in patients with COPD. CHEST Journal 2001; 119(4): 1043-8.
58. Çi□l E, Karasu I, Özsöz A, Özbi□lek E, Koparal H, Erbaycu Ae. Koah'li Hastalarda Gastroozafagi□al Reflü Hastaliği Sikliğinin Si□nti□grafi□ İle Değerlendi□.
59. Parsons JP, Mastronarde JG. Gastroesophageal reflux disease and asthma. Current opinion in pulmonary medicine

2010; 16(1) :60-3.
60. Almadi MA, Almousa MA, Althwainy AF, Altamimi AM, Alamoudi HO, Alshamrani HS, et al. Prevalence of symptoms of gastroesopahgeal reflux in a cohort of Saudi Arabians: A study of 1265 subjects. Saudi journal of gastroenterology: official journal of the Saudi Gastroenterology Association 2014; 20(4): 248.
61. Friedman M, Gurpinar B, Lin H-C, Schalch P, Joseph NJ. Impact of treatment of gastroesophageal reflux on obstructive sleep apnea-hypopnea syndrome. Annals of Otology, Rhinology & Laryngology. 2007;116(11):805-11.
62. Bakr RM, El-Mahalawy II, Elhabashy MM, Essa AS, Saad MT. Study of gastro-esophageal reflux disease among obstructive sleep apnea patients. Egyptian Journal of Chest Diseases and Tuberculosis 2015; 64(4): 903-6.
63. Kim HN, Vorona R, Winn M, Doviak M, Johnson D, Catesby Ware J. Symptoms of gastro-oesophageal reflux disease and the severity of obstructive sleep apnoea syndrome are not related in sleep disorders center patients. Alimentary pharmacology & therapeutics 2005; 21(9): 1127-33.

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Original article

A bioethical approach: vegan and vegetarian experiences*

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Summary. Veganism and vegetarianism are bioethical approaches as well as lifestyles and philosophies of life. There may be more than one reason why people choose to be a vegan/vegetarian. They can be discussed in terms of ethics, animal rights, respect for sentient beings, ecology and violence. In this study, veganism/vegetarianism is discussed in detail in terms of bioethics. A qualitative research was performed by using the snowball or chain sampling method and 40 people older than 18 years old and vegan/vegetarian for at least two months were interviewed. The interviews were conducted face-to-face with each participant from June 20 to October 24 in 2015. The data gathered in the study were evaluated in accordance with content analysis. Responses to open–ended questions were coded. Emerging themes were discussed and appropriate suggestions were made. The results of the study revealed reasons for adopting veganism/vegetarianism, ease and difficulties and benefits and harmful effects of these life-styles. It was concluded that veganism and vegetarianism could be dealt with in terms of bioethics.

Key words: vegan, vegetarian, ethics, bioethics, ecology, violence

Introduction

Veganism and vegetarianism, gaining popularity in the world (1-4) are considered not only a type of nutrition but also a lifestyle and philosophy of life (5). Thirty-five percent of the Indian population, 9% of the Italian and German populations and 4% of the American population are vegetarian and 2% of the American population is vegan (4, 6).

The word vegetarian originates from the Latin word vegetus. In opposition to what is generally thought, it does not stem from the word vegetable. Vegetus means full of life, healthy and alive. According to a definition made in 1842, vegetarianism refers to a type of nutrition in which red meat, chicken and fish are not consumed, but eggs, milk and dairy products are consumed depending on preferences (7). Its definition was improved by all members of the International Vegetarian Union in 2011 and vegetarianism was defined as a type of nutrition, the source of which is vegetables but which either includes or excludes animal produce like dairy products, eggs and honey (whether they are consumed or not depends on preferences) (8).

Veganism means not consuming animal produce at all (like yoghurt, milk and honey etc.). The diet of vegans involves vegetables, fruit, crops and nuts (e.g. walnut and hazelnut etc.) (9). Vegans also do not wear clothes made of animal products like wool, silk and leather, do not use products tested on animals (e.g. cosmetics, detergents and toothpaste etc.) and do not consume products containing milk such as chocolate, cake and pasta. They do not go to circuses since animals are used there and do not watch movies in which living animals are used (8, 10-14, 17). Veganism is dealt with separately although it is a part of vegetarianism (9). The word vegan was coined by Donald Watson and

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the first vegan society was established in England (The Vegan Society) in 1944. According to The Vegan Society, the definition of veganism updated in 1979, is as in the following: "*[…] a philosophy and way of living which seeks to exclude—as far as is possible and practicable—all forms of exploitation of, and cruelty to, animals for food, clothing or any other purpose; and by extension, promotes the development and use of animal-free alternatives for the benefit of humans, animals and the environment. In dietary terms, it denotes the practice of dispensing with all products derived wholly or partly from animals*" (15).

In the present study, reasons for adoption of veganism/vegetarianism, ease and difficulties, general benefits and harms, ecological benefits and harms and bioethical evaluations of these life-styles were dealt with.

Material and Methods

Study population and sample

This study had a qualitative design. A purposeful sampling, called snowball or chain sampling method, was used and vegans and vegetarians living in Ankara, Turkey, were contacted. The study population included people aged over 18 years and being a vegan/vegetarian for at least two months. The study sample comprised of 40 participants accepting to participate in the study.

Development and administration of data collection tool

Data were collected with an interview form prepared by the researcher in light of the literature (7, 8, 15-17). The form was composed of four parts and 18 questions. Part I included eight questions about demographic characteristics of the participants. Part II included two questions about descriptive characteristics of the participants in terms of veganism/vegetarianism (duration and type of veganism/vegetarianism). Part III included seven questions about opinions of the participants about veganism/vegetarianism (The reasons for vegan/vegetarian lifestyle, problems in social relationships because vegan/vegetarian lifestyle). Part IV included one question about whether the participants would like to add anything about the issue. They participants gave informed consent. The interviews, conducted with the participants, were recorded. Three participants declined voice recordings. Therefore, their interviews were only written down. All the interviews were held between 20 June and 24 October in 2015.

Data Analysis

Inductive content analysis was performed to evaluate data, which were obtained from responses to open ended questions. To achieve this, first, transcribed data were read by the researchers. Second, the data were coded and categorized by two researchers separately according to the predetermined themes based on the aim of the study (18). Then, the researchers discussed the themes, added the newly emerging themes and reached a consensus on them. This process allowed determining themes directly based on the data collected (n=21 for vegans and n=19 for vegetarians) and strengthening the credibility of the results.

Results

Most of the participants (72.5%) were young. In fact, they were aged 18-34 years. More than half of the participants were female. The majority of the participants was single and did not have any children. Almost all the participants were university graduates or university students. The majority of the participants were working in private sector. Half of the participants were vegans or vegetarians for 1-4 years (Table 1).

Twenty-one participants were vegan. Most of them commented that they became a vegan after experiencing vegetarianism, which they described as a transition period. Only three participants, all of whom were male, became a vegan without experiencing vegetarianism (Table 1).

The participants usually mentioned more than one reason for their vegan/vegetarian lifestyle. The most frequent reasons were objecting to animal exploitation and ethics, followed by respect for life of sentient beings, ecology and conscience. The reasons why the individuals participating in the present study adopted veganism/vegetarianism were considered under different headings.

Objecting to animal exploitation

The participants adopted veganism/vegetarianism as their lifestyle since they were against animal exploi-

Table 1. Descriptive characteristics of the participants (n=40)

Descriptive Characteristics	n	%
Age Groups (yrs.)		
18-34	29	72.5
35-49	9	22.5
50 and older	2	5.0
Gender		
Female	24	60.0
Male	16	40.0
Marital Status		
Married	4	10.0
Single	36	90.0
Having Children		
Yes	2	5.0
No	38	95.0
Education		
High school graduates	2	5.0
University students	4	10.0
University graduates	25	62.5
Attending an MA or a PhD program	9	22.5
Occupation		
Academician	3	7.5
Teacher	3	7.5
Civil servants	6	15.0
Yoga trainer	2	5.0
Having one's own business	5	12.5
Working in private sector	16	40.0
Student	5	12.5
Duration of being a vegan/vegetarian		
2-6 months	3	7.5
6 months-1 year	0	0.0
1 year-4 years	21	52.5
4-10 years	8	20.0
More than 10 years	8	20.0
Type of vegetarians		
Vegan	21	52.5
Lacto-ovo vegetarian	12	30.0
Lacto vegetarian	2	5.0
Ovo vegetarian	1	2.5
Pesco vegetarian	4	10.0
History of Becoming Vegetarian/Vegan		
Directly becoming vegan	3	7.5
First becoming vegetarian and then adopting veganism	18	45.0
Vegetarian	19	47.5
Towtal	**40**	**100.0**

tation. They argued that animal products should not be used at all. *"Animals suffer somewhere I have never seen. The process starts and finishes there and animals are changed into food, put into packages and served. I'm not involved in this process and I don't see it. I don't know about the connection between them?"* (39-year-old female vegan). It seems that the participants became a vegan/vegetarian since they were against animal exploitation and that especially the vegans did not consume animal products and adopted and maintained a lifestyle objecting to this exploitation.

Ethical views

The participants noted that people categorized animals into two: animals to love and animals to eat and that this categorization is unethical. They believe that just as people with disabilities have rights and are treated like normal people, animals should also have rights. This belief was expressed by the participants as in the following: *"I think animals cannot be considered as goods, but humans categorize animals into several types: those to love, to eat, less intelligent and more intelligent. There are humans with Down syndrome, lower intelligence and not as strong as we are. People do not find discrimination against them ethical, but they find categorization of animals according to their appearances ethical!"* (21-year-old male vegan). A vegan noted that even the fact that humans are the most intelligent beings in the world does not mean that they can exploit animals: *"Humans are meat-eaters. My ability to eat does not require me to eat it."* (19-year-old male vegan). They thought that veganism was a normal, expected and right attitude. "Being a vegan does not mean loving animals. Actually, it means doing what is right" (19-year-old male vegan). "Inability of animals to express their lack of consent influences me" (33-year-old male vegan).

Respect for life of sentient beings: all sentient beings are equal

The participants believed that all sentient beings and animals are equal. They argued that meat from cows and goats should not be eaten just as meat from cats and dogs are not. There is not a difference between animals. All of them are equally valuable.

Another reason why the participants became a vegan/vegetarian was that they respected life of sentient beings. Some participants explained it as follows: *"People eating meat violate the right (of animals) to live"* (31-year-old female vegan). "*The primary concern of a vegan should be to object to speciesism and the main principle of veganism is that all living things are equal*." (26-year-old female vegan).

Ecology

The participants also thought that vegan/vegetarian lifestyles were beneficial in terms of ecology. One of the comments made by the participants were as follows: *"Animal industry causes air pollution. I studied geography. I'm attending an MA program and searching for new information and I know about the issue"* (25-year-old female vegan).

Violence

The participants maintained that all types of exploitation should be considered collectively. They explained that violence against women and children and exploitation of children at workplaces should be considered together with exploitation of animals. Their argument was that one type of exploitation would lead to others and that none of them should be allowed to occur. One participant made the following comment about the issue: "*I'm in the opinion that all living things should have freedom. I think attempts to defend both animal rights and human rights should be made at the same time*" (33-year-old male vegan).

Conscience

Another issue discussed by vegans/vegetarians was related to suffering of animals during their slaughter. One participant commented as in the following: "The slaughter cannot be humane. If we approve of it, then we will have to accept human abuse" (24-year-old male vegan). Therefore, slaughter of animals should not be approved under any circumstances.

Discussion

The most striking finding of this study was that the participants adopted veganism/vegetarianism for ethical reasons. It has also been reported in the literature that individuals most frequently adopt veganism/

vegetarianism for ethical, ecological and health related reasons (19-23). But health was never the primary reason; this was seen in another study too (24).

In the present study, the participants underlined lack of competence in animals. They explained that inability of animals to speak and express their feelings and opinions does not mean that humans can use or consume them. Inability to speak is associated with competency. When human-beings undergo a procedure, for example, when they are exposed to a medical intervention, informed consent has to be obtained from them. So that they can give consent, they are expected to have competence. It means that so that they can make decisions, they communicate, understand information given, have certain values and goals and have an ability to think about a given decision. When people do not have competence (patients with dementia, infants and unconscious people etc.), their first-degree relatives make decisions on behalf of them. Extreme care is taken about these decisions since one makes it on behalf of another (16). This decision-making process also applies to animals. This suggests that humans should have an ethical principle about animals. Clearly, the participants in the current study argue that if an object has a value in it and for itself, that thing has an internal value (25). Therefore, humans should respect animals since they have an internal value.

Another important finding of this study was that the participants were against speciesism. It refers to prejudiced or biased treatment of a biological species for the benefits of another (17) or considering one's own benefits as more important than others' (26). The participants believed that all sentient beings (women, children, animals and patients) should be respected and treated fairly.

In the current study, the participants emphasized another reason for becoming vegan/vegetarian; i.e. minimization of damage to the environment. The aim of vegan/vegetarian lifestyles is to minimize damage to the environment. However, it is nearly impossible for humans not to give harm to the environment in this technological era. People use many objects, material and energy (like pens/pencils, paper, television, mobile phones, computers, electricity and motor vehicles etc.) during a day. Most of their sources come from nature and using them may damage nature. In addition, growing agricultural products causes damage to nature. In the present study, one participant admitted that she lives in a city and does not know how all products are manufactured exactly and what they contain.

It is not possible for humans to survive without eating vegetables, while it is possible to live without animal products. In other words, humans can survive without using animals. One participant in this study said that humans need to consume vegetables to survive and that being an omnivore does not require eating meat. When veganism/vegetarianism is considered as "sustainable nutrition" (27), it is clear that the issue involves responsibilities of humans regarding lives of future generations (4, 25).

In the current study, the vegan participants were against becoming vegetarian. They argued that vegetarianism has conflicting practices. They explained that although vegetarians respected animal rights, they ate secondary animal products like milk, yoghurt and eggs. Yourofsky, an animal rights activist and lecturer, has also advocated that one should become a vegan rather than a vegetarian (28).

While ethics tries to find answers to open-ended questions about human relations by making judgements "good or bad", responsibility, an important element of ethics, is restricted with humans. Bioethics allowed expansion of human responsibilities for their relations with the ecosystem including animals, plants, air and water. It examines ethical problems arising in a network of relationships extending from all sentient beings to the ecosystem. It has a wide scope involving quite different subjects such as artificial fertilization, donation of organs, tissues and cells, embryological studies, cloning, gene transmission, agricultural policies, genetically modified organisms and environmental problems. In general, the core of bioethics is life; not only the life of humans but also the life of all organisms available in nature (16). Accordingly, the subject of veganism and vegetarianism, directly related to the life of animals and ecology, is bioethics.

Conclusions and Recommendations

The participants became a vegan/vegetarian for more than one reason. The primary reasons were ob-

jection to animal exploitation and ethical concerns. Other reasons were respect for lives of sentient beings, conscience, ecology, violence and speciesism.

The results of the study elucidated the reasons why the participants adopted veganism/vegetarianism. They gave importance to equality between sentient beings and therefore were against speciesism and violence. They thought that vegetarianism and veganism in particular should exist to ensure equality between all sentient beings. All they are equal and have the right to live in the world. Veganism means objecting to all kinds of exploitation (exploitation of animals, women and children etc.) and believing that all sentient beings are equal. That humans use and eat animals is not considered ethical and inability of animals to express their lack of consent for procedures they are exposed to creates ethical concerns. It is obvious that animal exploitation is associated with ethics. In fact, the participants did not find animal exploitation ethical.

It can be concluded that veganism/vegetarianism has many aspects including animal exploitation, animal rights, ethics, ecology, respect for lives of sentient beings, equality between sentient beings, speciesism and violence and that all these features are the subjects of bioethics. This suggests that veganism/vegetarianism is both a nutrition style and a bioethical approach prioritizing life of sentient beings and arguing for equality between all sentient beings.

From the abovementioned point of view, it can be suggested that incorporation of human-animal-nature relations based on bioethics, animal rights and love for animals into preschool education can be useful. In addition, provision of education about bioethics, veganism and vegetarianism for university students, especially candidates of health professionals (veterinarians, doctors and nurses) will contribute to creation of awareness about the issue. Using V-Label on vegan/vegetarian products will facilitate selection of products during shopping and help create awareness about the issue in the society.

The present study has one limitation. Since the snowball or chain sampling method was used, the sample included individuals knowing each other and belonging to a certain group. Therefore, the results of the study are based on opinions of a certain group. As a result, further qualitative and quantitative studies involving different sections of the population in Turkey should be conducted.

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References

1. Clarys P, Deliens T, Huybrecht, I, Deriemaeker P, Vanaelst B, De Keyzer W, Hebbelinck M, Mullie P. Comparison of Nutritional Quality of the Vegan, Vegetarian, Semi-Vegetarian, Pesco-Vegetarian and Omnivorous Diet. Nutrients 2014; 6 (3): 1318-1332.
2. Dyett P, Rajaram S, Haddad EH, Sabate J. Evaluation of a Validated Food Frequency Questionnaire for Self-Defined Vegans in the United States. Nutrients 2014; 6: 2523-2539.
3. İsveç'te Etsiz Yaşam Yaygınlaşıyor. Life without meat becomes popular in Sweden. Veg & Nature Dergisi, Vejetaryen ve Vegan, Sürdürülebilir Yaşam ve Hayvan Özgürlüğü Dergisi. Journal of Veg & Nature, Vegetarian and Vegan, Journal of Sustainable Life and Animal Liberation. Türkiye Vegan ve Vejetaryen Derneği yayını, İstanbul, Nisan-Mayıs-Haziran, 2014.
4. Leitzmann C. Vegetarian Nutrition: Past, Present, Future. Am J Clin Nutr 2014; 100 (suppl): 496S–502S.
5. Pollan M. Etobur-Otobur İkilemi, Omnivore's Dilemma. The New York Times Bestseller, 1. Baskı, Türkçeden çeviren: İlke Önelge, Pegasus Yayınları, İstanbul, 2009
6. Tai Le L, Sabaté J. Beyond Meatless, the Health Effects of Vegan Diets: Findings from the Adventist Cohorts. Nutrients 2014; 6: 2131-2147.
7. Vejetaryen Kulübü, Vegetarian Club, http://www.vejetaryenkulubu.com. Accessed 27.4.2015.
8. Türkiye Vegan ve Vejetaryen Derneği, Vegan & Vegetarian Association of Turkey, http://tvd.org.tr Accessed 2.2.2016.
9. Yıldırım Z. 2015. Ebru Arıman: Vejetaryenlik bir tercih meselesi değil, bir zorunluluk, Ebru Arıman ile röportaj, An Interview with Ebru Arıman: Vegetarianism is not a matter of choice, it is a necessity, 7.10.2013, Yeşil Gazete, https://yesilgazete.org/blog /2013/10/07/ebru-ariman-vejetaryenlik-bir-tercih-meselesi-degil-bir-zorunluluk/ (1.12.2015). Accessed 1.12.2015.
10. Vegetarian Society. https://www.vegsoc.org. Accessed 25.9.2015.
11. Nuh'un Gemisinde Veganizm Etiği, Veganism Ethics in Noah's ark. 2014. Veg & Nature Dergisi, Vejetaryen ve Vegan, Sürdürülebilir Yaşam ve Hayvan Özgürlüğü Dergisi, Journal of Veg & Nature, Vegetarian and Vegan, Journal of Sustainable Life and Animal Liberation, Türkiye Vegan ve Vejetaryen Derneği yayını, İstanbul, Nisan-Mayıs-Haziran.

12. Çetin Z. 2015. Vejetaryen ve Vegan Beslenmesi, Vegetarian and Vegan Nutrition, 23 Temmuz 2014 tarihli yazı, medikal akademi. http://www.medikalakademi.com.tr /vejetaryen-ve-vegan-beslenmesi/ Accessed 12.4.2015
13. Kınıkoğlu M. Vegan Beslenme, Vegan Nutrition, 1. Baskı, Oğlak Yayıncılık, İstanbul, 2015.
14. Türkmen AB. Topyekûn ve Bütünsel bir Özgürlük Talebi: Veganlığın Felsefesi. An unconditional and holistic demand for freedom: vegan philosophy, Gaia Dergi, 19 Aralık 2015. https://gaiadergi.com/topyekun-ve-butunsel-bir-ozgurluk-talebi-veganligin-felsefesi/ Accessed 20.12.2015.
15. The Vegan Society https://www.vegansociety.com Accessed 31.3.2017.
16. Çobanoğlu, N. 2009. Kuramsal ve Uygulamalı Tıp Etiği, Theoretical and Practical Medical Ethics, Eflatun Yayınevi. Ankara.
17. Singer P. Hayvan Özgürleşmesi [Animal Liberation] 1. Basım, İngilizceden çeviren: Hayrullah Doğan, Ayrıntı Yayınları, İstanbul, 2005.
18. Yıldırım A. Şimşek H. Sosyal Bilimlerde Nitel Araştırma Yöntemleri, Qualitative Research Methods in Social Sciences, 8. Baskı, Tıpkı Basım, Seçkin Yayıncılık, Ankara, 2011.
19. Ruby Matthew B. Vegetarianism. A blossoming field of study. Appetite 2012; 58: 141-150.
20. Rothgerber H. A meaty matter. Pet diet and the vegetarian's dilemma. Appetite 2013; 68: 76-82.
21. Radnitz C, Beezhold B, DiMatteo J. Investigation of lifestyle choices of individuals following a vegan diet for health and ethical reasons. Appetite 2015; 90: 31-36.
22. Hoffman SR, Stallings SF, Bessinger RC, Brooks GT. Differences between health and ethical vegetarians. Strength of conviction, nutrition knowledge, dietary restriction, and duration of adherence. Appetite 2013; 65: 139-144.
23. Fox N, Ward K. Health, ethics and environment: A qualitative study of vegetarian motivations, Research Report. Appetite 2008; 50: 422-429.
24. Çiçekoğlu P, Tunçay GY. A Comparison of Eating Attitudes between Vegans/Vegetarians and Nonvegans/Nonvegetarians in Terms of Orthorexia Nervosa (Ortoreksiya Nervoza Açısından Vegan/Vejetaryen Olan Ve Olmayan Bireylerin Beslenme Alışkanlıkları) Archives of Nursing 2018, April 2018: 32 (2): 200-205. DOI: http://dx.doi.org/10.1016/j.apnu.2017.11. 002
25. Des Jardins JR. "Çevre Etiği" [Environmental Ethics] Çevre Felsefesine Giriş, Çev.: Ruşen Keleş, İmge Yayınevi, Ankara 2006, 149-192, 261.
26. Demirağ NG. Son Bakışta Hüzün: Merhamet Etiği Olarak Levinas ve Hayvan Hakları, Sadness at the last glance: Levinas and Animal Rights as Compassion Ethics, Yeşil ve Siyaset, Siyasal Ekoloji Üzerine Yazılar, Ed.: Orçun İmga, Hakan Olgun, Lotus Yayınevi, Ankara, 2012.
27. Taltekin A. Hayvanları Sevenlere İklim Değişikliğiyle Mücadele İçin 5 Neden, 5 Yöntem, Five Reasons and Five Methods about struggle against climate changes for animal lovers. Gaia Dergi, Gaia, Journal of Sustainable Life 7 Aralık 2015. https://gaiadergi.com/hayvanlari-sevenlere-iklim-degisikligiyle-mucadele-icin-5-neden-5-yontem Accessed 18.12.2015.
28. Gary Yourofsky ile röportaj. Gary Yourofsky: Veganlık Herkes içindir! An Interview with Gary Yourofsky. Gary Yourofsky: Veganism is for everyone! Gaia Dergi, Sürdürülebilir Yaşam Dergisi [Gaia, Journal of Sustainable Life] Haziran, 1, 2015.

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Original article

Dynamics of blood glucose concentration after a food determines subsequent energy consumption

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Summary. Obesity and its related comorbidities such as type 2 diabetes and cardiovascular disorders are global public health challenges. It is imperative to understand patterns of the dietary composition that regulate blood glucose concentration, satiety cues, and energy consumption. *Objectives:* This study elucidated the dynamics of blood glucose concentration after a test food as a crucial determinant of appetite and energy intake at a subsequent meal. *Methods:* Both low and high glycemic index (GI) foods in liquid and solid forms or fixed available carbohydrate and in equicaloric amounts were tested in healthy female volunteers (n=14/experiment) on blood-glucose and percent energy compensation (%EC). White wheat bread (solid, high-GI) was compared with chickpeas (solid, low-GI) at 50 g available-carbohydrate in Experiment 1 and Coca-Cola (liquid, high-GI) with skim milk (liquid, low-GI) and chickpeas (solid, low-GI) at equicaloric amounts in Experiment 2. Blood glucose and appetite were measured at baseline and over time up to two hours in Experiment 1 and one hour in Experiment 2. Caloric intake was estimated from a pizza-meal at the end of the studies and %EC calculated. *Results:* Both high GI foods had the largest glucose peaks; chickpeas had an intermediate and milk the smallest peak. Blood glucose concentration before meal time was associated with energy intake. The %EC was: chickpeas (70%) > bread (7%) in Experiment 1; and chickpeas (80%) > milk (40%) > Coca-Cola (32%) in Experiment 2. *Conclusions:* Intake of foods with low GI value would prove helpful in the prevention and controlling of obesity, hyperglycemia or hyperinsulinemia.

Key words: Coca-Cola, milk, chickpeas, bread, energy compensationy

Introduction

Metabolic regulation of body weight relays on the hypothalamic and other parts of the brain through programmed energy balance, known as 'Body weight set point' theory (1). However, body weight regulation is more complex, implicated not solely by the genetically programmed set point, but the obesogenic environment and the hedonic temptation of the abundance and variety of food play an integral role.

Excessive body weight is considered among the major risk factors for non-communicable dietary chronic diseases including diabetes. In the past 25 years, the prevalence of Type-2 diabetes has increased 120% worldwide and is projected to increase further (2). Health professionals are attempting hard to apprehend the mechanisms of causative dietary factors or patterns governing satiety cues and eating behaviour. It is well known that high glycemic index (GI) foods are known to encourage excess calories intake through blood glucose spike accompanying hyperinsulinemia that causes reactive hypoglycaemia, hunger and cellular fat gain (3). Nevertheless, it is argued that neither GI or insulin secretion is crucial, rather it is 'body weight set point' that regulates body fatness through the 'leptin-hypothalamus feedback loop' (4). The phenomenon to understand excessive energy consumption is further compounded by the conflicting results reported by various investigators. For example, some researchers suggest that the liquid form of foods

is less compensatory for the energy consumption at the next meal than the solid form of foods (5-7). Whereas, others reported vice versa (8-11). The reason for these controversial results may lie in the fact that most studies did not measure the blood glucose responses which could influence the feeling of hunger and consequently food consumption.

Glucose being a preferred energy source for the brain, fluctuations in the blood glucose concentration is monitored by the gut-brain axis via glucosensors in the hypothalamus (12). The blood level of glucose and its sustainability overtime is an important outcome of the type of food consumed by individuals. The postprandial blood glucose concentration, a consequence of several factors such as the ingested food form (liquid or solid) (5-11), GI (low or high) (3, 13), or nutrient composition (14-15), however, remains controversial on appetite and energy consumption. This study was designed to evaluate some of the common dietary determinants of blood glucose concentration influencing satiety and food intake in two short-term experiments. Experiment 1 compared up to 120 min the effect of the solid form of food with low and high GI, fed at equal amounts of available carbohydrate while Experiment 2 examined up to 60 min the effect of a solid versus a liquid form of food with low and high GI, fed in equicaloric amounts. In both experiments, participants were healthy young female subjects, a population group highly susceptible to excessive weight gain with age (16).

Material and Methods

Subjects

Volunteers (n=14/both experiments) were selected from a convenient sample of healthy female students, with a body mass index (BMI in kg/m^2) of 20-25 and ages 17-30 years. Subjects were recruited through flyers and by word of mouth from the College of Life Sciences at Kuwait University. According to the study exclusion criteria, volunteers with high fasting blood glucose, on medication, breakfast skippers or restrained eaters (scored ≥11 on the Eating Habits Questionnaire (17) were not recruited. Furthermore, no test session was scheduled during the menstrual cycle to avoid hormonal effects if any, on blood glucose or appetite (18). The study was conducted in the Human Nutrition laboratories of the Department of Food Science and Nutrition. A consent form was signed by all subjects. This study was implemented in accordance with the principles established by the Declaration of Helsinki. The study was approved by the Human Subjects' Review Committee, Ethics Review Office, Kuwait University. The sample size determination was based on the results from previously reported similar research studies that detected an 80% difference in the glycemic responses among the test treatments at an alpha level of $p<0.05$ using the statistical package of SPSS (19).

Test foods

The test foods - white wheat bread (Kuwait Flour Mills), canned chickpeas (Giant Chickpeas with Chillies, Al-Daniah, Kuwait), Coca-Cola (Classic), skim milk (Almarai Milk Company, Saudi Arabia) and bottled water (Aquafina) - were purchased from a local market. Weighed amounts corresponding to 50 g available carbohydrate (total carbohydrate - dietary fiber) of the white bread and canned chickpeas were used in Experiment 1 (Table 1). White bread was served immediately after toasting for 30 seconds and chickpeas were microwaved for 90 seconds before serving. Five gram of butter was served with both foods for taste. The water control was served at refrigerator temperature. Additional water was served with the test foods to equalize their volume in the stomach and to facilitate swallowing.

In Experiment 2, test foods were served at the temperatures they are usually consumed; Coca- Cola, skim milk and water at refrigerator temperature, and canned chickpeas were served as in Experiment 1. All test foods had contained 200 Kcal. The composition of test foods for both experiments is given in Table 1.

Experimental procedures

Both experiments had a cross-over study design. Each subject consumed the test foods for each experiment in a random order. The experimental procedure followed was similar to the other studies used in our lab. Blood glucose was measured in a finger prick sample by portable blood glucose monitoring system (One Touch Ultra, Life Scan Inc and Johnson & Johnson

Table 1. Nutrition composition of the test preloads of Experiments 1 and 2

Experiment 1: Test preloads fed at 50 g available carbohydrate			
	Water	**White Bread**	**Canned Chickpeas**
Energy (kcal)	0	262	307
Available Carbs (g)	0	50	50
Total Fat (g)	0	2.5	2.6
Protein (g)	0	10	21
Dietary Fiber (g)	0	0.7	20.8
Weight (g)	500	125	342
Water Served (mL)	500	375	158
Total Volume (mL)	500	500	500
Energy Density (kcal/g)	0	0.524	0.614

Experiment 2: Test preloads fed at equicaloric amounts				
	Water	**Coca Cola**	**Skim Milk**	**Canned Chickpeas**
Energy (kcal)	0	200	200	200
Available carbs (g)	0	53	27	35
Total Fat (g)	0	0	2.6	2.3
Protein (g)	0	0	17	10.8
Dietary Fiber (g)	0	0	0	8.2
Weight (g)	-	-	-	226
Volume (mL)	500	493	500	261
Water Served (mL)	-	7		239
Total Volume (mL)	500	500	500	500
Energy Density (Kcal/g)	0	0.4	0.4	0.4

Company, USA), and subjective appetite by a Visual Analogue Scale (VAS) questionnaire (20, 21) at baseline and at every 15 min for one hour after ingestion of the test food in both experiments and then at 30 min interval for the second hour of Experiment 1. At the end of each test, a pizza meal and a bottle of water were served. The subjects were asked to eat and drink until comfortably full.

Subjects came for the study sessions between 8.00 and 10.00 am after an overnight fast of 10-12 hours. Subjects were instructed to maintain a regular pattern of food intake and physical activity throughout the study.

Food intake

The pizzas prepared and served as described in our earlier studies at the end of each session (19, 20) were 5-inch round containing about 200 kcal, available in two varieties Briefly, the pizzas (Four Cheeses and Deep N Delicious Vegie Pizza; McCain Foods Ltd) were baked and cut into 4 pieces, served in consecutive trays within 6-7 minutes until the subjects refused to eat more. Food intake was assessed by weighing the cooked pizza before and after serving (left-over). The caloric consumption was calculated from the nutrition information provided by the manufacturer on the pizza labels.

Data analysis

Incremental area under the blood glucose response curves (AUC), ignoring any area below fasting, was determined for each test food for each subject. For analysis of VAS appetite responses, an average appetite score was calculated for each time point using the formula:

$$\text{Average Appetite} = [\text{Question 1} + \text{Question 2} + (100\text{-Question 3}) + \text{Question 4}]/4.$$

Percent energy intake compensation (%EC) at the second meal for the test food calories was calculated by the following formula:

% EC= [(Kcal intake at pizza meal after the Test food– Kcal intake at pizza meal after the Water Control)/Kcal from the Test Food] * 100.

Statistical analysis was conducted using SPSS (Statistical Package for Social Sciences). One-way repeated measures analysis of variance (ANOVA) was used to test for the effect of treatments on outcome variables, including changes from baseline in blood glucose concentrations and average appetite scores at each time point and incremental area under the curve (AUC) for these changes for the total test periods, and calorie intake and percent energy compensation at meals. Two-way repeated measures ANOVA was conducted on changes in appetite and blood glucose concentration scores at each test period to determine time and treatment effects and for a time by treatment interaction.

Tukey's posthoc tests were performed when treatment effects were statistically significant ($p<0.05$). All results presented are as mean ± standard error of the mean (SEM). Correlation analyses were conducted using the Pearson correlation coefficient.

Results

Blood glucose

Blood glucose changes were affected by both treatment ($p<0.001$) and time ($p<0.001$) with a time by treatment interaction ($p<0.001$). Peaks in blood glucose occurred at 30 min for all foods in both experiments followed by declines. In Experiment 1, white bread resulted in the most rapid increase in blood glucose, but then the levels declined by 120 min and fell below the baseline. Chickpeas reached a significantly lower rise at 30 min and maintained this rise above the baseline at 120 min, which was significantly different from white bread but not the water control. The calculated AUC for blood glucose was also significantly higher for white bread followed by chickpeas and water (Table 2).

In Experiment 2, blood glucose increases again peaked at 30 min with Coca-Cola showing significantly maximum rise than the milk, chickpeas or water and declined thereafter, with all treatments remained above the baseline at 60 min. The blood glucose AUC was significantly higher after Coca-Cola followed by chickpeas then milk and water. (Table 2).

Average appetite

The appetite scores at the baseline were not different for all the treatments in both Experiments. The average change in appetite was affected by treatment ($p<0.05$) and time ($p<0.05$), however, no treatment by time interaction was observed ($p>0.05$). The lowest values for appetite score change (e.g. least hunger), as expected, were observed between 30-60 min after the chickpeas preloads in both Experiments compared

Table 2. Change in blood glucose concentration from baseline with preload of 50 g of available carbohydrate (Experiment 1) or equicaloric amounts of low versus high GI foods (Experiment 2) as solid or liquid form

	Experiment 1			Experiment 2			
Time (min)	**Water**	**Bread mmol/L**	**Chickpeas**	**Water**	**Coca Cola mmol/L**	**Milk**	**Chickpeas**
15	0.04±0.12[a*]	1.05±0.12[b]	0.78±0.18[b]	0.11±0.01[a]	2.00±0.39[d]	0.40±0.41[b]	1.00±0.23[c]
30	0.06±0.10[a]	1.9±0.12[b]	1.39±0.22[c]	0.10±0.01[a]	3.00±0.24[d]	0.70±0.21[b]	1.5±0.34[c]
45	0.09±0.10[a]	1.50±0.12[b]	1.22±0.22[b]	0.06±0.02[a]	2.30±0.42[c]	0.37±0.55[a]	1.48±0.21[b]
60	0.04±0.11[a]	0.94±0.11[b]	0.44±0.15[a]	0.07±0.01[a]	1.40±0.43[b]	0.30±0.38[a]	0.57±0.27[a]
90	-0.08±0.11[a]	0.08±0.15[a]	0.16±0.15[a]				
120	-0.19±0.13[ab]	-0.38±0.09[b]	0.07±0.08[a]				
AUC[#]	32.56±10.03[a]	121.68±18.23[b]	86.66±17.30[ab]	12.00±2.00[a]	158.11±14.05[c]	35.40±34[a]	105.43±21.00[b]

** Data presented is as Mean ± SEM, n=14; Different superscript letters denote a significant difference at p<0.05 in the same row (Tukey's posthoc test).*

[#]AUC = Area under the curve (mmol min/L)

Table 3. Change in appetite from baseline with preload of 50 g of available carbohydrate (Experiment 1) or equicaloric amounts of low versus high GI foods (Experiment 2) as solid or liquid

	Experiment 1			Experiment 2			
Time (min)	**Water**	**Bread mm**	**Chickpeas**	**Water**	**Coca Cola**	**Milk mm**	**Chickpeas**
15	-1.82±0.76 a*	-3.52±1.09 a	-4.97±0.81 a	-2.50±1.63 a	-5.00±1.04 a	-5.50±0.80 a	-6.50±0.87 a
30	-0.46±0.87 a	-1.89±0.94 ab	-4.71±0.86 b	-0.05±0.89 a	-2.00±1.95 ab	-3.00±1.90 b	-4.50±0.90 b
45	0.41±0.55 a	-0.86±0.82 a	-3.51±0.88 b	0.50±1.50 a	-0.50±0.82 a	-2.00±0.88 ab	-3.50±0.80 b
60	0.49±0.43 a	-0.32±0.89 a	-1.80±0.74 a	2.00±1.30 a	0.50±0.92 a	-0.50±0.36 ab	-2.60±0.36 b
90	1.11±0.64 a	-0.02±1.82 a	-0.77±0.78 a				
120	1.32±0.74 a	0.87±0.61 a	0.23±0.71 a				

** Data presented is as Mean ± SEM, n=14; Different superscript letters denote a significant difference at p<0.05 in the same row (Tukey's posthoc test)*

to water, at 45 min compared to bread, and at 45-60 min compared to Coco-Cola but were not different from milk preloads (Table 3). AUCs for appetite score changes did not differ by test treatment in either of the Experiments (data not included).

Food intake

In Experiment 1, kcal intake at 120 min was significantly lower (p<0.0001) after chickpeas than white bread or water (Figure 1), and cumulative energy intake was significantly higher for white bread,

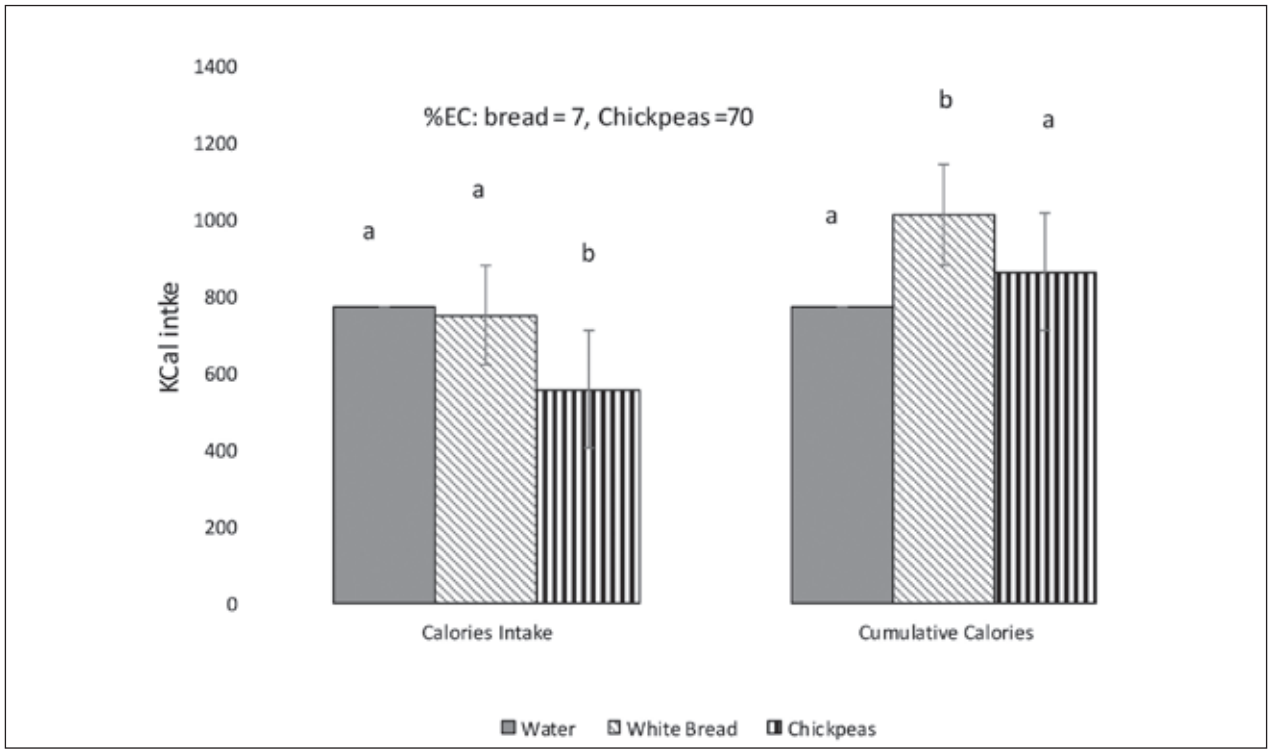

Figure 1. Experiment 1 – Effect of a similar available carbohydrate content on energy intake, cumulative energy intake and percent energy compensation (%EC)[$] in healthy females

Data presented is as Mean ± SEM, n=14; Different superscript letters denote a significant difference at p<0.05 in the same group of bars (Tukey's posthoc test)

[$]%EC= [(Kcal intake at meal after the Control (water) preload– Kcal intake at meal after the Test preload (white bread or chickpeas) / Kcal from the Test preload] * 100.

making the calculated %EC much higher for chickpeas. In Experiment 2, energy intake at 60 min was same for the three energy nutrients, whereas water was similar to milk and Coca-Cola, yet it was higher than the chickpeas. The cumulative energy intake was similar between milk and Coca-Cola, and both water and chickpeas were similar to each other yet significantly lower than Coca-Cola and milk (p<0.001) (Figure 2), making %EC the highest for chickpeas, and approximately double that of Coca-Cola and milk.

Relations among dependent measures

The average appetite AUC in each experiment had a positive correlation with Kcal intake (r=0.352, p<0.05 and r=0.330, p<0.01) in Experiment 1 and 2, respectively, supporting an increase in food intake with average increase in appetite scores as would be expected. On the other hand, blood glucose AUC for test foods in both experiments was not correlated with food intake or with average appetite AUC. However, the correlation between the final blood glucose before the meal and calories intake from the meal was time dependent, for example, there was a negative correlation at 120 min in Experiment 1 and no correlation at 60 min in Experiment 2

Discussion

These results demonstrate greatest energy compensation after the chickpeas in both experiments; the largest difference (70% vs. 7%) being with white bread of comparable carbohydrate content. When Kcal and preload energy density were equated, the energy intake after the three preloads was not significantly different, yet the differences in energy compensation were still

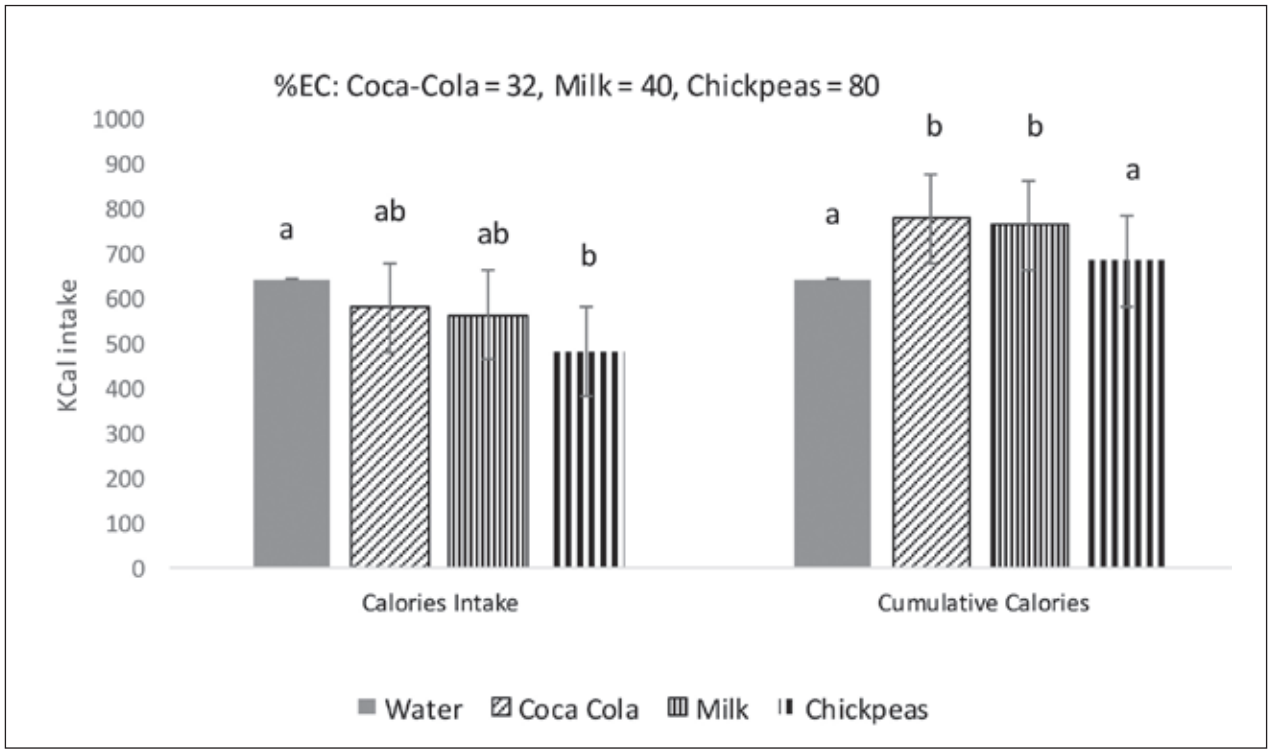

Figure 2. Experiment 2 - Similar caloric density preload effect on energy intake, cumulative energy intake and percent energy compensation (%EC)[$] in healthy females.
Data presented is as Mean ± SEM, n=14; Different superscript letters denote a significant difference at p<0.05 in the same group of bars (Tukey's posthoc test).
[$]%EC= [(Kcal intake at meal after the Control (water) preload– Kcal intake at meal after the Test preload (coca-cola, milk or chickpeas) / Kcal from the Test preload] * 100.

reasonably high for chickpeas when compared with milk (a liquid, low GI food) (80% vs 40%) and Coca-Cola (a liquid, high GI food) (80% vs 32%). This suggests that cooked chickpeas with their low GI are better in compensating energy intake when fed at equicaloric or available carbohydrate levels and whether the comparable food is a solid or a liquid. Chickpeas are low GI and reportedly are more satiating because of their high fiber and protein content and, therefore, with slow rate of digestion and absorption sustaining euglycemia for an extended period of time (22). When the two equicaloric liquid foods, milk and Coca-Cola, with low and high GI based on differences in their protein, fat and carbohydrate contents were compared, milk did not produce a significantly lower energy intake than Coca-Cola, despite its low GI (Figure 2). Other workers have also demonstrated no difference between milk and Coca-Cola in subsequent energy intake (15, 23, 24).

To further elucidate why the two solid foods of different GI, bread and chickpeas, fed at the same available carbohydrates and volume load responded differently on food intake at a test meal, but why the two liquid foods of different GI, milk and Coca-Cola, fed at the same caloric level and volume load responded similarly in food intake, we examined time trends in blood glucose patterns. Being low GI, both chickpeas and milk, resulted in a lower peak than for the high GI foods, white bread or Coca-Cola; yet at 120 min the blood glucose was significantly higher after the chickpeas compared to that after bread (Table 2), and was associated with less hunger and thus a lower energy intake. At 60 min the glucose level remained above the baseline after all four treatments (Table 2), the energy intake from the meal was not different among them except for the chickpeas that was lower only compared with the water control. Cumulative energy intake was, however, significantly lower after both chickpeas and the control. These results are supported by the higher blood glucose concentration after chocolate milk (sweeter) and not plain milk (not sweet) 30 min before meal that lead to reduced food intake (14).

When correlations performed, the final blood glucose response before meal time was implicated in the correlation of feeling of hunger and food intake; in Experiment 1, there was a positive correlation with both, but no correlation in Experiment 2. White bread, with blood glucose dropping below the baseline, showed only 7% EC, whereas the blood glucose after the Coca-Cola remained well above the baseline at one hour resulted in a much higher, 32% EC compared to white bread. Similarly, blood glucose concentration remaining above the baseline after milk preload resulted in 40% EC, only 8% more than after the Coca-Cola despite its low GI. Dove et al. (8) reported 8.5% compensation in energy consumption after skim milk compared to fruit juice. The pattern of blood glucose concentration and its influence on caloric intake might have changed after the high GI, Coca-Cola preload if the experiment time had extended beyond 60 min to allow a postprandial dip below baseline that would have triggered hunger signals by the brain to initiate eating.

The manipulating effect of blood glucose concentration on satiety and energy intake at the second meal therefore, emphasizes the need to measure blood glucose concentration simultaneously when measuring satiety and food intake. The concept of glucostatic theory presented earlier by Mayer (25) states that the transient decline in the blood glucose concentration when occurs at a correct magnitude and a correct time course is detected by the brain glucorecepters, thus initiates feeding and when the blood glucose concentration increases to a certain high level, feeding is terminated. The reason Maersk et al. (23) could not explain why they were not able to reproduce the results of a similar study conducted by Dove et al. (8) despite the same study design and testing of the same foods for the same intermeal interval was probably that Dove et al served the preloads with breakfast which contributed more calories and sustained blood glucose for longer while Maersk et al served the preloads alone (8, 23). But since the blood glucose concentration was not measured in either study, the differences in their results were hard to explain reflected in a conflicting message for the public. Likewise, lack of blood glucose measurements could not accurately explain why chocolate milk and Coca-Cola showed no difference in EC when food intake measured at 30 min or when 1% milk was compared with Coca-Cola and fruit juice tested at 145 minutes before lunch (15, 26).

Conclusions and recommendations

In conclusion, our results emphasize that it is not just the form of the foods such as solid or liquid, GI of a food such as low or high, its macronutrient composition or the energy density, it is rather the pattern of glucose that is imparted to the blood by a type of food consumed that might be influencing the feelings of hunger and thus the amount of calories consumption at the subsequent meal. Foods with low glycemic index containing dietary fiber and good quality of protein would encourage a sustained yet low peak glucose supply to the blood circulation as was seen in these two experiments. In order to prevent the occurrence of diabetes, monitoring body weight gain is imperative. Thus Consumption of foods with low GI value would prove beneficial in the prevention and management of obesity, hyperglycemia or hyperinsulinemia.

Acknowledgement

The voluntary participation by the subjects in the research is appreciated to make this work possible.

Ethical considerations:
Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

References

1. Yu YH, Vasseli JR, Zhang Y, Mechanick JI, Komer J, Peterli R. Metabolic vs. hedonic obesity: a conceptual distinction and its clinical applications. Obs Rev 2015; 16: 234-247.
2. Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Research and Clinical Practice 2014; 103: 137-149 http://dx.doi.org/10.1016/j.diabres.2013.11.002.
3. Ludwig DS. Clinical update: the low-glycemic-index diet. The Lancet 2007; 369:: 890-892.
4. Guyenet SJ, Schwartz MW. Regulation of food intake, energy balance, and body fat mass: implications for the pathogenesis and treatment of obesity. J Clin Endocrinol Metab 2012; 97: 745-755.
5. Cassady BA, Considine RV, Mattes RD. Beverage consumption, appetite, and energy intake: what did you expect? Am J Clin Nutr 2012; 95: 587-593.
6. Mourao DM, Bressan J, Campbell WW, Mattes RD. Effects of food form on appetite and energy intake in lean and obese young adults. Int J Obes (Lond) 2007; 31: 1688-1695.
7. Mattes RD, Campbell WW. Effects of food form and timing of ingestion on appetite and energy intake in lean young adults and in young adults with obesity. J Am Diet Assoc 2009; 109: 430-437.
8. Dove ER, Hodgson JM, Puddey IB, Beilin LJ, Lee YP, Mori TA. Skim milk compared with a fruit drink acutely reduces appetite and energy intake in overweight men and women. Am J Clin Nutr 2009; 90: 70-75.
9. Almiron-Roig E, Flores SY, Drewnowski A. No difference in satiety or in subsequent energy intakes between a beverage and a solid food. Physiol Behav 2004; 82: 671-7.
10. Almiron-Roig E, Chen Y, Drewnowski A. Liquid calories and the failure of satiety: how good is the evidence? Obes Rev 2003; 4: 201-212.
11. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. Am J Clin Nutr 2002; 76: 1023-1030.
12. Fournel A, Marlin A, Abot A, et al. Glucosensing in the gastrointestinal tract: impact on glucose metabolism. Am J Physiol Gastrointest Liver Physiol 2016. Doi:10.1152/ajpgi.00015.201.
13. Mattes R. Fluid calories and energy balance: the good, the bad, and the uncertain. Physiol Behav 2000; 89: 66-70.
14. Panahi S, Luhovyy BL, Liu TT, Akhavan T, ElKhoury D, Goff HD, Harvey AG. Energy and macronutrient content of familiar beverages interact with pre-meal intervals to determine later food intake, appetite and glycemic response in young adults. Appetite 2013; 60: 154-161.
15. Harper A, James A, Flint A, Astrup A. Increased satiety after intake of a chocolate milk drink compared with a carbonated beverage, but no difference in subsequent ad libitum lunch intake. Br J Nutr 2007; 97: 579-83.
16. Badr HE, Shah NM, Shah MA. Obesity among Kuwaitis aged 50 years or older: prevalence, correlates and comorbidities. The Gerontologist 2012; 53: 555-566.
17. Herman CP. Restrained Eating. Psychiatric Clinics of North America 1978; 1: 593-607.
18. Escalante PJM, Alpizar SM. Change in insulin sensitivity, secretion and glucose effectiveness during menstrual cycle. Arch Med Res 1999; 30: 19-22.
19. Zafar TA, Kabir Y. Chickpeas suppress postprandial blood glucose concentration and appetite and reduce energy intake at the next meal. J Food Sci Technol 2017; 54: 987-94.
20. Zafar TA, Kabir Y, Ghazaii C. Low glycemic index foods suppress glycemic response, appetite and food intake in young Kuwaiti females. Kuwait J Sci Eng 2011; 38: 111-23.
21. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Inter J Obesity 2000; 24: 38-48.

22. Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values. Am J Clin Nutr 2002; 76: 5-56.
23. Maersk M, Belza A, Holst JJ, Fenger-Gron M, Pedersen SB, Astrup A, Richelsen B. Satiety scores and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk and with non-caloric soft drink: a controlled trial. European Journal of Clinical Nutrition 2012; 66: S23-S29.
24. Soenen S, Westerterp-Plantenga MS. No difference in satiety or energy intake after high-fructose corn syrup, sucrose, or milk preloads. Am J Clin Nutr 2007; 86: 1586-1594.
25. Mayer J. Glucostatic mechanism of the regulation of food intake. N Engl J Med 1953; 249: 13-6.
26. Almiron-Roig E, Drewnowski A. Hunger, thirst, and energy intakes following consumption of caloric beverages Physiol Behav 2003: 79: 767-73.

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Original article

Knowledge levels and preference reasons regarding the doping of U23 Turkish National Team athletes

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Summary. The aim of this study was to determine the knowledge level and preference reasons of U23 Turkish National Team athletes about doping. The sample of the study was created by 100 U23 Turkish National Team athletes from different branches. 5-points likert-type scale and a questionnaire consisting of 29 questions was applied to the athletes. In the analysis of the obtained data, descriptive statistics (percentage and frequency) and chi-square test were used. As a result, it was determined that 47% of athletes had enough knowledge about doping, and 81% of them knew that doping had great damages on health, but they were indecisive about the usage of doping. Although athletes tend to use doping, this result may be due to the risk of being caught by the World Anti-Doping Agency.

Key words: doping knowledge, doping preference, health, world Anti-Doping Agency

Introduction

People's attitudes towards sports or tendency to participate it vary. This variation is shaped by the expectations of individuals from sports. Sometimes the reason for doing sports may be seen as a tool for education or health, and sometimes as a reason for gaining status in society. Moreover, the reasons for making sports differ from person to person may be related to the fact that sport is multifaceted (1). Recently, sports has been used as an effective tool in the development of relations between countries through national or international sports organizations and the popularity of sports. The rapid increase in the importance of sport in terms of general, cultural and prestige is considered to be the primary reason for the uplift of the contemporary world (2). Since the earliest times in history, the goal of the sport was to choose the faster and the stronger (3). For this reason, athletes have tried to find ways to achieve success for centuries, to increase sports performance, and to win victories (4) and they have concluded that the desired success has only been achieved by external factors (using doping). In this way, the sportive performance obtained by artificial ways will easily give the athlete the success and victory he/she desires (5). The usage of prohibited substances (Anabolic Agents, Peptide Hormones, Beta-2 Agonists, Hormone and Metabolic Modulators, Diuretics and Masking Agents, Stimulants, Narcotics, Cannabinoids, and Glucocorticoids) and / or methods (Manipulation of Blood and Blood Components, Chemical and Physical Manipulation, and Gene and Cell Doping) for unfairly increase the sportive performance of the athlete is considered to be a crime by the World Anti-Doping Agency (6,7). According to the law of the WADA, established in 1999 by the International Olympic Committee the substances must have the potential to improve the performance, to threat human health or to have the potential to do so in order to be considered as a crime. If the athlete uses two of

these three items on the grounds that it is against the spirit of the sport, the substances used are considered as doping (8). The use of substances to increase sportive performance and artificial power gain are not new. Doping is an old phenomenon that threatens human health and sports ethics as much as the physical struggle. As a result of the usage of substances, not only physical and psychological structures of the athlete be damaged, but also faces the fact that the results can extend to death. In addition to the understanding of champion athletes, it also causes corruption of moral values (5). As a result, modern sports can be said to occur with the completion of the process of transition from amateur to professional (9). When contests are examined in Ancient Greeks, it is stated that the prizes given to athletes represent a religious meaning more than a material gain. The prizes given to the winner are a crown made of an olive branch and the holiness given by the championship. For this reason, sports are mystical and religious values in which physical power competes in ancient Greeks. The only element motivating athletes at that time was spiritual values rather than material values (10,11). Nowadays, athletes who have achieved success in the Olympics are rewarded with gold medals instead of an olive branch, and getting good living conditions to motivates the athlete to win continuously (12). It is stated that the adoption of this understanding may be caused by the intertwining of sports and commercial relations and as a result, negativity is experienced in the sense of sports ethics and Fairplay. The most important reasons for the behaviours against sports ethics are the financial incentives and good conditions of the athletes. Moreover, when sports are in an economic process, corruption is experienced more (13). Doping is not only interested in athletes but also by researchers and politicians. This interest is not only limited to sports ethics and concerns about athletes' health, which is emphasized by WADA, but it is also associated with the spread of athletes to lower levels (8). Most of the studies in the literature showed that the age of using doping as low as the high school-level student-athletes, force training period used as a non-prescription (14), even before the age of 10 (15) and/or at some point of their lives, they obtained the knowledge that they used Anabolic androgen steroid (16,17). Doping has become a growing problem for all countries in the 20th century (9). Increases in the use of unconscious doping in many sports branches are arousing interest. Due to the use of the substances, the sport has moved away from its real purpose and damages the ethics of sports and the amateur spirit. Furthermore, to reveal the level of knowledge of young athletes about doping substances and to determine the reasons for their preference will contribute to the field of sports science. In this context, the aim of this study was to determine the knowledge level and preference reasons for U23 Turkish National Team athletes regarding doping.

Material and Method

Participants

Convenience Sampling Method was used in research. The research group consists of 100 (70 male and 30 female) athletes in the range of 18-23 years, from U23 Turkish National Teams of football, volleyball, karate, wrestling, and athletics.

Collection of Data

A questionnaire consisted of two phases, the validity and reliability (α = 0.797) were tested by Eröz (2007) (18). The first part of the questionnaire consists of questions about the gender of the participants and the second part about the doping knowledge levels and the reasons for their preference. The questionnaire applied to the participants for this study consists of a total of 19 questions 8 of which about the reasons for the preference of doping in sport and 11 of which about to determine the level of knowledge about doping and doping in sport (prohibited substance)".

Statistical Analysis

In addition to the descriptive statistics of the participants, the SPSS package program was used to compare the answers to the questionnaires according to gender. Chi-square test was used to investigate the differences of each dependent variable according to the independent variables. Significance level was accepted as p<,05.

Table 1. Analysis of the results of the athletes about the doping knowledge levels

Variables		Strongly disagree	Disagree	Neither agree or disagree	Agree	Strongly agree	χ^2	p
I have enough knowledge about doping.								
Man	**%**	10	11	16	27	6	3,136	,535
Woman	**%**	2	5	9	9	5		
Total	**%**	12	16	25	36	11		
Doping has great harm to health.								
Man	**%**	1	4	10	26	29	1,188	,880
Woman	**%**	0	1	3	13	13		
Total	**%**	1	5	13	39	42		
Most stimulants (caffeine, cocaine, etc.) are used in sports.								
Man	**%**	4	3	34	15	14	7,063	,133
Woman	**%**	1	2	14	12	1		
Total	**%**	5	5	48	27	15		
Mostly narcotic analgesics (morphine, etc.) are used in sports.								
Man	**%**	7	4	41	10	8	8,076	,089
Woman	**%**	3	7	14	5	1		
Total	**%**	10	11	55	15	9		
Mostly Anabolic-Androgenic steroids are used in sports.								
Man	**%**	3	6	38	16	7	5,171	,270
Woman	**%**	1	5	20	2	2		
Total	**%**	4	11	58	18	9		
Mostly Beta Blockers are used in sports.								
Man	**%**	3	4	46	10	7	11,453	**,022***
Woman	**%**	2	8	16	4	0		
Total	**%**	5	12	62	14	7		
Peptide Hormones (Erythropoietin, Growth Hormone, etc.) are mostly used in sports.								
Man	**%**	4	6	36	18	6	5,116	,276
Woman	**%**	2	5	19	3	1		
Total	**%**	6	11	55	21	7		
Mostly Masking Agents are used in sports.								
Man	**%**	8	8	39	12	3	1,459	,834
Woman	**%**	3	4	18	5	0		
Total	**%**	11	12	57	17	3		
Mostly Cannabinoids (marijuana, cannabis) are used in sports.								
Man	**%**	9	9	34	13	5	2,502	,644
Woman	**%**	5	4	16	5	0		
Total	**%**	14	13	50	18	5		
Mostly corticosteroids are used in sports.								
Man	**%**	8	9	42	9	2	1,295	,862
Woman	**%**	3	3	19	5	0		
Total	**%**	11	12	61	14	2		
Anti-estrogenic activity agents are mostly used in sports.								
Man	**%**	7	11	39	6	7	,969	,914
Woman	**%**	3	3	17	4	3		
Total	**%**	10	14	56	10	10		

***p<0,05**

Result

A total of 100 athletes from different sports branches participated in the study. The chi-square test was used to compare the answers to the questionnaires according to gender.

When the answers given by the athletes about doping information levels are examined, while the answers of the question "*most beta blockers are used in sport*" varies according to gender, the answers of other questions were determined not to differ statistically. In addition, although 47% of athletes have sufficient knowledge about doping and 81% know that doping has great harm to health when the responses are examined, they have been observed to be indecisive about the use of doping drugs. It is thought that this indecision is because of being forbidden in international sports organizations and that they will be penalized as a result of a positive sample but because they know that they can increase the performance with the use of doping at the maximum level.

According to the answers of the athletes in the study, while it was determined that the athletes' Doping preferences do not differ according to gender, it

Table 2. The analysis of the results about the athletes' preference reasons for doping

Variables		Strongly disagree	Disagree	Neither agree or disagree	Agree	Strongly agree	χ^2	p
A successful athlete wants to use doping to achieve higher performance.								
Man	**%**	11	9	15	18	17	5,840	,211
Woman	**%**	4	7	6	11	2		
Total	**%**	15	16	21	29	19		
A successful athlete wants to use doping to eliminate his/her fear of losing.								
Man	**%**	10	11	13	25	11	1,471	,832
Woman	**%**	5	7	6	9	3		
Total	**%**	15	18	19	34	14		
A successful athlete wants to use doping to calm his excitement.								
Man	**%**	21	13	19	11	6	4,845	,304
Woman	**%**	6	6	7	10	1		
Total	**%**	27	19	26	21	7		
A successful athlete wants to use doping because of his desire to win.								
Man	**%**	14	4	17	18	17	7,676	,104
Woman	**%**	5	6	4	11	4		
Total	**%**	19	10	21	29	21		
A successful athlete wants to use doping due to financial support.								
Man	**%**	16	11	12	23	8	1,339	,855
Woman	**%**	4	6	6	10	4		
Total	**%**	20	17	18	33	12		
A successful athlete wants to use doping to provide a social status and maintain this status.								
Man	**%**	14	7	24	15	10	5,439	,245
Woman	**%**	6	4	5	12	3		
Total	**%**	20	11	29	27	13		
A successful athlete wants to use doping to achieve a good standard of living.								
Man	**%**	17	15	21	11	6	1,999	,736
Woman	**%**	6	8	8	7	1		
Total	**%**	23	23	29	18	7		
A successful athlete wants to use doping to get club support.								
Man	**%**	17	10	16	20	7	5,536	,237
Woman	**%**	9	8	2	7	4		
Total	**%**	26	18	18	27	11		

was determined that athletes may use doping substances in order to achieve higher performance, to eliminate the fear of losing, due to his desire to win, to provide financial support and to provide social status and maintain this status.

Discussion

According to the main findings of this study, although 47% of athletes have enough knowledge about doping and 81% of them know that doping has great harm to health when the answers were examined, it was observed that the athletes related to the use of doping substances were generally indecisive. In their research on bodybuilding athletes, Yalnız ve Gündüz (2004) reported that athletes were using doping substances even though they know it had harmful effects (19). In another study, Eröz (2007) examined whether national athletes had enough knowledge about doping, these results were reported "strongly agree with 11.7%", "strongly disagree with a rate of 72.5%" and " neither agree or disagree 12.5%" (17). Ağırbaş (2002), the senior national team athletics in a study carried out to determine the level of knowledge of on doping "Do you know what is doping? " %75 of the answers was "no", 25% of the answer was "yes" (20). Şirin (2001), in his study which aimed to determine the level of knowledge about doping, which was applied to individual and team athletes, it was found that the athletes who said their information about doping sufficient lower than those who stated that their knowledge about doping was insufficient (21). In terms of whether the side effects of doping are known, this result was obtained, "I have less information" with the rate of 18.3% and "I have information" with the rate of 15.4%. These results showed that athletes are not knowledgeable about doping and have only dogmatic information (information heard from others).

Çetinkaya et al. (2007) in his study found that the knowledge and attitudes about doping in the students of the High school of Physical Education and Sports, while 1.4% of the students stated that they received training on doping, others 98.6% of them did not receive any education in this subject (22). Dallı et al. (2004) asked Do you have enough information about the performance-enhancing drug(doping)?', the answers were 62.5% partially, 11.7% "no" and 25.8% "yes" (23). Again, Öztürk et al. (2012) reported that most of the handball players do not have enough information about doping (24). In this respect, the results of the studies in the literature support our research results.

When the answers given to the questions about the doping knowledge and preference reasons of the athletes who participated in the research were examined, it was determined that they may use doping to gain the maximum performance, to have the means of victory. Eröz (2007), was stated that the participants› sports at the national level and from different sports branches (athletes, wrestling, judo and weightlifting); the question "A successful athlete wants to use doping due to his/her desire to win" answered back as "strongly agree with a total of 55.0%", "strongly disagree with 17.5%", and "13.3% of neither agree nor disagree". The answers about "A successful athlete wants to use doping to calm his excitement." was found "with the 15.8% of the strongly agree››, "strongly disagree with 63.3%›› "neither agree or disagree 8.3%››, they have expressed their opinion". Successful athletes want to use doping to provide a social status and maintain this status question, answered like "39.2% of the rate strongly agree", "15% of the rate strongly disagree", and "13.3% of the rate neither agree or disagree". "A successful athlete wants to use doping to achieve a good standard of living" question answered by strongly agree 67.5%, by strongly disagree 16.7%, and neither agree or disagree 7.5% (18).

Özel (1995) state that 65% of the sportsman using doping for extreme desire to win and 47.5% of them using doping because of increasing financial support in his work named researching of weightlifter›s doping using methods and widespread (2). According to Michigan state university's work on university sportsman in 1984, come up that in the last 14 month period %8 of the 2039 participants used amphetamine. This has been reported that while 61% of these participants using this medicine for personal or social causes, 37% of them using to improve performance (25).

Kim and Kim (2017) reported that approximately 50% of Korean national adolescent and adult athletes knew about doping substances for their respective sports in named Korean national athletes' knowledge,

practices, and attitudes of doping: a cross-sectional study. Morever, they found that there is a low level of doping knowledge among athletes (26). Moran et al. (2008) reported that 62.6% of athletes of various nationalities said that they had received information on banned substances in their sport, and 48.8% felt confident with their knowledge (27). Khidir and Mahmoud (2018), found that Sudanese athletes and sports players do not have culture of doping in sports, like in Europe or even those in western Africa (28).

Conclusion

As a result of the research, it was found that the athletes did not have sufficient knowledge level about doping and although they knew doping had great harm to health, they were not clear about the usage of the doping. It is thought that unsteadiness of the preference reasons for doping of the athletes is related to the desire to maximize performance, desire to win excess and satisfactory financial supports. According to these results that awareness events should conduct often to be gained the aim of the sport and sports ethics to sportsman and athlete health, doping and sports ethics.

References

1. Göral M. Determination of reasons of adults making sports in the sports facilities in kutahya province and their view points to sports. 9th International Sports Sciences Congress, 3-5 November 2006, Muğla, Turkey.
2. Özel R. Investigation of doping usage methods and prevalence in weightlifters, [Master Thesis]. Gazi University, Institute of Health Sciences, Ankara, Turkey, 1995.
3. Kargılı H. Sport morality and for the sportsman ote impressions of using drug and laborness of struggle against drug, [Master Thesis]. Dumlupınar University, Institute of Social Sciences, Kütahya, Turkey, 2002.
4. Erdemli A. İnsan,spor ve olimpizm. Sarmal Yayınevi, İstanbul, 1996. [In Turkish]
5. Öngel HB. Sporda etik değerler açısından doping. Gazi Üniversitesi Beden Eğitimi ve Spor Bilimleri Dergisi Yayınları, Ankara, 1997. [In Turkish]
6. Heuberger, J. A., & Cohen, A. F. Review of WADA prohibited substances: limited evidence for performance-enhancing effects. Sports Medicine 2019; 49(4): 525-539.
7. The World Anti-Doping Code, Prohibited List (2019). Retrieved from: https://www.wada-ama.org/sites/default/files/wada_2019_english_prohibited_list.pdf
8. Bloodworth AJ, Petróczi A, Bailey R, Pearce G, McNamee MJ. Doping and supplementation: the attitudes of talented young athletes. Scand J Med Sci Sports 2012; 22(2): 293-301.
9. Atasü T. Yücesir İ. Dopingin tarihçesi, doping ve futbolda performans arttırma yöntemleri. Form Reklâm Hizmetleri, İstanbul, 2004. [In Turkish]
10. Koryürek C. (1996). Olimpiyat Tarihi. Koç-Aktüel Yayınları, İstnabul,1996. [In Turkish]
11. Yesalis CE. Bahrke MS. History of doping in sport. International Sports Studies 2002; 24(1): 42-76.
12. Dinçer N. The determining of knowledge level related to doping in elite athletes, [Master Thesis]. Selçuk University, Institute of Health Sciences, Konya Turkey, 2010.
13. Şahin M. Spor ahlakı ve sorunları. Doğa Basın Yayın Tic. Ltd.Şti, İstanbul, 1998. [In Turkish]
14. Miller KE, Hoffman JH, Barnes GM, Sabo D, Melnick MJ, Farrell MP. Adolescent anabolic steroid use, gender, physical activity, and other problem behaviors. Subst Use Misuse 2005; 40(11): 1637-1657.
15. Stilger VG, Yesalis CE. Anabolic-androgenic steroid use among high school football players. J Community Health 1999; 24(2): 131-145.
16. Yesalis CE, Bahrke MS. Doping among adolescent athletes. Best Pract Res Clin Endocrinol Metab 2000; 14(1): 25-35.
17. Orhan Ö, Çetin E, Ertaş DB. Determining ergogenic aids and doping knowledge and using up status of Gazi university physical education department student. 9th International Sports Sciences Congress, 3-5 November 2006, Muğla/ Turkey.
18. Eröz F. Determination of sportmens knowledge level about doping and ergogenic help and views who have done athleticism, wrestling, judo and barbell at the national level. [Master Thesis]. Dumlupınar University, Institute of Social Sciences, Kütahya, Turkey, 2007.
19. Yalnız İ. Gündüz N. The investigation of the knowledge and the application levels of bodybuilding sportsmen about ergogenic aids in ankara. Gazi Journal of Physical Education and Sport Sciences 2004; 9(2): 33-42.
20. Ağırbaş İ. Atletizm genç milli takımının masaj-sauna-vitamin-mineral-doping üzerine bilgi düzeyleri, spora başlamalarında ve süresince etki eden faktörler. 7th International Sports Sciences Congress, 27-29 September 2002, Antalya, Turkey. [In Turkish]
21. Şirin EF. Knowledge level of sportsmen are determineted and compared about doping in individual and team sports, [Master Thesis]. Selçuk University, Institute of Health Sciences, Konya Turkey, 2001.
22. Çetinkaya G, Ağyar E, Dilbaz KÖ. Beden eğitimi ve spor yüksekokullarındaki öğrencilerin doping konusundaki bilgi düzeyleri ve tutumlarının incelenmesi (Akdeniz üniversitesi örneği). 4th International Mediterranean Sports Sciences Congress, 9-11 November 2007, Antalya, Turkey. [In Turkish]
23. Dallı M, Işıkdemir E, Bingöl E. Determination of doping knowledge level of physical education and sports college

students. International Journal of Science Culture and Sport 2004; 2(Special Issue 2): 11-20.
24. Öztürk EG, Suveren S, Çolakoğlu T. Doping in Turkey, assessment of knowledge level of players about doping (handball study case). Journal of Human Sciences 2012; 9(1): 249-260.
25. Kurdak SŞ. Sporda doping ve ilaç kullanımı. 1. Baskı, Sporsal Kuram Dizisi–7, Ankara, 1996. [In Turkish]
26. Kim T, Kim YH. Korean national athletes' knowledge, practices, and attitudes of doping: a cross-sectional study. Subst Abuse Treat Prev Policy, 2017; 12(1): 7.
27. Moran A, Guerin S, Kirby K, MacIntyre T. The development and validation of a doping attitudes and behaviour scale. World Anti-Doping Agency & The Irish Sports Council, 2008.
28. Khidir AM, Mahmoud AA. Doping awareness, and knowledge among sudanese athletes and sports players, World J Pharm Pharm Sci, 2018; 7(2): 1410-1417.

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Original article

The effects of dietary folate and iron supplementation on restless legs and preeclampsia in pregnancy

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Summary. *Introduction:* RLS in pregnant women at the beginning of the condition increases the risk of reduced sleep quality, systemic arterial pressure increase, iron and folate deficiency anemia. The most common causes are thought to be physical changes, decreased sleep quality, or organic factors (decreasing folate and iron level). *Aim:* The aim of this study was to evaluate the role of RLS and its interaction with preeclampsia, anemia, folate deficiency, and low sleep quality. *Methods:* Our study was conducted between July 2018 and February 2019 in Trakya University Medical Faculty Hospital, Obstetrics Department. Pregnant women with symptoms related to RLS were investigated in terms of sleep quality and preeclampsia. An interview form was completed with the pregnant women during face-to-face interviews. Sociodemographic characteristics were questioned in this form. The IRLSGG criteria were used for RLS evaluations. The sleep quality of the participants was assessed using the PSQI. The iron status of pregnant women was questioned. Pregnant women were asked whether they received prophylactic iron medication. *Results:* Three hundred twenty-four volunteer pregnant women who presented to the obstetrics clinic of Trakya University Medical Faculty Hospital were included in the study. The mean age of the pregnant women in the study was 29.18±6.19 years. There was no statistically significant difference when we compared pregnant women with and without RLS when asked about their BMI in pre-gestational periods. The average use of iron medication of the women with RLS was 3 days or less per week. There was a significant difference between women with and without RLS regarding iron prophylaxis. The evaluation of the relationship between RLS, sleep quality, and preeclampsia in the pregnant women showed that RLS and PSQI levels had statistically significant differences according to trimesters. *Discussion:* Our study evaluated the frequency of RLS, the relationship between RLS and preeclampsia, and the relationship between RLS and sleep quality in pregnant women. RLS is more common in pregnant women who do not receive iron support. Low iron levels contribute to the development of RLS. Clinical and laboratory (hemoglobin) analysis revealed some differences between the groups. As the hemoglobin levels and iron supplementation decreased, the incidence of RLS symptoms was found to increase. For the treatment of RLS, non-pharmacologic treatments in pregnant women should be considered first; however, the use of iron medication is usually recommended. After the iron requirement is met, additional treatment planning should be made by investigating whether the RLS symptoms have regressed. Therefore, it was concluded that RLS was related to BMI and hemoglobin level differences. During pregnancy, the recommended dietary allowance (RDA) for folate is 600 μg/day of dietary folate equivalent. Natural folate with foods and folate supplementation since pregnancy may help to prevent fetal morbidity. The major sources of dietary folate are citrus fruits and juices, legumes, whole-wheat bread, and green leafy vegetables. To prevent fetal morbidities, women planning childbirth or pregnant should consume 400 μg per day of synthetic folic acid from natural foods (cereals and other grains), or supplement drugs. Pregnant women may need advice from a physician or a qualified dietetics professional to follow nutritional guidelines, especially for folate and iron. Pregnant women; must be provided a wide range of nutrition quality and evaluation. We suggest that more studies are needed to assess the relationship

between low quality of sleep, iron and folate supports in nutrition, RLS symptoms, and/or preeclampsia. *Conclusion:* Our study demonstrates the need to establish quality care and interventions for the protection of both maternal and fetal health due to poor sleep quality and RLS symptoms during pregnancy. Pregnant women should be presented with a variety of evidence-based patient care interventions. In the presence of RLS, signs of systemic arterial hypertension and iron supplementation in pregnant women should be examined carefully and if necessary, pregnancy interventions should be added.

Key words: mineral content; calcium, magnesium, zinc, bone, DRI

Introduction

Restless leg syndrome (RLS) is a disorder characterized by an irresistible impulse to move the legs. In the literature, the prevalence of RLS in the population was found as 2-10%. RLS is twice as common in women as men. The risk of occurrence increases in pregnant women compared with the normal female population, increasing 2 to 3 times in pregnant women, affecting 15-25% of pregnant women (1, 2). The prevalence varied from 11% to 30% in studies performed in pregnant women from different societies (3-6). Early detection of severe RLS is very important to prevent maternal discomfort, poor sleep, and possible health risks.

The reason for the increased incidence of RLS during pregnancy is still not fully understood. RLS in pregnant women at the beginning of the condition increases the risk of reduced sleep quality, systemic arterial pressure increase, anxiety level increase, and iron deficiency anemia (2). In addition, symptoms develop with movement and lead to increased symptoms in the evening. Additional diagnostic criteria of RLS include symptoms presenting when people are not moving, and a progressive urge to move the legs.

Pregnancy is reported as a risk factor in the initiation and progression of RLS symptoms. The majority of affected pregnant women have not previously experienced RLS and almost all patients with pre-existing RLS had worsened symptoms during pregnancy (2, 7). Causes of RLS in pregnancy are various. According to the literature, the most common causes are thought to be hormonal factors (estrogen, progesterone), psychomotor factors, physical changes, decreased sleep quality, anxiety or organic factors (decreasing folate and iron level) (8-10). In most pregnant women, RLS often occurs in the last trimester. The presence of RLS in pregnancy is often a risk factor for chronic RLS that will develop later. Symptoms often disappear in the 6 to 18 weeks after birth. Early detection of severe RLS is important in pregnant women. Early intervention is very important to prevent discomfort, poor sleep quality, and possible health risks in pregnant women. RLS can significantly affect maternal health; it is a common disorder that can lead to negative consequences in the short and long term. RLS in pregnancy causes negative fetal and maternal effects (8, 11).

Many studies in the literature evaluated sleep quality using the Pittsburgh Sleep Quality Index (PSQI) in pregnant women (10, 12). 14-27% of pregnant women have low sleep quality. The decrease in sleep quality as measured using the PSQI during pregnancy is associated with a number of negative outcomes related to maternal health and fetal well-being. A reduction of sleep quality in pregnant women may increase the likelihood of premature birth. Low sleep quality leads to increased levels of systemic inflammation and is associated with shorter pregnancy duration. Pregnant women who have low sleep quality are more likely to have a caeserean section. Poor sleep quality is a potential risk factor for depression both before and after the birth (13-16).

Hypertension in pregnancy and preeclampsia are the leading causes of maternal and fetal mortality and morbidity worldwide. With preeclampsia there is an increased incidence of placental abruption, fetal growth restriction, and preterm births. Due to its potential severity and frequent occurrence, early diagnosis, knowledge of related parameters, and appropriate management are essential (11, 17).

The aim of this study was to evaluate the role of

RLS and its interaction with preeclampsia, anemia, folate deficiency, and low sleep quality.

Methods

Our study was conducted between July 2018 and February 2019 in Trakya University Medical Faculty Hospital, Obstetrics and Gynecology Department. Pregnant women with symptoms related to RLS were investigated in terms of sleep quality and preeclampsia. The recommendations of the International Restless Legs Study Group (IRLSSG) were considered in the performance of our study (11, 17).

The participants were informed about the study, after which each provided written informed consent. An interview form was completed with the pregnant women during face-to-face interviews. Sociodemographic characteristics (age, height, body weight, education, number of previous pregnancies) were questioned in this form. The IRLSGG criteria were used for RLS evaluations (11). Pregnancy hypertension was defined according to the American College of Obstetricians and Gynecologists (ACOG) criteria (15, 16). The sleep quality of the participants was assessed using the PSQI. The validity and reliability of the PSQI as a sleep assessment tool has been proven in the literature (13, 14).

The International Restless Legs Syndrome (IRLS) scale was evaluated using the four main criteria developed by the IRLSSG. The IRLS provides a good indication of the current situation in individuals, and is useful for clinical practice. The IRLS is a reliable, 10-question rating tool that has been adopted in the international literature (11, 18). The IRLS scale comprises questions about the typical symptoms of RLS; the scale is graded 0-4 on each of the typical symptoms of RLS and consists of 10 questions. In the evaluation, 0-10 points are mild, 11-20 points are in the middle, 21-30 points are serious, and 31-40 points indicate severe RLS. The literature on RLS in pregnancy supports the use of the IRLS scale in pregnant women (19-21). The IRLSGG criteria can be used during pregnancy. The most important factor suggesting the appropriateness of the of IRLSGG criteria in pregnancy is that the symptoms of RLS associated with pregnancy and idiopathic RLS are similar. A familial predisposition and the similarity of symptoms are common features of both. In addition, pregnancy is considered as an aggravating factor in families predisposed to RLS (22).

Patients with symptoms of leg cramps, venous stasis, leg edema, and stress were excluded from the study due to the risk of being confused with symptoms of RLS. Women with pre-pregnancy RLS symptoms or those with chronic hypertension were not included in the study.

To evaluate sleep quality, the PSQI was used, which is a commonly used instrument to evaluate sleep. It includes 19 questions and 7 clinical themes. It includes subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medication and daytime sleepiness (12, 23). A PSQI total score equal to or greater than 5 shows poor sleep quality with 90% sensitivity and 67% specificity. The PSQI can be used safely in the examination of sleep quality in pregnant women and mothers in the first six months after birth (14, 24).

The iron status of pregnant women was questioned. Pregnant women were asked whether they received prophylactic iron medication (at least one dose per day at least 5 times in a week).

After evaluation the pregnant women in our study, if we detected a risk for RLS we reported this to the clinic's physicians and nurses. Pregnant women with symptoms of RLS were contacted during the postpartum period to determine whether there was regression in RLS findings.

The study was approved by the Ethics Committee of Scientific Research of Trakya University Faculty of Medicine (Decision No: 2018-343-21). Official permission was obtained from the management of Trakya University Medical Faculty. Written informed consents were obtained from the participants of this study.

All statistical analyses were performed using the SPSS 21.0 package program. Normal distribution of the data was tested using the Shapiro-Wilk test. Bivariate group comparisons were performed using Student's t-test and the Mann-Whitney U test. The data are summarized with appropriate descriptive statistics. Mean and standard deviation were evaluated for numerical variables, and frequency and percentage were evaluated for categorical variables. One-way analysis of variance

(ANOVA) was used for the comparison of multiple groups. The Chi-square test was used for the associations between categorical variables. Level of significance for all statistical analyses was accepted as 5%.

Results

Three hundred twenty-four volunteer pregnant women who presented to the obstetrics clinic of Trakya University Medical Faculty Hospital were included in the study.

The sociodemographic and clinical characteristics in pregnant women and presence of preeclampsia of mothers with and without RLS symptoms were compared.

The mean age of the pregnant women in the study was 29.18±6.19 (range, 18-45) years. The mean age of patients with no RLS symptoms was 28±6.27 years. The mean age of patients with RLS symptoms was 30.04±5.90 years (Table 1). The body mass index (BMI) of pregnant women with RLS was 33.6 kg/m^2. The mean BMI of pregnant women with no RLS symptoms was 30.90 kg/m^2 *(p= 0.011)*. There was no statistically significant difference between the two groups in terms of BMIs in pregnant women with and without RLS. The mean Hgb (hemoglobin) of the pregnant women with RLS was 11.21 g/dL (±1.75), and the average use of iron medication was 3 days or less per week. The mean values of those without RLS symptoms were as follows: Hgb 11.71 g/dL (±1.55); the use of iron prophylaxis was 4 days or more per week. There was a significant difference between women with and without RLS regarding hemoglobin levels, but it was not clinically important. Low hemoglobin was more frequent in patients with RLS findings and iron prophylaxis was less frequent (Table 2).

Among the patients with RLS and without RLS symptoms, there was a significant difference in the level of alanine aminotransferase (ALT) *(p= 0.002)* (Table 3). Women with RLS (n=90) were compared with pregnant women without RLS (n=234) and there was no difference between the groups in terms of PSQI, aspartate transaminase (AST), and number of child births. However, pregnant women with RLS generally scored higher on the PSQI than women without RLS.

The evaluation of the relationship between RLS, sleep quality, and preeclampsia in the pregnant women showed that RLS and PSQI levels had statistically significant differences according to trimesters *(p=0.019 and p<0.001)* (Table 4). As the trimesters of pregnancy progressed, the incidence of RLS symptoms increased and sleep quality decreased. There were statistical differences between women with preeclampsia and those with RLS. Symptoms of preeclampsia and RLS increased as the trimesters of the participants advanced. Patients with RLS symptoms had more frequent preeclampsia findings (Table 5).

It was observed that 27.77% of the pregnant women met the RLS diagnostic criteria. The mean IRLSSG score of the pregnant women in our study was 15.59. RLS symptoms in previous pregnancies were examined and 31% of all the pregnant women reported having RLS previously.

Discussion

Our study evaluated the frequency of RLS, the relationship between RLS, preeclampsia and sleep quality in pregnant women. In this respect, it is among the first of its kind in the literature. RLS is a frequently encountered disorder in pregnant women but it remains unrecognized and undiagnosed.

Our rate of RLS in pregnancy was 27.7%, which is consistent with data in the literature, citing 11-30%. In recent studies, the frequency of RLS in the last trimester of pregnancy has been shown to increase to 36% (4, 12, 25). It was determined that the severity and frequency of RLS increased in the last months of pregnancy. Among the pregnant women in our study, the difference between the second and third trimesters was significant in terms of RLS *(p= 0.019)*.

RLS is more common in pregnant women who do not receive iron support. Low iron levels contribute to the development of RLS. However, there may be some differences. RLS may occur in patients with preeclampsia without low iron levels. The mean serum ferritin concentration and hemoglobin levels may be higher in patients with preeclampsia than in healthy pregnant women (26, 27). Clinical and laboratory (hemoglobin) analysis revealed some differences between the groups.

Table 1. RLS and some differences in pregnant women

Mean (SD)	Parameters	Restless Legs Syndrome (-)	Restless Legs Syndrome (+)	*p*
	Age	28.00 (6.27)	30.04 (5.90)	*0.123**
	BMI[1]	30.90 (7.12)	33.06 (5.51)	0.011*
	Hgb[2]	11.21 (1.75)	11.71 (1.55)	0.013*

BMI[1]: Body Mass Index; Hgb[2]: Hemoglobin; *statistical significance

Table 2. Comparison of pregnant women with and without restless leg symptoms

Paramaters	Range	Restless Legs Syndrome (-)	Restless Legs Syndrome (+)	p
Folate intake	≥ 400 mcg/ day	193 (82.5%)	65 (72.2%)	*0.040**
	< 400 mcg/ day	41 (17.5%)	25 (27.8%)	
Leukocyte	high	192 (82.1%)	83 (92.2%)	*0.022**
	normal	42 (17.9%)	7 (7.8%)	
Preeclampsia	Available	220 (94%)	83 (92.2%)	*<0.001**
	No	14 (6%)	7 (7.8%)	
Taking iron prophylaxis	insufficient	77 (32.9%)	45 (50%)	*0.004**
	Sufficient	157 (67.1%)	45 (50%)	

*statistical significance

Table 3. Some differences according to the RLS symptoms in terms of PSQI, AST, and number of child births.

RLS symptoms	Percentiles	ALT	AST	Number of child births	PSQI
RLS (-) (n= 234)					
	25	8.75	16.00	1.00	3.00
	50	12.00	20.00	2.00	5.00
	75	15.00	24.00	3.00	10.00
RLS (+) (n= 90)					
	25	9.00	15.00	1.00	3.00
	50	14.00	20.00	2.00	8.00
	75	36.25	30.00	3.00	13.00
P		*0.002**	*0.513*	*0.404*	*0.174*

*statistical significance; ALT: Alanine aminotransferase, AST: Aspartate transaminase, RLS: Restless legs syndrome, PSQI: Pittsburgh Sleep Quality Index

Table 4. Evaluation of the relationship between restless leg syndrome, sleep quality and preeclampsia in pregnant women in our study, according to gestational weeks

Parameters	1st trimester n (%)	2nd trimester n (%)	3rd trimester n (%)	Total N (%)	*P*
Restless legs syndrome (RLS)					*0.019**
Present	2 (2.2%)	7 (8.8%)	81 (90%)	90 (100%)	
Absent	23 (9.8%)	31 (13.2%)	180 (76.9%)	234 (100%)	
Sleep quality disorder (PUKI)					*<0.001**
>6 (unwell)	2 (8%)	23 (60.5%)	148 (56.7%)	173 (53.3%)	
≤5 (good)	23 (92%)	15 (39.5%)	113 (43.3%)	151 (46.6%)	
Total	25 (100%)	38 (100%)	261 (100%)	324 (100%)	

*statistical significance; RLS: Restless legs syndrome, PSQI: Pittsburgh Sleep Quality Index

Table 5. Preeclampsia and restless leg syndrome evaluation in the participants

Parameters		1st trimester n (%)	2nd trimester n (%)	3rd trimester n (%)	Total N (%)	*P*
Pre-eclampsia (-)	Restless leg syndrome (-)	23 (92%)	30 (78.9%)	167 (64%)	220	*0.067*
	Restless leg syndrome (+)	2 (8%)	6 (15.8%)	70 (26.8%)	78	
Pre-eclampsia (+)	Restless leg syndrome (-)	0	1	13	14	
	Restless leg syndrome (+)	0	2 (53.%)	24 (20.9%)	26	
	Total	25 (100%)	38 (100%)	261 (100%)	324 (100%)	

As the hemoglobin levels and iron supplementation decreased, the incidence of RLS symptoms was found to increase *(p=0.013; p= 0.004)*. Iron deficiency in pregnant women should be eliminated and re-evaluated. For the treatment of RLS, non-pharmacologic treatments in pregnant women should be considered first; however, the use of iron medication is usually recommended. After the iron requirement is met, additional treatment planning should be made by investigating whether the RLS symptoms have regressed. Therefore, it was concluded that RLS was related to BMI and hemoglobin level differences. Anemia and iron deficiency increase the tendency to sleep in pregnant women. However, more extensive studies are needed.

During pregnancy, the recommended dietary allowance (RDA) for folate is 600 μg/day of dietary folate equivalents (28, 29). Natural folate with foods and folate supplementation since pregnancy may help to prevent fetal morbidity. The major sources of dietary folate are citrus fruits and juices, legumes, whole-wheat bread, and green leafy vegetables. To prevent fetal morbidities, women planning childbirth or pregnant women should consume 400 μg per day of synthetic folic acid from natural foods (cereals and other grains), or supplement drugs. Research indicates that abnormal folate metabolism and malnutrition may also play a role in birth defects (30).

RLS is usually associated with insomnia and sometimes excessive daytime sleepiness (31). It was determined that the frequency of RLS increased in pregnant women with poor sleep quality. In some (53.3%) patients with RLS, sleep quality was low, as measured using the PSQI. Also, according to the trimesters, pregnant women with RLS expect more sleep problems. We found worse sleep quality in the third trimester than in the second trimester. The mean PSQI score worsened as the gestational week progressed *(P<0.001)*.

However, RLS symptoms and PSQI levels, independent from trimester, was not found statically significant. The median PSQI score was 8 (3-13) in RLS (+) pregnant women. The median PSQI score was 5 (range, 3-10) in RLS (-) pregnant women *(p=0.174)*.

The discussion regarding the PSQI cut-off value in pregnant women continues in the literature. In a recent meta-analysis, the PSQI score in pregnancy was 6.07, and 45.7% of pregnant women were reported to have a PSQI score of 5 or more, expressing poor sleep quality. The results of this study show that the average PSQI score of the pregnant women increased by 1.68 points from the second trimester to the third trimester (12, 32). In our study, the findings showed that low sleep quality was common in pregnant women. In order to preserve maternal and fetal health, it is recommended to give importance to sleep quality and to add to health services.

The findings of our study show that future research should determine whether higher global cut-off scores are more appropriate in pregnancy. Our study of the PSQI in pregnant women shows the necessity of providing different cut-off values for variables such as gestational age, the sociocultural characteristics of pregnant women, and the presence of comorbid RLS symptoms.

Although the data were limited in our study, there was no specific sociodemographic differences between the number of child births and patients with and without RLS symptoms *(p= 0.404)*.

When the literature is examined, it is shown that preeclampsia has increased in the same direction with RLS in various countries (30, 33, 34). There are different hypotheses that the incidence of preeclampsia development tends to increase with RLS. One of the most commonly held is that hypertension and increased sympathetic activation causes disturbances in blood pressure regulation, which converge in RLS and preeclampsia. Increased systemic arterial pressure is

seen in both RLS and preeclampsia (16). Our study shows that there is a positive relationship between RLS and preeclampsia during pregnancy. Although not statistically significant, it presents evidence that there is an increasing trend in preeclampsia and RLS with increased trimesters *(p= 0.067)*. More pregnant women with RLS had more preeclampsia than non-RLS pregnant women.

Factors that contribute to the reduction of sleep quality during pregnancy should be evaluated in pregnant women, especially when they attend healthcare facilities for routine checks.

Pregnant women may need advice from a physician or a qualified dietetics professional to follow nutritional guidelines, especially for folate and iron. Pregnant women; must be provided a wide range of nutrition quality and evaluation.

We suggest that more studies are needed to assess the relationship between low quality of sleep, iron and folate supports in nutrition, RLS symptoms, and/or preeclampsia. Care should be taken with regard to the development of preeclampsia in pregnant women with RLS symptoms.

We recommend further research on how to distinguish pregnant women in need of care in order to preserve maternal and fetal health during pregnancy.

Limitations

Our study had some limitations. Pregnant women who had RLS symptoms were referred to the clinical responsible health care providers. However, their treatment process could not be followed because it was not initiated by the responsible clinicians; without different consultations for women's symptoms.

Additional imaging and laboratory evaluations for sleep disturbances could not be performed because the clinician was not authorized.

Iron prophylaxis was evaluated, but serum iron, folate, ferritin laboratory values were not because the women could not be evaluated for them in the clinic by their physicians. The diagnosis of preeclampsia was made according to diagnostic criteria; however, no detailed data were available regarding the medical treatments used for preeclampsia.

Conclusion

Our study demonstrates the need to establish quality care and interventions for the protection of both maternal and fetal health due to poor sleep quality and RLS symptoms during pregnancy. RLS findings and assessment of sleep quality should be included in the follow-up evaluations of pregnant women. Pregnant women should be presented with a variety of evidence-based patient care interventions.

In the presence of RLS, signs of systemic arterial hypertension and iron supplementation in pregnant women should be examined carefully and if necessary, pregnancy interventions should be added. Care should be taken in terms of preeclampsia in the presence of RLS symptoms.

References

1. Allen RP, Walters AS, Montplaisir J, Hening W, Myers A, Bell TJ, et al. Restless legs syndrome prevalence and impact: REST general population study. Archives of internal medicine. 2005;165(11):1286-92.
2. Manconi M, Govoni V, De Vito A, Economou NT, Cesnik E, Mollica G, et al. Pregnancy as a risk factor for restless legs syndrome. Sleep medicine. 2004;5(3):305-8.
3. Picchietti DL, Hensley JG, Bainbridge JL, Lee KA, Manconi M, McGregor JA, et al. Consensus clinical practice guidelines for the diagnosis and treatment of restless legs syndrome/Willis-Ekbom disease during pregnancy and lactation. Sleep medicine reviews. 2015;22:64-77.
4. Alves DAG, de Carvalho LBC, de Morais JF, do Prado GF. Restless legs syndrome during pregnancy in Brazilian women. Sleep medicine. 2010;11(10):1049-54.
5. Panvatvanich S, Lolekha P. Restless Legs Syndrome in Pregnant Thai Women: Prevalence, Predictive Factors, and Natural Course. Journal of Clinical Neurology. 2019;15(1):97-101.
6. TelaroviŞS, ŞəndiŞL. Frequency of iron deficiency anemia in pregnant and non-pregnant women suffering from restless legs syndrome. Hematology. 2019;24(1):263-7.
7. Trotti LM, Becker LA. Iron for the treatment of restless legs syndrome. Cochrane Database of Systematic Reviews. 2019(1).
8. Chen S-J, Shi L, Bao Y-P, Sun Y-K, Lin X, Que J-Y, et al. Prevalence of restless legs syndrome during pregnancy: a systematic review and meta-analysis. Sleep medicine reviews. 2018;40:43-54.
9. Dunietz GL, Lisabeth LD, Shedden K, Shamim-Uzzaman QA, Bullough AS, Chames MC, et al. Restless legs syndrome and sleep-wake disturbances in pregnancy. Journal of Clinical Sleep Medicine. 2017;13(07):863-70.

10. Morker M, Gossett D, McKnight T, Patel B, Patel M, Attarian H. Serum folic acid level and its relationship to gestational willis ekbom disease (Restless legs syndrome): A Pilot study. Journal of Reproductive Medicine. 2017;62(6):593-7.
11. Allen RP, Picchietti DL, Auerbach M, Cho YW, Connor JR, Earley CJ, et al. Evidence-based and consensus clinical practice guidelines for the iron treatment of restless legs syndrome/Willis-Ekbom disease in adults and children: an IRLSSG task force report. Sleep medicine. 2018;41:27-44.
12. Sedov ID, Cameron EE, Madigan S, Tomfohr-Madsen LM. Sleep quality during pregnancy: A meta-analysis. Sleep medicine reviews. 2018;38:168-76.
13. Manzar MD, BaHammam AS, Hameed UA, Spence DW, Pandi-Perumal SR, Moscovitch A, et al. Dimensionality of the Pittsburgh Sleep Quality Index: a systematic review. Health and quality of life outcomes. 2018;16(1):89.
14. Qiu C, Gelaye B, Zhong Q-Y, Enquobahrie DA, Frederick IO, Williams MA. Construct validity and factor structure of the Pittsburgh Sleep Quality Index among pregnant women in a Pacific-Northwest cohort. Sleep and Breathing. 2016;20(1):293-301.
15. Practice ACoO. ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. American College of Obstetricians and Gynecologists. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2002;77(1):67.
16. Allen RP, Picchietti D, Hening WA, Trenkwalder C, Walters AS, Montplaisi J. Restless Legs Syndrome Diagnosis and Epidemiology workshop at the National Institutes of Health; International Restless Legs Syndrome Study Group. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. Sleep Med. 2003;4(2):101-19.
17. Allen RP, Picchietti DL, Garcia-Borreguero D, Ondo WG, Walters AS, Winkelman JW, et al. Restless legs syndrome/Willis–Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group (IRLSSG) consensus criteria–history, rationale, description, and significance. Sleep medicine. 2014;15(8):860-73.
18. Xu X-M, Liu Y, Jia S-Y, Dong M-X, Cao D, Wei Y-D. Complementary and alternative therapies for restless legs syndrome: An evidence-based systematic review. Sleep medicine reviews. 2018;38:158-67.
19. Shang X, Yang J, Guo Y, Ma S, Jia Z, Xue R. Restless legs syndrome among pregnant women in China: prevalence and risk factors. Sleep and Breathing. 2015;19(3):1093-9.
20. Neau J-P, Porcheron A, Mathis S, Julian A, Meurice J-C, Paquereau J, et al. Restless legs syndrome and pregnancy: a questionnaire study in the Poitiers District, France. European neurology. 2010;64(5):268-74.
21. Plancoulaine S, Flori S, Bat-Pitault F, Patural H, Lin J-S, Franco P. Sleep trajectories among pregnant women and the impact on outcomes: a population-based cohort study. Maternal and child health journal. 2017;21(5):1139-46.
22. Cesnik E, Casetta I, Turri M, Govoni V, Granieri E, Strambi LF, et al. Transient RLS during pregnancy is a risk factor for the chronic idiopathic form. Neurology. 2010;75(23):2117-20.
23. Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry research. 1989;28(2):193-213.
24. Zhong Q-Y, Gelaye B, Sánchez SE, Williams MA. Psychometric properties of the Pittsburgh Sleep Quality Index (PSQI) in a cohort of Peruvian pregnant women. Journal of Clinical Sleep Medicine. 2015;11(08):869-77.
25. Ramirez J, Cabrera S, Hidalgo H, Cabrera S, Linnebank M, Bassetti C, et al. Is preeclampsia associated with restless legs syndrome? Sleep medicine. 2013;14(9):894-6.
26. Siddiqui IA, Jaleel A, Al Kadri HM, Al Saeed W, Tamimi W. Iron status parameters in preeclamptic women. Archives of gynecology and obstetrics. 2011;284(3):587-91.
27. Uglane MT, Westad S, BACKE B. Restless legs syndrome in pregnancy is a frequent disorder with a good prognosis. Acta obstetricia et gynecologica Scandinavica. 2011;90(9):1046-8.
28. Cuskelly GJ, McNulty H, Scott JM. Effect of increasing dietary folate on red-cell folate: implications for prevention of neural tube defects. The Lancet. 1996;347(9002):657-9.
29. Kaiser L, Allen LH. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. J Am Diet Assoc. 2008;108(3):553-61.
30. Wilson RL, Gummow JA, McAninch D, BiancoŞMiotto T, Roberts CT. Vitamin and mineral supplementation in pregnancy: evidence to practice. Journal of Pharmacy Practice and Research. 2018;48(2):186-92.
31. Kallweit U, Siccoli MM, Poryazova R, Werth E, Bassetti CL. Excessive daytime sleepiness in idiopathic restless legs syndrome: characteristics and evolution under dopaminergic treatment. European neurology. 2009;62(3):176-9.
32. Tomfohr LM, Buliga E, Letourneau NL, Campbell TS, Giesbrecht GF. Trajectories of sleep quality and associations with mood during the perinatal period. Sleep. 2015;38(8):1237-45.
33. Innes KE, Kandati S, Flack KL, Agarwal P, Selfe TK. The relationship of restless legs syndrome to history of pregnancy-induced hypertension. Journal of Women›s Health. 2016;25(4):397-408.
34. Wilson DL. Sleep-disordered breathing in gestational hypertension and preeclampsia: impact on maternal and fetal outcomes 2018.

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Original article

Anthropometric and metabolic parameters in relation to high sensitivity C-reactive protein in Montenegrin population with type 2 diabetes

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Summary. *Background/Aim:* High sensitivity C-reactive protein (hsCRP) in widely recognized inflammation marker associated with increased cardiovascular disease (CVD) risk. Since CVD is a common complication in type 2 diabetes (DM2), and since there is a high prevalence of obesity and DM2 in Montenegro, we aimed to examine the association of hsCRP with anthropometric and metabolic parameters in the cohort of individuals with DM2. Additionally, we aimed to examine the gender difference in CVD risk as determined with hsCRP levels. *Methods:* A total of 184 participants with DM2 (of them 47.3% females) were recruited in this cross-sectional study. Fasting glucose, glycated hemoglobin, lipid parameters and hsCRP were measured. Anthropometric parameters were obtained. Participants were stratified into low (hsCRP <1 mg/L), intermediate (1 mg/L≤ hsCRP <3 mg/L) and high risk CVD category subgroup (hsCRP ≥3 mg/L). *Results:* Significantly higher number of females were in the high hsCRP subgroup, compared with males (χ^2=12.80, p<0.001). Also, significantly higher number of obese individuals were in the high risk subgroup compared with low risk subgroup (χ^2= 18.68, p<0.001). Multiple linear regression analysis showed that waist circumference (WC) (Beta=0.205, p=0.045) and glucose (Beta=0.305, p=0.003) in males, and WC in females (Beta=0.405, p=0.003), were the independent predictors of hsCRP levels. *Conclusion:* Females exhibited higher CVD risk than males, as measured with hsCRP. Also, unlike some other anthropometric indices, WC is independently associated with hsCRP in both gender, suggesting that this simple parameter could be a reliable and cost-effective tool for evaluating CVD risk in population with DM2.

Key words: C-reactive protein, diabetes, inflammation, obesity, waist circumference

Introduction

Inflammation is regarded to be the underlying pathophysiological mechanism in many metabolic disturbances such as metabolic syndrome (1), non-alcoholic fatty liver disease (2), polycystic ovarian syndrome (3), type 2 diabetes mellitus (DM2) (4), and cardiovascular disease (CVD) (5). Since obesity is an established risk factor for the occurrence of all these disorders, it is suggested that inflammation may be the link between hyperthrophied adipose tissue and a broad spectrum of metabolic disturbances (6, 7).

Namely, it was shown that adipose tissue is an endocrine organ that secrets a wide range of pro-inflammatory cytokines and adipokines, thus leading to increased chronic low-grade inflammation state (6). Inflammation may, at the same time, be a contributing factor to both, insulin resistance and endothelial dysfunction, which may also explain the high risk of CVD in individuals with diabetes (6).

Although males are regarded to have a greater risk of CVD than females (8), some studies report that this gender difference gets lost in patients with DM2 (9, 10). In addition, considering the fact that majority of indi-

viduals with DM2 are with overweight or obesity (11), it is not well elucidated which one anthropometric parameter could be the best predictor of unfavorable cardiometabolic profile given the contradictory results of various studies in different population groups (12-15).

Since there is a high prevalence of obesity and DM2 in Montenegro (7, 16), we aimed to examine the association of high sensitivity C-reactive protein (hsCRP) with anthropometric and metabolic parameters in the cohort of Montenegrin adults with DM2. Additionally, we aimed to examine the gender difference in CVD risk as determined with hsCRP levels.

Materials and Methods

Study population

A total of 184 individuals with DM2 (of them 47.3% females) who volunteered to participate in this cross-sectional study were enrolled. Patients with DM2 were consecutively recruited by the endocrinologist in the Primary Health Care Center in Podgorica, Montenegro, for their regular check-up in a period from March 2018 to September 2018.

Inclusion criterion for participation in the study was DM2. Diabetes cases were defined using criteria of American Diabetes Association (17).

Exclusion criteria were: participants with a previous history of CVD, type 1 diabetes mellitus, acute inflammatory disease, hsCRP > 10 mg/L, malignancy, hepatic disease other than steatosis, kidney disease except for diabetic nephropathy, pregnancy, alcohol consumption, usage of anti-inflammatory medicines in the last 6 months, as well as participants who were unwilling to enter the study.

All the participants provided written informed consent. The study protocol was approved by the Institutional Review Board of Primary Health Care Center in Podgorica, Montenegro and the research was carry out following the principles of the Declaration of Helsinki.

Anthropometric measurements

Basic anthropometric measurements: waist circumference (WC) (cm), body height (cm), and body weight (kg) were obtained, and body mass index (BMI) was calculated.

Participants with $18.5 \leq$ BMI < 25 kg/m^2, $25 \leq$ BMI <30 kg/m^2 and BMI ≥ 30 kg/m^2, were regarded as subjects who were normal weight, subjects with overweight and with obesity, respectively. There were no underweight participants in our study (i.e., BMI < 18.5 kg/m^2).
Skinfold (SF) thicknesses were measured on four sites with a Harpenden calliper (i.e., at biceps, triceps, iliac, and subscapular sites). All readings were measured to the nearest mm and by the same examiner.

Biochemical analyses

The blood samples were taken between 7-9 hours a.m., after at least 12 hours of fast. Samples were centrifuged at 3000 rpm for 10 minutes, after clotting for 30 minutes. Serum samples were then immediately analyzed. Another aliquot was collecting as a whole blood in K_2EDTA for determination of glycated hemoglobin (HbA1c). Levels of hsCRP and Hba1c were determined immunoturbidimetrically (Roche Cobas c501 analyzer, Mannheim, Germany), whereas levels of glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), and triglycerides (TG), were measured spectrophotometrically on the same analyzer. Low density lipoprotein cholesterol (LDL-c) was calculated using Friedewald formula, as follows: LDL-c = TC - HDL-c - TG / 2.2 (18). Non-HDL-c was calculated as follows: Non-HDL-c = TC - HDL-c. Additionally, TG/HDL-ratio was calculated, also.

Since we aimed to test the association of anthropometric and metabolic parameters with the hsCRP, as well as with CVD risk, all participants were stratified into low (hsCRP <1 mg/L), intermediate (1 mg/L≤ hsCRP <3 mg/L) and high CVD risk category subgroup (hsCRP ≥3mg/L) (19).

Statistical analysis

Statistical package SPSS (version 15.0 for Windows, SPSS, Chicago, IL, USA) was used for statistical analyses. Data are presented as mean ± standard deviation (if normally distributed), median (interquartile range) (if non-normally distributed), or with counts and percentages (categorical variables). Student's t test or one-way ANOVA (for normally), Mann-Whitney test and Kruskal-Wallis non-parametric analysis of variance (for non-normally distributed parameters), were used for the evaluation of differences between

examined groups. The differences between categorical data were analyzed with Chi-squared test. All variables with skewed distribution were logarithmically transformed. The relationship between log hsCRP and other variables was determined with Pearsons's (r) correlation coefficient. Multiple linear regression analysis was performed to identify independent variables affecting log hsCRP. In all analyses p value of < 0.05 was regarded to be statistically significant.

Results

Table 1 shows the general demographic, clinical and biochemical characteristics of the studied participants with diabetes. With exception to WC (p=0.708), females displayed significantly higher anthropometric indices [i.e., BMI (p=0.001) and all four skinfold thickness measures (p<0.001, respectively)]. Also, females had higher TC (p=0.005), HDL-c (p<0.001), TG/HDL-ratio (p=0.056), as well as hsCRP (p=0.004). More males than females were on insulin therapy (p=0.008) and were classified as smokers (p=0.042), whereas more females were on antihypertensive therapy (p=0.046), and were classified as obese (p=0.005). There was no gender difference in age, fasting glucose, HbA1c, LDL-c, non-HDL-c, TG, oral antihyperglycemic and hypolipidemic therapy usage, as well as in duration of diabetes, between the groups (Table 1).

Significantly higher number of females were in the high hsCRP subgroup, compared with males (χ^2=12.80, p<0.001). Also, significantly higher number of obese individuals were in the high risk subgroup as compared with low risk subgroup (χ^2=18.68, p<0.001). Furthermore, we found significantly higher values of all examined anthropometric parameters [e.g., BMI and WC (p<0.001, respectively), SF biceps, SF triceps, SF suprailiac and SF subscapular thicknesses (p=0.003, p=0.002, p=0.026 and p=0.003, respectively)] in the high risk subgroup, compared to low and intermediate subgroup (Table 2).

Pearson's correlation analysis was performed in order to examine potential relationship between hsCRP and anthropometric and metabolic parameters in participants with DM2. As shown, hsCRP correlated positively with anthropometric parameters (i.e., BMI and WC) and fasting glucose both, in males and females. Also, hsCRP correlated positively with age, SF subscapular thickness and TG/HDL-c ratio only in females (Table 3).

All variables that showed a significant predictive value in Pearson's correlation (i.e., WC and glucose in both gender, and age, SF subscapular thick-

Table 1. General characteristics of studied participants with diabetes

Characteristics	Women (n=87)	Men (n=97)	p
Age (years)	63.2±10.9	61.3±11.3	0.233
BMI (kg/m²)#	30.1 (27.7-33.9)	28.3 (26.0-30.9)	0.001
WC (cm)	107±11.7	106±12.0	0.708
SF biceps (mm)#	18.0 (15.0-22.0)	12.0 (8.0-15.0)	<0.001
SF triceps (mm)#	26.5 (21.0-32.0)	13.0 (10.0-18.7)	<0.001
SF suprailiac (mm)	33.9±6.22	29.8±9.20	<0.001
SF subscapular (mm)	33.1±6.63	27.6±8.59	<0.001
Fasting glucose (mmol/L)	7.72±2.20	8.51±3.30	0.414
HbA1c (%)#	6.50 (5.80-7.67)	6.70 (5.80-8.10)	0.339
TC (mmol/L)#	5.43 (4.86-6.27)	5.07 (4.41-5.70)	0.005
HDL-c (mmol/L)#	1.29 (1.10-1.51)	1.01 (0.84-1.32)	<0.001
LDL-c (mmol/L)#	3.28 (2.77-3.95)	3.02 (2.49-3.68)	0.148
TG (mmol/L)#	1.83 (1.47-2.45)	1.82 (1.28-2.49)	0.897
Non-HDL-c (mmol/L)#	4.19 (3.47-5.00)	3.93 (3.33-4.71)	0.150
TG/HDL-c ratio#	1.41 (0.94-2.30)	1.89 (1.07-2.63)	0.056
hsCRP (mg/L)	1.98 (1.10-4.79)	1.42 (0.76-2.46)	0.004
Smoking habits (No/Yes), n (%)	73/14 (83.9/16.1)	68/29 (70.1/29.9)	0.042
Antihypertensives (No/Yes), n (%)	18/69 (20.7/79.3)	34/63 (35.1/64.9)	0.046
Hypolipidemics (No/Yes), n (%)	49/38 (56.3/43.7)	55/42 (56.7/43.3)	0.923
Antihyperglycemics (No/Yes), n (%)	6/81 (6.9/93.1)	15/82 (15.5/84.5)	0.138
Insulin (No/Yes), n (%)	78/9 (89.7/10.3)	71/26 (73.2/26.8)	0.008
Duration of diabetes (years)#	4.00 (2.00-7.75)	5.00 (2.00-10.00)	0.259
Normal weight/ Overweight/Obese, n (%)	6/35/46 (6.9/40.2/52.9)	15/49/33 (15.5/50.5/34.0)	0.005

Data are presented as mean ± standard deviation or # median (interquartile range), or counts (percentages); BMI-Body mass index; WC-Waist circumference; SF-skinfold thickness; HbA1c-glycated hemoglobin; TC-Total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; hsCRP-high sensitivity C-reactive protein

Table 2. Anthropometric and metabolic parameters in subgroups according to cardiovascular risk level, as estimated with hsCRP

Parameter	Low risk group (hsCRP < 1 mg/L), n=54 (29.4 %)	Intermediate risk group (1 ≤ hsCRP <3 mg/L), n=77 (41.8 %)	High risk group (hsCRP ≥ 3 mg/L), n=53 (28.8 %)	p
Age (years)	63.7±9.99	61.7±10.09	61.4±13.49	0.483
BMI (kg/m²)#	27.7 (25.2-30.2)^{aaa,b}	28.7 (27.2-31.7)^{aa}	28.6±3.68	<0.001
WC (cm)	100±11.1^{aaa,b}	105±10.6^{aa}	111±12.4	<0.001
SF biceps (mm)#	13.0 (8.3-17.8)^{aa,b}	14.0 (11.0-19.8)	17.0 (13.0-22.0)	0.003
SF triceps (mm)#	14.0 (10.3-22.5)^{aa,b}	20.0 (13.3-26.8)	24.0 (14.3-30.0)	0.002
SF suprailiac (mm)	29.4±9.74^{a}	32.0±7.15	33.7±7.51	0.026
SF subscapular (mm)	27.4±8.33^{aa,b}	30.2±7.70	32.8±7.98	0.003
Glucose (mmol/L)	7.08±1.50	8.00±2.63	8.52±3.42	0.513
HbA1c (%)#	6.50 (5.77-7.87)	6.70 (5.77-8.00)	6.60 (5.97-7.70)	0.598
TC (mmol/L)#	5.20 (4.74-5.78)	5.22 (4.55-6.00)	5.26 (4.56-6.12)	0.812
HDL-c (mmol/L)#	1.21 (0.92-1.52)	1.14 (0.93-1.37)	1.18 (0.93-1.30)	0.598
LDL-c (mmol/L)#	3.00 (2.63-3.75)	3.14 (2.55-3.89)	3.10 (2.62-3.93)	0.869
TG (mmol/L)#	1.68 (1.26-2.13)	1.86 (1.39-2.56)	2.11 (1.49-2.91)	0.177
Non-HDL-c (mmol/L)#	4.04 (3.45-4.68)	4.03 (3.28-4.84)	4.11 (3.35-5.02)	0.667
TG/HDL-c ratio#	1.31 (0.98-2.33)	1.58 (0.99-2.54)	1.91 (1.18-2.57)	0.317
Duration of diabetes (years)#	5.50 (2.00-10.00)	4.00 (2.00-8.00)	4.00 (1.75-7.00)	0.290
Normal weight, n (%)	11 (20.4)	6 (7.8)	4 (7.5)	**χ2= 18.68** <0.001
Overweight, n (%)	26 (48.1)	43 (55.8)	15 (28.3)	
Obese, n (%)	17 (31.5)	28 (36.4)	34 (64.2)	
Gender (F/M), n (%)	16/38 (29.6/70.4)	37/40 (48.1/51.9)	34/19 (64.2/35.8)	χ^2= 12.80 <0.001

*^{a} – p<0.05, ^{aa} – p<0.01, ^{aaa} -p<0.001 vs. High risk group, ^{b} – p<0.05, ^{bb} – p<0.01, ^{bbb} – p<0.001 vs. Medium risk group. #- data with non-Gaussian distribution are shown as median values (interquartile range) *P value from one-way ANOVA or Kruskal-Wallis non-parametric analysis of variance, followed by Student's t-test or non-parametric Mann-Whitney U test, where appropriate; BMI-Body mass index; WC-Waist circumference; SF-skinfold thickness; HbA1c-glycated hemoglobin; TC-total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; hsCRP-High sensitivity C–reactive protein; F-Females; M-Males*

ness and TG/HDL-c ratio only in females) were further analyzed in multiple linear regression analysis analysis for hsCRP prediction. The backward selection enabled to find the best model consisted of 2 parameters [e.g., WC (Beta=0.205, p=0.045 and glucose (Beta=0.305, p=0.003)] in males, and only WC in females (Beta=0.405, p=0.003), (Table 4).

Discussion

The finding of the current study shows that females with DM2 have higher inflammation level, as well as higher CVD risk than males, as assessed with hsCRP. Furthermore, hsCRP is significantly associated with anthropometric indices in patients with DM2, but central adiposity, as measured with WC is its better predictor, compared to general adiposity measure (e.g., BMI) and four skinfold thickness measures (e.g., biceps, triceps, subscapular and suprailiac) in individuals with DM2 (Table 4).

We previously showed that WC was the independent predictor of hsCRP level in normal weight and overweight, otherwise healthy postmenopausal women (7), and that WC correlated better than BMI with cardiometabolic parameters even in younger healthy population (20, 21). Current study extends those observations, suggesting that this simple and

Table 3. Pearson's correlation coefficients (r) of log hsCRP with studied parameters

Variable	Females r	p	Males r	p
Age (years)	-0.233	0.030	0.038	0.709
BMI (kg/m²)	0.549	<0.001	0.193	0.058
WC (cm)	0.496	<0.001	0.200	0.050
SF biceps (mm)	0.185	0.096	0.065	0.530
SF triceps (mm)	0.192	0.084	-0.023	0.822
SF suprailiac (mm)	0.215	0.053	0.015	0.888
SF subscapular (mm)	0.252	0.022	0.095	0.360
Fasting glucose (mmol/L)	0.286	0.007	0.271	0.007
HbA1c (%)	0.177	0.101	0.139	0.176
TC (mmol/L)	0.104	0.337	-0.154	0.132
HDL-c (mmol/L)	-0.121	0.266	-0.197	0.053
LDL-c (mmol/L)	0.051	0.638	-0.126	0.217
TG (mmol/L)	0.174	0.108	0.025	0.806
Non-HDL-c (mmol/L)	0.146	0.179	-0.123	0.230
TG/HDL-c ratio	0.267	0.012	0.041	0.687
Duration of diabetes (years)	0.022	0.838	-0.092	0.370

BMI-Body mass index; WC-Waist circumference; SF-skinfold thickness; HbA1c-glycated hemoglobin; TC-total cholesterol; HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG-Triglycerides; hsCRP-high sensitivity C–reactive protein

Table 4. Multiple linear regression analysis for the association of several parameters with log hsCRP as dependent variable

Coefficients					
Model for males	Unstandardized Coefficients		Standardized Coefficients	t	p
	B	Std. Error	Beta		
(Constant)	-8.187	2.925		-2.799	0.006
Glucose (mmol/L)	0.251	0.081	0.305	3.096	0.003
WC (cm)	0.046	0.023	0.205	2.034	0.045

WC-Waist circumference

Coefficients					
Model for females	Unstandardized Coefficients		Standardized Coefficients	t	p
	B	Std. Error	Beta		
(Constant)	-14.020	4.582		-3.060	0.003
WC (cm)	0.107	0.034	0.405	3.134	0.003

WC-Waist circumference

cost-effective anthropometric parameter could be a reliable tool for evaluating CVD risk, not only in healthy, but also in population with diabetes. In addition, a majority of population with diabetes in our study were with overweight/obesity (88.6%), (Table 1), which is in line with previous studies, pointing out obesity as a key determinant for DM2 occurrence and progression (6). Moreover, adipocytes and macrophages from hyperplastic and hypertrophied adipose tissue secrete a broad spectrum of adipokines and pro-inflammatory cytokines [e.g., CRP, Interleukin-6, Tumor necrosis factor-alpha] which impair insulin signaling within the cells and lead to consequent obesity-related comorbidities, such as metabolic syndrome, DM2, CVD (6, 7, 22).

In the current study, about 42% of individuals with DM2 had CRP levels ≥2 mg/L, and about one third (29%) of participants had hCRP levels ≥3mg/L (Table 2), which categorized them in intermediate and high risk category for CVD, respectively (23). Similarly, Halcox et al. (24) reported that approximately 50% of non-diabetic patients had CRP levels ≥2 mg/L, and about 30% had CRP levels ≥3 mg/L. Morever, Salazar et al. (25) observed that individuals with hsCRP >3 mg/L had doubled CVD risk, whereas those with hsCRP 1–3 mg/L displayed 50% higher risk of CVD, compared to those with hsCRP <1 mg/L.

Women with DM2 free of previous known CVD in our study displayed higher hsCRP levels compared to males (Table 1), which is similar to other studies (1, 24), but contrary to another ones (26). These discrepancies may be explained by different sample size of studied groups, as well as different age of participants in those studies. Furthermore, with exception to WC, women in our study had higher anthropometric indices than men. Also, significantly higher number of females with DM2 were in the high hsCRP subgroup, compared with males (Table 2). Although males are regarded to have a greater risk of CVD than females (8), there are studies showing that this gender difference gets lost in patients with DM2 (9, 10). In addition, this gender difference in CVD risk gets diminished in postmenopause, since women in that period tend to have more atherogenic profile, and show redistribution of adipose tissue toward visceral region, compared to women in premenopause (27). The majority of wom-

en in our study were considered to be postmenopausal, and were with obesity, which can explain higher hsCRP, as well as higher CVD risk in females than in males with DM2.

Since this study has cross-sectional design, the causalty between obesity, inflammation and CVD risk in population with diabetes cannot be established. Thus, large-scale longitudinal studies are needed to confirm this possible causalty, as well as to confirm the benefits of reduction of inflammation in decreasing the risk of diabetes complications, such as CVD.

In conclusion, females with DM2 have higher CVD risk, as measured with hsCRP than males. Furthermore, among other examined anthropometric indices, WC is independently associated with hsCRP, both in males and females, suggesting that this simple parameter could be a reliable and cost-effective tool for evaluating CVD risk in population with DM2.

References

1. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13(4):851–63.
2. Yu E, Hsu HY, Huang CY, Hwang LC. Inflammatory Biomarkers and Risk of Atherosclerotic Cardiovascular Disease. Open Med (Wars). 2018;13:208-13.
3. Rojas J, Chávez M, Olivar L, et al. Polycystic ovary syndrome, insulin resistance, and obesity: navigating the pathophysiologic labyrinth. Int J Reprod Med. 2014;2014:719050.
4. Vargas-Uricoechea H, Cáceres-Acosta MF. Control of Blood Pressure and Cardiovascular Outcomes in Type 2 Diabetes. Open Med (Wars). 2018;13:304-23.
5. Adukauskienė D, Čiginskienė A, Adukauskaitė A, Pentiokinienė D, Šlapikas R, Čeponienė I. Clinical relevance of high sensitivity C-reactive protein in cardiology. Medicina (Kaunas). 2016;52:1-10.
6. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci. 2015;11:463-82.
7. Klisic AN, Vasiljevic ND, Simic TP, Djukic TI, Maksimovic MZ, Matic MG. Association between C-reactive protein, anthropometric and lipid parameters among healthy normal weight and overweight postmenopausal women in Montenegro. Lab Med. 2014;45:12-6.
8. Karam C, Beauchet A, Czernichow S, et al. Trends in Cardiovascular Disease Risk Factor Prevalence and Estimated 10-Year Cardiovascular Risk Scores in a Large Untreated French Urban Population: The CARVAR 92 Study. PLoS ONE. 2015;10:e0124817.
9. McEwan P, Williams JE, Griffiths JD, et al. Evaluating the performance of the Framingham risk equations in a population with diabetes. Diabet Med. 2004;21:318-23.
10. Coleman RL, Stevens RJ, Retnakaran R, Holman RR. Framingham, SCORE, and DECODE risk equations do not provide reliable cardiovascular risk estimates in type 2 diabetes. Diabetes Care. 2007;30:1292-3.
11. Kavaric N, Klisic A, Ninic A. Are Visceral Adiposity Index and Lipid Accumulation Product reliable indices for metabolic disturbances in patients with type 2 diabetes mellitus? J Clin Lab Anal. 2018;32:e22283.
12. Oliveira-Santos J, Santos R, Moreira C, et al. Ability of Measures of Adiposity in Identifying Adverse Levels of Inflammatory and Metabolic Markers in Adolescents. Child Obes. 2016;12:135-43.
13. Bauer KW, Marcus MD, El ghormli L, Ogden CL, Foster GD. Cardio-metabolic risk screening among adolescents: understanding the utility of body mass index, waist circumference and waist to height ratio. Pediatr Obes. 2015;10:329-37.
14. Dagan SS, Segev S, Novikov I, Dankner R. Waist circumference vs body mass index in association with cardiorespiratory fitness in healthy men and women: a cross sectional analysis of 403 subjects. Nutr J. 2013;12:12.
15. Hou X, Lu J, Weng J, et al. Impact of waist circumference and body mass index on risk of cardiometabolic disorder and cardiovascular disease in Chinese adults: a national diabetes and metabolic disorders survey. PLoS One. 2013;8(3):e57319.
16. Klisic A, Kavaric N, Jovanovic M, et al. Association between unfavorable lipid profile and glycemic control in patients with type 2 diabetes mellitus. J Res Med Sci. 2017;22:122.
17. American Diabetes Association. Standards of Medical Care in Diabetes. Diabetes Care. 2016;39(Supplement 1):S1-S2.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.
19. Yousuf O, Mohanty BD, Martin SS, et al. High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link? J Am Coll Cardiol. 2013;62(5):397-408.
20. Klisic A, Kavaric N, Soldatovic I, Bjelakovic B, Kotur-Stevuljevic J. Relationship between cardiovascular risk score and traditional and nontraditional cardiometabolic parameters in obese adolescent girls. J Med Biochem. 2016;35(3):282-92.
21. Klisic A, Kavaric N, Bjelakovic B, Soldatovic I, Martinovic M, Kotur-Stevuljevic J. The association between retinol-binding protein 4 and cardiovascular risk score is mediated by waist circumference in overweight/obese adolescent girls. Acta Clin Croat. 2017;56:92-8.
22. Martinac M, Pehar D, Karlović D, Babić D, MarČinko D, Jakovljević M. Metabolic syndrome, activity of the hypothalamic-pituitary-adrenal axis and inflammatory mediators in depressive disorder. Acta Clin Croat. 2014;53:55-71.

23. Pearson TA, Mensah GA, Alexander RW, et al. Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003;107:499-511.
24. Halcox JP, Roy C, Tubach F, et al. C-reactive protein levels in patients at cardiovascular risk: EURIKA study. BMC Cardiovasc Disord. 2014;14:25.
25. Salazar J, Martínez MS, Chávez M, et al. C-reactive protein: clinical and epidemiological perspectives. Cardiol Res Pract. 2014;2014:605810.
26. Wang Z, Wang X, Chen Z, Zhang L, Zhu M. Distribution of High-Sensitivity C-Reactive Protein and Its Relationship with Other Cardiovascular Risk Factors in the Middle-Aged Chinese Population. Int J Environ Res Public Health. 2016;13:872.
27. Lovejoy J, Champagne C, de Jonge L, Xie H, Smith S. Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes (London). 2008; 32: 949-58.

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Original article

Parenting strategies for eating and activity scale (PEAS): Turkish validity and reliability study

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Summary. The aim of this research was to determine Turkish validity and reliability of the Parenting Strategies for Eating and Activity Scale (PEAS). The study group consisted of 1004 parents, who had children at the ages of 6-12 years old and were referred to the children's health and diseases service of a state hospital. The PEAS and the Child-Feeding Questionnaire (CFQ) that is used to measure structure validity were used to collect data. The PEAS consists of five sub-dimensions and 26 items. Internal consistency coefficient and item-total score correlation coefficients of the subscales of the PEAS were calculated for the reliability analysis. Scope and structure validity analyses were conducted for validity. A moderate and more than positive correlation was found between the scores of all sub-dimensions in the structural validity analysis. The correlation between the scores obtained from the PEAS and the CFQ scale was found to be significant in the criterion validity analysis. Cronbach's alpha reliability coefficient value was 0.91. This study showed that the PEAS is a valid and reliable measurement tool to measure parental approaches toward nutrition and activities of children in Turkey. PEAS is suggested to be used by the healthcare professionals working with children and their families to determine the factors related to the parents' approaches regarding children's nutrition and activities.

Key words: child health, nutrition, activity, parental control

Introduction

In childhood, the lack of adequate-balanced nutrition and regular physical activity lead to various health problems and affect the individual's life negatively (1,2). Parenting strategies are of great importance in children's eating and physical activities. Parents' eating habits directly affect children's food selections and eating habits (3,4). Lo et al (2015) have determined that the parental control over the feeding behaviors of children is effective on children's consumption of more fruits and vegetables as well as consumption of high-calorie foods less often (5). In the study of Blissett and Haycraft (2008) examining the effect of parental attitude on eating behavior, it was found that the permissive attitude of parents had less control over the unhealthy food consumption of children (6). Applying a moderate level of control, such as monitoring unhealthy food consumption, is a healthy strategy for parents in order to manage their children's food consumption (7). There is some variation in the findings concerning the degree of negative impact that excessive control has on child weight (8). The research suggests that highly restrictive feeding practices have been most consistently associated with child weight gain (8), and monitoring feeding practices have been associated with slower weight gain (9).

The evaluation of parental approaches toward children's nutrition and activities is important to raise parental awareness about the growth, development and well-being of children (10). However, in most of the studies conducted, valid and reliable measuring

instruments were not used to determine parental approaches toward children's nutrition and activities, and instead, questionnaires prepared by researchers were used (11,12). Hence, it is necessary to use powerful, valid and reliable measurement tools that allow evaluation of parents' approach. In Turkey, the studies which assess parents' approaches towards children's nutrition and activities are limited. In this study, it was aimed to adapt the Parenting Strategies for Eating and Activity Scale (PEAS) to Turkish and to determine its validity and reliability.

Material and Method

Participants

This study was planned methodologically and its population consisted of parents who applied to the Child Health and Diseases Service of a State Hospital within a year and had children under the age of 18 years old. The sample was composed of 1004 Turkish literate parents living together with their children between the ages of 6-12 years old and agreed to participate in the survey between August 2015 and December 2016. Parents who were not literate in Turkish, did not have children aged 6-12, did not live with their children, wanted to be excluded from the study at any phase, had physical or psychological problems that affect written or verbal communication were excluded from the study.

The sample size was calculated by taking into consideration the number of scale items, Likert type and the factor analysis that is to be made. There are several suggestions in the literature about the calculation of sample size for factor analysis. Gorusch (1983) and Hatcher (1994) proposed to researchers approximately (5-10): 1 subject-item ratio for factor analysis (13-15). Another suggestion in factor analysis about the number of subjects is the assessment of 200 subjects as "moderate", 300 subjects as "good", 500 subjects as "very good", and 1000 subjects "excellent"(16,17). In our study, there were 26 items in the scale that we tested for its reliability and Turkish validity and the results were evaluated as a 5 point-Likert scale. Based on all of these suggestions, in our study, we aimed to reach 1000 participants, rated as "excellent" considering that accurate results were obtained in factor analysis, and a sufficient sample size was achieved with 1004 participants.

Ethical Considerations

We first received permission via e-mail from Sandra E Larios, who developed the scale (PEAS) that we tested for validity and reliability for the Turkish version in our study. Ethical approval was received from the Ethics Committee of Yıldırım Beyazıt University prior to conducting the study. Written informed consent was obtained from each patient who met the inclusion criteria.

Data Collection Tools

In this study, an introductory information form developed by the researchers and the Parenting Strategies for Eating and Activity Scale were used as data collection tools, and the Child Feeding Questionnaire (CFQ) was used to test the validity of the structure.

Introductory information form

This form consisted of nine questions making up the first part of the data collection form and was prepared to designate some personal features (age, gender, income status, family type, etc.) of parents and children.

The Parenting Strategies for Eating and Activity Scale (PEAS)

The PEAS was developed by Larios et al (2009) and it assesses families' attitudes towards children's nutrition and activities. The scale consists of five subdivisions and contains 26 items (14 items are nutrition-limiting and 12 items are activity-limiting):

Monitoring: Seven items (five items about nutrition and two items about activity) measured monitoring frequency of families regarding their children's healthy behaviors.

Discipline: Five items measured (three items about nutrition and two items about activity) the frequency of disciplinary practices of families about restricted unhealthy nutrition (e.g. soft drink consumption) and sedentary life behaviors (e.g. watching TV) of their children. Control: Six items (five items about nutrition and one item about activity) measured the control approaches used by families.

Restrictions: Six items (two items about nutrition and four items about activity) assessed the appropriate restrictions used by families about unhealthy nutrition and sedentary lifestyle behaviors of their children.

Supporting: Two items (one item about nutrition and one item about activity) measured the use of praise for children when they consumed healthy snacks or when they were busy with activities.

The five-point Likert scale, where '1 = never' to '5 = always', was used in the subdivisions of monitoring, discipline and support. The five-point Likert scale, where '1 = I do not agree' to '5 = I agree', was used in the subdivisions of control and restriction. The scores of the sub-scales were equal to the sum of the scores received in items 1-5. As the scores received in the sub-scales increased, parents' had positive approach and as the decreased, parents had negative approach, except for the control sub-scale (18).

The Child Feeding Questionnaire (CFQ)

The CFQ, developed by Camcı 2010, consists of 7 sub-scales; out of which 3 sub-scales (Restriction, Monitoring, and Pressure to Eat) evaluate parental controls over child feeding; and 4 sub-scales (Perceived Responsibility for Child Feeding, Interest on Children's Weight, Perceived Children's Weight, Perceived Parent's Weight) evaluate parental attitudes and behaviors about child feeding. The items in the questionnaire are evaluated with the 5-point Likert scale. One of the sub-scales included in the questionnaire, 'Perceived Children's Weight', rates parental perception' about the overweight situation of their child at various ages between 1 (thin) through 5 (overweight) (19).

The Application of Data Collection Tools

Preliminary application

The Turkish version of the scale, with its language and content validated, was applied as a preliminary application to 20 parents who applied to the child health and illness polyclinic where the study was carried out. In the preliminary application performed with the parents, it was aimed to identify the items that were not understood and not suitable. The data collection form of the study consisted of the short, clear and applicable final states of the questions in the questionnaire. No items were removed from the scale and the preliminary application data were not included in the study.

Application

Data collection was carried out in the child health and illness clinic, an environment where the interviews would not be interrupted. The data were collected in one-time face-to-face interviews based on self-reports of the individuals. Each interview lasted approximately 10-15 minutes. In order to ensure the privacy of the participants, specific numbers were written on the forms in place of the patients' names.

Data Analysis

The validity of Parenting Strategies for Eating and Activity Scale was considered by testing the language and scope validity, structural validity and criterion validity. Kendall W analysis was used for doing the compatibility analysis of expert opinions stated about the language (scope) validity of the scale. Structural validity was evaluated by using exploratory factor analysis. Criterion validity was determined by using Spearman correlation test with the evaluation of the correlation between the means of the Child Feeding Questionnaire and Parenting Strategies for Eating and Activity Scale. Compliance of the data for factor analysis was evaluated by measuring the sample adequacy with Kaiser-Meyer-Olkin test and Bartlett's globalism test. In order to determine the reliability, internal consistency analysis and test-retest methods were used. The data were analyzed by using the Statistical Package for Social Sciences (SPSS) for Windows 15.0 software (SPSS Inc., Chicago, IL, USA) and by receiving assistance of bio-statistics specialists. The descriptive statistics are presented as frequency, percentage, means and standard deviations. p value ≤ 0.05 was considered statistically significant.

Results

The mean age of the parents was 36.72 ± 7.64 (min: 20.01-max: 74.05), 74.1% were mothers, 42.1% had two children and 37.4% were high school graduates. The parents mostly (84.3%) lived in nuclear families and 54.3% of them were employed and their

mean monthly income was 2824.44 ± 1642.21 TL (n = 1004).

Validity analysis results of the scale

Language and content validity

To ensure language validity of the scale, the first questionnaire form was prepared by translating the questionnaire to the target language and retranslating it to the original language (English). Initially, each item in the scale was translated to Turkish and evaluated by three different English linguists. For the Turkish version, the most appropriate translation of each item was used. Then, the completed new Turkish version was translated to the original language by three different English linguists. The final Turkish and English versions of the questionnaire were compared with the original English version and 5 academic lecturers, who were literate both in Turkish and English and specialized in health field, determined whether they were compatible. In the evaluation of expert opinions, Content Validity Index (CVI) was calculated by using Davis technique. The experts were given the original scale and its translation and were asked to evaluate each item as 1 = not relevant, 2 = item need some revision, 3 = relevant but need minor revision, 4 = very relevant. To obtain CVI for relevancy of each item (I-CVI), the number of those judging the item as relevant (rating 3 or 4) was divided by the number of content experts. The I-CVI expresses the proportion of agreement on the relevancy of each item, which is between 0 and 1(20) and I-CVI in our study was calculated to be 0.94. The mean score assigned by the five experts for the scale items was determined as 3.78 ± 0.2 (min 3-max 4). According to Kendall's Coefficient of Concordance Test (Kendall's W) analysis made to evaluate the compatibility of the expert opinions, the difference between the scores given by the experts to the scale items was found to be statistically insignificant (Kendall W = 0.07, p = 0.44).

Structural validity

In our study, Kaiser-Meyer-Olkin (KMO) 0.91, p <0.001 and Bartlett Test (approximately x2) = 11864.30, were determined as p <0.001. Accordingly, it was found that the data obtained from the scale in the study were perfectly compatible for doing factor analysis (Table 1).

Factor analysis was made by using the principal component method and the varimax axis rotation method in order to test the construct validity of the scale. It was found in the analysis that there were 5 factors as similar to the original scale.

The factor loads introduced by the items and the correlations of each item with the total scores are shown in Table 3. All of the factors explain 59.36% of the variance. The factors in this study are called "monitoring, discipline, control, restriction, and support" by remaining loyal to the original scale (Table 2). Based on the factor analysis result, it is possible to see that the supporting sub-dimension of the 2 items (item 5 and item 7) of the monitoring sub-dimension is distributed (Table 3). The table shows that there was a positive, intermediate and higher correlation between the mean of all sub-dimensions (p <0.001).

Criterion validity

In the examination of the criterion validity of the scale and considering the relation between the scores obtained from the CFQ and the scores obtained from the PEAS, the correlation between all of the sub-dimensions was found significant (p <0.001) (Table 3). Moreover, it was found that the highest correlation coefficients were obtained between the sub-dimensions questioning the similar items. There was a strong correlation between the mean score of the monitoring sub-dimension of the PEAS and the monitoring sub-dimension of the CFQ (r = 0.643, p <0.001). There was a moderate correlation between the mean scores of the control sub-dimension of the PEAS and the Restriction and Pressure to Eat sub-dimensions of the CFQ (r = 0.441, p <0.001; r = 0.489, p <0.001). Furthermore, there was a moderate and high correlation between the mean scores of the Restriction sub-dimension of the PEAS and the Concern about Child's Weight, Restric-

Table 1. KMO and Bartlett's test statistics of PEAS

Statistic Test	Test Value
Kaiser-Meyer-Olkin (KMO) Sampling Compliance Measure	0.91
Bartlett's Test Approximate chi-square (x^2)	1186430
Degree of Freedom (df)	325; p< 0.001

Table 2. The factor analysis results of PEAS

Scale Items	Factors				
	1	2	3	4	5
Monitoring question 1		.831			
Monitoring question 2		.849			
Monitoring question 3		.765			
Monitoring question 4		.654			
Monitoring question 5		.169		.716	
Monitoring question 6		.648			
Monitoring question 7		.189		.652	
Discipline question 8					.667
Discipline question 9					.666
Discipline question 10					.412
Discipline question 11					.730
Discipline question 12					.677
Discipline question 13			.520		
Discipline question 14			.620		
Discipline question 15			.512		
Control Question 16			.645		
Control Question 17			.685		
Control Question 18			.663		
Restriction 19	.511				
Restriction 20	.466				
Restriction 21	.755				
Restriction 22	.782				
Restriction 23	.774				
Restriction 24	.787				
Supporting 25				.589	
Supporting 26				.652	

Note: Rotation Method: Varimax with Kaiser Normalization

tion, Pressure to Eat and Monitoring sub-dimensions of the CFQ (respectively, r=0.446, p<0.001; r=0.646, p<0.001; r=0.431, p<0.001; r=0.407, p<0.001) (Table 4).

Reliability analysis results of the scale

The reliability level of the scale was studied by examining its internal consistency. None of the items had a negative effect on the reliability based on the Cronbach's alpha reliability coefficient calculation. Therefore, no item was excluded. Cronbach's alpha reliability coefficient α= was calculated as 0.91 for the entire Turkish form of the scale. Cronbach's alpha reliability coefficients for the sub-dimensions were calculated as 0.80 for the monitoring sub-dimension; α= 0.75 for the discipline sub-dimension; α= 0.81 for the control sub-dimension; α= 0.86 for the restriction sub-dimension; and α= 0.82 for the supporting sub-dimension (Table 5).

Discussion

We achieved a large sample with the large number of participants (n=1004) for the validity and reliability evaluation, and our study results show that the Turkish form of the PEAS is a reliable and valid tool. The mean age of the parents we reached in this study were above middle age and nearly half of them had two children. In addition, our study was conducted with participants who applied to a state hospital with their children, and it is very important that parents living in middle-income families, which make up a large portion of the Turkish society, reflect Turkish culture in the best way and that the results are generalized to the population.

The purpose of the content validation is to generate an entirety consisting of meaningful items by asking a specialist group to examine whether the items on the scale represent the area that is to be measured (21). For calculating the content validity, the number of the experts marking option A and option B is divided to

Table 3. The correlation coefficients between the sub-dimensions of the PEAS

	Monitoring	Discipline	Control	Restriction	Supporting
Monitoring	-				
Discipline	r=0.475*	-			
Control	r=0.516*	r=0.555**	-		
Restriction	r=0.577**	r=0.532*	r=0.629**	-	
Supporting	r=0.410*	r=0.438*	r=0.516*	r=0.683**	-
Scale Total	r=0.759**	r=0.752**	r=0.807**	r=0.874**	r=0.709**

*Note. r= Spearman correlation; * Moderate level correlation coefficients, p <0.001; ** High level correlation coefficients, p <0.001.*

Table 4. Correlation between the mean scores of the sub-dimensions of the PEAS and CFQ

The sub-dimensions of the PEAS	Child Nutrition Questionnaire Sub-Dimensions						
	Perception of Responsibility	Parents' Weight Perception	Child's Weight Perception	Anxiety About the Child's Weight	Restriction	Pressure to Eat	Monitoring
Monitoring	r=0.367	r=0.140	r=0.125	r=0.372	r=0.396	r=0.341	r=0.643**
Discipline	r=0.267	r=0.223	r=0.246	r=0.167	r=0.272	r=0.302	r=0.155
Control	r=0.393	r=0.170	r=0.125	r=0.333	r=0.441*	r=0.489*	r=0.299
Restriction	r=0.375	r=0.166	r=0.131	r=0.446*	r=0.646**	r=0.431*	r=0.407*
Supporting	r=0.342	r=0.139	r=0.167	r=0.288	r=0.294	r=0.315	r=0.253

*Note. r= Spearman correlation, * Moderate level correlation coefficients p <0.001, ** High level correlation coefficients p <0.001.*

Table 5. The mean scale scores of the parents' approaches on nutrition and activity and calculated Cronbach's alpha reliability coefficient (n = 1004)

The sub-dimensions of the PEAS	Total Scores Mean±SD	Item Scores Mean±SD	Cronbach's Alpha
Monitoring sub-dimension	24.37±5.39	3.48±0.77	0.80
Discipline sub-dimension	16.18 4.47	4.05±1.14	0.75
Control sub-dimension	20.75±4.88	3.45±0.81	0.81
Restriction sub-dimension	27.26±6.53	4.54±1.08	0.86
Supporting sub-dimension	6.67±2.23	3.33±1.11	0.82
Scale Total	95.27±18.92	3.66±0.72	0.91

the total number of the experts and the CVI value is obtained. In the literature, the value of 0.80 CVI is recommended as the criterion (22,23). In this study, it was found that the expert opinions were compatible with each other concerning whether the PEAS items that were translated to Turkish were appropriate to the language and culture. Based on these results, it can be said that the statements of the PEAS that were translated to Turkish are suitable to Turkish culture and that the content validity is achieved.

In the construct validity study, it is shown that a KMO value of 0.60 or higher sample size is suitable for the factor analysis in order to evaluate whether the sample is sufficient for factor analysis. Moreover, Bartlett test result should be p <0.05 (17,24). In this study, KMO value of the PEAS was greater than 0.60 and the sample size was sufficient for the factor analysis. The factor analysis is the most commonly used method for adaptation of intercultural scales and it is used to evaluate whether the items of the scale are gathered under different sub-dimensions (22,24). As a result of the exploratory factor analysis made in our study, it was observed that the areas measured by the items were generally related with each other. Only the "supporting" sub-dimension distribution of the two items belonging to the "monitoring" sub-dimension was observed; (Item 5 "Does your child have to ask for your permission before getting snacks; Item 7 " How seriously do you follow your child's activity / exercise extent?". Despite these two items are related with and similar to the items in the supporting sub-dimension, these situations need to be monitored and supported. When parents support their children for choosing healthy snacks and monitor and support the amount of their movements and exercises, this facilitates their children to adopt healthy lifestyle behaviors as a form of behavior at an early age (18,25). There is a literature which suggests that parents' feeding practices are broadly linked with their parenting styles (26), and that parenting styles are good predictors of children's healthier eating and physical activity behaviours (27). In the study conducted on the development of the PEAS scale, Larios et al. 2009 reported that in case the support sub-scale consisted of only 2 items, this could restrict the support sub-dimension. Hence, evaluation of these two items that are included in the monitoring sub-dimension of the original scale in the supporting sub-dimension may enhance the supporting sub-dimension. In this case, in the Turkish form of the scale, the support sub-scale will consist of 4 items and the monitoring sub-scale will consist of 5 items. Minimum and maximum that the participants can receive from the monitoring sub-dimension are 5 and 25, re-

spectively; the minimum and maximum that they can receive from the support sub-dimension are 4 and 20, respectively (18).

In order to determine the effectiveness of the scale, the criterion validity examines the relationship between the scores received from the scale and the determined criteria (20,22). In our research, the strong correlation between the mean scores received from the monitoring sub-dimension of the PEAS and the monitoring sub-dimension of the CFQ, which is determined as the criterion, shows the compliance validity. Similarly, the moderate correlation between the mean scores received from the Control sub-dimension of the PEAS and the Restriction and Pressure to Eat sub-dimensions of the CFQ's explains the compliance validity. The medium and high level of correlations between the mean scores of the Restriction sub-dimension of the PEAS and the sub-dimensions of Concern about Child Weight, Restriction, Pressure to Eat and Monitoring of the CFQ also indicate that there is compliance validity.

Cronbach's alpha reliability coefficient was used to evaluate the internal consistency of a measurement tool. The coefficient is considered as a determinant of the internal consistency of the questions in the test and is expected to take a value between 0-1. The high reliability coefficient of a measuring instrument indicates that the items in the scale evaluate the same character and they are consistent with each other (21). In our study, the Cronbach's alpha reliability coefficient of the PEAS was found to be 0.91 and it was determined that the internal consistency of the scale was highly reliable. Larios et al (2009), who developed the Parenting Strategies for Eating and Activity Scale, found Cronbach's alpha reliability coefficient of the five sub-scales of the scale as between 0.81 and 0.82 in their original research. Cronbach's alpha reliability coefficient of the sub-scales of the PEAS were also between 0.75 - 0.86 in this study and it had a fairly reliable internal consistency similar to the original study results (18).

Conclusions

The data obtained from this study also reveal that PEAS is a valid and reliable measurement tool in measuring parental approaches regarding nutrition and activities of children in Turkey. In this direction, PEAS is suggested to be used by the healthcare professionals working with children and their families to determine the factors related to the parents' approaches regarding children's nutrition and activities. The scale also can be used in making more effective programs and evaluating programs by providing valid and reliable information about children's healthy nutrition and activity programs. Along with that, this scale is suggested as a possible guide for healthcare professionals in preparing nutrition and diet programs for children by revealing parents' attitudes towards nutrition and activity.

References

1. Yalvac S, Erkan T, Erginöz E, Çokuğraş FÇ, Kutlu F. The evaluation of nutritional status of children by antropometric measurements living in day nursery of Bahçelievler Original Article. Turkish Archives of Pediatrics 2008;43:89-93. http://www.turkpediatriarsivi.com/sayilar/224/buyuk/89-931.pdf
2. Erkan T, Yalvaç S, Erginöz E, Çokuğraş FÇ, Kutlu T. The evaluation of nutritional status of children, by anthropometric measurements, attending the day nursery of Cerrahpasa Medical School. Turkish Archives of Pediatrics 2007;42:142-147. http://www.turkpediatriarsivi.com/sayilar/227/buyuk/142-1471.pdf
3. Birch LL, Davison KK, et al. Family environmental factors influencing the developing behavioral controls of food intake and childhood overweight. Pediatric Clinics of North America 2001; 48: 893-907. https://doi.org/10.1016/S0031-3955(05)70347-3
4. Kobak C, Pek H. Comparison of Mother and Child Health and Nutrition Habits of the Nursery School Kids in Preschool Period (Aged 3-6). H.U. Journal of Education 2015; 30:42-55. http://www.efdergi.hacettepe.edu.tr/yonetim/icerik/makaleler/6-published.pdf
5. Lo K, Cheung C, Lee A, Tam WSW, Keung V. Associations between parental feding styles and childhood eating habits: a survey of Hong Kong pre-school children. Plos One 2015;10:1-11. https://doi.org/10.1371/journal.pone.0124753
6. Blissett J, Haycraft E. Are parenting style and controlling feeding practices related? Appetite 2008; 50: 477–485. https://doi.org/10.1016/j.appet.2007.10.003
7. Birch LL, Fisher JO, Davison KK. Learning to overeat: Maternal use of restrictive feeding practices promotes girls' eating in the absence of hunger. American Journal of Clinical Nutrition 2003; 78(2): 215–220. doi: 10.1093/ajcn/78.2.215

8. Clark HR, Goyder E, Bissell P, Blank L, Peters J. How do parents' child-feeding behaviours influence child weight? Implications for childhood obesity policy. Journal of Public Health 2007; 29:132–141.
9. Faith MS, Berkowitz RI, Stallings VA, et al. Parental feeding attitudes and styles and child body mass index: Prospective analysis of a gene–environment interaction. Pediatrics 2004; 114(4): 429–436. doi: 10.1542/peds.2003-1075-L
10. Ayala G, Ibarra L, Arredondo E, et al. Promoting healthy eating by strengthening family relations: design and implementation of the Entre Familia: Reflejos de Salud intervention. Matto H, Strolin-Goltzman J, Ballan M, Elk R, Landrine H. Cancer Disparities: Causes and Evidence-Based Solutions. American Cancer Society Springer Publishing Company. 2011; 237-245.
11. Palfreyman Z, Haycraft E, Meyer C. Development of the Parental Modelling of Eating Behaviours Scale (PARM): links with food intake among children and their mothers. Maternal and Child Nutrition 2014; 10: 617–629. doi: 10.1111/j.1740-8709.2012.00438.x.
12. Economos CD, Bakun JP, Herzog BJ, et al. Children's perceptions of weight, obesity, nutrition, physical activity and related health and socio-behavioural factors. Public Health Nutrition 2012; 17: 170–1378. https://doi.org/10.1017/S136898001200479X
13. Osborne JW, Costello AB. Sample size and subject to item ratio in principal components analysis. Practical Assessment, Research & Evaluation 2004;9(11):1-9. https://pareonline.net/getvn.asp?v=9%26n=11
14. Hatcher LA. Step-by-Step approach to using the SAS system for factor analysis and structural equation modeling. Cary, North Carolina, SAS Institute, Inc. 1994;325-339.
15. Gorsuch RL. Factor Analysis. 2. Edition, Hillsdale, NJ, Lawrence Erlbaum Associates 1983.
16. Streiner DL, Kottner J. Recommendations for Reporting the Results of Studies of Instrument and Scale Development and Testing. Journal of Advanced Nursing 2014;70: 1970–1979. https://doi.org/10.1111/jan.12402
17. Tavşancıl E. Attitude Measurement and Data Analysis with SPSS. Ankara, Nobel Publishing. 2014;19-51.
18. Larios SE, Ayala GX, Arredondo EM, Baquero B, Elder JP. Development and validation of a scale to measure Latino parenting strategies related to children's obesigenic behaviors. The parenting strategies for eating and activity scale (PEAS). Appetite 2009;52:166-172. doi: 10.1016/j.appet.2008.09.011.
19. Camcı N, Bas M, Buyukkaragoz AH. The psychometric properties of the Child Feeding Questionnaire (CFQ) in Turkey. Appetite 2014;78:49-54. https://doi.org/10.1016/j.appet.2014.03.009
20. Zamanzadeh V, Rassouli M, Abbaszadeh A, et al. Details of Content Validity and Objectifying It in Instrument Development. Nursing Practice Today 2014;1:163-171. http://npt.tums.ac.ir/index.php/npt/article/view/24/75
21. Harrington D. Confirmatory Factor Analysis. Tripodi T. United States Of America, Oxford University Press 2009.
22. Esin MN. Reliability and Validation of Data Collection Tools. Erdoğan S, Nahcivan N, Esin MN. Research Process, Practice and Criticism in Nursing. Istanbul, Nobel Medical Bookstores. 2014;216-229.
23. LoBiondo-Wood G, Haber J. Realibility and Validity. LoBiondo-Wood G, Haber J. Nursing Research. New York, Elsevier Health Sciences, 2014;289-309.
24. Sencan H. Validity and Reliability in Social and Behavioral Measures. Ankara, Seckin Publishing. 2005.
25. O'Connor TM, Hingle M, Chuang JR, et al. Conceptual Understanding of Screen Media Parenting: Report of a Working Group. Childhood Obesity 2013;9:110-118. doi: 10.1089/chi.2013.0025.
26. Hughes SO, Power TG, Fisher JO, Mueller S, Nicklas TA. Revisiting a neglected construct: Parenting styles in a child-feeding context. Appetite 2005;44:83–92. doi: 10.1016/j.appet.2004.08.007
27. Rhee KE, Lumeng JC, Appugliese DP, Kaciroti N, Bradley RH. Parenting styles and overweight status in first grade. Pediatrics 2006; 117: 2047–2054. doi: 10.1542/peds.2005-2259

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Original article

Developing a meal-planning exchange list for commonly consumed Pakistani dishes

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Summary. In the present study, thirty commonly consumed Pakistani dishes were prepared using standard recipes and analyzed for their proximate composition. Meal planning exchange lists were then developed for those dishes. Significant variations in macronutrient content were observed among the dishes. The fat content varied from 5.2% in chicken biryani to 20.7% in bitter gourd. The protein content ranged from 1.24% in okra to 18.06% in fish while carbohydrate content ranged from 4.83% in fish to 28.9% in simple rice. The energy value ranged from 128 kcal/100 g in haleem to 280.5 kcal/100 g in chapli kabab. Correlation analysis between macronutrient content obtained from proximate analysis and those obtained from nutrient analysis software showed significant correlation for carbohydrate r = 0.80 ($P < 0.01$) and protein content r = 0.682 ($P < 0.01$) and non significant correlation for fat content r = 0.295 ($P = 0.113$). Data from the present study can be used as a meal planning tool by dietitians and nutritionists in planning normal and therapeutic diets. Further research on the nutritional contributions of other traditional dishes in the region is needed.

Key words: traditional dishes, meal planning, exchange list, macronutrient content, diabetes

Introduction

The prevalence of chronic conditions including diabetes and obesity is increasing worldwide with diet plays an important role in their development and prevention (1). Therefore planning a healthy diet may help to prevent development of these conditions. A number of food guidance systems are available for planning diet for diabetic and obese individuals including the carbohydrate counting system and exchange list system (2).

Exchange list system is a food guided tool for meal planning that enables people to exchange foods without disturbing their macronutrient or energy content (3, 4). The American Diabetes Association and the American Dietetic Association developed it for the first time in 1950. Since that the food exchange system has been used in meal planning and dietary counseling for people with diabetes and those on weight loss programs (5).

The exchange list system has been modified from time to time and according to the need of individuals new exchange lists have been developed in other countries (3, 6, 7). For example, Samoans are known to be the most overweight population in the world and an exchange list has been developed based on this need using different Samoan foods (7). Similarly, exchange lists have been developed for different traditional dishes, desserts and appetizers commonly consumed in Jordan (3, 8). In another study, a food exchange list based on West African local foods has been developed which is used by diabetes educators in West Africa (9). However, no such national or regional exchange lists have yet been developed for commonly consumed tra-

ditional dishes in Pakistan. The lack of regional food exchange lists has made it difficult for nutritionist and dietitians to incorporate traditional dishes when planning meals for diabetic individuals.

Therefore, the objective of this study was to determine the proximate composition of traditional dishes commonly consumed in Khyber Pakhtunkhwa (KP), Pakistan and to develop meal planning-exchange lists for these dishes based on the results obtained from the proximate composition.

Materials and methods

Selection of dishes

For the selection of dishes, one hundred and fifty housewives were randomly selected from different areas of KP. Each housewife was requested to provide the names of at least twenty dishes she commonly cook at home. The names of the dishes were recorded in a questionnaire. Most frequently named dishes (N = 30) were chosen to be included in the present study.

Dish recipe and preparation method

In order to minimize the variation in dish recipe and preparation method, a sub sample of five housewives was randomly selected and asked to provide a detailed recipe and method of preparation for each dish. The five recipes for each dish were then averaged using the summation method. Each ingredient in the average recipe was reported in both kitchen and standard measurements. Major ingredients of the dishes are presented in Table 1.

Dishes preparation

Dishes were prepared using the averaged recipes and were cooked under optimized cooking conditions (cooking time and temperature). Researchers used the same facilities and utensils for dishes preparation. The aim of cooking was done to get exact weight of the ingredients and net weight of the cooked dish.

Proximate composition of dishes

Proximate composition of the dishes was carried out in Food Technology and Agriculture Chemistry laboratory of Agricultural Research Institute, Tarnab, Peshawar and Human Nutrition laboratory at The University of Agriculture Peshawar, Pakistan. Association of Official Analytical Chemists (AOAC) procedures were used for the determination of moisture, ash, protein, fat and carbohydrate content of the dishes. Moisture content was determined by drying the sample until constant weight in an air oven at 105 ^{0}C, as described in the approved method No. 930.15 (10). Moisture content was calculated by the difference in wet and dry sample weights. For further analysis the dried samples were ground using a commercial grinder. The ground samples were stored in sealed plastic jars for further analyses. Ash content was determined by the official method No. 942.05 of AOAC, (2003) using a muffle furnace. Determination of crude protein was done by official Kjeldahl method No. 984.13 of AOAC (2003). Total fat (ether extract) was analyzed by official method No. 920.39 of AOAC (2003) using a Soxhlet apparatus. Nitrogen free extract (total carbohydrate) was calculated by difference. The energy content from macronutrients was calculated by multiplying the amount of carbohydrate, fat and protein in grams by factors 4, 9 and 4, respectively (11).

Exchange lists development.

For determination of number of exchanges for fat, protein and carbohydrate the values of macronutrients per 100 g of food that were obtained from proximate analysis were used. Rounding off method proposed by Wheeler and colleagues in literature were used for fitting dishes into exchange list (6).

A food portion was not counted as a serving if it had 1-5 g carbohydrate. If it contained 6-10 g carbohydrate, it was considered as half a serving and if it contained 11-20 g carbohydrate, it was counted as one carbohydrate serving. A food portion with 0-2 g fat was not counted as serving. If it contained 3 g fat, it was considered as half a serving and if there were 4-7 g fat, it was counted as one fat serving. A food portion was not considered to be a serving if it had 0-3 g protein from meat substitutes and meat lists while food portion with 4-10 g was counted as one serving.

Statistical analysis

All data were expressed as mean ± standard deviation of duplicate analyses. Statistical analysis was

Table 1. Major ingredients of the dishes

Local name	Common Name	Major Ingredients
Aloo gosht	Potato meat	Potato, beef, onion, tomato, garlic & ginger paste, green chili, cooking oil, spices, coriander, salt, water
Gosht korma	Beef korma	Beef, slit chick pea lentils, onion, tomato, garlic & ginger paste, green chili, cooking oil, spices, salt, coriander, water
Baingan	Bringels	Bringles, onion, tomato, green chili, cooking oil, salt, spices, coriander
Bhindi	Okra	Okra, onion, tomato, green chili, cooking oil, salt, spices, coriander
Channa daal	Slit chick pea lentils	Slit chick pea lentils, onion, tomato, garlic & ginger paste, green chili, cooking oil, spices, salt, coriander, water
Chapli kabab	Chapli kabab	Minced beef, onion, tomato, ginger & garlic paste, spices, egg, maize flour, cooking oil, coriander, salt
Chicken biryani	Chicken biryani	Rice, chicken, onion, tomato, yogurt, green chili, cooking oil, salt, ginger & garlic paste, biryani masala, water
Chicken karahi	Chicken karahi	Chicken, tomato, ginger & garlic paste, green chili, coriander, cooking oil, salt, spices
Vegetable rice	Vegetable rice	Rice, capsicum, cabbage, carrot, peas, green onion, vinegar, chili sauce, soya sauce, china salt, salt, chicken cube, cooking oil, black pepper, garlic paste, water
Choley	Gram/ Chick pea	Chick pea, onion, tomato, green chili, ginger & garlic paste, spices, salt, cooking oil, coriander, soda, water
Daal kadu	Slit chick pea lentil with bottle gourd	Bottle gourd, slit chick pea lentils, onion, tomato, ginger & garlic paste, green chili, salt, spices, cooking oil, coriander, water
Daal mash	Mash bean	Mash beans, onion, tomato, green chili, garlic & ginger paste, cooking oil, salt, spices, coriander, water
Machli	Fish	Fish, fish masala, lemon, ginger & garlic paste, salt, cooking oil, vinegar
Haleem	Haleem	Meat, onion, mash bean, masoor lentils, wheat, slit chick pea lentils, moong lentils, ginger & garlic paste, green chili, haleem masala, salt, cooking oil, coriander, lemon, tomato, water
Kachalo qeema	Colocassia with minced beef	Colocassia, minced beef, onion, tomato, green chili, ginger & garlic paste, spices, salt, coriander, cooking oil
Kaleji	Liver	Liver, tomato, green chili, ginger & garlic paste, spices, cooking oil, salt, coriander, lemon
Karella qeema	Minced beef with bitter gourd	Bitter gourd, minced beef, onion, tomato, salt, cooking oil, ginger & garlic paste, spices, green chili
Kadhi pakora	Yogurt curry with pakoras	Yogurt, garlic & ginger paste, onion, tomato green chili, cooking oil, spices, salt, chick pea flour, potato, coriander, soda, water
Koftay	Meat balls curry	Ground beef, onion, ginger & garlic paste, green chili, salt, cooking oil, spices, tomato, coriander, egg, chick pea flour, yogurt, water
Lobia	Kidney beans	Kidney beans, tomato, onion, garlic paste, salt, cooking oil, coriander, spices, green chili, water
Macroni qeema	Macroni with minced beef	Macroni, minced beef, onion, tomato, green chili, cooking oil, salt, spices, capsicum, black pepper, vinegar, soya sauce, chili sauce, ginger & garlic paste, coriander, water
Peeti	Mix masoor and moong lentils	Moong lentils, masoor lentils, onion, tomato, ginger & garlic paste, salt, cooking oil, green chili, coriander, water
Mix sabzi	Mix vegetable	Peas, cauliflower, carrot, potato, onion, tomato, salt, cooking oil, green chili, spices, coriander, water
Mutton karahi	Mutton karahi	Mutton, tomato, ginger & garlic paste, green chili, salt, cooking oil, spices, coriander, water
Nihari Gosht	Beef nihari	Beef, onion, tomato, ginger & garlic paste, green chili, nihari masala, wheat flour, coriander, cookimg oil, salt, water
Palak	Spinach	Spinach, onion, tomato, garlic paste, green chili, salt, cooking oil, spices
Pulao gosht	Beef pulao	Rice, beef, onion, tomato, green chili, salt, cooking oil, ginger & garlic paste, spices, water
Shimla mirch qeema	Minced beef with capsicum	Capsicum, ground beef, onion, tomato, green chili, salt, cooking oil, ginger & garlic paste, spices
Chawal	Simple rice	Rice, onion, tomato, garlic & ginger paste, salt, cooking oil, china salt, water
Tori	Ridge gourd	Ridge gourd, onion, tomato, salt, spices, green chili, coriander, garlic paste, cooking oil

performed using SPSS for windows (version 20; SPSS Inc., Chicago, Illinois, USA). Association between the values obtained from the results of proximate analysis and nutrient analysis software was carried out using Pearson's correlation. One-way analysis of variance (ANOVA) and Fisher's least significant difference *post hoc* test were used to determine significant differences in proximate composition and energy data among the dishes.

Results

Proximate composition

Data on the proximate composition per 100 g of each dish is presented in Table 2. Moisture content ranged from 52.5 in chapli kabab to 75% in mix vegetable. Moisture content was moderately high in all the vegetable dishes analyzed in the present study. The protein content ranged from 1.24% in okra to 18.06% in fish. In general all the vegetable based dishes had low

Table 2. Proximate analysis of the dishes (g per 100 g wet basis)*

Dish name	Moisture	Ash	Fat	Protein	Carbohydrate
Potato meat	67.5±0.71	1.71±0.06	15.23±1.04	4.76±0.01	10.80±0.39
Beef korma	65.50±0.71	1.28±0.07	12.21±0.20	7.85±0.38	13.16±0.05
Bringels	69.5±0.71	1.46±0.08	18.50±0.28	1.93±0.24	8.61±0.59
Okra	73±1.41	1.97±0.03	11.64±0.25	1.24±0.14	12.15±1.00
Slit chick pea lentils	63.5±0.71	1.37±0.00	17.74±0.40	2.48±0.06	14.91±0.37
Chapli kabab	52.5±0.71	2.21±0.07	19.52±0.31	12.16±0.32	13.61±0.77
Chicken biryani	65±1.41	1.49±0.08	5.23±1.15	6.43±0.48	21.85±0.30
Chicken karahi	57.5±0.71	2.19±0.23	15.85±0.02	13.37±0.49	11.09±0.42
Vegetable rice	67.7±1.41	1.52±0.05	5.52±0.19	3.54±0.32	21.99±1.04
Gram/ Chick pea	64±2.83	2.72±0.24	9.88±0.70	4.15±0.00	19.26±1.89
Slit chick pea lentil with bottle gourd	69.5±0.71	1.27±0.05	13.11±0.13	2.33±0.23	13.79±0.75
Mash bean	53±1.41	1.46±0.02	17.98±0.44	6.36±0.37	21.21±1.37
Fish	65±1.41	3.08±0.22	9.04±0.66	18.05±0.28	4.83±0.24
Haleem	73±1.41	1.65±0.05	5.38±0.19	5.38±0.36	14.60±0.82
Colocassia with minced beef	65.5±0.71	0.93±0.07	11.71±1.31	5.42±0.08	16.45±0.59
Liver	53±4.24	1.74±0.09	19.09±1.92	16.04±1.71	10.13±0.52
Minced beef with bitter gourd	61±1.41	1.14±0.04	20.74±1.57	7.70±0.93	9.42±1.04
Yogurt curry with pakoras	71.5±0.71	1.44±0.02	13.85±0.26	2.92±0.03	10.29±0.46
Meat balls curry	60.5±0.71	1.96±0.12	18.28±1.30	11.14±0.09	8.13±0.80
Kidney beans	70.5±0.71	1.12±0.07	11.60±0.44	3.67±0.02	13.11±0.21
Macroni with minced beef	67.5±0.71	1.20±0.07	9.25±0.32	4.70±0.31	17.35±0.15
Mix masoor and moong lentils	67±1.41	1.57±0.09	11.49±0.63	4.90±0.00	15.05±0.69
Mix vegetable	75±1.41	1.80±0.17	9.81±0.61	2.34±0.11	11.04±0.75
Mutton karahi	62.5±0.71	2.33±0.10	20.35±0.52	9.02±0.41	5.81±0.31
Beef nihari	71.5±0.71	1.99±0.01	13.21±0.31	6.24±0.86	7.06±0.47
Spinach	73.5±0.71	2.36±0.03	14.18±0.34	2.49±0.14	7.47±0.20
Beef pulao	63.5±0.71	0.82±0.04	7.65±0.84	5.82±0.02	22.21±0.16
Minced beef with capsicum	71.5±0.71	0.39±0.07	15.19±0.66	5.99±0.31	6.93±0.33
Simple rice	59±1.41	1.29±0.02	6.94±0.08	3.86±0.54	28.91±0.78
Ridge gourd	77±1.41	1.65±0.04	10.45±0.56	1.36±0.15	9.54±0.67

**Values are mean ± SD of duplicate analysis*

protein content while all the meat based dishes had high protein content. The fat content ranged from 5.2% in chicken biryani to 20.7% in bitter gourd. The average ash content ranged from 0.38%-3.08%. There were extensive differences in the carbohydrate content among the analyzed dishes. The carbohydrate content of the dishes ranged from 4.83% in fish to 28.9% in rice.

Energy content

The energy content (per 100 g wet basis) of the dishes is presented in Table 3. The energy density of the dishes ranged from 128 kcal/100 g (haleem) to 280.5 kcal/100 g (chapli kabab).

Correlation between macronutrient content

Significant correlation was found between proxi-

Table 3. Energy content of the dishes (per 100 g wet basis)

Dish	Protein		Fat		Carbohydrate		Total Energy*
	Kcals	% of total	Kcals	% of total	Kcals	% of total	Kcals
Potato meat	19.05	9.56	137.04	68.76	43.23	21.69	199.34±7.81
Beef korma	31.40	16.19	109.92	56.67	52.65	27.14	193.95±3.54
Bringels	7.75	3.71	166.48	79.80	34.40	16.49	208.63±3.91
Okra	4.97	3.14	104.85	66.21	48.55	30.65	158.33±6.79
Slit chick pea lentils	9.93	4.33	159.65	69.65	59.64	26.02	229.22±4.80
Chapli kabab	50.40	17.97	175.70	62.63	54.44	19.40	278.76±1.53
Chicken biryani	25.69	16.04	46.94	29.31	87.50	54.64	160.22±11.07
Chicken karahi	53.47	22.23	142.67	59.32	44.37	18.45	240.51±3.88
Vegetable rice	12.74	8.47	49.72	33.05	87.96	58.48	150.42±7.13
Gram/ Chick pea	16.63	9.11	88.94	48.72	76.97	42.17	182.52±13.86
Slit chick pea lentil with bottle gourd	9.33	5.11	118.04	64.67	55.14	30.21	182.51±3.27
Mash bean	25.47	9.36	161.80	59.47	84.79	31.17	272.06±7.95
Fish	72.24	41.80	81.27	47.02	19.32	11.18	172.85±8.07
Haleem	21.49	16.75	48.48	37.77	58.37	45.48	128.34±6.40
Colocassia with minced beef	21.67	11.24	105.26	54.61	65.83	34.15	192.81±9.12
Liver	64.11	23.19	171.74	62.12	40.61	14.69	276.51±26.22
Minced beef with bitter gourd	37.95	14.00	186.66	68.87	46.43	17.13	271.04±13.68
Yogurt curry with pakoras	11.69	6.58	124.66	70.23	41.15	23.19	177.49±4.08
Meat balls curry	44.56	18.45	164.42	68.08	32.55	13.48	241.56±8.88
Kidney beans	14.69	8.57	104.34	60.85	52.45	30.59	171.49±4.78
Macroni with minced beef	18.79	10.96	83.22	48.54	69.42	40.50	171.43±4.67
Mix masoor and moong lentils	19.60	10.70	103.36	56.43	60.19	32.86	183.16±8.46
Mix vegetable	9.40	6.63	88.31	62.26	44.14	31.12	141.86±8.01
Mutton karahi	36.08	14.88	183.09	75.53	23.25	9.59	242.42±5.02
Beef nihari	24.91	14.48	118.89	69.09	28.27	16.43	172.07±4.33
Spinach	9.96	5.95	127.60	76.20	29.89	17.85	167.45±4.43
Beef pulao	23.29	12.87	68.82	38.03	88.84	49.10	180.98±6.89
Minced beef with capsicum	23.94	12.71	136.71	72.56	27.76	14.73	188.43±5.84
Simple rice	15.42	7.97	62.45	32.27	115.66	59.76	193.52±5.99
Ridge gourd	5.50	3.99	94.08	68.32	38.13	27.69	137.64±8.32

**Values are mean±SD of duplicate analysis. Data are expressed for kcal from protein, fat and carbohydrates and kcal/100 g on a fresh weight basis for energy.*

mate analysis and nutrient analysis software values for carbohydrate r = 0.80 ($P < 0.01$) and protein r = 0.682 ($P < 0.01$) while non significant correlation was found for fat content r = 0.295 ($P = 0.113$).

Exchange list

Table 4 shows the number of fat, protein and carbohydrate exchanges calculated from the macronutrient values obtained from proximate analysis in 100 g of each dish. Most of the dishes contained at least one exchange of carbohydrate. Potato meat, bringles, liver, yogurt curry with pakoras, meat balls, beef nihari, spanich, minced beef with capsicum and ridge gourd contained 6-10 g of carbohydrate per 100 g portion and provided half exchange of carbohydrate. Fish and mutton karahi contained less than 6 g of carbohydrate

Table 4. Meal planning exchange lists for the dishes

	Macronutrients (g/100g)			Number of exchange per 100 g food			Amount of food that provides 1 exchange (g)		
DISH	**CHO**	**PRTN**	**FAT**	**CHO**	**PRTN**	**FAT**	**CHO**	**PRTN**	**FAT**
Potato meat	10.81	4.76	15.23	½	1	3	139	147	33
Beef korma	13.16	7.85	12.21	1	1	2	114	89	41
Bringels	8.60	1.94	18.50	½	*	3 ½	174	361	27
Okra	12.14	1.24	11.65	1	*	2	124	564	43
Slit chickpea lentils	14.91	2.48	17.74	1	*	3 ½	101	282	28
Chapli kabab	13.61	12.60	19.52	1	1 ½	3 ½	110	56	26
Chicken biryani	21.88	6.42	5.22	1 ½	1	1	68	109	96
Chicken karahi	11.09	13.37	15.85	1	1 ½	3	135	52	32
Vegetable rice	21.99	3.19	5.52	1 ½	1	1	76	220	91
Gram/ Chick pea	19.24	4.16	9.88	1	1	1 ½	79	168	51
Slit chick pea lentil with bottle gourd	13.79	2.33	13.12	1	*	2 ½	109	300	38
Mash bean	21.20	6.37	17.98	1	1	3 ½	71	110	28
Fish	4.83	18.06	9.03	*	2 ½	1 ½	313	39	55
Haleem	14.59	5.37	5.39	1	1	1	103	130	93
Colocassia with minced beef	16.46	5.42	11.70	1	1	2	91	129	43
Liver	10.15	16.03	19.08	½	2	3 ½	143	44	26
Minced beef with bitter gourd	11.61	9.49	20.74	1	1	4	129	74	24
Yogurt curry with pakoras	10.29	2.92	13.85	½	*	2 ½	146	240	36
Meat balls curry	8.14	11.14	18.27	½	1	3 ½	185	63	27
Kidney beans	13.11	3.67	11.59	1	*	2	115	191	43
Macroni with minced beef	17.00	4.70	9.25	1	1	1 ½	88	149	54
Mix masoor and moong lentils	15.05	4.90	11.48	1	1	2	100	143	44
Mix vegetable	11.04	2.35	9.81	1	*	1 ½	136	298	51
Mutton karahi	5.81	9.02	20.34	*	1	4	259	78	25
Beef nihari	7.07	6.23	13.21	½	1	2 ½	211	112	38
Spinach	7.47	2.49	14.18	½	*	2 ½	200	281	35
Beef pulao	22.21	5.82	7.65	1 ½	1	1	68	120	65
Minced beef with capsicum	6.94	5.99	15.19	½	1	3	217	117	33
Simple rice	28.92	3.85	6.94	1 ½	*	1	52	182	72
Ridge gourd	9.53	1.38	10.45	½	*	2	158	509	48

** Too small to be counted as a serving.*

for each 100 g portion and was too small to be counted as serving. The rice dishes including chicken biryani, vegetable rice, beef pulao and simple rice contained the highest number of carbohydrate exchanges (1 ½ exchanges per 100 g portion). Vegetable dishes and other dishes including slit chick pea lentils, slit chick pea lentil with bottle gourd, yogurt curry with pakoras, kidney beans and rice contained less than 4 g of protein per100 g portion and thus these dishes were not considered as sources of protein exchanges. Fish had the highest number of protein exchanges (2 ½ protein exchanges per 100 g portion). All the dishes had at least 1 exchange of fat while mutton karahi and minced beef with bitter gourd contained the maximum number of fat exchanges (4 fat exchanges per 100 g portion).

Discussion

In the present study a total of 30 different dishes of KP were analyzed for their proximate composition and meal planning exchange lists were than developed for these dishes. To our knowledge, the data on nutritional composition of frequently consumed KP traditional dishes is very limited. No study has yet been reported the nutritional composition of KP traditional dishes that collected averaged recipes, cooked dishes according to standardized recipe and developed meal planning exchange lists for these dishes.

Strong correlation was found between proximate analysis and nutrient analysis software values for carbohydrate and protein content and not for fat content. An explanation for no correlation of fat content is not fully understood. It is important to note that in the nutrient analysis software ingredients entered were in uncooked, raw form; whereas proximate analysis results were obtained in lab using cooked dishes. In other words, the data from nutrient analysis software was obtained from the addition of all constituents in their crude, uncooked form. For that reason the differences may be obviously seen in dishes that need extensive heat up and cooking treatment.

Significant variations were observed in the nutritional composition of the studied dishes due to different method of preparation and variety of ingredients. In general all the dishes included in the present study showed high fat, adequate protein and variable carbohydrate content. Among the thirty dishes, the moisture content was moderately high in all the vegetable dishes. It has been shown that the moisture content in various foods mainly depends on the ingredients used in their preparation and on the cooking methods (12). Therefore, the recipe and cooking method may have resulted in such differences. Regarding protein content, the fish dish showed the highest protein content and is in agreement with a previous study in which fish based dishes contained the highest protein content among the other dishes consumed in the State of Kuwait (11). Beef korma, chapli kabab, chicken biryani, chicken karahi, fish, liver, minced beef with bitter gourd, meat balls, mutton karahi, beef nihari, beef pulao and minced beef with capsicum are considered to be adequate in protein content. In general all the meat based dishes showed high protein content. The addition of other ingredients like rice and vegetables to meat based dishes lowered the protein content of these dishes as shown by chicken biryani, beef pulao, potato meat and other vegetables with minced beef.

The fat content among the dishes ranged from 5.2% (chicken biryani) to 20.7% (bitter gourd). Variations in fat content among the dishes could be due to the different quantities of hydrogenated oil used for processing and fatty acid profile of the ingredients. Most of the dishes were cooked in hydrogenated oil which resulted in a considerable contribution from fat to the total energy. This finding is similar to that observed among fried foods in other cultures (13). Foods fried in hydrogenated vegetables oils contain trans fatty acids and their consumption have been shown to increase the risk for cardiovascular diseases (14, 15). Therefore, dietary intervention such as alternative cooking method should be considered in this community to reduce high fat consumption.

Among the analyzed dishes carbohydrate content was high in rice based dishes whereas the meat dishes had low carbohydrate content. A comparative high estimation of carbohydrate in rice has been recorded in previous studies conducted in Saudi Arabia (16) and Oman (17). The present data is comparable with the data from the previous studies conducted on rice dishes in Pakistan and Saudi Arabia (18, 19). Chapli

kabab, a traditional KP dish contained 62.63% calories from fat, 19.40 calories from carbohydrate and 17.97 calories from protein and was the most energy-dense among all the dishes.

Previous studies have also shown the nutrient content of some frequently consumed Pakistani dishes however, meal planning exchange lists for those dishes were not provided (20, 21). The meal planning exchange lists developed in the present study can now be use to develop culture specific meal plans.

A limitation of the present study was that no information was provided about the type of fat (monounsaturated, polyunsaturated and saturated) and fatty acid profiles of the studied dishes. The consideration of total carbohydrate instead of available carbohydrate was another limitation. To get available carbohydrates as an energy providing nutrients, the fibre content should have been excluded. We were not able to measure any nutrient loss during cooking process. The mineral content of the dishes was also not investigated in the present study. Despite these limitations, the present research adds to the scientific literature on nutrient composition of the KP dishes by providing for the first time the proximate composition of thirty traditional dishes. Moreover, from the results of proximate analysis meal planning exchange lists were calculated to facilitate meal planning for diabetic and obese individuals. The present data may also help in assessing nutrient intake in epidemiological studies. The reported protein-rich dishes could serve for healthful dietary practices.

Conclusion

Exchange list for thirty traditional dishes of KPK is currently available and would serve as the basis for planning normal and therapeutic meals. It would help individuals to make better choice by comparing dishes for their macronutrient content. Data on the nutritional composition of KP dishes can also be used by health care workers, nutritionists and dietitians to calculate energy and nutrient intakes. In general, the nutritive qualities assessed in the present study recommend that most of the dishes have variable carbohydrate, adequate protein and high fat content. The total dietary fat of the majority of the dishes surpassed 30% of the total energy.

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References

1. IDF (International Diabetes Federation): "IDF Diabetes Atlas." 6th ed. 2014. International Diabetes Federation, Basel, Switzerland. Accessed at: http://www.idf.org/diabetesatlas
2. Gillen LJ, Tapsell LC. Development of food groupings to guide dietary advice for people with diabetes. Nutr Diet 2006; 63(1):36-47.
3. Bawadi HA, Al-Sahawneh SA. Developing a meal-planning exchange list for traditional dishes in jordan. J Am Diet Assoc 2008; 108(5):840-6. doi: 10.1016/j.jada.2008.02.016.
4. Wheeler ML, Daly A, Evert A, et al. Choose Your Foods: Exchange Lists for Diabetes, Sixth Edition, 2008: Description and Guidelines for Use. J Am Diet Assoc 2008; 108(5):883-8. doi: 10.1016/j.jada.2008.02.002.
5. Benezra LM, Nieman DC, Nieman CM, et al. Intakes of most nutrients remain at acceptable levels during a weight management program using the food exchange system. J Am Diet Assoc 2001; 101(5): 554-61.
6. Wheeler ML, Franz M, Barrier P, Holler H, Cronmiller N, Delahanty LM. Macronutrient and energy database for the 1995 exchange lists for meal planning: a rationale for clinical practice decisions. J Am Diet Assoc 1996; 96(11):1167-71.
7. Shovic AC. Development of a Samoan nutrition exchange list using culturally accepted foods. J Am Diet Assoc 1994; 94(5):541-3.
8. Bawadi HA, Al-Shwaiyat NM, Tayyem RF, Mekary R, Tuuri G. Developing a food exchange list for Middle Eastern appetizers and desserts commonly consumed in Jordan. Nutr Diet 2009; 66(1):20-6. doi: 10.1111/j.1747-0080.2008.01313.x.
9. Coulibaly A, O'Brien H, Galibois I. Development of a Malian food exchange system based on local foods and dishes for the assessment of nutrient and food intake in type 2 diabetic subjects. South Afr J Clin Nutr 2009; 22(1).
10. AOAC. Official methods of analysis (17th ed.). Gaithersburg MD, USA: Association of Official Analytical Chemists 2003.
11. Dashti B, Al-Awadi F, Khalafawi M, Al-Zenki S, Sawaya

W. Nutrient contents of some traditional Kuwaiti dishes: proximate composition, and phytate content. Food Chem 2001; 74(2):169-75.
12. Musaiger AO, Al-Jedah JH, D'Souza R. Nutritional profile of ready-to-eat foods consumed in Bahrain. Ecol Food Nutr 2007; 46(1):47-60.
13. Sharma S, Cao X, Gittelsohn J, Ethelbah B, Anliker J. Nutritional composition of commonly consumed traditional Apache foods in Arizona. Int J Food Sci Nutr 2008; 59(1):1-10.
14. Spearing K, Kolahdooz F, Lukasewich M, Mathe N, Khamis T, Sharma S. Nutritional composition of commonly consumed composite dishes from rural villages in Empangeni, KwaZulu-Natal, South Africa. J Hum Nutr Diet 2013; 26(3):222-9. doi: 10.1111/jhn.12001.
15. Motard-Bélanger A, Charest A, Grenier G, et al. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. Am J Clin Nutr 2008; 87(3):593-9.
16. Al Nagdy S, Abd-El Ghani SA, Abdel-Rahman M. Chemical assessment of some traditional Qatari dishes. Food Chem 1994; 49(3):261-4.
17. Musaiger AO, Ahmed MA, Rao MV. Chemical composition of some traditional dishes of Oman. Food Chem 1998; 61(1):17-22.
18. Khan M. Nutritional value of cereals in relation to human need. Pak J Agric Res 1981; 2:241-246..
19. AlşKanhal M, AlşMohizea I, AlşOthaimeen A, Khan MA. Nutritive value of various rice based dishes in Saudi Arabia. Ecol Food Nutr 1999; 38(3):223-35.
20. Ullah N, Alam I, Zeb A, Iqbal N, Khan A. Proximate composition of the prepared dishes of District Mardan and Peshawar. ARPN J Agric Biol Sci 2006; 8(5):364-72.
21. Hussain T. Food composition table for Pakistan. Government of Pakistan, Ministry of Planning & Developing Islamabad, Pakistan. 2001.

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Original article

Addition of firik improves the antioxidant and quality characteristics of steamed rice cake (*Sulgidduk*)

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Summary. Firik (or Frekeh) is a green wheat with high antioxidant activity and dietary fibers. Our aim was to analyze the antioxidant activities, quality characteristics, retarding retrogradation effect and sensory properties of *Sulgidduk* then to suggest the optimum proportion of firik in *Sulgidduk*. Five different samples were prepared with different amounts of firik powder (0%, 5%, 10%, 15%, and 20%). Firik has higher water holding capacity than rice: 401.50% and 221.74% respectively. The swelling power was varied with the temperature. Antioxidant activities of *Sulgidduk* significantly increased (p<0.05). The *L*-value and *a*-value significantly decreased, whereas *b*-value and Δ*E* increased with the increase in the amount of firik powder (p<0.05). Hardness, cohesiveness, springiness, chewiness, and gumminess decreased but not in a gradual manner (p<0.05). Retrogradation retardation revealed the strongest effect for the group treated with 5-10% firik powder that showed the lowest Avrami exponent. The group treated with 5% powder had the highest score in sensory evaluation test. These results suggest that the addition of 5% firik powder is suitable for improving the consumer acceptability and functionality of *Sulgidduk*.

Key words: antioxidant activity, firik, quality, retarding retrogradation, sensory evaluation

Introduction

Firik, cultivated in Cyprus, Middle East, Greece, Turkey, and North Africa, is known as a traditional functional food (1). After it is harvested during the immature stage, it is burned and dried in the sunlight (1). Firik which belongs to wheat family is usually produced in the Middle East and firik is a traditional cuisine in Anatolia, which is used as a substitute for rice or bulgur in pilav. (2). Firik generally comprises 77% of carbohydrates, 12.7% of proteins, and 16.5% of dietary fibers as nutritional components. Furthermore, firik has a low fat content and blood sugar index and is a rich source of vitamin A, B1, B2, C, and E, zeaxanthin, lutein, and minerals such as phosphorus, magnesium, zinc, iron, and calcium (1). Dietary fibers and fructo-oligosaccharides present in firik impart health-promoting characteristics, while the antioxidant activities of the phenolic compounds contribute to antimicrobial, anticancer, antitumor, and anti-inflammatory effects (3). These polymers decrease as the wheat ripen (4). During wheat ripening, the levels of soluble fiber decrease and those of insoluble fibers increase (3). In comparison with mature wheat, firik is rich in water-soluble cellulose that binds to high amount of water, thereby imparting water-binding ability, swelling power, viscosity, and gel formation characteristics; all these properties are favorable for the application of firik in food (3).

Prior studies about firik have investigated the physical, chemical change by maturation time of wheat, (4), and water-uptake properties as per the maturation process, processing method (3), physical characteristics (5), chemical components (1), and functional ingredients (6). As a wheat cultivar, firik is preferred over

common wheat owing to the cost of production (4). However, no study has evaluated the fiber content, antioxidant activities, and various biological components of firik for its application in food industry.

Steamed rice cake (*Sulgidduk*), a traditional rice cake in Korea, is still used by many people as a meal substitute (7). Rice cake is made by mixing various materials such as rice powder, potato powder, or starch as main ingredients and fruits and vegetables as other ingredients (8). Rice cakes are classified into steamed, boiled, fried, or kneaded rice cakes according to method of cooking (8). *Sulgidduk* is the most typical steamed rice cake prepared with a simple recipe using only rice; hence, it lacks nutrients and easily undergoes retrogradation, posing difficulties in commercial applications (8). Thus, there is an unmet need to retard the process of retrogradation and improve the nutritional value using health-promoting ingredients. Prior research about retrogradation retardation in *Sulgidduk* has been conducted with the supplementation of wheat powder (9), rice bran (10), oligosaccharides (11), and apple pomace dietary powder (12).

In this study, we analyzed the antioxidant activities, quality characteristics, retrogradation retardation of *Sulgidduk* supplemented with firik.

Materials and methods

Materials

Each ingredient was prepared by pulverization of rice powder (500 g) through a 40-mesh sieve. Before pulverization, rice (Uiseong, Korea) and firik (Health and Food Co., Ltd, Seoul, Korea) were washed five times and soaked in water for 24 h then drying for 1 h at room temperature (25°C). The grains were treated with 1% salt (CJ Cheiljedang Co., Ltd) and 10% water (distilled water) and subjected to grinding using a roll mill (DK 101, Donggwang Co., Ltd, Daegu, Korea). The powder was prepared with pass through a 20-mesh sieve. Sugar (CJ Cheiljedang Co., Ltd, Incheon, Korea) and salt were purchased from a local market.

Preparation of Sulgidduk

The steaming temperature was set to 100°C and the samples were steamed in a steamer (25 ´ 25 ´ 10 cm) for 20 min then cooling at room temperature (25°C) for 10 min. Samples were prepared with different amounts of firik and designated as F0 (without firik powder), F5 (5% firik powder), F10 (10% firik powder), F15 (15% firik powder), and F20 (20% firik powder). *Sulgidduk* formula was determined based on the studies on the retardation of retrogradation by the addition of wheat powder (9) and effects of black carrot powder on *Sulgidduk* (7). The formula of the *Sulgidduk* shown in Table 1. To prepare *Sulgidduk,* each powder was mixed and sugar and water were added to the mixture.

Physical analysis of firik

Water-holding capacity and swelling power

The water-holding capacity of firik was measured by the modified method of Song et al (7). Before measuring the water-holding capacity, firik was freeze-dried for 48 h and determined by dissolving 1 g of the sample in 20 mL of distilled water at room temperature 25°C in a 45-mL test tube then vortexing for 1 h. Three replicates were prepared for each sample. Mixed materials were centrifuged for 20 min at 4,000 rpm (Universal 32R, Hettich, Tuttlingen, Germany) and the water-holding capacity was calculated using the following formula:Water-holding capacity (%) = (Precipitate (g) – Sample (g))/Sample (g)

Swelling power was measured by the method of Schoch et al (13) with some modifications. A total of 0.5 g of sample was mixed in 25 mL of water in a centrifuge tube, and the sample was heated at 60, 70, 80, and 90°C for using a water bath for 20 min. The supernatant was poured into a container that had been preheated and weighed. Swelling power was estimated

Table 1. Formulation of *Sulgidduk* with different amounts of firik powder

Ingredients	Firik powder content (%)				
	Control[1)]	F5	F10	F15	F20
Rice powder	500	475	450	425	400
Firik powder	0	25	50	75	100
Water	50	50	50	50	50
Sugar	50	50	50	50	50
Salt	5	5	5	5	5

[1)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.

using the below equation:

Swelling power (%) = Weight of sediment (g)/ weight of sample (g) ´ 100

Antioxidant activities of firik

Preparation of sample

Before sample extraction, each sample was freeze-dried for 96 h and then pulverized using a high speed grinder. A total of 1 g of powder was extracted for 3 h with 15 mL of distilled water. Centrifugation was performed for 10 min at 3,000 rpm (Universal 32R, Hettich, Tuttlingen, Germany) and the samples were filtered through the Whatman No. 1 filter paper.

Total phenol content

The total polyphenol content was analyzed using the method of Folin-Denis with slight modification, as suggested by Akay et al (14). A solution containing 0.9 M Folin-Ciocalteu reagent (Junsei Chemistry, Tokyo, Japan) and 20% (w/v) sodium carbonate solution (Merck kGaA, Darmstadt, Germany) was prepared and 10 μL of samples were vortexed with 790 μL of distilled water for 1 min. A total of 50 μL of 0.9 N Folin-Ciocalteu reagent (Junsei Chemistry, Tokyo, Japan) was added to 150 μL of 20% sodium carbonate solution (Merck kGaA, Darmstadt, Germany) and the reaction mixture was incubated for 30 min at room temperature in the dark. The absorbance of each sample was measured at 750 nm wavelength using a microplate reader (Infinite 200 PRO, Tecan, Mannedorf, Switzerland). Gallic acid (Merck kGaA, Darmstadt, Germany) was used as a standard and the absorbance of each sample was converted to gallic acid equivalent (GAE).

Flavonoid content

The flavonoid content was measured as previously described (15) with slight modifications. A total of 1 mL of sample was vortexed with 150 μL of 5% sodium nitrite (Junsei Chemistry, Tokyo, Japan) for 6 min in the dark (25°C), followed by the addition of 300 μL of 10% aluminum chloride (Junsei Chemistry, Tokyo, Japan) and incubation in the dark (25°C) for 5 min. The solution was reacted by adding 1 mL of 1 N sodium hydroxide solution (Daejung Chemicals & Metals, Gyeonggi, Korea), Each sample was measured at 520 nm. Absorbance calibration curves were prepared using quercetin (Sigma-Aldrich Co., Ltd, Mo, USA) as a standard, and the total flavonoid content of each sample was converted to quercetin equivalent (QE).

Ferric-reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power was measured method by "authors names of (16)" with some modifications. The reagents used included 0.2 M phosphate buffer (pH 6.6), 1% potassium ferricyanide (Merck kGaA), 10% trichloroacetic acid (Sigma-Aldrich Co., Ltd, Mo, USA), and 0.1% ferric chloride ($FeCl_3$) (Junsei Chemistry, Tokyo, Japan). A total of 250 μL of sample and 250 μL of 0.2 M phosphate buffer and 1% potassium ferricyanide were allowed to react for 30 min at 50°C and the mixture was treated with 250 μL of 10% trichloroacetic acid. A total of 0.5 mL of this mixture was treated with 0.5 mL of distilled water and 0.1% $FeCl_3$. The absorbance of each solution was measured at 700 nm.

Quality characteristics of Sulgidduk

Moisture content

The moisture content of each sample was measured using a moisture analyzer (MB35, OHAUS, Zurich, Switzerland). A total of 5 g of sample was obtained from the central part of each sample.

pH

To determine the pH value of each sample, 10 g of sample was mixed with 90 mL of distilled water in a 250-mL beaker and homogenized (Unidrive 1000D, CAT M. Zipperer GmbH, Staufen, Germany) for 1 min. The pH of the solution was measured after 15 min.

Color

To measure the color of samples, the surface of each sample was evaluated using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) according to Hunter's color value system. The parameters *L* (brightness), *a* (redness), and *b* (yellowness) were measured. The value of ΔE (total color difference) was calculated using the following equation:

ΔE=√((L_sample-L_standard)2+(a_sample-a_standard)^2+(b_sample-b_standard)2)

where *ΔL, Δa, and Δb* are the differences in *L, a,* and *b* values between calibration white board (*L*: 96.62, *a*: −0.04, *b*: 1.63) and sample, respectively.

Texture analysis

After cooling for 10 min, the samples were cut at a uniform size (2 × 2 × 2 cm). Textural properties (hardness, chewiness, gumminess, springiness, and cohesiveness) of samples were measured using a rheometer (Sun rheometer Compac-100 II, Sun Scientific Co., Ltd, Tokyo, Japan). The texture profile was set with the two-bite compression test using the following operation conditions: No. 1 Φ20 mm prove; maximum weight, 2 kg; distance, 33%; table speed, 120 mm/min; 2 bites.

Retrogradation analysis from isothermal crystallization kinetics

Retrogradation rate of each sample was measured with the previously described method (17) using a rheometer (Sun rheometer Compac-100 II, Sun Scientific Co. Ltd, Tokyo, Japan). To measure the hardness, samples preserved at 20°C for 0, 3, 6, 9, 12, 24, and 48 h were analyzed by the Avrami equation as follows: $\theta = (E_L-E_t)/(E_L-E_0) = e^{-kt^n}$

where θ is the fraction of non-crystallized material, E_L represents maximum hardness, E_0 is the hardness at 0 h, E_t indicates hardness at t h, k represents the rate constant, n is Avrami exponent, and t indicates the storage time (h).

To obtain the values of hardness and time, each sample was measured at a given time. The equation is shown below. Avrami exponent (n) was calculated using the slope of the plot represented by the equation given below. The rate constant (k) was determined by the y-intercept value. Time constant ($1/k$) is the reciprocal of the rate constant (k).

$$\log[-\ln(E_L-E_t)/(E_L-E_0)] = \log k + n \log t$$

Sensory evaluation

A panel of 50 individuals (20-30 years of age, except those with celiac disease) participated in this test and analyzed the appearance, flavor, texture, sweetness, and overall acceptability of samples using the 9-point scale method (strong dislike = 1 and strong like = 9). The panels received five samples (2 × 2 × 2 cm) on a white plate.

Statistical analysis

All data were expressed as the mean ± standard deviation of triplicate experiments. All data obtained from measurements were evaluated by one-way analysis of variance (ANOVA) using SPSS ver. 23.0 (SPSS InC., Chicago, IL, USA). Significance between the means of measured experimental values was analyzed by Duncan's multiple range test ($p<0.05$).

Result and discussion

Water-holding capacity and swelling power

The water-holding capacity was measured at 25°C, while the swelling were measured at 60, 70, 80, and 90°C. The results are presented in Table 2. The water-holding capacity and swelling power were affected by amylose, amylopectin structure of starch, proteins, soluble fibers, and insoluble fibers (18-20). The higher the content of long chain amylopectin molecule is, the swelling power increased. Firik had higher water-holding capacity than rice, suggesting that rice

Table 2. Water-holding capacity and swelling power of firik and rice

Properties	Temperature (°C)	Firik	Rice
Water-holding capacity (%)	25	401.50 ± 5.61	221.74 ± 4.24
Swelling power (%)	60	6.83 ± 0.58^{d}	0.68 ± 0.16^{c}
	70	7.58 ± 0.09^{c}	2.43 ± 0.43^{b}
	80	10.51 ± 0.3^{b}	2.45 ± 0.21^{b}
	90	18.73 ± 0.42$^{a1)}$	4.2 ± 0.31^{a}

[1)]Values are expressed as mean ± standard deviation

[a-d]Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p<0.05$)

has more compact structure and firik has more fiber content. The higher the water-binding capacity is, the longer is the moisture retention in the food; hence, the moisture content would be high for a longer time and affect retrogradation (7). In addition, firik had higher swelling power than rice that increased at higher heating temperature during gelatinization. This observation correlated with the results of water-holding capacity analysis. The swelling power increased for both firik and rice with an increase in the temperature. The better water-holding capacity of firik may be attributed to its thin and loose seed coat as compared to the compact seed coat of rice so that addition of firik makes structure of *Sulgidduk* loose than control.

Antioxidant activities

Polyphenols comprise various molecules with many phenol structure and several subgroups of phenolic compounds (21). The polyphenols are components of yellow, green, red orange and purple pigments found in plant and they interact with the lipids, proteins, and carbohydrates that decrease lipid absorption and oxidation, and then create an antioxidative environment that prevent the effects of various reactive oxygen species (22-23). Antioxidant compounds act as anticancer and antimicrobial agents and reduce the risk of cardiovascular and gastrointestinal disorders (24). Firik has many health benefit compounds such as dietary fibers, fructose-rich polymers and fructo-oligosaccharides and phenolics, which may act as antioxidants (6). The total phenolic content of firik varied according to the maturation degrees and stages (16). During the process of ripening, the content of total phenolics as well as the antioxidant activity were low (6). Total phenolic and flavonoid contents of samples are shown in Table 3 and these values slightly increased with the increase in the amount of firik. The results of FRAP analysis were in line with those of total phenol and flavonoid content analyses. Although the difference was small, the control group showed the lowest value of 0.08 mg/mL, while the F20 group showed the highest value of 0.12 mg/mL for FRAP. A similar result was also confirmed in rice cake containing Asparagus powder (24) or Moringa oleifera leaf extract (8). Rice and firik had a polyphenol content of 2.80 and 11.53 μg GAE/mg, respectively, and a flavonoid content of 87.45 and 105.38 g QE/mg, respectively. The value of FRAP was 0.11 mg/mL for rice and 0.21 mg/mL for firik. This discrepancy is likely due to differences in the antioxidant compound content of each grain.

Moisture content and pH values

The moisture content and pH values of *Sulgidduk* are shown in Table 4. F20 group showed the lowest value (38.20%), while the control and F5 groups had the highest values (41.36%); however, no significant difference was observed between different groups except F20 group. Similar results were also observed with rice cake treated with Aronia powder (25) and cabbage powder (26). The moisture content affects the texture and retrogradation of food (7). Lower moisture content correlated with higher hardness values and quick retrogradation (7). The pH value of the sample increased with an increase in the amount of firik powder. The pH of samples ranged from 4.38 to 5.48, but no significant difference was observed. The pH affects the texture alkaline pH may result in larger pores and lower hardness

Table 3. Antioxidant activities of *Sulgidduk* with different amounts of firik powder

Samples	Firik powder content (%)					F-value (*p* value)
	Control[2)]	F5	F10	F15	F20	
Total phenolic content (g GAE/mg)	1.32 ± 0.10$^{b,1)}$	1.32 ± 0.10^{b}	1.71 ± 0.46^{a,b}	1.99 ± 0.39^{a,b}	2.41 ± 0.72^{a}	3.531* (0.048)
Flavonoid content (g QE/mg)	0.25 ± 0.11^{b}	0.32 ± 0.19^{b}	0.92 ± 0.22^{a}	1.10 ± 0.25^{a}	1.28 ± 0.23^{a}	15.313*** (0.000)
Ferric-reducing antioxidant power assay (mg/mL)	0.08 ± 0.01^{d}	0.09 ± 0.01^{c}	0.10 ± 0.00^{b}	0.11 ± 0.00^{b}	0.12 ± 0.00^{a}	21.059*** (0.000)

[1)] Values are expressed as mean ± standard deviation

[a-d] Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p<0.05$)

$^{*}p < 0.05$, $^{***}p < 0.001$

[2)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.

Table 4. Moisture content and pH values of *Sulgidduk* with different amounts of firik powder

Properties	Firik powder content (%)					F-value (*p* value)
	Control[2)]	F5	F10	F15	F20	
Moisture (%)	41.36 ± 0.10[a1)]	41.36 ± 0.11[a]	40.52 ± 0.19[b]	40.34 ± 0.13[b]	38.20 ± 0.02[c]	339.709[***] (0.000)
pH	4.38 ± 0.12[c]	5.18 ± 0.04[b]	5.37 ± 0.12[a]	5.43 ± 0.07[a]	5.48 ± 0.01[a]	91.590[***] (0.000)

[1)]Values are expressed as mean ± standard deviation
[2)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.
[a-c]Different superscripts indicate significant differences between values in the same row according to Duncan's range test (p<0.05)
[***]p < 0.001

values (7). On the other hand, acidic pH may result in smaller pores and compact texture, causing an increase in hardness (7). We observed that firik (pH 7.48) had higher pH than rice (pH 5.36) and considered that the pH of firik contribute to pH of *Sulgidduk*.

Color properties

The *L*-values (brightness) slightly decreased with the amount of firik powder increased (Table 5 and Fig. 1) ($p<0.05$). The color values were as follows: Rice (*L*: 96.37, *a*: −1.18, *b*: 4.67) and firik (*L*: 76.60, *a*: 3.73, *b*: 20.63). The control group had a value of 81.82, while the F20 group had a value of 79.39 ($p<0.05$). The *a*-values (redness) for the control was −1.39; the addition of firik resulted in a decrease in the *a*-values. The *b*-values (yellowness) increased for firik-treated samples (from 4.79 to 14.47) as compared with the control sample (4.79). Δ*E*- value was significantly affected by firik; the control group showed the lowest value of 0.07, while F20 group had the highest value of 10.04 ($p<0.05$). These results are in accordance with those reported in *Chlorella* powder (27) added or *Asparagus* powder added *Sulgidduk* (24). In these studies in which powder containing chlorophyll or carotenoid was added, similar results were observed, showing that *L* and *a* values decreased and *b* value increased as the content of powder increased. The difference in the ingredients and the content of chlorophyll and carotenoid may affect the results.

Table 5. Color values of *Sulgidduk* with different amounts of firik powder

Color value	Freekeh powder content (%)					F-value (*p*-value)
	Control[2)]	F5	F10	F15	F20	
*L**	81.82±0.10[a1)]	81.23±0.52[b]	80.47±0.37[c]	79.57±0.36[d]	79.39±0.41[d]	45.997[***] (0.000)
*a**	-1.39±0.11[a]	-2.29±0.11[b]	-2.35±0.13[bc]	-2.44±0.27[cd]	-2.49±0.52[d]	253.194[***] (0.000)
*b**	4.79±0.15[d]	11.02±0.23[c]	13.83±0.77[b]	14.31±0.77[ab]	14.47±0.13[a]	629.047[***] (0.000)
ΔE	0.07±0.59[d]	6.32±0.03[c]	9.20±0.72[b]	9.84±0.04[a]	10.04±0.19[a]	718.964[***] (0.000)

[1)]Values are expressed as mean ± standard deviation
[2)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.
[a-d]Different superscripts indicate significant differences between values in the same row according to Duncan's range test (p<0.05)
[***]p < 0.001

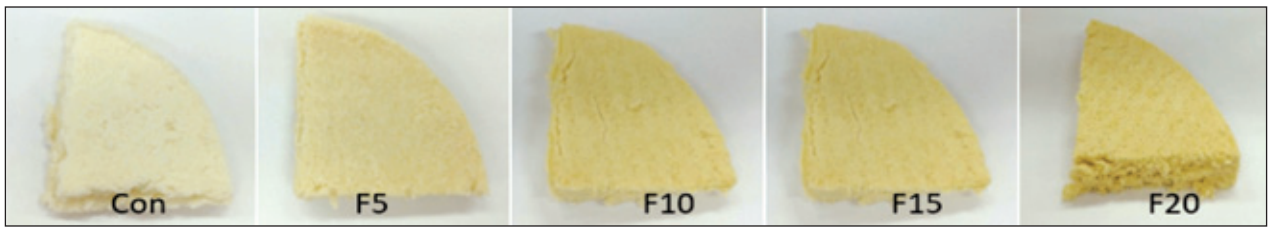

Figure1. Photographs of *Sulgidduk* containing different amounts of firik powder.

Textural properties

Hardness, cohesiveness, springiness, chewiness, and gumminess were compared between samples (Table 6). The control group showed the highest value of hardness (3.43 N), while the values reported for the samples containing firik powder were lower than the value of the control group. However, F5 group showed the lowest value (2.16 N) among the firik-treated samples, while the highest value was reported for F20 group (3.07 N). Cohesiveness is related to the degree of stickiness of rice cake and is a characteristic attributed to the restoring force against binding force or deformation required to maintain the shape or form of food (28). Cohesiveness decreased with an increase in the amount of firik (p<0.05). Thus higher the amount of firik is, it makes easily brittle the product. Springiness is a property that helps the product gain its original shape upon removal of force (28). Springiness value was the lowest for F15 and F20 (7.43 and 7.60 mm, respectively) groups. Gumminess is a collective texture that is related to hardness and cohesiveness. F5 (1.68 N) group had the lowest value for gumminess, as observed with the hardness results. Chewiness value which is the energy required to chew was lower for samples supplemented with firik than the control samples, and F5, F10, F15, and F20 groups had lower values (30). The texture of rice cake depends on the quantity and size of starch, ratio of amylose to amylopectin, gelatinization or retrogradation degree of starch, and fiber content of sub-ingredients (31). Firik has 16.5% dietary fiber that imparts good prebiotic effects and other health-promoting benefits (32-33). The report published by USDA (2017) revealed that rice and firik had a fiber content of 2.4 and 15.6 g/100 g, respectively. It is suggested that the hardness and chewiness decreased with the presence of these dietary fibers, which may interfere with the binding of amylose or amylopectin and water absorption capacity (29). This result was similar to the sesame leaf powder (30) containing high dietary fiber. It was reported that addition of sesame powder reduces the hardness and chewiness of rice cake. The opposite results were observed with the addition of pumpkin leaf powder, wherein hardness, springiness, and chewiness increased as the amount of pumpkin leaf powder increased (31). These results reported that addition of sub-ingredients which have lower moisture content than the main ingredient give more hard texture to the rice cake by competing with the main ingredient. Then the main ingredient loses their moisture to sub ingredient (31). The major factors affecting the texture were addition of the firik powder to *Sulgidduk* widens the intermolecular gap thereby resulting in the decrease in the hardness.

Evaluation of retrogradation by Avrami equation

Starch undergoes gelatinization upon cooking or processing and starch retrogradation during cooling or storage affects the texture, digestibility, shelf-life, and consumer preference of starch-containing food (32).

Table 6. Textural properties of *Sulgidduk* with different amounts of firik powder

Properties	Freekeh powder content (%)					F-value (p-value)
	Control[2)]	F5	F10	F15	F20	
Hardness (N)	3.43±0.35[a1)]	2.16±0.31[c]	2.44±0.06[c]	2.85±0.08[b]	3.07±0.12[ab]	15.722[***] (0.000)
Cohesiveness (%)	0.80±0.31[a]	0.78±0.02[a]	0.71±0.02[b]	0.66±0.03[bc]	0.61±0.02[c]	21.193[***] (0.000)
Springness (mm)	8.69±0.33[a]	8.81±0.34[a]	8.59±0.05[a]	7.43±0.46[b]	7.60±0.44[b]	10.345[***] (0.001)
Chewiness (N*mm)	23.64±1.09[a]	14.76±1.95[b]	14.87±0.69[b]	14.03±1.12[b]	14.27±0.97[b]	33.093[***] (0.000)
Gumminess (N)	2.72±0.10[a]	1.68±0.24[b]	1.73±0.08[b]	1.89±0.07[b]	1.88±0.05[b]	33.383[***] (0.000)

[1)] Values are expressed as mean ± standard deviation

[2)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.

[a-c] Different superscripts indicate significant differences between values in the same row according to Duncan's range test (p<0.05)

[***] p < 0.001

Upon crystallization, starchy food exhibits an increase in rigidity and firmness and the polymer and solvent phases undergo separation, a process called as syneresis that produces undesirable effects on starch-based products (19). To retard the process of retrogradation of starch, food company usually control the temperature, moisture content, pH, and starch type and add saccharides that inhibit the decrease in the moisture content or emulsifying agents that reduce the stiffening speed. The retardation in the retrogradation of *Sulgidduk* supplemented with firik was analyzed using the Avrami equation at 20°C for 0, 3, 6, 9, 12, 24, 36, and 48 h. The values of Avrami exponent (*n*) represent the crystallization state of *Sulgidduk* and were 0.98, 0.73, 0.93, 0.98, and 0.96 for the control, F5, F10, F15, and F20 groups, respectively. Lower Avrami exponent (*n*) and rate constant values and higher time constant value corresponded to stronger retrogradation retardation effects (34). Thus, F5, F10, and F20 had stronger retrogradation retardation effects than the control group, with F5 group showing the strongest effects as shown in Table 7. Retardation of retrogradation was dependent on the amount of additives. A previous study (7) showed similar results, wherein the retardation of retrogradation was different based on the ratio of additives added to the rice cake. The value was 0.17 for the control group and 0.06, 3.80, and 2.73 for 0.5%, 3%, and 4% groups. These results show that 5% firik had better retardation of retrogradation effects than the other groups. Time constant (1/k) was used as the reciprocal of rate constant (k) and indicated the rate of retrogradation; the value was 19.67 (lowest value) for the control group and 28.69, 42.13 (the highest value), 27.20, and 23.95 for F5, F10, F15, and F20 groups, respectively. Thus, the addition of 5% or 10% of firik powder maximized the retardation of retrogradation in *Sulgidduk*. We used Avrami equation to analyze retrogradation retardation and observed results similar with those reported by previous studies using wheat (11) and chia seed (29), wherein low values of Avrami exponent and rate constant and high values of time constant were observed for the groups supplemented with wheat or chia seed. Kim & Chung (11) found that the addition of 10% and 20% wheat may retard retrogradation. We found that the addition of 5% and 10% firik may retard retrogradation to a level similar to that observed with the addition of less than 5% wheat.

Sensory evaluation

The results of the sensory evaluation test of *Sulgidduk* containing firik powder were shown in Table 8. The control group had the highest score (7.34) for appearance, while F5 group showed the highest score for flavor, texture, sweetness, and overall acceptability. No significant difference was observed in the flavor among different samples. F5 (6.00) and F10 (6.62) groups had higher values than the control (6.10) group ($p<0.05$), while F15 (5.78) and F20 (5.58) groups had lower values than the control group. The scores of overall acceptability were higher for F5 (7.14) and F10 (6.58) groups than for the control group (6.42). F15 (5.60) and F20 (5.50) groups had lower scores than the control group but had same scores for flavor, texture, sweetness, and overall acceptability.

Table 7. Avrami exponent (n), rate constant (k), and time constant (T) of *Sulgidduk* with different amounts of firik powder

Avrami equation analysis	Freekeh powder content (%)				
	Control[3)]	F5	F10	F15	F20
Avrami exponent(n)[1)]	0.9838	0.7264	0.9324	0.9763	0.9577
Rate constant (k)[2)]	5.01×10^{-2}	3.49×10^{-2}	2.37×10^{-2}	3.68×10^{-2}	4.17×10^{-2}
Time constant (h) (1/k)	19.67	28.69	42.13	27.20	23.95

[1)] Values obtained from the slope of plot

[2)] Values obtained from the slope of plot $\ln(E_L-E_t)$ vs time

[3)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.

Table 8. Sensory preference scores for *Sulgidduk* with different amounts of firik powder

Sensory preference	Freekeh powder content (%)					F-value (p-value)
	Control[2)]	F5	F10	F15	F20	
Appearance	7.34±1.32[a1)]	6.64±1.26[b]	5.98±1.17[c]	5.58±1.20[d]	5.10±1.40[d]	24.028[***] (0.000)
Flavor	6.00±1.23[b]	6.62±1.18[a]	6.10±1.36[a]	5.78±1.40[b]	5.58±1.47[b]	4.350[***] (0.002)
Texture (Chewiness)	5.92±1.69[c]	7.22±1.13[a]	6.54±1.55[b]	5.6±1.43[cd]	5.30±1.25[d]	14.593[***] (0.000)
Sweetness	6.12±1.42[b]	6.82±1.26[a]	6.52±1.61[ab]	5.40±1.32[d]	5.60±1.50[cd]	8.804[***] (0.000)
Overall preference	6.42±1.46[b]	7.14±1.26[a]	6.58±1.39[b]	5.60±1.32[c]	5.50±1.30[c]	13.182[***] (0.000)

[1)] Values are expressed as mean ± SD of triplicate observations

[a-d]Different superscripts indicate significant differences between values in the same row according to Duncan's range test ($p<0.05$)

[***]$p < 0.001$

[2)] Control: without firik powder, F5: 5% firik powder, F10: 10% firik powder, F15 :15% firik powder, F20: 20% firik powder.

Conclusion

The purpose of this study was to apply firik to the Korean traditional food, *Sulgidduk*, and examine the antioxidant activities, quality characteristics, retarding retrogradation effect and sensory evaluation. The water holding capacity of each grain was higher for firik at 401.50% and rice showed lower value at 221.74%. The swelling power was varied with the temperature. The results of antioxidant activity showed a significant increase with addition of firik and the color values of *Sulgidduk* (*L* and *a*) decreased with the addition of firik but the *b* value increased as the amount of added firik increased. The hardness and gumminess decreased but not gradually whereas the cohesiveness, springiness and chewiness decreased with the increasing level of firik. F5 received the highest score in the results of Avrami and sensory evaluation. Collectively, these results suggest that the most optimal level of firik to improve the quality characteristics and retard the retrogradation effect was found to be 5% for the production of *Sulgidduk*. This study will allow the value of Sulgidduk increased for improving the quality properties and consumer preference.

References

1. Carsanba E, Ismail A, Mahir T. Examination of firik produced in hatay region in terms of nutritional aspect. Gida J Food 2017; 42: 726-30.
2. Maskan M. Effect of maturation and processing on water uptake characteristics of wheat. J Food Eng 2001; 47: 51-7.
3. Ozkay B, Ozkaya H, Eren N, Unsal AS, Koksel H. Effects of wheat maturation stage and cooking method on physical and chemical properties of firiks. Food Chem 1999; 66: 97-102.
4. D'Edigio MG, Cecchini C, Desiderio E, Cervigni T. Immature wheat grains as functional food. In: Cereals for Human Health and Preventive Nutrition, Brno (Czech Republic), Mendelova Zemedelska a Lesnicka Univerzita, 1998.
5. Mohammed A, Shazad H, Abdellatif M, Akram AAQ, Mohamed I. A study on the effect of black cumin extract on the swelling power, textural, and pasting properties of different starches. Starch-Starke 2016; 68: 1233-43.
6. Ozkaya B, Turksoy S, Ozkaya H, Baumgartner B, Ozkeser I, Koksel H. Changes in the functional constituents and phytic acid contents of firiks produced from wheats at different maturation stages. Food Chem 2018; 246: 150-5.
7. Song KY, O H, Zhang Y, Joung KY, Choi DW, Kim YS. Effect of black carrot (*Daucus carota* L.) powder on quality characteristics and retarding retrogradation by shelf-life of Sulgidduck (rice cake). Prog Nutr 2017; 19: 442-9.
8. Choi EJ, Kim EK. Effect of moringa oleifera leaf on antioxidant and quality characteristics of the korean traditional rice cake sulgidduk. J Food Preserv 2015;41:e12820.
9. Kim SS, Chung YH. The effects of wheat powder addition on retarding retrogradation in rorean rice cakes (karedduk). J Korean Sci Food Nutr 2007; 22: 185-91.
10. Choi EH, Lee JH. Quality characteristics of garaedduk with roasted rice bran. Korean J Culinary Res 2010; 16: 277-86.
11. Kim SS, Chung YH. Texture profiles and retarding retrogradation analysis of a korean rice cake (karedduk) with addition of oligosaccharides. Korean J Food Sci Nutr 2012; 41: 533-8.
12. Park YK, Kim HS, Park HY, Kim HG, Kim MH. Retarded retrogradation effect of garaetteok with apple pomace di-

etary fiber powder. Korean J Food Culture 2011; 26: 400-8.
13. Schoch TJ. Swelling power and solubility of granular starches. In: Methods in Carbohydrate Chemistry. Whistler RL, ed. NY, USA: Academic Press. 1964; p. 106-108.
14. Akay S, Alpak I, Yesil-Celiktas O. Effects of process parameters on supercritical CO2 extraction of total phenols from strawberry (*Arbutus unedo* L.) fruits: An optimization study. J Sep Sci 2011; 34: 1925-31.
15. Vosoughi N, Gomarian M, Pirbalouti AG, Khaghani S, Malekapoor F. Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. Ind Crops Prod 2018; 117: 366-74.
16. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239: 70-6.
17. Rojas JA, Rosell CM, de Barber CB. Role of maltodextrins in the staling of starch gels. Eur Food Res Technol 2001; 212: 364-8.
18. Alamri M, Hussain S, Mohamed A, Qasem AAA, Ibraheem M. A study on the effect of black cumin extract on the swelling power, textural, and pasting properties of different starches. Starch-Starke 2016; 68: 1233-43.
19. Vandeputte GE, Derycke V, Geeroms J, Delcour JA. Rice starches. II. Structural aspects provide insight into swelling and pasting properties. J Cereal Sci 2003; 38: 53-9.
20. Anderson RA. Water-absorption and solubility and amylograph characteristics of roll-cooked small grain product. Cereal Chem 1982; 59: 265-9.
21. Jakobek L. Interactions of polyphenols with carbohydrates, lipids and proteins. Food Chem 2015; 175: 556-67.
22. Watanabe M, Musumi K, Ayugase J. Carotenoid pigment composition, polyphenol content, and antioxidant activities of extracts from orange-colored Chinese cabbage. LWT-Food Sci Tech 2011; 44: 1971-5.
23. Xu JG, Hu QP, Wang XD, Luo JY, Liu Y, Tian CR. Changes in the main nutrients, phytochemicals, and antioxidant activity in yellow corn grain during maturation. J Agr Food Chem 2010; 58: 5751-6.
24. Zhang Y, Kim JH, Song KY, O H, Kim YS. Quality characteristics and antioxidant activities of sulgidduck with asparagus (*Asparagus officinalis* L.) powder. J East Asian Dietary Life 2016; 26: 63-72.
25. Hwang YR, Hwang ES. Quality characteristics and antioxidant activity of *sulgidduk* prepared by addition of aronia powder (*Aronia melanocarpa*). Korean J Food Sci Technol 2015; 47: 452-9.
26. Yang MO. Quality characteristics of sulgidduk added with cabbage powder. East Asian Dietary Life. 2009; 19: 729.
27. Park MK, Lee JM, Park CH, In MJ. Quality Characterisitics of Sulgidduk Containing *Chlorella* Powder. J Korean Soc Food Sci Nutr 2002; 31: 225-229.
28. Kim MY, Kim SJ, Huang Y. Development and characterization of easily chewable korean rice cake (*Garaedduk*) for Elderly. J Korean Sci Food Nutr 2018; 31: 80-8.
29. O H, Choi BB, Kim YS. Quality characteristics and antioxidant activities of *sulgidduk* (rice cake) added with chia (*Salvia hispanica* L.) seed powder. J Korean Sci Food Nutr 2017; 46: 61-4.
30. Choi BS, Kim HY. Quality characteristics of Sulgidduk added with perilla Leaves. Korean J Culinary Res 2010; 16: 299.
31. Song KY, O H, Zhang Y, Joung KY, Kim YS. Effects of pumpkin (*Cucurbita moschata* Duch.) leaf powder on quality characteristics, antioxidant activities, and retarding retrogradation by shelf-life of sulgidduck (rice cake). J Korean Food Sci Nutr 2016; 45: 1792-8.
32. Bird AR, Mular M. Product analysis: green wheat Firik. CSIRO Health Sciences and Nutrition, 2003; Product Analysis Report: 4.
33. Pool-Zobel BL. Health-promoting properties of inulin and oligofructose - Part 1: Dietary fibre effects, prebiotic properties, improvement of absorption of calcium and associated secondary effects. Ernahrungs Umschau, 2007; 54: 8
34. Biliaderis CG. The structure and interactions of starch with food constituents. Can J Physiol Pharmacol 1991; 69: 60-78.

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Original article

Quality of packed baby kiwi cultivar 'Hortgem Tahi®' and 'Hortgem Rua®'

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Summary. Baby kiwi fruits (*Actinidia arguta*) represents a relatively new product in the fresh italian market and the crop is grown in recent years. They are similar to berries and their storage period is different from other kiwi. The aim of this work is to evaluate the evolution of some quality parameters of packaged fruits of two cultivar actually marketed under the Nergi® brand (Hortgem Tahi®and Hortgem Rua®). We monitored weight loss, colour, total soluble solid content, titratable acidity, dry matter, firmness, textural parameters and total polyphenol content of fruits stored at low temperature (1±1°C) up to 60 daysWe can state that baby kiwi are very susceptible to pulp firmness decrease. Generally samples of cv Hortgem Tahi® and Hortgem Rua® packed with the lid have maintained an higher pulp if compared with the control values. The stability of the peel colour and the limiting of the weight losses during the whole storage period are successfully preserved in this preliminary study.

Key words: *Actinidia arguta*, post-harvest, storage, quality, punnet

Introduction

Fruits of *Actinidia arguta* (Siebold et Zucc), also known as baby kiwi, kiwi berries, mini kiwi, hairless kiwi and hardy kiwifruit, are the smaller version of the renowned green-fleshed (*Actinidia deliciosa*) and yellow-fleshed (*Actinidia chinensis*) kiwifruit. The region of origin of this species includes Russian Siberia, Northern China, Japan and Korea (1, 2) and different varieties and cultivars are cultivated in the word. Cultivars Issai, Weiki, Geneva, Hortgem Tahi®, Hortgem Rua®, Hortgem Toru®, Hortgem Wha®, Jumbo and Ananasnaya are the most important in Europe. The production of *Actinidia arguta* has increased in the last few years, as a result of a growth in consumer demand.

Due the rich content of bioactive compounds (3, 4) the sweet and aromatic taste and the high vitamin C content (5), they are known as healthy fruits. They are marked as berries because the small size and the nakedness makes their management during the post-harvest chain similar to those of the other soft fruits such as blueberries or raspberries (6).

Fruits of Actinidia arguta show a short shelf life (1-2 months) (7,8) if compared to other kiwi (9) and the quality at the harvesting time is fundamental to influence the storage process (10). In fact the green kiwifruit (Actinidia deliciosa) have an indicative shelf-life of 6 to 8 months and the gold kiwifruit (Actinidia chinensis Planch.) of 4 to 6 mouths (7,11). The rapid dehydration and softening process (12,13) cause important economic losses during the during the commercialisation because the consumer rejection (14,15) so, their management after harvest is crucial. The baby kiwi softening can be limited by low temperature but limited studies about the evolution of the quality parameters during the post-harvest are reported. Edible coating solutions maintaining fruits under cold temperature could be improve the shelf life of cv. Ananasnaya of three weeks (16) while Latocha et al. (9) studied the post-harvest life of *A. arguta* and various hybrids, un-

der air and controlled atmosphere for 2 months. From field to fresh market sector the importance of packaging solution is well known. Considering the baby kiwi edibility with peel it's necessary to minimize the treatments. In this context the use of good packaging solutions is fundamental. Rigid containers such as plastic basket or ventiled clamshell are able to protect berries from damage and plastic overwrape are usefull to resist environmental contaminants. On the contrary, unsuitable packaging can cause an accumulation of moisture inside the package and a possible development of spoilage by microorganisms (17). Considering the limited knowledge about the quality of these fruits during post-harvest period the aim of this work is to evaluate the quality and the storability of the baby kiwi of two cultivar (Hortgem Rua® and Hortgem Tahi®) actually marketed under the Nergi® brand in the italian market.

Materials and methods

Fruit source and experimental field location

The baby kiwi orchard was localised at Revello (Cuneo, Piedmont, Italy). Two different cultivars Hortgem Rua® and Hortgem Tahi® (marketed under the Nergi® brand) of *A. arguta* (Siebold et Zucc.) were used for the work. Both cultivars are new patented varieties and come from New Zealand (18,19). Fruits of each cultivar were collected at the harvesting maturity stage, placed inside a plastic fruit box and transported to the Agrifrutta Cooperative warehouse (Peveragno, Cuneo, Piedmont, Italy) for the storage process at 1±1°C in normal atmosphere.

Experimental design and sampling procedure

The selected baby kiwi fruits, of each cultivar, were packed inside plastic polyethylene terephthalate punnets (5 × 10 × 3.7 cm) each one contained 0.125 kg of fruit. The fruits were packaged in the punnets without a lid (control) and in the punnets with lid.. The *A. arguta* were stored in a cold room (1±1°C), for up to 60 days. For each cultivar and for each type (control and lids) a total of 72 punnets were prepared at the start beginning. The quality parameters of the samples were analysed the day of packing the fruit (day 0) and after 20, 40 and 60 days of storage. For each sample and for each analysis of quality, six punnets were randomly selected and analysed regarding the weight losses, the quality parameters (total soluble solids (TSS), titratable acidity (TA), and dry matter (DM)), colour parameters (luminosity, chroma and hue angle), firmness, texture profile analysis (TPA) (hardness, cohesiveness, gumminess and springiness) and the total phenolic content (TPC). All analysis were performed according with the responsible of the warehouse integrating to the analysis on kiwi (13) the most important analysis that are usually performed on berry fruits in the post-harvest (20-22).

Weight loss

Weight loss (%) was determined using an electronic balance (model SE622, VWR Science Education, Radnor, Pennsylvania, USA), with a 10^{-2}g accuracy. Weight was monitored during the whole storage period and it was calculated as difference between initial and final punnet weights.

Quality parametres

Total soluble solids (TSS) were evaluated with a digital refractometer Atago® Pal-1 (Atago Co. Ltd., Tokyo, Japan) and expressed as °Brix. For each quality control the instrument was calibrated with distillate water. The titratable acidity (TA) was measured using an automatic titrator (Titritino 702, Methrom, Herisav, Switzerland) and it was determined potentiometrically using 0.1N NaOH to the end point of 8.1 in 5 mL of juice diluted in 25 mL of distilled water.

Dry matter (DR) was measured on 10 whole fruits. The fruits were placed in an oven at 70 ± 2 °C for 24 h. Initial and final weights were measured using an electronic balance and the value were expressed as %, according to Mc Glone et al. (23).

Colour parameters

For each cultivar and sample the colour measurement was performed on the middle of peel of 20 fruits, using a tristimulus CR-400 chromameter (Konica Minolta, Langenhagen, Germany), according to the Commission International d'Eclairage (CIE) L*a*b* system. L^* refers to the lightness and ranged from L^* = 0 (black) to L^* = 100 (white). A negative and positive values of a^* indicates green and red color, respectively

while positive and negative *b** indicate yellow and blue color, respectively (24). These values were used to calculate the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$), which denotes the intensity or colour saturation, and the hue angle (h° = arctangent(b*/a*)), where 0° = red to purple, 90° = yellow, 180° = bluish to green and 270° = blue (25).

Firmness and Textural parameters

The firmness and the texture profyle analysis (TPA) were performed with the Texture Analyser TA.XT.PLUS (Stable Micro Systems USA) (30 Kilo Load Cell). For the firmness measurement a compression test on 20 whole fruits for each sample and cultivar was performed with a 30-mm aluminium flat tipped probe (P/3) to an 10% strain, with a pre test speed of 1 mm/s; test speed 1 mm/s; post test speed 5 mm/s and 5 g trigger force. For the TPA analysis, the instrument was equipped with a 75-mm aluminium compression plate (P/75) and the instrument settings were as follows: setting strain 25%, pre-test speed of 1 mm/s, test-speed 5 mm/s and trigger force 5 g. The parameters analysed were the hardness, the cohesiveness, the gumminess and the springiness.

Total phenolic content (TPC)

For each cultivar an extract of the baby kiwi fruits was obtained using 10 g of sample added to 25 ml of extraction buffer (500 ml methanol, 23.8 ml deionised water and 1.4 ml of 37% hydrochloric acid). After 1 hour in the dark at room temperature, the samples were thoroughly homogenised for few minutes, with an Ultra-Turrax (IKA, Staufen, Germany) and then centrifuged at 3000rpm for 15 minutes. The supernatant obtained by centrifugation, was collected and transferred into glass test tubes and stored at –20°C, until analysis. The TPC was measured using the Folin–Ciocalteu reagent with gallic acid as a standard, at 765 nm, based on Slinkard and Singleton (26). The results were expressed as mg of gallic acid equivalents per 100 g of fresh weight (mgGAE/100g).

Statistical analysis

All the pooled data were analysed using SPSS Statistics 24 (2017, IBM, Milan, Italy) for MAC. Analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test, when the differences were significant. Also, multivariate analysis was carried out, to verify a possible interaction between the packaging and the storage days and, also, a possible single effect between the packaging and the storage day.

Results and Discussion

Weight loss

The weight loss (figure 1) is correlated to the water loss and to the dehydration process during the post-harvest storage, consequently, the evolution of this parameter directly affects the marketability of the product (27). Although a rapid pre-cooling was performed before the packaging process to reduce the respiration rate (28) weight losses occurred for both the cultivars considered up to the end of storage but all the samples fruits maintained with the lid showed lower

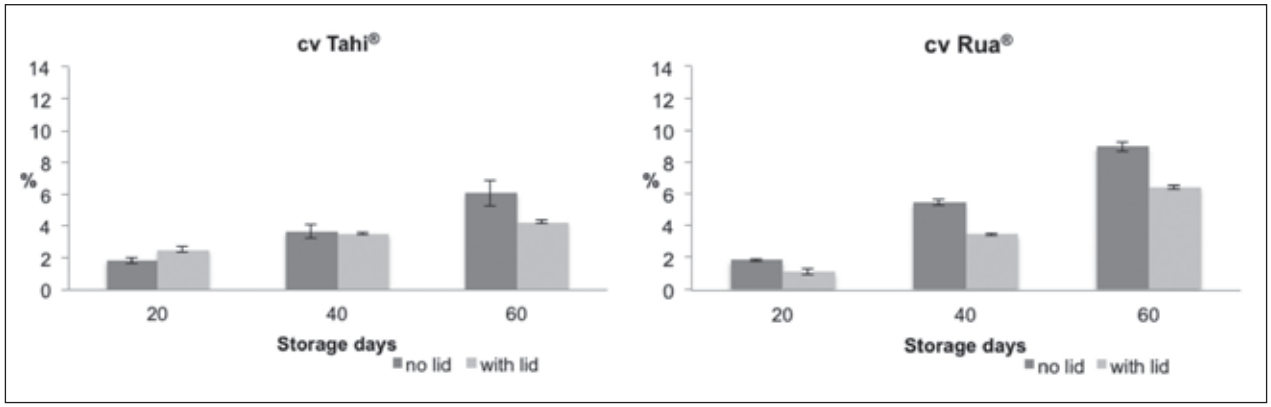

Figure 1. Weight loss (%) for the cv Horgem Tahi® and cv Hortgem Rua during storage

water losses. In fact the lids by retaining moisture and reducing pathological deterioration and consequently metabolic activities of the fruits have maintained an higher hydratation of fruits. Considering the cv Hortgem Tahi® is possible to observe a stabilization of the weight losses between 40 and 60 days of storage (respectively in a range of 5.08±0.32% and 5.11±0.19% for the control and 3.29±0.12% and 3.59±0.14% for packaging with lid). The cv Hortgem Rua® generally showed an increase of the weight losses reaching the maximum at the end of the storage with more than 6% for the control and around 4 % for fruits packed with lid.

Quality parameters

Quality parameters reported in this study included total soluble content (TSS), titratable acidity (TA) and dry matter content (DM) of the fruits. The TSS of *A. arguta* has proved to be to be predominantly represented by sucrose, with small contents of glucose and fructose (29). Regarding the TA, the citric and malic acids are reported to be the more representative but in a lower concentration respect to *A. deliciosa* and *A. chinensis* (30). About the dry matter (DM), *A. arguta* has a lower content than other species of Actinidia because *A. arguta* is richer in non-structural carbohydrates (31). All the quality parameters monitored are reported in Table 1. The TSS content at the packaging day (0 days) was

Table 1. Averages and multivariate analysis (single effects and interactions) of the evolution of the quality parameters (total solid soluble, titratable acidity and dry matter) for the cv Hortgem Tahi® and cv Hortgem Rua®.

	Storage days	TSS (°Brix)			TA (meq L^{-1})			DM (%)		
		Average	S.E.		Average	S.E.		Average	S.E.	
cv Hortgem Tahi®	control									
	20	13.93	0.28	ns	14.92	0.03	ns	19.35	0.48	a
	40	15.53	0.15		14.74	0.48		17.77	0.57	a
	60	14.23	0.75		14.15	0.21		15.77	0.11	b
	with lid									
	20	14.87	0.15	ns	14.76	0.04	ns	18.11	0.49	ns
	40	14.23	0.01		14.56	0.76		15.92	0.36	
	60	14.80	0.56		14.90	0.11		15.74	1.33	
	Single effects/interaction	Sig.			Sig.			Sig.		
	Packaging	0.001			0.020			0.270		
	Storage days	0.001			0.001			0.010		
	Packaging*Storage days	0.001			0.060			0.200		
cv Hortgem Rua®	control									
	20	14.73	0.34	a	10.75	0.15	a	18.21	0.26	a
	40	14.53	0.20	a	8.13	0.69	b	16.32	0.05	b
	60	12.60	0.42	b	9.37	0.21	ab	17.92	0.34	a
	with lid									
	20	13.90	0.38	ns	9.27	0.30	ab	17.78	0.25	a
	40	13.73	0.03		8.98	0.15	b	15.58	0.27	b
	60	14.83	0.54		9.98	0.13	a	17.07	0.11	a
	Single effects/interaction	Sig.			Sig.			Sig.		
	Packaging	0.050			0.010			0.250		
	Storage days	0.020			0.010			0.001		
	Packaging* Storage days	0.930			0.050			0.710		

All data are expressed as average value and the standard error of 15 different fruits. Different letters within the same column indicate significant differences among every harvesting time (Tukey test; p< 0.05).

respectively of 7.31 °Brix and 6.25 °Brix for the cultivar Hortgem Tahi® and Hortgem Rua® (data not showed). In both cultivars onsidered and all samples (control and packaging with lid) is possible to observe an increase in the TSS content if compared with the starting values. According with previous studies of Krupa et al. (32) this happens because the starch in the baby kiwi is used for the fruit respiration (33). In fact the increase in the TSS content would be correlated with the glycolytic enzyme activity that would cause the starch degradation and its conversion in sucrose. Cultivars of *A. deliciosa* and *A. chinensis* contain similar amounts of glucose and fructose, with lower levels of sucrose, whereas *A. arguta* species are particularly high in sucrose with lower glucose and fructose contents (34). Choudhury et al. (35) identified a different activity of the glycolytic enzyme based on the cultivar but, in this current study no important differences were found between the cultivars considered. If compared with *A. deliciosa* and *A. chinensis* cultivars the levels of organic acid in fruits of *A. arguta* are reported to be lower due the lower levels of the quinic acid (30). The ratios of the acids vary as the fruit matures and the total concentration is important for the sugar-acid balance and its influence on the organoleptic qualities. About the titratable acidity (TA) 16.61 meq L^{-1} and 14.66 meq L^{-1} values were observed respectively for Hortgem Tahi® and Hortgem Rua® as initial values (data not showed). All samples of both cultivars show a general decrease during the storage period and the multivariate analysis reported the interaction of the packaging and the storage factors in affecting the TA content ($p<0.05$). About the dry matter (DM) is well known that its value is correlated with a good flavour (36) and fruit with an higher content should be more acceptable to the consumer. Considering that the initial DM content by the two cultivar was of 19.2% and 18.9 % (data not showed) it's possible to assert that in all samples the content was maintained at high levels until the end of storage. The multivariate analysis shows as the packaging and its interaction with the storage time didn't affect statistically the DM values in any cultivar.

Colour parameters

Colour is an important attribute of fruits and influences the consumer's choice and preferences. The parameters describing this attribute evolve during growth, maturation and post-harvest handling of fruit (25). All the colour parameters observed during the storage are reported in table 2. The external colour of *A. arguta* varies according to the genotype of the fruit (3). Based on the current experiment, however, no important differences were found between the cultivars, regarding the luminosity, chroma and hue angle at the starting time (data not showed) and during the storage period. For the cultivar Hortgem Tahi® the initial L*, *C** and hue angle values were of 67.99, 36.61 and -1.04 (data not showed) while for the cultivar Hortgem Rua®, the parameters observed were of 65.88 (L*), 37.58 (*C**) and -1.10° for the hue angle. Only the single effect of the storage days affects statistically the L and chroma values for both the cultivars ($p<0.05$).

Firmness and Textural parametres

Changes in firmness and texture properties are largely attributed to alterations in the composition and structure of cell wall polysaccharides and they have a great commercial importance because these modifications shall not be accepted by consumers. The firmness and the texture parameters were monitored during the post-harvest storage because softening is one of the main issues for the *A. arguta* and models of softening decay are suggested for these fruits (13). The firmness value is the most important parameter for the control of *A. arguta* quality and the decrease of this attribute is due to the polygalacturonase activity (32). All the values measured at the harvesting time (data not showed) are within the range documented by Firsk et al. (16) and as expected for both the cultivars is possible to observe a statistical decrease of the pulp firmness and hardness during the storage period (table 3). Generally samples of cv Hortgem Tahi® and Hortgem Rua® packed with the lid have maintained an higher pulp if compared with the control values. Considering all the parameters with the exclusion of the gumminess for the Hortgem Rua® is possible to confirm that the storage duration influences statistically the TPA values.

Total phenolic content (TPC)

The content of phenolic compounds (figure 2) including phenolic acids, anthocyanins, and flavonoids in the fruits depends on the species and cultivars of Actinidia (32) and the *A. arguta* is a rich source of these bio-

Table 2. Averages and multivariate analysis (single effects and interactions) of the evolution of the color parameters (luminosity, chromacity and hue angle) for the cv Hortgem Tahi® and cv Hortgem Rua® for the 2016 season.

	Storage days	Luminosity (L)			Chroma (C*)			Hue angle (h°)		
		Average	S.E.		Average	S.E.		Average	S.E.	
cv Hortgem Tahi®	control									
	20	57.06	0.86	ns	32.53	0.53		-1.05	0.01	ns
	40	58.24	1.22		28.39	0.38		-1.05	0.01	
	60	58.41	1.04		25.09	1.84		-1.07	0.01	
	with lid									
	20	55.49	0.77	ns	32.06	0.56	a	-1.05	0.01	ns
	40	57.07	0.62		27.73	0.42	b	-1.04	0.01	
	60	58.55	3.96		23.84	2.36	b	-1.05	0.01	
	Single effects/interaction		Sig.			Sig.			Sig.	
	Packaging		0.89			0.71			0.98	
	Storage days		0.06			0.00			0.78	
	Packaging*Storage days		0.32			0.76			0.81	
cv Hortgem Rua®	control									
	20	57.75	0.98	ns	31.45	0.54	a	-1.07	0.01	ns
	40	57.67	0.54		24.42	1.13	b	-1.12	0.03	
	60	56.24	1.59		26.78	0.68	b	-1.10	0.01	
	with lid									
	20	57.75	0.98	ns	30.99	0.58	a	-1.08	0.01	ns
	40	57.67	0.54		26.32	0.59	b	-1.08	0.01	
	60	56.24	1.59		25.20	0.83	b	-1.11	0.02	
	Single effects/interaction		Sig.			Sig.			Sig.	
	Packaging		0.19			0.82			0.54	
	Storage days		0.04			0.04			0.09	
	Packaging*Storage days		0.05			0.96			0.69	

All data are expressed as average value and the standard error of 15 different fruits. Different letters within the same column indicate significant differences among every harvesting time (Tukey test; p< 0.05).

actives. These compounds are considered to be among the best antioxidants in helping the human organism and their function is similar to vitamins effect (4). Due to the unstable structure, phenolic compounds are strongly affected by environmental condition such as light, pH, oxygen, storage temperature and time (37). Due the varietal difference the TPC content of Hortgem Tahi® is greater than those of Hortgem Rua®. Hortgem Tahi® samples packaged with lid maintained highest values if compared with control samples respectively of 12%, 14% and 20% after 20 , 40 and 60 days of storage. On the contrary no differences were observed among samples of Hortgem Rua® wich TPC content was mantained stable in the range of 1346,67 and 1969,14 mq GAE 100 kg^{-1}

Conclusions

The baby kiwi represents a new product in the italian fruit marke. The consumer demand is due to the high quality properties of fruit and to his edibility unpeeled, like a berry fruit and the edibility of the whole fruit with the peel like a berry fruit.

The extension of the conservation in terms of time, the containment of the product losses, the shelf life, improvement, the maintenance of quality and nutritional aspects are key elements for the success of their supply chain. The post-harvest management of *A. arguta* is crucial to maintain the quality of the fruits and the use of packaging with lid can be suggested to

Table 3. Average and multivariate analysis (single effects and interactions) of the evolution of the mechanical property firmness, hardness, cohesivness, gumminess, springness) for the cv Hortgem Tahi® and cv Hortgem Rua®

	Storage days	Fimness (N)			Hardness (N)			Cohesivness			Gumminess			Springness		
		Average	S.E.		Average	S.E.		Average	S.E.		Average	S.E.		Average	S.E.	
								control								
	20	0.33	0.03	a	17.70	22.83	a	0.39	0.01	b	696.19	81.24	a	0.67	0.01	ns
	40	0.17	0.02	b	12.35	89.77	a	0.40	0.02	b	503.09	38.88	a	0.63	0.02	
	60	0.21	0.02	b	4.30	32.66	b	0.53	0.00	a	234.36	17.16	b	0.70	0.01	
								with lid								
cv Hortgem Tahi®	20	2.91	0.27	ns	17.89	21.76	a	0.38	0.01	b	691.52	86.56	a	0.67	0.02	a
	40	2.10	0.11		11.28	88.15	b	0.38	0.01	b	432.85	31.92	b	0.62	0.01	b
	60	2.13	0.46		3.57	68.56	c	0.55	0.02	a	193.33	33.52	b	0.71	0.02	a
	Single effects/ interaction		Sig.			Sig.			Sig.			Sig.			Sig.	
	Packaging		0.180			0.280			0.600			0.120			0.200	
	Storage days		0.100			0.002			0.000			0.003			0.030	
	Packaging* Storage days		0.810			0.860			0.380			0.750			0.080	
								control								
	20	0.24	0.03	a	20.91	16.74	a	0.41	0.01	b	867.45	58.58	a	0.69	0.01	ns
	40	0.11	0.02	b	12.52	11.37	b	0.37	0.02	b	479.09	44.08	b	0.65	0.02	
	60	0.17	0.02	b	3.90	35.28	c	0.53	0.01	a	210.13	18.81	c	0.69	0.01	
								with lid								
	20	0.24	0.04	a	23.41	21.77	a	0.25	0.05	b	612.14	14.26	ns	0.55	0.04	b
cv Hortgem Rua®	40	0.23	0.02	b	15.99	11.15	b	0.35	0.01	b	565.61	41.16		0.64	0.01	ab
	60	0.33	0.02	b	5.21	49.51	c	0.54	0.01	a	283.56	24.14		0.70	0.01	b
	Single effects/ interaction		Sig.			Sig.			Sig.			Sig.			Sig.	
	Packaging		0.080			0.240			0.002			0.100			0.002	
	Storage days		0.010			0.010			0.003			0.420			0.003	
	Packaging* Storage days		0.860			0.830			0.001			0.070			0.003	

All data are expressed as average value and the standard error of 15 different fruits. Different letters within the same column indicate significants differences among every harvesting time (Tukey test; p< 0.05).

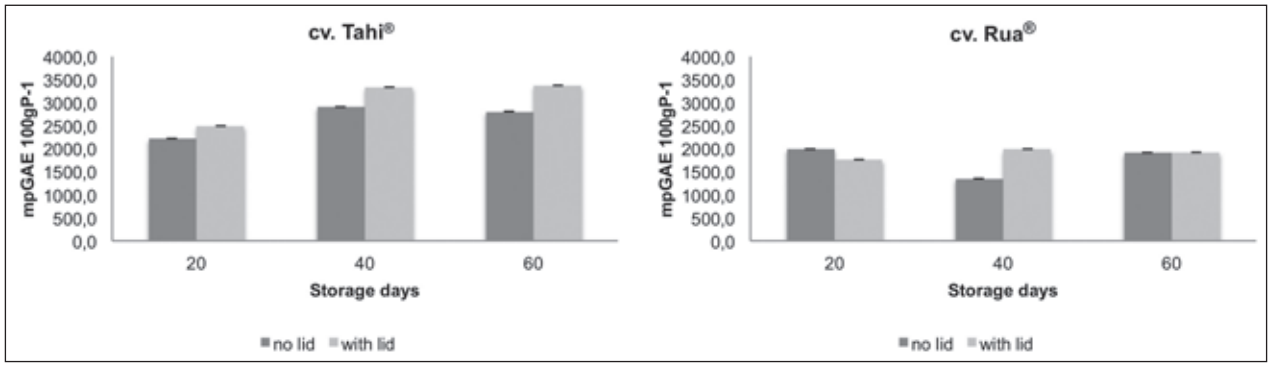

Figure 2. Total phenol content (TPC) for the cv Horgem Tahi® and cv Hortgem Rua® during storage

store fruits at low temperature (1±1°C) over long periods such as 60 days. The marketability of Hortgem Tahi® and Hortgem Rua® depends on different quality index such as the stability of peel colour during the storage period, the maintenance of the firmness pulp and the limiting of the weight losses and all these parameters are successfully preserved in this preliminary study. Considering the emergent market for these fruits the potential of the research in post-harvest sector to support the supply chain process is necessary to improve the knowledge on some post-harvest technology treatment such as the use of MAP, of controlled atmosphere, of ozone and the use of edible coating considering also the safety issues.

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Litterature cited

1. Cossio F, Debersaques F, Latocha P, Kiwiberry (*Actinidia arguta*): New Perspectives for a Great Future. Acta Hortic 2014,1096: 423–434.
2. Debersaques F, Mekers O, Decorte J, Van Labeke MC, Schoedl-Hummel K, Latocha P, Challenges faced by commercial kiwiberry (actinidia argute planch.) Production. Acta Hortic 2014, 1096: 435–442.
3. Latocha P, Jankowski P, Radzanowska J, Genotypic difference in postharvest characteristics of hardy kiwifruit (Actinidia arguta and its hybrids), as a new commercial crop Part I. Sensory profiling and physicochemical differences. Food Res Int 2011, 44(7): 1936–1945.
4. Latocha P, Wołosiak R, Worobiej E, Krupa T, Clonal differences in antioxidant activity and bioactive constituents of hardy kiwifruit (Actinidia arguta) and its year-to-year variability. J Sci Food Agric 2013, 93(6): 1412–1419.
5. Calvez E, Neuberger S, Wagner K, Schoedl-Hummel K, Debersaques F, Management strategies for minikiwi (Actinidia arguta): practical experiences of european growers. Acta Hortic 2014,1096: 451–454.
6. Baudino C, Giuggioli NR, Briano R, Massaglia S, Peano C, Integrated methodologies (SWOT, TOWS, LCA) for improving production chains and environmental sustainability of kiwifruit and baby kiwi in Italy. Sustainability 2017, 9, 1621: doi:10.3390/su9091621.
7. Ritenour MA, Crisosto CH, Garner DT, Cheng GW, Zoffoli JP, Temperature, length of cold storage and maturity influence the ripening rate of ethylene-preconditioned kiwifruit. Postharvest Biol Technol 1999, 15(2): 107-115.
8. Lim S, Han SH, Kim J, Lee HJ, Lee JG, Lee EJ, Inhibition of hardy kiwifruit (Actinidia aruguta) ripening by 1-methylcyclopropene during cold storage and anticancer properties of the fruit extract. Food Chem. 2016,190: 150–157.
9. Latocha P, Krupa T, Jankowski R, Radzanowska J, Changes in postharvest physicochemical and sensory characteristics of hardy kiwifruit (Actinidia arguta and its hybrid) after cold storage under normal versus controlled atmosphere. Postharvest Biol Technol 2014, 88: 21–33.
10. Oh SB, Muneer S, Kwack YB, Shin MH, Kim JG, Characteristic of fruit development for optimal harvest date and postharvest storability in 'Skinny Green'baby kiwifruit. Scientia Hortic 2017, 222: 57–61.
11. Yilmaz D, Yildirim I , Effects of different storage techniques on rupture properties of kiwifruits J. Food Measurement Characterization, 2016 10(3): 539–545.
12. Strik BC, Hummer KE, Ananasnaya'hardy kiwifruit. J Am Pomol Sci 2006, 60(3): 106–112.
13. Giuggioli NR, Girgenti V, Baudino C, Giacalone G, Chiabrando V, Prediction models for the softening of new commercial Actinidia cultivars. J Food Process Preserv 2017, DOI: 10.1111/jfpp.13245.
14. Kitinoja L, Saran S, Roy SK, Kader A.A., Postharvest technology for developing countries: challenges and opportunities in research, outreach and advocacy. J Sci Food Agric 2011, 91(4), 597–603.
15. Bovi GG, Caleb OJ, Linke M, Rauh C, Mahajan PV, Transpiration and moisture evolution in packaged fresh horticultural produce and the role of integrated mathematical models: A review. Biosyst Eng 2016, 150: 24–39.
16. Fisk CL, Silver AM, Strik BC, Zhao Y, Postharvest quality of hardy kiwifruit (*Actinidia arguta 'Ananasnaya'*) associated with packaging and storage conditions. Postharvest Biol Technol 2008, 47(3): 338–345.
17. Powers T, Calvo WJ, 2003. Moisture regulation. RAhvenainen, Woodhead Publishing Limited, England.
18. Mcneilage MA, Beatson RA, Macrae EA (2001). Patent US 2001/0042258 P1, United States Plant Patent, pp. 1–3.
19. Mcneilage MA, Beatson RA, Macrae, EA (2004). Patent N8 US PP14,625 P3 United States Plant Patent, pp. 126.
20. Peano C, Girgenti V, Giuggioli NR, Change in quality and volatile constituents of strawberries (cv. Evie2) under MAP storage. Journal of Food, Agriculture and Environment, 2014, 12:93-100.
21. Giuggioli NR, Girgenti V, Peano C, Qualitative performance and consumer acceptability of starch films for the

blueberry modified atmosphere packaging storage, Pol J Food Nutr Sci, 2017, 67:129-136.
22. Giuggioli NR, Briano R, Girgenti V, Peano C, Quality effect of ozone treatment for the red raspberries storage. Chemical Engineering Transactions, 2015, 44:25-30.
23. Mc Glone VA, Jordan RB, Seelye R, Clark CJ. Dry matter-a better prediction of the post-storage soluble solids in apple?. Postharvest Biol Technol 2003, 28: 431–435.
24. McGuire RG, Reporting of objective color measurements. Hort Sci., 1992, 27: 1254–1255.
25. Pathare PB, Opara UL, Al-Said FAJ, Colour measurement and analysis in fresh and processed foods: a review. Food Biopr Technol 2013, 6(1): 36–69.
26. Slinkard K, Singleton VL, Total phenol analysis: automation and comparison with manual methods. Ame J Enol Vitic 1977, 28 (1): 49–55.
27. Prusky D, Reduction of the incidence of postharvest quality losses, and future prospects. Food Sec 2011, 3(4): 463–474.
28. Brosnan T, Sun DW, Precooling techniques and applications for horticultural products—a review. Int J Refrig 2001, 24(2): 154–170.
29. Henare S.J. The Nutritional Composition of Kiwifruit (Actinidia spp.). Nutrit. Composition Fruit Cultivars, 2015. 337.
30. Nishiyami II, Fukuda T, Shi-mohashi A, Oota T,. Sugar and organic acid composition in the fruit juice of different Actinidia varieties. Food Sci Technol Res 2008, 14: 67–73.
31. Boldingh H, Smith GS, Klages K, Seasonal concentrations of non-structural carbohydrates of five Actinidia species in fruit, leaf and fine root tissue. Ann Bot 2000, 85(4): 469–476.
32. Krupa T, Latocha P, Liwińska A, Changes of physicochemical quality, phenolic and vitamin C content in hardy kiwifruit (Actinidia arguta and its hybrid) during storage. Sci Hortic 2011, 130(2): 410–417.
33. MacRae E, Quick WP, Benker C, Stitt M, Carbohydrate metabolism during postharvest ripening in kiwifruit. Planta 1992, 188(3): 314–323.
34. Henare SJ, The Nutritional Composition of Kiwifruit (Actinidia spp.), 337-370.
35. Choudhury SR, Roy S, Sengupta DN, A comparative study of cultivar differences in sucrose phosphate synthase gene expression and sucrose formation during banana fruit ripening. Postharvest Biol Technol 2008, 54(1): 15–24.
36. Harker FR, Carr BT, Lenjo M, MacRae EA, Wismer WV, Marsh KB, Gunson FA, Consumer liking for kiwifruit flavour: A meta-analysis of five studies on fruit quality. Food Qual Prefer 2009, 20(1): 30–41.
37. Kim AN, Kim HJ, Chun J, Heo HJ, Kerr WL, Choi SG, Degradation kinetics of phenolic content and antioxidant activity of hardy kiwifruit (*Actinidia arguta*) puree at different storage temperatures. LWT, 2018, 89: 535–541.

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Original article

A study on the feed value, *in vitro* digestibility and methane production of tobacco (*Nicotiana tabacum* L.) field waste

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Summary. This study aims to determine the nutrient contents, forage quality, *in vitro* true digestibility and methane production of the pellets prepared using straw and meal of tobacco (*Nicotiana tabacum* L.). Tobacco stalks (straws) and seeds collected from 25 different locations were used in this study. Daisy incubator was used to determine the *in vitro* true digestibility. *In vitro* gas production of the feed was determined using Hohenheim gas test while infrared methane analyzer was used to determine methane production. The study was designed in accordance with the completely randomized design. The results of the study showed that tobacco meal offers higher values in terms of crude protein and ether extract contents, while it offers lower ash, nitrogen free extract contents and cell wall fiber components when compared to tobacco straw (P<0.001). The crude protein content of the feed was found to be 4.69% for tobacco straw and 38.61% for tobacco meal. Tobacco meal outperformed tobacco straw with respect to methane production (P<0.05), organic matter digestibility, energy values and *in vitro* true digestibility (P<0.001). With respect to relative feed value, tobacco straw is classified under "5-reject" quality class. The condensed tannin contents of tobacco wastes were found to be below the risk limits for ruminants. The results suggest that tobacco straw can be used as an alternative to wheat straw and poor quality hay, while tobacco meal can be used as an alternative for some meals such as cottonseed meal and sunflower seed meal.

Key words: *In vitro* true digestibility, methane, pellet, tobacco straw, tobacco meal

Introduction

Ruminants require the provision of quality and cheap forage on a regular basis. However, fodder crop cultivation in the world is not sufficiently able to meet the forage need which translates into significant amounts of forage deficit. Price hikes of the poor quality forage (straws, etc.) in some countries in times when there is a shortage of forage have a negative impact on the livestock production economy. Therefore, the importance of alternative resources to be used to close the forage gap comes to prominence. Today, several studies are investigating the potential use of tree leaves, prickly weeds and shrubs, industrial waste and harvest waste as forage sources (1). With the use of a number of waste which are offering high nutritional values in animal nutrition, reintegration of such waste to the economy is of importance. Pehlevan and Ozdogan (2) reported that tobacco harvest waste of graze by small ruminants in a field after tobacco harvest. However, consumption of the animals was not at a satisfactory level. In this context, it can be seen that tobacco harvest waste cannot be used economically. Nevertheless, tobacco straws (TS) can be repurposed as forage source in animal nutrition while tobacco seeds can be used as concentrates feed source (tobacco seed meal = TM) after the oil is extracted for its use in biodiesel production.

Now the advantages of pelleting is becoming clear and straws, alfalfa, grape pomace, sugar beet leaves and straws are pelleted in order to be used in ruminant nutrition. In addition to physical benefits of pelleting, ease of transportation, homogeneity of the feed, longer shelf life, elimination of feed loss due to scatter, increased feed consumption, reduction in the pathogen microorganisms due to the heat component of the pelleting process are some of the other advantages of pelleting (1, 3, 4).

Ruminants on Earth produces 80 to 115 million ton of methane gas annually which leads to environmental issues and waste of valuable feed energy. Methane production in ruminants is more common when poor quality forages are consumed. Recent studies on rumen metabolism commonly focus on reduction of the methane production (5). *In vitro* gas production technique is often used in such studies. Wright et al. (6) reported that processed forage and pelleting reduce methane production while Gulecyuz (7) found that pelleting increases the digestibility. Current study aims to define the nutrient compositions, *in vitro* gas production, methane production and *in vitro* true digestibility (IVTD) in relation with the consumption of TS and TM, two tobacco harvest wastes.

Materials and Methods

In the experiment we used stalks and seeds of tobacco *(Nicotiana tabacum L.)* plants collected from 25 different locations in 5 different geographical regions of Turkey. After being dried and ground to a size able to pass through 4mm sieve (tobacco straw=TS), TS was pelleted. However, TM was pelleted without any additives after the oil available in the seeds was extracted using a press for biodiesel production.

Rumen fluid used in *in vitro* studies with approximately 150-200 grams rumen content collected from three Simmental X Holstein breed bulls (Average 24 months age and 650-750 kg Live weight) slaughtered at a local slaughterhouse. Then it was brought to the laboratory within 15-20 minutes in thermos (39 °C). Rumen content mixed and it was taken under CO_2 and were filtered through two layers of cheesecloth.

Determining Chemical Composition of Samples

All the TS and TM were dried. Then, dried TS and TM's were milled in a hammer mill through a 1 mm sieve for determining chemical compositions, *in vitro* gas production parameters and IVTD*'s* assays. The samples were analysed for ash, dry matter (DM) and crude protein (CP) contents were analysed according to AOAC (8). Kjeldahl N and CP was calculated by multiplying N by 6.25. The crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) analysis were done according to Van Soest *et al.* (9) using Ankom 2000 semi-automated fiber analyser. Besides, ether extract (EE) content was determined using Ankom ^{XT15} analyzer (10). The contents of organic matters (OM=DM-ash), nitrogen free extract (NFE=DM-(CP+ash+EE+CF)), cellulose (Cel= ADF-ADL) and hemicellulose (Hcel = NDF-ADF), were determined by calculation. Condensed tannin contents were determined according to Makkar et al. (11). All chemical analyses of samples were carried out in triplicate.

Determining rumen fluid pH, volatile fatty acids (VFA) and ammonia-N (NH_3-N) contents

The pH value of rumen fluid before using *in vitro* studies was determined using digital pH meter (Hanna I. 1332) with 3 replications. The volatile fatty acids analysis of rumen fluid were done using a gas chromatography (Agilent Technologies 6890N gas chromatography, Cat. 11023, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 um df. Maximum temperature: 260°C) according to Wiedmeier et al. (12). Rumen fluid amonnia-N analysis were done using Kjeldahl methods according to Blümmel et al. (13) in 3 replicates.

Determining in vitro gas production of TS and TM

In this study, approximately 200 mg dry weight of samples (TS and TM) were weighed into 100 ml calibrated glass syringes following Hohenheim gas test procedures of Menke and Steingass (14). The syringes were warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39 °C. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Five repetitions of each sample were used in the *in vitro* gas production experiment. Net gas produc-

tions of TS and TM were determined at 24 h after incubation and corrected for blank and hay standard (University of Hohenheim, Germany). Cumulative gas production data were fitted to the model of Ørskov and McDonald (15) by the NEWAY computer package programme: $y = a + b(1-exp^{-ct})$

where: a, gas production from the immediately soluble fraction (ml), b, gas production from the insoluble fraction (ml), a + b, potential gas production (ml), c, gas production rate constant for the insoluble fraction (ml/h), t, incubation time (h), y, gas produced at time 't'.

Organic matter digestibility (OMD), metabolic energy (ME) and net energy lactation (NE_L) contents of TS (1) and TM (2) were estimated using equations given below:

- OMD, % = 14.88+ 0.8893GP + 0.448CP + 0.651 ash (16)....(1)
- OMD, %= 0.7602GP + 0.6365CP + 22.53 (17)....(2)
- ME, MJ/kg DM = 2.20+0.136GP + 0.057CP + 0.002859 EE^2 (16)....(1)
- ME, (MJ/kg KM) = 1.06+0.157GP + 0.084CP + 0.22 EE - 0.081 ash (14)....(2)
- NE_L, MJ/kg DM = 0.101GP + 0.051CP + 0.11EE (14)....(1)
- NE_L, (MJ/kg DM) = 0.075GP + 0.087CP + 0.161EE + 0.056 NFE-2.422 (14)....(2)

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%), NFE: Nitrogen free extract (%)

Determination of in vitro true digestibilities

Daisy incubator makes *in vitro* NDF disappearance study easy and efficient because it use an equipment which was designed with four rotating digestion jar and maintains constant, uniform heat and agitation within a controlled (39.5 °C) chamber (9; 18). Daisy incubator instrument contains 4 cylinder incubators which 1 cylinder need 1600 ml buffer solution and 400 ml rumen fluid as inoculums and bag filter (25 pieces). Filter bags F57 could be placed inside the each other cylinder with solution. The cylinder was bubbled with CO_2 immediately before closed with lid of cylinder well and placed into incubator for 48 h. After incubation, filter bags was cleaned under water flow and dried. The bags was analyzed for NDF digestibility with fibre analyzer. *In vitro* true digestibilities of TS and TM samples were estimated as follows;

IVTD, % =100 – ((W3-(W1xC1))*100)/W2

Where : W1: Weight of filter bag, W2: Weight of sample, W3: Final weight after NDF analysis, C1: The bag without sample was prepared also for correction.

Determining methane production of TS and TM

Methane contents (%) of total gas produced at 24 h fermentation of TS and TM were measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath Germany) (19). After measuring gas produced at 24 h incubation, gas samples was transferred into inlet of the infrared methane analyzer with the syringe. Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) X the percent of methane (%)

Determination of releative feed values (RFV)

Because of, the chemical contents of TM were similar concentrates, it was not calculated the RFV for TM. However, RFV of TS were calculated as follows (20).

Dry matter digestibility (DMD, %)= 88.9-(0.779 x ADF%)

Dry matter intake (DMI, live weight, %)= 120/(NDF%)

Relative feed value (RFV,%)= (DMD x DMI)/1.29

Statistical Analysis

The data obtained from the experiments is analyzed using SPSS 20.0 software package program. Nutrient content, *in vitro* gas production and IVTD data of the feeds investigated in this study were analyzed in accordance with the completely randomized design controlling for normality and variance homogeneity. Permutatational multivariate analysis of variance method was used to analyze condensed tannin content of the feeds using the NPMANOVA software (21).

Results

Nutrient contents of the TS and TM tested in the experiment are shown in Table 1. It was found that the OM content of TS was 91.09% while the ash content of TS was 8.91%. The OM content of TM was

determined to have an average of 93.86%, while the ash content of TM was found 6.14%. According to these results, it can be said that TM is a forage source with high OM content. With respect to the CP content which is one of the most important nutrient when assessing forages, TS was found to have an average of 4.49% while TM was found to have an average of 38.61%. It was also seen that TS has a higher CF content (49.88%) when compared to TM (27.73%).

According to Table 1, some of the important contents of TS are as follows: NDF content was 70.02%; ADF content was 51.13%; ADL content was 17.82%; HCel content was 18.89% and Cel content was 33.31%. For tobacco meals, on the other hand, NDF content was 43.44%, ADF content was 32.41%, ADL content was 17.82%, HCel content was 11.03% and Cel content was 17.39%. Results showed that TS has higher cell wall fiber components when compared to TM ($P<0.001$).

The pH, NH_3-N and VFA contents of the rumen fluid used for in vitro studies were found as pH: 5.70 (5.65-5.80), total volatile fatty acid (TVFA): 95.35± 0.85 mmol/L, acetic acid: 49.7± 0.28 mmol/L, propionic acid: 24.15± 0.76 mmol/L, butyric acid: 18.12± 0.61 mmol/L, isobutyric acid: 2.09± 0.34 mmol/L, isovaleric acid: 1.07± 0.04 mmol/L and valeric acid: 1.21± 0.08 mmol/L and rumen fluid NH_3-N contents: 29.17± 1.16 mg/100 ml.

Table 2 shows the *in vitro* gas production and pH value after 96 h for both TS and TM. According to the Table 2, however there are differences between TS and TM in terms of *in vitro* gas production at the onset, no significant difference was found between the two after 12 hours incubation. In terms of pH value at the end of 96 h incubation, lower values were found for TM when compared to TS ($P<0.05$).

Table 3 shows the *in vitro* gas production parameters, methane production, OMD, ME, NE_L and IVTD for both TS and TM. Tobacco meal outperformed TS with respect to OMD, ME, NE_L and IVTD ($P<0.001$). In addition, results showed that TM has significantly higher IVTD values when compared to TS ($P<0.001$). A significant difference was found between the TS and TM analyzed in this study with respect to the gas production rate constant "c value" ($P<0.001$) TM was found to have a higher gas production rate constant. Nevertheless, there were no significant differences between TS and TM in terms of total gas production, "a+b value". However, TM outperformed TS with respect to methane production ($P<0.05$) OMD, ME, NE_L and IVTD contents ($P<0.001$).

Table 4 shows the RFV, DMD, DMI forage quality class and condensed tannin content of TS and TM. Adopted in many developed countries in the marketing and pricing of forages, RFV was found at an average of 65.88 for TS tested in this study. According to the RFV results TS was classified under "5-reject" quality class. It was also found that the CT contents of TS and TM were below 5% and there was no significant difference between CT results.

Discussion

According to Table 1, it can be seen that tobacco straws have a DM content similar to straws and hay commonly used in animal nutrition while tobacco meals have a DM content similar to grains and meals commonly used in animal nutrition (22). As there is no research in the literature on the assessment of tobacco as forage, TS was compared to straws and poor quality hay with similar nutrient content, while TM was compared to sunflower seed meal and cotton seed meal. In this context, the ash content of wheat straws was found in the range of 7.0-12.3% in a number of studies (7, 22-24). It can be said that the ash content of TS found in this study is partially similar to the ash content of wheat straws. Feeds with higher OM content and lower ash content are considered to have a higher nutritional value (22). Nevertheless, it is expected that TS with high ash content will also have a higher mineral content while offering lower energy content and digestibility. Another reason behind the high ash content available in a feed is material mixed in the feed such as soil, sand, clay, etc. In this respect, attention must be paid in order to avoid impurities in tobacco waste.

It can be seen that TS has a similar CP content when compared to wheat straws (7, 22, 24-26). Crude protein content of wheat straws was reported by a number of authors (22, 23, 27) in the range of 2.9 - 4.1%. Looking into the CP content of TM, it can

be said that TM is an alternative to some expensive concentrates sources such as cottonseed meal (26.0-41.0% CP) and sunflower seed meal (30.0-36.0% CP). Moreover, it was suggested that the CP content depends on the oil extraction process applied to the seeds and that the extraction process leads to reduced oil content of the meal while the CP content is increased (22). Kara (28) reported the nitrogen content of the tobacco waste, a mix of TS and tobacco seed, in a range of 1-3%. This N content is the equivalent of CP content in the range of 6.25-18.75% which is between the values of TS and TM in this study. This can be accounted for by the use of mix of straws and seeds. In addition, it is reported that factors such as feed type, soil structure, fertilization, harvest time, ratio of straws and seeds in the hay, etc. can account for the differences in the CP content of samples collected from different locations (24).

It was found that the EE content of TS is average 1.44% and this result is similar to the CP content of wheat straws (1.3-2.5%) (22, 29). On the other hand, EE content of TM was found as average 10.64%. In this study, meal was obtained after tobacco seeds were crushed under the press and the EE content of the meal remained at a higher level. It can be said that TM is a nutrition source with high nutritional value in terms of its CP and EE contents. However, it can be seen that TS is poor in energy due to its low EE content.

High CF content has adverse effects on the forage quality. Therefore, TS should not be used as a single forage in ruminant nutrition. The CF content of wheat straws was found in the range of 37.1-42.5% in a number of studies (22-24). These results can be interpreted as TS (49.88% CF) offers a lower forage value when compared to wheat straws. In addition, Table 1 shows that the CF content of TM was found to have an average of 27.73% and these results were similar to the CF contents obtained from pressed cotton seed meal (19.0%-25.0%) and sunflower meal (19.0%-21.0%) (22).

Nitrogen free extract is an indicator of the easily fermented carbohydrate content available in the feed. It is considered that energy conversion of the feed with higher NFE content is faster. The NFE content of the TS tested in this study was found at an average of

Table 1. Nutrient compositions of tobacco straws (TS) and tobacco meals (TM), DM,%

	DM*	OM	Ash	CP	EE	CF	NFE	NDF	ADF	ADL	HCel	Cel
TS	93.66±0.05	91.09±0.18	8.91±0.18	4.69±0.25	1.44±0.07	49.88±0.48	35.08±0.56	70.02±0.64	51.13±0.44	17.82±0.31	18.89±0.25	33.31±0.31
TM	93.11±0.04	93.86±0.08	6.14±0.08	38.61±0.23	10.64±0.20	27.73±0.40	16.89±0.43	43.44±0.30	32.41±0.19	15.03±0.17	11.03±0.26	17.39±0.17
Significant	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

**As feed, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, CF: Crude fibre, NFE: Nitrogen free extracts, NDF: Neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, HCel:hemicellulose, Cel: cellulose*

Table 2. In vitro gas productions (ml/200 mg DM) and pH values after 96.h incubation for TS and TM

	3, ml	6, ml	9, ml	12, ml	24, ml	48, ml	72, ml	96, ml	pH*
TS	13.03±0.77	19.49±1.00	23.65±1.12	27.10±1.19	29.95±1.31	33.40±1.51	35.56±1.58	37.83±1.60	6.40±0.02
TM	17.79±0.94	24.71±1.24	28.81±1.39	31.26±1.49	33.73±1.67	35.97±1.89	37.86±1.98	39.58±2.04	6.47±0.03
Significant	0.000	0.001	0.004	0.030	0.077	0.290	0.365	0.502	0.019

**Measured pH values after 96.hours incubation*

35.08%. Nitrogen free extract content of wheat straws was reported in the range of 37.7-52.3% by Kutlu and Celik (22), Sehu et al (30) and Mohamoud Abdi (24) and the minimum value is similar to the one found for TS. But, TS having slightly lower NFE content from wheat straws. Moreover, the low NFE content found in TM (16.89%) is due to the high CP and EE contents.

It was reported that feed with high NDF content is less appetizing for the animals; and feed with high ADF content offers low digestibility (20). In this study, the NDF contents found for TS were similar to the ones found for the wheat straws (54.4-78.89%) (23, 24, 27). Furthermore, it can be seen that TM also has fiber components which is similar to many concentrates (22).

The ash (8.39%), CP (15.52%), CF (32.30%), NDF (50.36%) and ADF (42.53%) contents reported by Pehlevan and Ozdogan (2) for tobacco wastes with varying TS and tobacco seed ratios are in the ranges reported in the current study. However, the reason behind the high EE (15.29%) content reported by Pehlevan and Ozdogan (2) is that the tobacco waste contains tobacco seeds. On the other hand, we have used the TM remaining after the pressing process for biodiesel production in this study.

It was found that the gas production (66.69 ml/200 mg DM) as measured by Gulecyuz (7) for wheat straws at the end of 96 h incubation was higher than the average (37.83 ml/200 mg DM) found for TS in this study. Pelleting process may account for the lower gas production. The pH value measured at the end of 96 h incubation indicates the functionality of the tampon used in the injectors, if the pH is acidic then the tampon was consumed and could not perform its duty. However, the pH values obtained in this study do not indicate that the tampon is consumed (31). The gas production of TM in 96 hours were close to the ones reported by Kilic and Saricicek (32) for hazelnut kernel oil meal (49.07%) and canola meal (45.83%) and similar to the ones reported by Kilic and Boga (33) for sunflower seed meal (30.21-43.30%). Kilic and Boga (33) used sunflower seed meal (32%-44%) with different CP contents and the one with the highest CP content was also the one with the highest gas production. In this study, TM had a CP content of 38.61% and its gas production was in the range between these two sunflower seed meals.

According to Table 3, the results obtained for a+b value which is the total gas production were lower than the one reported by Gulecyuz (7) for wheat straws (77.54 ml). Methane production of TS was found to be 4.62 ml. Methane production of wheat straw, on the other hand, was reported to be 6.57 ml (7). As poor quality forage would lead to increased methane production, it is believed that it would be best to combine TS with quality forages. Furthermore, methane production is a function of digestible organic matter and it must be considered the fact that feed with lower digestibility will lead to higher methane production. Therefore, considering the fact that the OMD content of tobacco straws are found to have an average of 49.42% while OMD content of wheat straws was found at an average of 56.80% (7), it can be said that TS with lower digestibility will lead to increased methane production. However, it was found that wheat straws as a poor quality forage will give results similar to TS.

Table 3 shows that OMD, ME, NE_L and IVTD contents of TM are similar to the other meals. Kilic and Boga (33) reported c value of 0.008 ml/h and a+b value in a range of 30.26-43.36 ml for sunflower seed meal. This report is in compliance with the results of this study. The ME and NE_L contents of wheat straws are reported as 7.73 MJ/kg DM and 4.21 MJ/kg DM, respectively (7). Accordingly, it can be said that wheat straws have a slightly higher energy content when compared to tobacco straws. Moreover, it was also found that the IVTD content of tobacco straws (52.05%) is

Table 3. *In vitro* gas production parameters, methane productions, OMD, ME, NE_L and IVTD values for TS and TM

	c, ml/h	a+b, ml	Methane, ml	OMD, %	ME, MJ/kg DM	NE_L, MJ/kg DM	IVTD, %
TS	0.10±0.01	36.37±1.59	4.62±0.30	49.42±1.13	6.55±0.17	3.42±0.13	52.05±0.68
TM	0.13±0.00	37.54±1.98	5.75±0.44	66.17±1.48	9.32±0.22	6.55±0.17	74.79±0.31
Significant	0.000	0.647	0.034	0.000	0.000	0.000	0.000

c: the gas production rate constant for the insoluble fraction (ml/h), a+b: total gas production, OMD: organic matter digestibility, ME: Metabolizable energy, NE_L: Net energy lactation, IVTD: In vitro true digestibility

higher than the digestibility values reported by Mohamoud Abdi (24) for wheat straws (39.06%), sorghum straws (49.02%) and soybean straws (46.06%). In addition, tobacco straws were found to be of poor quality with the values found for DMD at 49.07% and DMI at 1.72 % LW.

ME and NE_L contents of Tobacco meals found in this study were higher than the ME (5.47-7.28 MJ/kg DM) and NE_L (4.49-6.14 MJ/kg DM) reported by Kilic and Boga (33) for sunflower seed meal while OMD contents (53.6-68.4%) were similar in both studies. ME contents reported by Seven et al., (34) for sunflower seed meal and cotton seed meal (8.92 MJ/kg DM and 8.75 MJ/kg DM, respectively) were lower than the ME contents found in this study for TM. This was a result of the higher EE and CP contents (which are used in ME calculations) found for TM in this study along with the differences in species tested, soil structure, fertilization, harvest time, meal production methods, etc. (31).

The reason behind the higher "c value" for the gas production of TM when compared to TS is that TM is classified under concentrates. Therefore, higher methane production, OMD, ME and NE_L contents found for TM when compared to TS may lead to the recommendation of using TM for animal nutrition as forages with lower methane production has also lower OMD and energy contents. That is why the most suitable forage combination must be achieved in animal nutrition.

As shown in Table 4, forage quality of TM was defined in order to be compared to TS. However, a classification under forages is not suitable for TM in terms of its nutrient content. The IVTD was found as average 74.79% for TM and better results obtained when compared to forages are marked findings. According to the RFV index, TM was classified under "2-Good" quality. Significant differences ($P<0.001$) were reported for all the nutrient contents explored in this experiment for TS and TM. It can be said that it will be better to address TM under concentrates as it is rich in CP and EE content while being poor in cell wall fiber components.

Kilic and Mohamoud Abdi (4) reported that the lowest IVTD among grape pomace pellets were found for the ones with the highest condensed tannin content. No statistically significant differences were found between TS and TM with respect to their CT contents. However, TS was found to have a slightly lower CT content and a lower IVTD value. These findings are similar to the literature. It was suggested that a tannin content of 5-10% results in disgust, reduces forage consumption and live weight gain, decreases the digestibility level and absorption of nutrition, reduces performance and lead to toxicity related effects (35). As the CT contents of both TS and TM used in this study were below 5%, it is believe that this will not have an adverse effect on the feed consumption. Nevertheless, it is likely that pelleting of tobacco waste will reduce the adverse effects of CT.

The CT contents found in TS and TM were significantly lower than expected. Therefore, TS and TM can be used as forage. It is observed that small ruminants, especially goats, consume tobacco seeds as field waste while TS is not commonly preferred due to its stalk's thickness. However, it is possible to use TS after being ground into the size of straws and being pelleted as a portion of straws or poor quality hays in animal nutrition. Ruminants show difference tolerances against condensed tannin and the tolerance of goats are significantly higher than sheep. Thus, it is believed that goats can tolerate 8-10% tannin in ratio and that goats are the most suitable animals for the use of tobacco harvest waste (4). Furthermore, future research

Table 4. Rleative feed values, quality class and condensed tannin contents of TS and TM

	RFV	DMD, %	DMI, % LW	RFV Quality Class	CT, %
TS	65.88±1.04	19.07±0.34	1.72±0.02	5 (Reject)	4.62 ± 0.39
TM	136.86±1.15	63.65±0.15	2.77±0.02	2 (Good)	3.28 ± 0.53
Significant	<0.000	<0.000	<0.000		0.122

According to the Quality Grading Standard assigned by The Hay Marketing Task Force of the American Forage and Grassland Council, the RFV were assessed as forages based on prime >151 , 1 (premium) 151-125, 2 (good). 124-103. 3 (fair). 102-87, 4 (poor). 86-75 , 5(reject).< 75. RFV: Relative feed value, DMD: Dry matter digestibility, DMI; Dry matter intake, CT: Condensed tannin.

may focus on the use of additive in order to increase the pellet quality and to improve the nutritional value of tobacco waste and on the use of TM in a specific ratio with cottonseed meal or sunflower seed meal, which it can be an alternative to.

According to the findings of this study, it should be taken into consideration that TS has nutrient content similar to wheat straws while its consumption will be at a lower level due to its higher NDF and ADF contents. TM, on the other hand, proves to be advantageous when compared to TS as it has nutrient content similar to concentrates and has higher OMD, ME, NE_L and IVTD values. It must be remembered that oil extraction process may alter the nutrient content of seed meals. It was found that the method used to obtain meals has a direct impact on the CP and EE contents among nutrient contents. Extraction method is recommended as it is the method to almost fully extract the oil content of oily seeds (22). Thus, TM can be considered as an alternative for a number of forages such as expensive sunflower seed meal and cotton seed meal.

Conclusions

In conclusion, TS and TM are recommended to be pelleted as the methane production of pelleted forages is reduced. However, an economic analysis in which the costs of seed collection, seed processing, and their use as fertilizers in the field must be conducted. The use of additives such as molasses, soybean meal, clay minerals etc. is recommended in order to improve nutritional values and pellet quality in the pelleting of TS. In addition, it is possible to ensile TS after being ground to the size of straw with a suitable humidity content (60-70% humidity). It is believed that this process helps remove the possible phenolic components during fermentation which then be safely used in animal nutrition.

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References

1. Gulecyuz E, Kilic, U. Pelleting forage and usability in ruminant nutrition. 12. National Zootechnia Student Congress, 2016, 9-11 May 2016. Pp: 138. Isparta-Turkey.
2. Pehlevan F, Ozdogan M. Comparison between chemical and near infrared reflectance spectroscopy methods for determining of nutrient content of some alternative feeds. Journal of Tekirdag Agricultural Faculty, 2015, *12*(2): 1-10.
3. Basmacioglu H. The factors affecting pellet quality in mixed feed production. Journal of Animal Production. 2004, 45 (1): 23-30.
4. Kilic U, Mohamoud Abdi A. Determination of *in vitro* true digestibilities and relative feed values of wine industry grape residues as alternative feed source. Journal of The Faculty of Veterinary Medicine, Kafkas University, 2016, 22 (6): 895-901.
5. Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, De Haan C. Livestock's long shadow: environmental issues and options. Food and Agriculture Organization of the United Nations, 2006, p.82-114.
6. Wright A.G., Williams, A.J., Winder B, Christophersen C.T., Rodgers S.L., Smith, K.D. Molecular Diversity of rumen methanogens from sheep in Western Australia. Appl. Environ. Microbiol., 2004, 70: 1263–1270.
7. Gulecyuz E. Determining in vitro digestibility and methane production of wheat straw and soybean straw pelleted with different additives. Ondokuz Mayıs University, Sci. Enstitute, (MSc Thesis). Samsun-Turkey, 2016
8. AOAC. Officinal Methods of Analysis. 16th Edition, AOAC International, Gaithersburg, MD. 1998.
9. Van Soest P.V., Robertson J.B., Lewis B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 1991, 74(10), 3583-3597.
10. AOCS. Official procedure, approved procedure Am 5-04, Rapid determination of oil/fat utilizing high temperature solvent extraction. J Am Oil Chem Soc, Urbana, IL. 2005.
11. Makkar H.P.S., Blummel M, Becker, K. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and their implication in gas production and true digestibility *in vitro* techniques. Br J Nutr, 1995, 73 (6) 897-913.
12. Wiedmeier R.D., Arambel M.J., Walters J.L. Effect of yeast culture and Aspergillus oryzae fermentation extract on ruminal characteristics and nutrient digestibility. J. Dairy Sci. 1987, 70: 2063- 2068.
13. Blummel M, Makkar H.P.S., Becker K. In Vitro Gas Production- A Technique Revisied. Journal of Animal Physiology and Animal Nutrition. 1997, 77:24–34.
14. Menke K.H., Steingass H. Estimation of the Energetic Feed Value Obtained from Chemical Analysis and in vitro Gas Production Using Rumen Fluid. Anim. Res. Devl., 1988, Separate Print, 28:7-55.
15. Ørskov E, Mcdonald I. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb. 1979, 92:

499–503.
16. Menke K.H., Raab L, Salewski A, Steingass H, Fritz D, Schneider W. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro". J. Agric. Sci. Camb. 1979, 93:217–222.
17. Close W, Menke K.H. Selected topics in animal nutrition. A manuel prepared for the 3rd Hohenheim course on animal nutrition in the tropics and semi-tropics. 2nd edition. University of Hohenheim, Stuttgart, Germany, 1986.
18. Ankom Technology, *In vitro* true digestibility using th Daisy II Incubator. Available from http://www.ankom.com/media/documents/ IVDMD_0805D200.pdf. 2005, Accessed:13.09.2010.
19. Goel G, Makkar H.P.S., Becker, K. Effect of Sesbania sesban and Carduus pycnocephalus leaves and Fenugreek (Trigonella foenum-graecum L) seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. Anim Feed Sci Technol, 2008, 147 (1-3): 72-89.
20. Yavuz M. Determination of Some Ruminant Feeds' Relative Feed Value and In vitro Digestion Values. Journal of Agricultural Faculty of Gaziosmanpasa University, 2005, 22 (1): 97-101.
21. Onder H, Using Permutation Tests to Reduce Type I and II Errors for Small Ruminant Research, J. Appl. Anim. Res., 2007, 32 (1), 69 – 72.
22. Kutlu H.R., Celik L. Feeds Knowledge and Feed Technology. Cukurova University, Agricultural Faculty, General Publish Number:266, Lesson Books. Adana-Turkey.2014.
23. Stanton T.L., LeValley S. Feed Composition for Cattle and Sheep. Factsheet No. 1.615, Colorado State University Cooperative Extension. 2006.
24. Mohamoud Abdi A. Effect of lignin peroxidase enzyme on feed values of different straws. Ondokuz Mayıs University, Sci. Enstitute (MSc Thesis) Samsun-Turkey. 2016
25. Kalkan H, Filya, I. Effects of Cellulase Enzyme on Nutritive Value, in vitro Digestion Characteristics and Microbial Biomass Production of Wheat Straw. Journal of The Faculty of Veterinary Medicine, Kafkas University, 2011, 17(4): 585-594.
26. Akcil E, Denek N. Investigation of Different Levels Eucalyptus (Eucalyptus camaldulensis) Leaves Effect on In Vitro Methane Production of Some Roughages. Harran Univ. Vet. Fak.Journal. 2013, 2(2):75-81.
27. Can A, Denek N, Yazgan, K. Effect of urea and molasses supplementation on nutrient intake and digestibility of sheep fed with straw. Journal of Animal Veterinary Advances, 2004, 3 (7): 466-469.
28. Kara E.E. The effect of tobacco-waste on nitrogen contents and biological activities of soils. Anadolu Journal of AARI, 1996, 6 (2):100–111.
29. Kamalak A. Comparision of some forages in terms of gas production parameters and metabolizable energy contents. KSU Journal of Natural Sciences, 2005, 8(2), 116-20.
30. Sehu A, Yalcin S, Onol A.G., Kocak D. Prediction of Dry Matter Intake and Live Weight Gain in Lambs by Some Characteristics of Roughages. Tr. J. of Veterinary and Animal Sicences, 1998, 22: 475–483.
31. Kilic U, Sarıcicek B.Z. Factors affecting the results of gas production technique. Journal of Animal Production .*2006, 47* (2):54-61.
32. Kilic U, Saricicek B.Z. Effects of some concentrates on *in vitro* gas production parameters. 5. National Zootechnia Scientific Congress. 2007, 05-08 Sep. P:70 Van-Turkey.
33. Kilic U, Boga, M. Determining the effects of sunflower varieties with different protein contents on results of in vitro gas production and enzyme techniques. TUBAV (Turkish Science) Journal. 2009, 2 (2):231-238.
34. Seven P.T., Cerci H, Azman M.A. The Different Between Methods and Determining of Metabolisable Energy Levels with Enzyme and Gas Techniques in Concentrate Feeds. Firat University, Journal of Healt. Sci., 2007, 21(4):159-162.
35. Kamalak A. Feed Additives and Some Chemical Treatment to Decrease the Negative Effect of Condensed Tannin. KSU Journal of Natural Sciences. 2007, 10(2):144-150.

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Original article

The concentration of selected ions in bottled, commercial zamzam, and household water in Riyadh city and its effect on bone mineral content in growing rabbits

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Summary. *Objective*: Drinking safe water is an internationally accepted human right. This objective of this study was to evaluate some of the minerals responsible for bone health in various types of water and to assess the effect of bottled water, commercial zamzam water, and tap water on bone mineral content (Ca, Mg, and Zn) in growing rabbits, as well as to identify the association between the water mineral content and bone mineral content of rabbits that had consumed various water types. *Method*: Samples were collected locally. Water samples were analyzed directly by the inductively coupled plasma spectrometry (ICPS), while bone (femur) samples were digested in a microwave digestion system for 30 min and then aspirated into inductively coupled plasma mass spectrometer. *Result:* Highest concentration of calcium was observed in commercial zamzam water followed by bottled and tap water and the zinc concentration was highest in tap water followed by commercial zamzam and bottled water. Highest concentration of magnesium was found in one of the bottled water (B3) followed by commercial zamzam, than other bottled water and least in tap water. Statistically significant differences in mineral content were observed between the various water types ($p \leq 0.05$). All variety of water except zamzam provides magnesium and calcium in an amount of less than 10% of the amount recommended for children and in older people, these products supply even lower levels of the DRI of this component. Commercial zamzam and tap water provides high percentage of zinc. In bone (femur) concentration of calcium was highest in commercial zamzam water and least in bottled water. We detected significant differences when comparing the calcium content of commercial zamzam water and the other tested types ($p \leq 0.05$). But the differences in the mean concentration of magnesium and zinc were statistically insignificant ($p \geq 0.05$) between all varieties. A strong and highly positive correlation ($p \leq 0.05$) was observed between bone and water calcium ($R^2=0.9286$) and slight positive correlation was observed between bone and water magnesium ($R^2=0.2576$). *Conclusion*: This study inferred that the concentration of all analyzed elements was within the guidelines set by various agencies. A large variation in various mineral concentrations has been observed in bottled water. Zamzam water provides essential minerals required for bone health. This study will help in providing more focused design of further research.

Key words: mineral content; calcium, magnesium, zinc, bone, DRI

Introduction

Drinking safe water is an internationally accepted human right (1), and it is an elementary requirement for healthy living. The choice of water for consumption can vary, but it must be "clear, tasteless, odorless, colorless and absence of any harmful and pathogenic microorganisms and chemicals dangerous to humans" (2). Due to ostensible risks of tap water and the favored taste, and the convenience and the safety of bottled water (BW) (3), in the past decade there has been an enormous increase in the consumption of

BW (4). This surge in demand has produced one of the most vibrant sectors in the food and beverage industries (5). BW is defined as "water that is intended for human consumption, and that is sealed in bottles or other containers with no added ingredients except that it may potentially contain safe and suitable antimicrobial agents" (6). BW has turn out to be the main types of drinking water, second only to tap water; so, assessment of its safety and impact on health is now necessary (7,8).

In adults, approximately 60% of the total body weight is composed of water, which is distributed into intracellular and extracellular fluid compartments (9, 10). In the circulatory system, water functions as a carrier of nutrients and various other substances (2). Water is an essential source of mineral elements for human skeletal health (11). Although numerous chemicals might occur in drinking water, only a few of them are significant (12). Various metal ions, such as magnesium, calcium, and sodium, are important for the proper growth, development, and functioning of the human body as they comprise the material for bones, regulate the water-electrolyte balance and affect the course of metabolic processes (13). Certain trace elements (e.g., zinc) are also essential for optimal growth, development, and reproduction (12). Among the minerals found in BW, emphasis has been given to calcium and magnesium (14) because these mineral have a beneficial effect on bone metabolism. Various epidemiological studies have shown that the dietary intake of calcium is far below its recommended amount (12) and Ca-rich mineral waters are a substitute for dairy products (2). This study aims to evaluate some of the minerals responsible for bone health in various types of water and to assess the effect of BW, commercial zamzam water, and tap water on bone mineral content (Ca, Mg, and Zn) in growing rabbits, as well as to identify the association between the water mineral content and bone mineral content of rabbits that had consumed various water types.

Materials and methods

Sample Collection and Preparation

BW of different manufacturing dates and commercial zamzam water were purchased from the local shops in Riyadh. BW samples were given a code, and analytical grade chemicals and reagents were used. The bottles used for collecting samples were soaked in 0.5% nitric acid overnight followed by washing with household water and rinsing with deionized water.

Animals and Care

Eighteen weanlings New Zealand white rabbits (6 weeks old, weighing 700–1300 g) were used in this study. Animals were obtained from the Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Saudi Arabia. This study is in accordance with the Animal Ethics Committee of the College of Science, King Saud University. The rabbits were randomly divided by weight into six groups (three in each group) and housed individually in stainless steel cages under controlled temperature (25 ± 2°C) and relative humidity (50 ± 5%), with a 12-h light/dark cycle.

Diets formulation and preparation

The Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine; King Saud University, Saudi Arabia provided the basal diet. Each rabbit was fed 500 g of basal diet and 500 ml of water (according to treatment group) every alternate day. Food cups were refilled every 2 days, and food provided, and the remaining food was weighed to calculate daily food consumption.

Collection of blood

At the end of the experiment, on the 100th day, animals were food deprived overnight and anesthetized under chloroform. Blood was collected from the retro-orbital plexus in the heparinized tube and centrifuged at 3500 rpm for 15 min for plasma separation and stored at 5-7°C for further analysis.

Determination of Ca, Mg, and Zinc in various types of water and rabbit's bone by inductively coupled plasma spectrometry (ICPS)

A small portion of the samples was separated and acidified in 0.5% nitric acid for the analysis of Ca, Mg, and Zn in bottled, commercial zamzam, and tap water by ICPS. Samples were warmed at ambient temperature before analysis and then aspirated directly into ICPS.

Bones (femur) were obtained as previously described by Dekel *et al.* (15). Briefly, the femur was excised and carefully freed of soft tissue by gentle scraping with a scalpel, rinsed with normal saline (0.9% NaCl). Then, the femur was dried using a lint-free paper towel, and stored in a plastic container at −20°C until elicitation of bone marrow. All procedures were accepted by the Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. The bones were thawed at room temperature; both bone epiphyses were removed using a fine saw, an incision was made along the bone using a sharp non-serrated knife, bone marrow was removed and weighed to the nearest 0.0001 g (AL204, Mettler-Toledo Inc., Shanghai). Finally, bone samples were again rinsed with normal saline, dried using a lint-free paper towel, and then stored in a plastic tube at −20°C until used for minerals analysis.

To determine calcium (Ca), magnesium (Mg), and zinc (Zn) contents, femur samples (300 mg) were digested in 6 ml of concentrated nitric acid (HNO3) and 2 ml hydrogen peroxide (H_2O_2) for 30 min in a microwave digestion system (START D, Milestone, Italy). Ca, Mg, and Zn concentrations were measured using an inductively coupled plasma mass spectrometer (ICP-MS 7500A, Agilent Technologies, Inc., Santa Clara, CA, USA).

Statistical Analysis

The data are presented as the average of replicates ± SD. The data of water and bone mineral content was subjected to statistical analysis by analyzing variance (ANOVA), using the SPSS software package (version 9.0). The significant differences identified using Turkey HSD tests, and p-values of < 0.05 were considered significant.

Results and discussion

Elemental analysis of water

Water consumption is related to most diseases that cause morbidity and mortality in humans, so water quality is an important aspect of human life (16). Water is a significant source of mineral intake because the gastrointestinal tract easily absorbs the minerals in water as they are available in an ionic form (17). Elemental analysis of water and bone was carried out using ICPS. Table 1 shows the mineral content of the water types given to the rabbits. The concentration of calcium was highest in commercial zamzam water, followed by bottled and tap waters, and the concentration of zinc was highest in tap water, followed by commercial zamzam and BW. The concentration of magnesium was highest in one of the BW (B3), followed by commercial zamzam, then other BWs, with the least in tap water. Statistically significant differences in mineral content were observed between the various water types ($p \leq 0.05$). All analyzed elements were in agreement with the maximum contaminants limits set by the regulatory agencies (18, 19, 20). In a previous study, compared to bottled and tap (municipal), relatively higher concentrations of anion and cations were reported in zamzam water (6). Doha *et al.* (21) reported previously that commercial zamzam water has a rich mineral profile, which is in accordance with the results obtained in this study. However, unlike this study, the previous study reported a higher concentration of major cations in tap water (21). This difference might be due to the regional differences; wide variations in mineral content have been noted when comparing regions (21). The concentration of calcium in BW was previously reported as between 0.3 and 40.0 mg/L, with an average value of 14.4 mg/L, while the concentration of magnesium ranged between 0.01 to 25.0 mg/L, with an average value of 4.7 mg/L (22). The mean mineral concentrations in BW reported in this study are higher (18.37 mg/L Ca and 5.83 mg/L Mg) than those reported previously (22). Saudi Arabia uses conventional (surface water and groundwater) and unconventional (desalination of seawater and treated wastewater) water resources to fulfill its continuous increasing water demand (23) and is the world's largest producer of desalinated water, which accounts for 70% of the total water demand (24).

Several factors (e.g., the geological settings, rock mineralogy, the source of climate, treatments, length of storage, and type of material used for storage) might be responsible for the difference in the chemical composition between the samples (25,26). BW goes through a purification process that incorporates reverse osmosis; ultraviolet and ozone sterilization. Municipal tap water goes through a purification process that incorporates desalination, followed by filtration (reverse osmosis),

and zamzam water is filtered through a series of sand filters and cartridge filters and then sterilized by UV irradiation at these treatment plants, before distribution to consumers. Filtration is less effective in removing solids or dissolved particles that are smaller than the filters pore size (27). In this study, the pH of all water (bottled, tap and commercial zamzam) was ≥ 7.00 (data not shown), which is within the values recommended by the WHO (6.5-8.0) (28). Previously, it has been reported that drinking water with a pH of ≥ 7.0 reduced the risk of forearm fractures relative to drinking water with a pH < 7.0 (29). The ratio of calcium and magnesium is important for optimal utilization of minerals in the body; the optimum value of calcium and magnesium ratio is 2:1(Ca:Mg) (30). In this study, none of the water samples had an optimum calcium-magnesium proportion (Table 1). Gątarska *et al*. (14) reported that only a few of the many tested samples featured the optimum calcium-magnesium proportion.

Calcium, magnesium, and zinc intake from water relative to DRIs

The issue of the adequate amount of water intake is not clear (31). Proper hydration is necessary to sustain the body water equilibrium, and its demand varies from individual to individual and largely depends on various factors, such as age, physical activity, personal circumstances, weather conditions, and other sources of fluids (2). Gątarska *et al*. (14) reported that for determination of various minerals in water, the assumption of daily consumption of the 1-liter volume of the product allows for the estimation of a given water percentage in the RDA of a given mineral. Dietary Reference Intake (DRIs) includes the perception of preventing nutrient deficiencies as well as risk reduction for chronic conditions (32). The results reported here can be used to estimate the amounts of ingested elements (22). To determine the clinical importance of mineral intake from drinking water, mineral level in bottled, commercial zamzam water, and tap water has been compared to DRIs (33). Comparison between DRI and mineral intake from drinking water is made using a reference volume of 1 L. It has been assumed that adults typically consume about 1 L of water per day (about 500 ml for children), and the remaining drinking water requirement is fulfilled by other sources, such as tea and juices. From Table 2, it can be concluded that all variety of water, except commercial zamzam, provide cations (Ca and Mg) in an amount of less than 10% of the amount recommended for children, and the contribution of tap water was found to be very low. In older people (relative to children and other population age groups), these products supply levels lower than the DRI. Variation has been observed between the mineral contents of the samples. Commercial zamzam and tap waters provide 50% and 70% of the recommended amount of zinc for children up to 3 years and more than 32% and 42% of the amount recommended for children under 8 years, respectively. Zinc was not detected in two BWs, and the rest of the BWs provide less than 3% of the amount recommended for children under 8 years old, and even lower levels of the DRI of this component were found in adolescents and adults. Gatarska *et al*. (14) also analyzed three mineral waters with different mineral levels (low, medium and high) and reported that daily consumption of 1 L of these waters provides a good percentage of the RDA in younger children, but in older and special need persons these products supply lower level of RDAs of various minerals, which is quite similar to the results reported in this study.

Table 1: Concentration of Ca, Mg and Zn in bottles, tap and commercial zamzam water.

	Ca (mg/L)	Mg (mg/L)	Zn (mg/L)	Ca: Mg
B1	34.96 ± 0.157^{e}	4.74±0.010^{c}	0±0^{a}	7.37:1
B2	12.597±0.169^{c}	3.39±0.001^{b}	0±0^{a}	3.71:1
B3	11.007± 0.074^{b}	12.99 ±0.047^{e}	0.066±0.0003^{c}	0.84:1
B4	14.92±0.039^{d}	2.21±0.036^{a}	0.012±0.0003^{b}	6.75:1
Zamzam	51.03±0.346^{f}	11.06±0.049^{d}	1.65±0.00089^{d}	4.61:1
Tap	10.082 ±0.0977^{a}	10.08±0. 053^{d}	2.118± 0.0007^{e}	1:1

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript ^{abc} indicate significant differences among various groups as indicated by ANOVA followed by Turkey HSD test.

Table 2: The percentage of daily recommended index (DRI) for calcium, magnesium and zinc at the assumed consumption of 1 liter of water in different age group of consumer

Life stage								
	Mineral* (mg/d)	**DRI**	**B1**	**B2**	**B3**	**B4**	**Zamzam**	**Tap**
Children								
1-3 years	Ca	500	6.99	2.51	2.20	2.98	10.21	2.02
1-3 years	Mg	80	5.93	4.23	16.24	2.76	13.82	12.6
1-3 years	Zn	3	0	0	2.3	0.4	54.87	70.6
4-8 years	Ca	800	4.37	1.57	1.38	1.87	6.38	1.26
4-8 years	Mg	130	3.65	2.61	9.99	1.7	8.51	7.75
4-8 years	Zn	5	0	0	1.32	0.24	32.92	42.36
Males								
9-13 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
9-13 years	Mg	240	1.98	1.41	5.41	0.92	4.60	4.2
9-13 years	Zn	8	0	0	0.83	0.15	20.58	26.475
14-18 years	Ca	1300	2.69	0.967	0.85	1.15	3.93	0.78
14-18 years	Mg	410	1.16	0.83	3.17	0.53	2.69	2.46
14-18 years	Zn	11	0	0	0.6	0.11	14.96	19.25
19-30 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
19-30 years	Mg	400	1.19	0.85	3.25	0.55	2.76	2.52
19-30 years	Zn	11	0	0	0.6	0.11	14.96	19.25
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
31-50 years	Zn	11	0	0	0.6	0.11	14.96	19.25
51-70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
51-70 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
51-70 years	Zn	11	0	0	0.6	0.11	14.96	19.25
>70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
>70 years	Mg	420	1.13	0.81	3.09	0.53	2.63	2.4
>70 years	Zn	11	0	0	0.6	0.11	14.96	19.25
Females								
9-13 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
9-13 years	Mg	240	1.98	1.41	5.41	0.92	4.61	4.2
9-13 years	Zn	8	0	0	0.83	0.15	20.58	26.475
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
14-18 years	Zn	9	0	0	0.73	0.13	18.29	23.53
19-30 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
19-30 years	Mg	310	1.52	1.09	4.19	0.71	3.57	3.25
19-30 years	Zn	8	0	0	0.83	0.15	20.56	26.475
31-50 years	Ca	1000	3.49	1.26	1.10	1.49	5.10	1.101
31-50 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
31-50 years	Zn	8	0	0	0.83	0.15	20.56	26.475

Table 2: (Continued) The percentage of daily recommended index (DRI) for calcium, magnesium and zinc at the assumed consumption of 1 liter of water in different age group of consumer

Life stage								
	Mineral* (mg/d)	**DRI**	**B1**	**B2**	**B3**	**B4**	**Zamzam**	**Tap**
51-70 years	Ca	1200	2.92	1.05	0.92	1.24	4.25	0.840
51-70 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
51-70 years	Zn	8	0	0	0.83	0.15	20.56	26.475
>70 years	Ca	1200	2.91	1.04	0.92	1.24	4.25	0.840
>70 years	Mg	320	1.48	1.06	4.06	0.69	3.46	3.15
>70 years	Zn	8	0	0	0.83	0.15	20.56	26.475
Pregnancy								
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	400	1.19	0.85	3.25	0.55	2.76	2.52
14-18 years	Zn	12	0	0	0.55	0.1	13.72	17.65
19-30 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
19-30 years	Mg	350	1.35	0.97	3.71	0.63	3.16	2.88
19-30 years	Zn	11	0	0	0.6	0.11	14.96	19.254
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
31-50 years	Zn	11	0	0	0.6	0.11	14.96	19.254
Lactation								
14-18 years	Ca	1300	2.69	0.97	0.85	1.15	3.93	0.78
14-18 years	Mg	360	1.32	0.94	3.61	0.61	3.07	2.8
14-18 years	Zn	13	0	0	0.51	0.09	12.66	16.292
19-30 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
19-30 years	Mg	310	1.53	1.09	4.19	0.71	3.57	3.25
19-30 years	Zn	12	0	0	0.55	0.1	13.72	17.65
31-50 years	Ca	1000	3.49	1.26	1.1	1.49	5.10	1.101
31-50 years	Mg	320	1.48	1.06	4.05	0.69	3.46	3.15
31-50 years	Zn	12	0	0	0.55	0.1	13.72	17.65

Elemental analysis of rabbit bones (femur)

Table 3 reports the mineral content of the bone (femur) of rabbits that consumed bottled, commercial zamzam, and tap waters. The concentration of calcium was highest in commercial zamzam water and least in BW. We detected significant differences when comparing the calcium content of commercial zamzam water and the other tested types ($p \leq 0.05$). Even though the concentrations of magnesium and zinc were highest in BW, these failed to reach statistical significance ($p \geq 0.05$). Even though many different chemicals are found in drinking water, only a few are significant in any given

Table 3. Concentration of Ca, Mg and Zn in bone (femur) of male rabbits consuming bottled, zamzam and tap water

	Ca (mg/L)	**Mg (mg/L)**	**Zn (mg/L)**
B1	46±22.596^{ab}	7.57±0.541^{a}	4.01±0.671^{a}
B2	22.67± 0.963^{a}	7.29±0.4413^{a}	3.25± 0.011^{a}
B3	20.59±1.944^{a}	7.89±0.314^{a}	4.21±0.450^{a}
B4	36.71±8.701^{a}	7.49±0.589^{a}	4.30±0.576^{a}
Zamzam	78.37±4.586^{b}	7.37±0.076^{a}	3.87±0.364^{a}
Tap	25.22± 15.492^{a}	7.49± 1.007^{a}	4.14± 0.57^{a}

Data are expressed as the mean ± standard deviation; Model ANOVA, p values < 0.05 are significant. Superscript ^{abc} indicate significant differences among various groups as indicated by ANOVA followed by Turkey HSD test.

circumstances (12). Bone plays a significant role in mineral homeostasis regulation, and it is the core reservoir for minerals (34). The repetitive cycle of destruction and rebuilding maintains the bone mass, as well as the balance between bone formation and bone resorption, which are controlled by osteoblasts and osteoclasts respectively (35). Magnesium and calcium play significant roles in human dietary needs (36, 37). The skeleton stores calcium and thus regulates blood calcium levels, but a dearth of calcium leads to depletion of the bone reservoirs, causing brittleness in the bones and greater risk of fractures (osteoporosis) (38). Various epidemiological, clinical, and animal studies have reported an inverse relationship between calcium intake and incidence of osteoporosis (39, 40). Deficiency in calcium intake is generally combined with a vitamin D insufficiency (41), which leads to decreased calcium absorption. This causes a reduction in ionized extracellular fluid calcium, which in turn stimulates parathyroid gland secretion. Augmented parathyroid secretion upsurges bone turnover, which is the main determining factor for bone loss and fracture risk (42). Various authors have studied the bioavailability of calcium from mineral waters, concluding that it is very high (43, 44). Previous studies have reported an increase in bone mineralization, considering both femoral and spinal bone mineral density with consumption of calcium-rich mineral water (45, 46). Magnesium (Mg) is the second most abundant in the intracellular fluid and the fourth most abundant cation in the human body (47). Absorption of waterborne Mg is almost 30% faster and better than Mg from food (48) and its bioavailability increases with a light meal (49). An appropriate magnesium intake maintains adequate bone mineral density (50, 51). Relative to dairy products and other sources, waterborne calcium and magnesium are advantageous because unlike them neither it has any side effects nor it brings any, calorie, or lipid supplements (52).

Zinc is an essential trace metal, and its deficiency leads to a decrease in bone weight, retardation of bone growth and maintenance, and delays growth in bone metabolism (53). Zinc stimulates cellular protein synthesis by activating aminoacyl tRNA synthetase in osteoblastic cells and inhibits osteoclastic bone resorption by inhibiting osteoclast-like cell formation from marrow cells (54). Drinking water contains this trace metal in minute quantities, which might decrease the likelihood of its deficiency in the diet. However, its deposition in the human body causes harmful effects, such as acceleration of anemic condition (55). As compared to BW, higher levels of zinc have been reported in tap water, which is similar to the result obtained in this study (12).

Correlation between water and bone mineral contents in rabbits

A strong and highly positive correlation ($p \leq 0.05$) was observed between bone and water calcium levels ($R^2 = 0.9286$), and a slight positive correlation was observed between bone and water magnesium levels ($R^2 = 0.2576$). The correlation was very weak between bone and water zinc levels ($R^2 = 0.0169$) (Figure 1 A-C). The associations observed in this study provide further evidence of the links between nutrients and bone metabolism (56). These results might reflect the positive influence of the consumption of water on bone health.

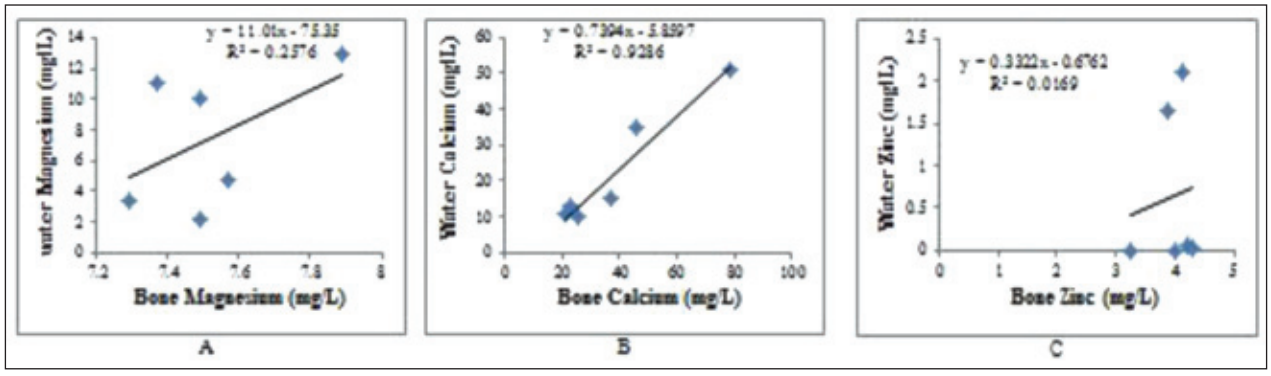

Figure1. Pearson's correlation coefficient between ions in water and bone (femur) of male rabbits.

Conclusion

In conclusion, this study shows that the concentration of all analyzed elements was within the guidelines set by various agencies. A large variation in various mineral concentrations was detected among BWs. We found that commercial zamzam water provides the essential minerals required for bone health. Further studies are required to understand the biochemical pathways involved in health. This study will help in supporting a more focused design of further research and can be considered as a baseline for future reference.

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References

1. Scanlon J, Cassar A, Nemes, N. Water as a human right? IUCN Environmental policy and law paper no.51, IUCN, Gland, Switzerland and Cambridge, UK, 2004; 1-53.
2. Quattrini S, Pampaloni B, Brandi ML. Natural mineral waters: chemical characteristics and health effects. Clin Cases Miner Bone Metab 2016; 13:173-180.
3. Saylor A, Prokopy LS, Amberg S. What's wrong with the tap? Examining perceptions of tap water and bottled water at Purdue University. Environ Manage 2011; 48: 588–601.
4. Ballantine PW, Ozanne LK, Bayfield R. Why Buy Free? Exploring Perceptions of Bottled Water Consumption and Its Environmental Consequences. Sustainability 2019; 11: 757.
5. Semerjian LA. Quality assessment of various bottled waters marketed in Lebanon. Environ Monit Assess 2011; 172:275–285.
6. Alfadul SM, Khan MA. Water quality of bottled water in the kingdom of Saudi Arabia: A comparative study with Riyadh municipal and Zamzam water. J Environ Sci Heal A 2011; 46:1519-1528,
7. Ward LA, Cain OL, Mullally RA, Holliday KS, Wernham AGH, Baillie PD Greenfield SM . Health beliefs about bottled water: a qualitative study. BMC Public Health 2009; **9**:196.
8. Qian N. Bottled Water or Tap Water? A Comparative Study of Drinking Water Choices on University Campuses. Water 2018; 10:1-12.
9. Casado A, Ramos P, Rodriguez J, Moreno N, Gil P. Types and characteristics of drinking water for hydration in the elderly. Crit Rev Food Sci Nutr. 2015; 55:1633-41.
10. Petraccia L, Liberati G, Masciullo SG, Grassi M, Fraioli A. Water, mineral waters and health. Clin Nutr. 2006; 25:377-85.
11. Vitoria I, Maraver F, Ferreira-Pêgo C, Armijo F, Moreno Aznar L, Salas-Salvadó J. The calcium concentration of public drinking waters and bottled mineral waters in Spain and its contribution to satisfying nutritional needs. Nutr Hosp 2014; 30: 188–199.
12. El-Harouny M, El-Dakroory S, Attalla S, Hasan N, Hegazy R. Chemical quality of tap water versus bottled water:Evaluation of some heavy metals and elements content of drinking water in Dakhlia Governorate – Egypt. Internet J Nutr Wellness 2009; 9:1-7.
13. Błaszczyk U., Tuszyş ki T.Mineral waters and their meaning in wholesome prophylaxis. Lab 2007; 4:20-23 (in Polish).
14. Gş arska A, Elş bieta Toş ka, Joanna Ciborska. Natural mineral bottled waters available on the polish market as a source of minerals for the consumers. Part 1. Calcium and Magnesium. Rocz Panstw Zakl Hig 2016 ; 67:1-8
15. Dekel S, Lenthall G, Francis, MJO. Release of prostaglandins from bone and muscle after tibial fracture: An experimental study in rabbits. J. Bone joint Surg. Br. 1981; 63-B: 185-189.
16. Shayo NB, Chove BE, Gidamis AB, Ngoma OB. The quality of water in small community supplies of Kingolwira village, Morogora, Tanzania. Tanzan Health Res Bull Jav 2007; 9:56-60.
17. Azoulay A, Phillippe Garzon, MJ.Eisenberg. Comparison of the Mineral content of tap water and bottled water. J Gen Intern Med 2001, 16:168-175.
18. Saudi Arabian Standards Organization (SASO). Bottled and un-bottled drinking water, 1994; Standard no. 409.
19. International Bottled Water Association (IBWA). Bottled water code of practice. 1700 Diagonal Road, Alexandria, VA, 2016; https://www.bottledwater.org/education/codes-of-practice
20. U.S. Environmental Protection Agency (USEPA). National Pri-mary drinking water regulations, 2011; http://www.epa.gov/safewater/mcl.html
21. Doha Al Nouri, Badriah Al Abdulkarim, Shaista Arzoo, Zubaida Abdel Nabi Bakeet. Quality characteristics of commonly consumed drinking water in riyadh and effect of domestic treatments on its chemical constituents. J Food Nutr Res 2014; 2:25-33.
22. Ghrefat HA. Classification and evaluation of commercial bottled drinking waters in Saudi Arabia. Res J Environ Earth Sci 2013; 5: 210-218.
23. Ouda OKM. Towards assessment of Saudi Arabia public awareness of water shortage problem. Resources and Environment 2013; 3: 10-13.
24. Ahmad M, Bajahlan AS. 2009. Quality comparison of tap water vs. bottled water in the industrial city of Yanbu (Saudi Arabia). Environ Monitor Assess. 159: 1-14.
25. Mokthar M, Aris AZ, Abdullah MA, Yusoff MK, Abdullah MP, Idris AR, Raja Uzir RI. A pristine environment and water quality in perspective: Maliau Basin, Borneo's mysterious world. Water Environ J 2009; 23:219–228.
26. Gu¨ler C, Thyne GD, McCray JE, Turner AK. Evaluation of

graphical and multivariate statistical methods for classification of water chemistry data. Hydrogeol J 2002; 10:455–474.
27. Sagara J. Study of filtration for point-of-use drinking water treatment in Nepal. MSc thesis, Massachusetts Institute of Technology, Cambridge, MA, USA, 2000; pp:1-140.
28. World Health Organization (WHO). Guidelines for drinking-water quality, 2nd ed. Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva, 1996.
29. Dahl C, Søgaard AJ, Tell GS, Flaten TP, Krogh T, Aamodt G, NOREPOS Core Research Group. Is the quality of drinking water a risk factor for self-reported forearm fractures? Cohort of Norway. Osteoporos Int. 2013; 24: 541–551.
30. Wojtaszek T. Prophilactic - wholesome function mineral waters. J. Elementol. 2006; 11:119-126 (in Polish, English abstract).
31. Morr S, Cuartas E, Alwattar B, Lane JM. How much calcium is in your drinking water? A survey of calcium concentrations in bottled and tap water and their significance for medical treatment and drug administration. HSSJ 2006; 2: 130–135.
32. Elizabeth A Yetley, Amanda J MacFarlane, Linda S Greene-Finestone,Cutberto Garza, Jamy D Ard, Stephanie A Atkinson, Dennis M Bier, Alicia L Carriquiry,William R Harlan, Dale Hattis,Janet C King, Daniel Krewski, Deborah L O'Connor, Ross L Prentice, Joseph V Rodricks, and George A Wells. Options for basing Dietary Reference Intakes (DRIs) on chronic disease endpoints: report from a joint US-/Canadian-sponsored working group. Am J Clin Nutr 2017; 105(Suppl):249S–85S.
33. Edelstein S. Dietary Reference Intake. In: Life cycle Nutrition: An evidence based approach. 2nd edition, Jones and Barlett Learning, Burlington, MA, 2014; pp. 1-559.
34. Monarca S, Donato F, Zerbini I, Calderon RL, Craun GF. Review of epidemiological studies on drinking water hardness and cardiovascular diseases. Eur J Cardiovasc Prev Rehabil. 2006; 13: 495–506.
35. Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature 2003; 423:349-55.
36. Vannucci L, Fossi C, Quattrini S et al. Calcium Intake in Bone Health: A Focus on Calcium-Rich Mineral Waters. Nutrients 2018; 10:1930.
37. Cashman KD. Diet, Nutrition, and Bone Health. J Nutr 2007; 137: 2507S–2512S
38. Rylander R. Drinking water constituents and disease. J Nutr 2008; 138: 423S–425S.
39. U.S. Department of Health and Human Services, Public Health Service. The surgeon general's report on nutrition and health: summary and recommendations. DHHS (PHS) Publication No. 88-50211. 1988.
40. Varenna M, Binelli M, Casari S,Zucchi F, Sinigaglia L. Effects of dietary calcium intake on body weight and prevalence of osteoporosis in early postmenopausal women. Am J Clin Nutr 2007; 86:639-44.
41. Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, Garnero P, Meunier PJ. Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the DECALYOS II study. Osteoporos Int 2002; 19:257–264.
42. Marcocci C, Cianferotti L, Cetani F. Bone disease in primary hyperparathyroidism.Ther Adv Musculoskelet Dis. 2012; 4:357-368.
43. Bacciottini L, Tanini A, Falcheti A, Masi L, Franceschelli F, Pampaloni B, Giorgi G, Brandi ML. Calcium bioavailability from a calcium-rich mineral water, with some observations on method. J Clin Gastroenterol 2004; 38:761-766.
44. Greupner T, Schneider I, Hahn A. Calcium Bioavailability from Mineral Waters with Different Mineralization in Comparison to Milk and a Supplement. J Am Coll Nutr 2017; 36:386-390.
45. Heany RP. Absorbability and utility of calcium in mineral waters. Am J Clin Nutr 2006; 84: 371–374.
46. Taj V, Leung W, Grey A, Reid IR, Bolland AJ. Calcium intake and bone mineral density: systematic review and meta-analysis. BMJ 2015; 351: h4183.
47. Maraver F, Vitoria I, Ferreira-Pêgo C, Armijo F, Salas-Salvadó J. Magnesium in tap and bottled mineral water in Spain and its contribution to nutritional recommendations. Nutr Hosp. 2015; 31:2297-2312.
48. Alfonso JF, De Alvarez RR. Effects of mercury on human gestation. Am J Obstet Gynecol. 1984; 75:18-24.
49. Sabatier M, Arnaud MJ, Kastenmayer P, Rytz A, Barclay DP. Meal effect on magnesium bioavailability from mineral water in healthy women. Am J Clin Nutr 2002; 75:65-71.
50. Ryder KM, Shorr RI, Bush AJ, Kritchevsky SB, Harris T, Stone K, Cauley J, Tylavsky FA. Magnesium intake from food and supplements is associated with bone mineral density in healthy older white subjects. J Am Geriatr Soc 2005; 53:1875-80.
51. Miggiano GA, Gagliardi L. Diet, nutrition and bone health. Clin Ter 2005; 156:47-56.
52. Meunier PJ, Jenvrin C, Munoz F, de la Gueronnie`re V, Garnero P. Consumption of a high calcium mineral water lowers biochemical indices of bone remodeling in postmenopausal women with low calcium intake. Osteoporos Int 2005; 16: 1203–1209.
53. Seyedmajidi, Seyedmajidi M, Moghadamnia Aliakbar, Haghanifar S, Ziaei R, Zaheshpasha S, Arash V, Gholamali J, Halalkhor S. Effect of zinc-deficient nutrition on craniofacial bone growth in rats. Dent Res J (Isfahan) 2014; 11: 475–480.
54. Yamaguchi M. Role of nutritional zinc in the prevention of osteoporosis. Mol Cell Biochem 2010; 338:241-54.
55. Tayyeb ZA, Farid SM, Otaibi KA. Trace element concentration of commercially available drinking water in Makkah and Jeddah. JKAU: Eng Sci, 2004; 15: 149 – 154.
56. New SA, Bolton-Smith C, Grubb DA, Reid DM. Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. Am J Clin Nutr 1997; 65:1831–1839.

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Original article

Identification of bacteria species isolated from soil and investigation of optimum conditions: application in food industry and biotechnological fields

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Summary. In this study, soil samples were taken. Isolation of bacteria was carried out by dilutions technique. Identification and biochemical tests of the obtained bacteria were performed. Bacteria were identified to be *Bacillus cereus* WYS01, *Bacillus niacini* PRPB1, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P, *Bacillus cereus* TG16. As a result of biochemical tests, all bacteria showed gram (+), urease tests (-) and amylase activity (+) results. Bacteria reproduced in different feed-lots showed most reproduction in Nutrient Agar and MRS Agar. By determining the optimum reproducing conditions of the bacteria, all bacteria were found to be the most reproductive at 40°C, pH 7.0 within 24 hours.

Key words: bacteria, isolation, identification, food industry

Introduction

Microorganisms are spread out from the deepest part of the seas to the highest levels of the sky. The soil being one of these areas has water, air, oxygen, mineral matter, carbon and nitrogen resources that are necessary for the life of microorganisms (1). Soil is a complex and dynamic biological system. The number of bacteria in one gram of soil is estimated to be 10 billion and to have thousands of species (2). In addition to individual studies, global projects are also conducted to determine soil diversity (3, 4). As developing technology, microorganisms can be detected in laboratory by new generation 16S rRNA and 18S rRNA sequencing metagenomics studies. As a result of analyzes using new generation sequencing technologies, the maximum number calculated is 8.3 million (5). Physicochemical structure of the soil, climate changes and agricultural practices significantly affect the distribution and diversity of bacteria (2,3,6). This distribution and diversity offers a wide range of resources for biotechnological applications. Soil is generally poor and limited for microorganisms in terms of nutrients and energy sources compared to in vitro conditions (6). Besides, there shows up a competition between microorganisms both due to high number of microorganisms and variety. In this competition, microorganisms try to have factors such as larger area, organic matter and water for themselves by using the toxic substances they produce to take the advantage for life. An important part of the soil microflora is bacterium of the genus *Bacillus*. *Bacillus* species in the family Bacillaceae is aerop and facultative anaerope, gram positive endospore bacteria (7-9). *Bacillus* species is used to produce many industrially important enzymes. Because most of its species do not show pathogenic properties, they have a wide genetic diversity and are easy to develop (10). Typical enzyme production by fermentation can be carried out in a short time with *Bacillus* species and with much lower costs of nitrogen and carbon sources (11). About half of commercial enzyme production is produced by *Bacillus* species. These enzymes include enzymes such as proteases, α-amylases, glucose isomerases and pullunases. *Bacillus* species are in a significant group

among microbial rennet producing bacteria. Among these species, the enzyme secreted by the *Bacillus polymyxa* has very good properties (12). Most of the enzymes used in beer production, baking and textile industry are amylase and ş-glucanase enzymes produced by *Bacilllus* spp (13). Amylase produced by *Bacillus mojavensis* SO-10 is used in many fields (14). Alkaline polygalacturonase lyase (PGL) enzyme is the enzyme pectin depolymerase. PGL B, which is used in many fields such as tea and coffee fermentation, oil extraction, fruit juice and textile, is produced by *B. subtilis* (12). Proteases isolated from *Bacillus* are also used in detergent production and leather industry (13). It has been determined that the protease enzymes obtained from *Bacillus subtilis* and *Bacillus cereus* bacteria are an alternative to chemical use in the leather industry and thus it is an effective method for reducing environmental pollution (15). In addition, various foods are obtained from microorganisms (milk products such as yoghurt, kouz; all alcoholic beverages, vinegar, various products such as far eastern origin soy sauce, fermentation of bread, single cell protein).

Due to such effects of bacteria, in this study, soil samples were taken and bacterial isolation was performed by dilution technique. Bacterial identification and biochemical tests were performed. The optimum conditions of the identified bacteria were determined and it was aimed to be used both in biotechnological studies and in various industrial fields.

Material and Method

Biological material

Bacteria isolated from soil were used.

Isolation of microorganisms

To obtain microorganisms from the soil, 1 gram of sample was taken and 9 ml of sterile water was left on that sample. By that method, a suspension of 10^{-1} was made. By similar way, as sequential transfers were performed, and 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilutions were obtained. In order to obtain a single colony, smear sowing was done from the samples made of serial dilution instead of Nutrient Agar (NA) and left to incubation at 37°C for 1 night.

Identification of bacteria species

Isolated bacteria were partially subjected to 16S rRNA analysis by EpiGen.

Biochemical tests

Biochemical tests were performed in order to identify the selected bacterial samples. As biochemical tests such as gram staining, hemolysis, mobility, catalase, amylase, coagulase, urease tests were applied on bacteria separately.

The Effect of different medium on bacterial development

In order to determine the growing of bacteria on solid medium, medium such as Nurient Agar (NA), MRS Agar, Plate- Count Agar, EMB Agar, Endo Agar which were prepared on sterilized petris were experimented. Growing of bacteria was observed as being planted in these medium.

Effect of temperature, pH and incubation time on microorganism growing

25 ml of Nutrient Broth (NB) liquid medium were prepared and 100 ml of glass bottle were autoclaved and 2 ml of bacterial cultivation was performed from fresh overnight culture. It was incubated in shaking in the water bath at 120 rpm as starting from 30°C to 60°C in increments of 10°C.

The effects of pH on the development of bacteria were investigated. For that purpose, 25 ml NB liquid medium were autoclaved by adjusting the pH to be 4.0-11.0 and 2 ml of planting was done from fresh culture. The bacteria were incubated at 120 rpm in shaking in the water bath at the optimum temperature of the bacteria.

In order to investigate the effect of incubation time on the development of bacteria, bacteria was produced in shaking in water bath at 120 rpm at the optimum pH and temperature of the bacterium by 2 ml planting from the fresh culture instead of 25 ml of NB liquid medium. Absorbance of the bacteria samples taken at 12, 24, 36, 48, 60 and 72 hours at 460 nm by spectrophotometer was measured.

Results

Identification of microorganism and biochemical tests

5 bacteria species isolated from soil were subjected to partially 16S rRNA analysis by EpiGen. The results showed that the bacteria were *Bacillus cereus*

WYS01, *Bacillus niacini* PRPB1, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P, *Bacillus cereus* TG16. There found base strains as bacteria *Bacillus cereus* WYS01 891, *Bacillus niacini* PRPB1 900, *Bacillus thuringiensis* VITLW1 879, *Bacillus cereus* TG16 883, *Bacillus thuringiensis* ES2I3P 874. Some of base strains of bacteria are shown as follows.

CCCATGCGGCGTACTCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACT…(*Bacillus cereus* WYS01)

CATGCTGGCGTACTCCCAGGCGGAGTGCTTATGCGTTAGCTGCAGCACTAAAGGGGAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAAAGCCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTTTCCTCTTCTGTACTCAAGTCCCCCAGTTTCCAATGACCCTCCACGGTTAGCCGTGGGCTTTCACATCAGACTTAAAGGACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGGCAGTTACTCCGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGACCCGAAGGCCTTCATCGCTCACGCGGCGTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGATCTGGGCCGTGTC…(*Bacillus niacini* PRPB1)

CATTGCGTCGTACTCCCAGGCGGAGTGCTTATGCGTTAACTTCAGCACTAAAGGCGGAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGGTTGCTCCGTCAGACTTTCG…(*Bacillus thuringiensis* VITLW1)

CCATGGCGCGTACTCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTACACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCC…(*Bacillus thuringiensis* ES2I3P)

CCTTGGCTGCCGTACTCCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCG…(*Bacillus cereus* TG16)

Various biochemical tests were applied to bacteria identified and which strain analysis had been conducted. It was determined that all bacteria were gram (+) during gram coloring. It is found that *Bacillus niacini* PRPB1 and *Bacillus thuringiensis* ES2I3P were inactive and the others were active. It was determined that all microorganisms performed hemolysis. All of the bacteria were found to have negative urease activity but positive amylase activity. Detailed information on biochemical tests and properties is shown at Table 1.

The effect of different medium on bacterial growing

In order to determine bacteria growing in different medium, medium such as Nurient Agar (NA),

Table 1. Biochemical Tests

Features	*Bacillus cereus* WYS01	*Bacillus niacini* PRPB 1	*Bacillus thuringiensis* VITLW1	*Bacillus thuringiensis* E S2I3P	*Bacillus cereus* TG16
Gram	+	+	+	+	+
Hemolysis	+	+	+	+	+
Mobility	+	-	+	-	+
Growth					
Temperature range	30-40°C	30-40°C	30-40°C	30-50°C	30-40°C
pH range	4.0-8.0	5.0-8.0	4.0-8.0	5.0-9.0	5.0-8.0
Activity					
Ureaz	-	-	-	-	-
Katalaz	+	-	+	+	+
Kuagülaz	+	-	-	-	-
Amylase	+	+	+	+	+

MRS Agar, Plate- Count Agar, EMB Agar, Endo Agar were examined. As a result of that study, it was found that *Bacillus cereus* WYS01 shows less growing in Endo and EMB Agars but its growing in other ones was good. It is determined that *Bacillus niacini* PRPB1 bacteria does not grow at all in Endo and EMG Agars, but contrary to those medium, it showed a great growing in Nutrient and MRS Agars. It is also found that *Bacillus thuringiensis* ES2I3P bacteria does not grow in Endo Agar. The growth of microorganisms in solid medium was shown in detail at Table 2.

Effect of temperature, pH and incubation time on microorganism growing

For the temperature effect on the bacterial reproduction experiment was conducted between 30°C and 60°C. In the analysis, it is found that *Bacillus cereus* WYS01 showed the highest growing among the other bacteria at 30 °C. It is also found that all bacteria showed a good reproductive growth from 30°C and 4°C but there showed decreases in the reproduction due to the increasing temperature. The growth of bacteria is shown at Figure 1.

The other important factor in the growth of microorganisms is pH. All of the bacteria produced at pH ranges between 4 and 11 were found to have the best growth rate at pH 7.0. It is found that the microorganism showing the best growing in bacteria was *Bacillus cereus* WYS01. Bacterial reproducing grows up respectively *Bacillus cereus* WYS01, *Bacillus cereus* TG16, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P and *Bacillus niacini* PRPB1. Incubation time has a great effect on bacterial growth like as the other parameters. The bacterial growth is observed between 12–72 hours. When checked the growing of bacteria at 12 hour, it is found that the most was *Bacillus cereus* WYS01. After that, it is found that there was respectively *Bacillus niacini* PRPB1, *Bacillus cereus* TG16, *Bacillus thuringiensis* VITLW1 and *Bacillus thuringiensis* ES2I3P as to their growing rate. As

Table 2. The effect of different medium on bacterial growing

Microorganisms	Nutrient Agar	Endo Agar	EMB Agar	Plate- Count Agar	MRS Agar
Bacillus cereus WYS01	+++	+	++	+++	+++
Bacillus niacini PRPB1	+++	-	-	+	+++
Bacillus thuringiensis VITLW1	+++	+	+	+++	+++
Bacillus thuringiensis ES2I3P	+++	-	+	+++	+++
Bacillus cereus TG16	+++	+	++	+++	+++

+, positive result or growth; -, negative result

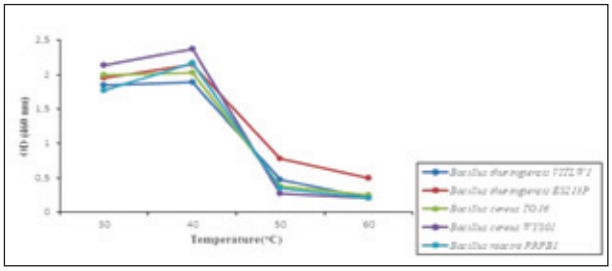

Figure 1. Effect of temperature on microorganism growing

the time that all microorganisms showed the most reproduction was found as the 24th hour. After that time, a proportional decrease was observed in bacterial growth. It is determined that *Bacillus cereus* WYS01 was the most growing microorganism at the 24th hour. The effect of time on the growth of microorganisms is shown at Figure 3 in detail.

Discussion and Conclusion

In our study, isolation of bacteria from soil was carried out. As a result of isolation, a partial 16S rRNA analysis was conducted and it was found that all bacteria were the *Bacillus* species. As a result of biochemical tests, bacteria was found as gram (+). Damodara Chari et al (16) detected that all bacteria they had obtained from soil was gram (+). As a similar result, Ortakaya et al (17) declared that *Bacillus simplex* microorganism was gram (+) in their study which was conducted as isolating bacteria from soil. The optimum conditions of bacteria identified in our study was found as 40°C, pH 7.0 and the 24th hour. Hamilton et al (18) found that the conditions of *Bacillus* sp. IMD 43 were 40°C and the 41st hour. Behal et al (19) conducted reproduction of *Bacillus* sp. AB 04 between 24 and 36 hours at 40°C. Asgher et al (20) detected that incubation time was 48 hours, temperature was 50°C and pH was 7.0 for thermophilic *Bacillus subtilis* JS-2004. Saxena et al (21) carried out reproduction of *Bacillus* sp. PN5 after incubation for 60 hours, at 60°C and pH 7.0. Hmidet et al (22) produced *Bacillus licheniformis* NH1 at 48 hours, 37°C and pH 7.0. Ozdemir et al (14) produced *Bacillus mojavensis* microorganism at 35°C, pH 7.0 and 36 hours for use in biotechnological studies. The temperature of the environment greatly affects the growth of microorganisms. Microorganisms can usually reproduce and grow within their specific temperature limits. These limits include the optimal temperature at which the growth occurs. On the other hand, pH of medium should be within the optimal limits for reproduction of microorganisms. Among the physical parameters, the pH of the growing environment plays an important role in the morphological changes in the microorganism and consequently in the increase of industrial and biotechnological production (20).

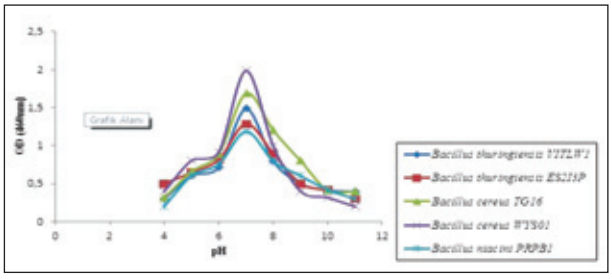

Figure 2. Effect of pH on microorganism growing

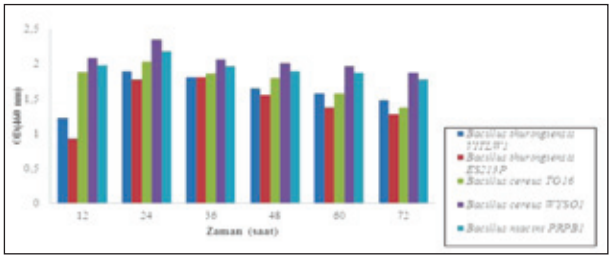

Figure 3. Effect of incubation time on microorganism growing

When we examine the properties of the bacteria obtained in our study, there are advantages both in terms of biochemical test characteristics and optimal conditions. Bacterial growth in a short term and providing low-temperature growth are the significant advantageous for industrially important issues, such as the short duration and electricity savings.

References

1. Başkaya Y, Kocabaş A. Determination of Antimicrobial Substance Production Potentials of Soil Isolate Microorganisms. KSU J Nat Sci 2016; 19(4): 393–398.
2. Torsvik V, Ovreas L. Microbial Diversity and Function in soil: from Genes to Ecosystems. Curr Opin Microbio 2002; 5: 240–245.

3. Roesch LFW, Fulthorpe, RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FA, Farmerie WG, Triplett EWPyrosequencing Enumerates and Contrasts Soil Microbial Diversity, The ISME J 2007;1: 283–290.
4. Gilbert JA, Jansson JK, Knight R. The Earth Microbiome Project: Successes and Aspirations. BMC biol 2014;12: 69–72.
5. Gans J, Wolinsky M, Dunbar J. Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. Sci 2005; 309:1387–1390.
6. Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G. Microbial Diversity and Soil Functions. Eur J Soil Sci 2003;54: 655–670.
7. Topçal F, Dığrak M, Gündoğan R. Identification of Bacillus Species Isolated from Soil and Determination of Bacteriocin Production. Adıyaman Uni J Sci 2014; 4(2): 57–67.
8. Logan NA, Turnbull PCB. *Bacillus* and Recently Derived Genera. In:PR Murray, Rogel Berkeley, Blackwell Science Ltd. Blackwell Publishingh, 2002; 305.
9. Berkeley RCW, Logan N. *Bacillus*, *Alicyelobacillus* and *Paenibacillus*, Principles and Practice of Clinical Bacteriology, Chichester: Wiley 1997; 185.
10. Claus D, Berkeley, CW. The genus *Bacillus* In: Bergey's Manual of Systematic Bacteriology. 1986; 2 (34): 1105–1139.
11. Arellano-Carbajal F, Olmos-Soto J. Thermostable α-1,4- and α-1,6-glucosidase enzymes from *Bacillus* sp. isolated from marine environment. World J Microbiol Biotechnol 2002;18: 791–795.
12. Zhang J. Kang Z, Ling Z, Cao W, Liu L, Wang M, Dub G, Chen J. High-level extracellular production of alkaline polygalacturonate lyase in *Bacillus subtilis* with optimized regulatory elements. Biores Technol 2013;146: 543–548.
13. Demain AL, Solomon N. In Industrial Microbiology. Sci 1981; 214 (4524): 987–995.
14. Ozdemir S, Aguloglu Fincan S, Karakaya A, Enez B. A Novel Raw Starch Hydrolyzing Thermostable α-Amylase Produced by Newly Isolated *Bacillus mojavensis* SO-10: Purification, Characterization and Usage in Starch Industries. Braz Arch Biol Technol 2018;61:e18160399.
15. Antelo SC, No ICF, Böhme K, Alnakip ME, Baluja MQ, Velazquez JB, Mata PC. Genetic discrimination of food borne pathogenic and spoilage *Bacillus* subsp. based on three house keeping genes. Food Microbiol 2014; 46: 288–298.
16. Damodara Chari K, Subhash Reddy R, Triveni S, Trimurtulu N, Durga Rani CHV, Sreedhar M. Isolation and Characterization of Abiotic Stress Tolerant Plant Growth Promoting *Bacillus* spp. from Different Rhizospheric Soils of Telangana. Bıoscı Bıotechnol Research Asia 2018; 15(2): 485–494.
17. Ortakaya V, Aguloglu Fincan S, Enez B. α-Amylase From *Bacıllus Sımplex*- Production, Characterization and Partial Purification. Fresen Environ Bull 2017; 26 (7): 4446–4455.
18. Hamilton LM, Kelly CT, Fogarty WM. Production and properties of the raw strach digestig amylase of *Bacillus* sp. IMD 435. Process Biochem 1999;35: 27–31.
19. Behal A, Sharma MK, Puri P, Singh J, Batra N. Characterization ofalkaline α-amylase from *Bacillus* sp.AB 04. Int J Agric Biol 2006; 8: 80–83.
20. Asgher M, Asad MJ, Rahman SU, Legge RL. A thermostable α-amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing. J Food Eng 2007; 79:950–955.
21. Saxena RK, Dutt K, Agarwal L, Nayyar PA. Highly thermostable and alkaline amylase from a *Bacillus* sp. PN5. Bioresour Technol 2007; 98:260–265.
22. Hmidet N, Bayoudh, A, Berrin JG, Kanoun S, Juge N, Nasri M. Purification and biochemical characterization of a novel a-amylase from *Bacillus licheniformis* NH1 Cloning, nucleotide sequence and expression of amyN gene in *Escherichia coli*. Process Biochem 2008;43: 499–510.

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Original article

Determination of selected vitamin, mineral and trace element content of *Laserpitium carduchorum* Hedge & Lamond

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Summary. In our study, selected vitamin (A, E), mineral (K, Mg) and trace element (Fe, Cu, Zn, Mn) levels of the *Laserpitium carduchorum* Hedge & Lamond plant which is endemically grown in Van Lake basin were examined. When the results of the study were evaluated; Vitamin A level of *Laserpitium carduchorum* Hedge & Lamond plant was found to be 0.61±0.063 μg g^{-1} and vitamin E level was 0.77±0.067 μg g^{-1}. When the mineral and trace element contents of *Laserpitium carduchorum* Hedge & Lamond were evaluated, K (Potassium) was 868.83± 72.92 μg g^{-1}, Mg (Magnesium) 180.77±18.47 μg g^{-1}, Fe (Iron) 11.74±2.37, Cu (Copper) 9.59±1.1 Zn (Zinc) 23.69±2.46 Mn (Manganese) 34.45±5.35 μg g^{-1}. It was detected that the plant was rich in minerals and trace elements such as K (potassium), Mg (Magnesium), Mn (Manganese) and Zn (zinc).

Key words: *Laserpitium carduchorum* Hedge, mineral, trace element, vitamin

Introduction

Plants have been used in many fields including medicine, nutrition, flavoring, soft drink, cosmetics, parfumes, painting cigarettes and other industrial goals. Since the prehistorical period, plants have formed the basis of almost all medical treatments until the development of synthetic drugs in the nineteenth century (1). Oxidative stress indicates the overproduction of products called free radicals and reactive oxygen species (ROS) and imbalance of ROS and antioxidant chemicals. They are harmful when free radicals are not scavenged by endogenous systems. Normally, oxidative stress is caused by an imbalance between reactive oxygen species and endogenous antioxidant systems (2). It has been stated that reactive oxygen / nitrogen species produced in the human body may reason oxidative damage and this damage is related to many degenerative diseases (3). Antioxidants play a critical role in protective the human body against the damage of ROS (4). Most industrial and trading used pharmaceuticals are crops of secondary metabolism in microbial or plant systems (5). Most of the aromatic and spicy plants comprise chemical compounds that exhibit antioxidant properties (6). Medicinally used parts of plants are generally rich in flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignins, phenols and other phenolic compounds (7). They may play an important role in adsorbing and neutralizing free radicals, singlet and triplet oxygen quenching or peroxides decomposition. Many of these phytochemicals have important antioxidant capacities associated with lower rates of human disease and lower mortality rates (4). About 35.000 of the 350.000 plant species known to date have been used all the world for medicinal purposes and less than 0.5% of them have been chemically investigated (5). For this reason, research studies to understand the effects of medicinal plants on their metabolism have increased. Apiaceae Lindl. (Umbelliferae Juss.) is an important family of flowering plants with 300-455 genus and 3.000-3.750 species (8). Members of this family are greatly distributed in tem-

perate climates (9). The Apiaceae family contains the majority of bioactive polyacetylenes and in addition to the well-known medicinal and toxic plants, food plants such as carrots, celery and parsley (10). The members of the Apiaceae family have several compounds with many biological activities. Some of the main features are apoptosis, antibacterial, hepatoprotective and vasoactive properties, cyclooxygenase inhibitory effect and the ability to induce antitumor action (11). The genus *Laserpitium* L. (Apiaceae) contains 20 aromatic species deployed in the northern hemisphere from the Canary Islands in the west to Siberia and Iran in the East. In vegetation Europaea, this genus is represented by 14 species. Some greatly deployed species of this genus such as *L. siler* L. and *L. latifolium* L. have been used in the traditional medicine of some European countries (12). *Laserpitium carduchorum* Hedge & Lamond is an important species that grows endemic in Van Lake basin (13). In addition to the use of *Laserpitium carduchorum* Hedge & Lamond as a spice has been reported to be used in folk medicine to treatment urinary tract infections in particular (14). In this study, it was aimed to evaluate selected vitamin (A, E) mineral (K, Mg) and trace element (Fe, Cu, Zn, Mn) contents of the *Laserpitium carduchorum* Hedge & Lamond plant growing endemic in Van Lake basin.

Materials and Methods

Plant Material

Laserpitium carduchorum plant examples used in the research were gathered from B9 Bitlis: Kotum (küçüksu) Karz mountains Kerner locality at 2.200 m. Plant samples were dried in room conditions without exposure to direct sunlight after collection. The identification of *Laserpitium carduchorum* Hedge & Lamond was carried out by Assoc. Prof. Dr. Fevzi Özgökçe. *Laserpitium carduchorum* Hedge & Lamond plant samples are kept in Van Herbarium with the code of VANF F13882.

Vitamin A, E Analysis

Laserpitium carduchorum Hedge & Lamond plant aerial parts were dried and grinded for Vitamin A (Retinol) and E (α-tocopherol) determination (15-17). Plant samples were extracted with a mixture of n-hexane and ethanol. 1% BHT was added and kept in a dark environment for one day. At the end of this period, centrifugation was carried out at 4000 rpm (+4°C) for 10 min. The obtained supernatant was filtered by the help of whatman filter paper and 0.5 mL of n-hexane was added. Drying was then performed using nitrogen gas. The residue in the tubes was dissolved in a methanol + tetrahydrofuran mixture. Analyzes were carried out in Thermo Scientific Finnigan Surveyor model high performance liquid chromatography (HPLC) and in amber glass vials on Tray autosampler using PDA array detector. Results were expressed as μg g^{-1}.

Determination of Minerals and Trace Elements

The levels of minerals and trace elements of the *Laserpitium carduchorum* Hedge & Lamond plant were measured using dry ashing method (18). 5 g of dry plant samples were kept in the stove at 105°C for approximately 4-5 hours. Then 2 mL of ethyl alcohol-sulfuric acid mixture was adding to the samples taken from the oven. The temperature of the plant samples added to the mixture of ethyl alcohol and sulfuric acid was increased from 250 °C to 550° C. The ash samples were removed from the furnace and added to the samples at a 3 N concentration of hydrochloric acid solution. Analysis of the Fe, Cu, Zn, Mn, K and Mg elements of the samples, which were adjusted to 12.5 mL with the final volume of double-distilled water, was evaluated using the inductively coupled plasma optical emission spectroscopy (ICP-OES) (Thermo iCAP 6300 DUO). Results were expressed as μg g^{-1}.

Statistical Analysis

The results are expressed as mean ± the standard error of the mean ($\bar{X}$± SEM).The descriptive statistical analysis was performed by using the SPSS®, version 22 statistical software (SPSS Inc. Chicago Illinois).

Results

Laserpitium carduchorum Hedge & Lamond. vitamin A and vitamin E levels were evaluated and given in Table 1 and Figure 1. The vitamin A content of the plant was 0.61±0.063 μg g^{-1} and vitamin E levels was

Table 1. Vitamin A and E levels of *Laserpitium carduchorum* Hedge & Lamond.

Sample	Vitamin A (µg g^{-1})	Vitamin E(µg g^{-1})
Laserpitium carduchorum Hedge&Lamond.	0.61 ± 0.063	0.77 ± 0.067

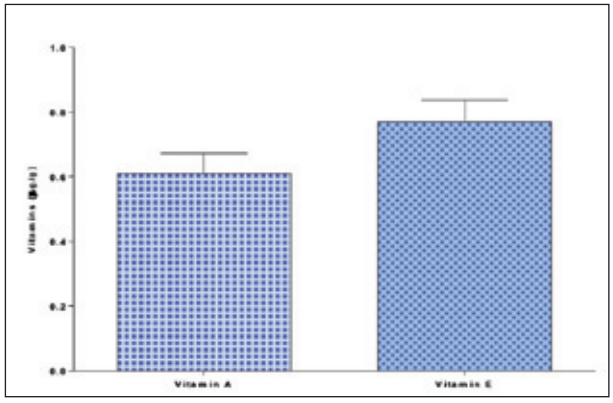

Figure1. Vitamin contents *Laserpitium carduchorum* Hedge & Lamond

0.77±0.067 µg g^{-1}. Vitamin E levels of the *Laserpitium carduchorum* Hedge & Lamond plant was found to be in a high level.

When the mineral and trace element amounts of the *Laserpitium carduchorum* Hedge & Lamond. plant are evaluated; K (potassium) was 868.83±72.92 µg g^{-1}, Mg (magnesium) was 180.77±18.47 µg g^{-1} and trace element levels Cu (copper) was 9.59±1.1 µg g^{-1}, Fe (iron) was 11.74±2.37 µg g^{-1}, Mn (manganese) was 34.45±5.35 µg g^{-1} and Zn (Zinc) was 23.69 ± 2.46 µg g^{-1} (Table 2). Mineral and trace element content was found to be ranked as potassium (K)> magnesium (Mg)> manganese (Mn)> zinc (Zn)> iron (Fe)> copper (Cu) (Figure 2 and Figure 3).

Table 2. Mineral and trace element levels of *Laserpitium carduchorum* Hedge &Lamond.

Minerals and Trace Elements µg g^{-1}	*Laserpitium carduchorum* Hedge&Lamond
K	868.83 ± 72.92
Mg	180.77 ± 18.47
Cu	9.59 ± 1.1
Fe	11.74 ± 2.37
Mn	34.45 ± 5.35
Zn	23.69 ± 2.46

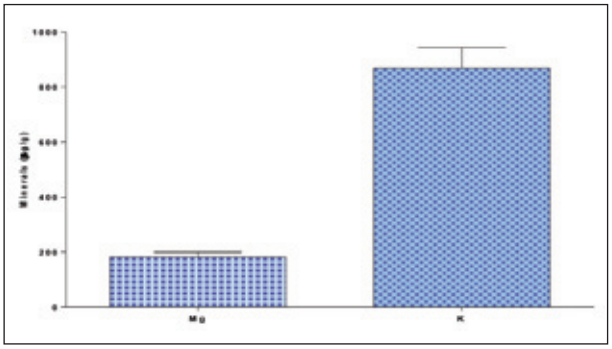

Figure2. Mineral contents *Laserpitium carduchorum* Hedge & Lamond

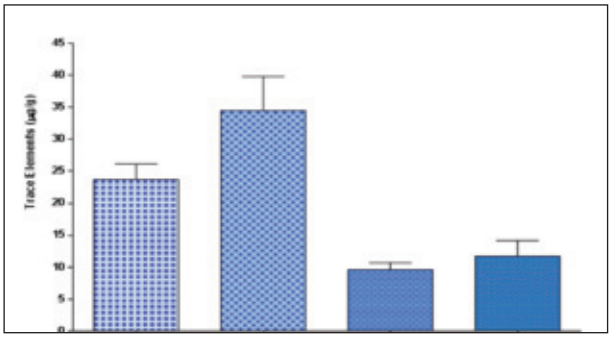

Figure3. Trace element contents *Laserpitium carduchorum* Hedge & Lamond

Discussion

Medicinal herbs play an important role in traditional medicine and are broadly used up as home remedies. In recent years has seen a considerable rise in the use of herbal medicines due to its least side effects, availability and acceptability to the majority of third-world countries (19). Today, the use of medicinal plants to weaken and cure the disease by a lot of people is quite widespread in the world because of its mild properties and low side effects (20). The plants are abundant in natural antioxidants such as phenolic compounds tochopherols, carotenoids and ascorbic acid (21). Epidemiological studies have shown that the intake of natural antioxidants reduces the risk of many diseases (22). In general, vitamins such as tochopherols, carotenoids and ascorbic acid are bioactive components that show protective effects (23). It has been shown that there is a correlation between the consumption of fruit in the diet and the reduction of the risk of chronic disease (24). It has been

reported that the intake of large amounts of fruits and vegetables has protective effects against various diseases, especially cardiovascular diseases (23). Vitamin E acts as a chain-breaking antioxidant that prevents the spread of free radical reactions in various disease processes (25). The concept vitamin A defines a group of retinoid compounds with the biological activity of all-trans retinol. Vitamin A plays an important role in many physiological functions including vision, growth, reproduction, hematopoiesis and immunity (26). In particular, vitamins A and E (a-tocopherol) have been shown as chemopreventive agents for some types of cancer (27). Considering the important effects on metabolism, *Laserpitium carduchorum* Hedge & Lamond vitamin A and vitamin E contents were 0.61±0.063 μg g^{-1} and vitamin E content as 0.77±0.067 μg g^{-1}, respectively. Vitamin E content of the plant was found to be better than vitamin A. Minerals in general; Ca, Mg, K, Na and P are known as macro elements and are generally included in anatomical structures, but they are also in the ionized active form (28). Trace elements; in living organisms, plants and soil, low concentrations are defined as molecules (29). Trace elements have important effects on vital processes for vitality. Many studies have revealed that trace elements are associated with many diseases (30). The trace elements that have been extensively studied during the last decade contain copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), boron (B), cobalt (Co), nickel (Ni) (29). When the minerals are evaluated; Potassium is the main cation in the intracellular fluid and acts in the acid-base balance, arrangement of osmotic pressure, delivery of nerve impulse, muscle contraction, especially cardiac muscle, cell membrane function and Na+/K+ -ATPase. Potassium is needed during glycogenesis (31). The Mg mineral is the second dominant component, which is the co-factor of more than 300 basic metabolic reactions, including those that produce or utilize the Mg-ATP complex, an important regulator of cellular processes in the intracellular compartment. However, its functionality is related to tissue components, growth and thermogenesis and tyrosine kinase activity in glucose metabolism (32). Zinc (Zn), which has an important take place among trace elements, is widely distributed in plant and animal tissues and is seen in all living cells (31). It functions as a cofactor and forms the component of many enzymes such as lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase. Zn-dependent enzymes are involved in macronutrient metabolism and cell replication (33). Manganese (Mn), which is effective in the growth of skeletal growth, nucleic acids, proteins and hemoglobin, and the metabolism of lipids and carbohydrates, also has anti-allergic properties. It acts as a calcium antagonist to the nervous system and is as effective as Mg^{2+} to inhibit the release of neuromediators into the synaptic area (28). Copper (Cu), one of the important trace elements, is essential for the development of connective tissue, nerve coatings and bone. Cu tasks as a reductant in superoxide dismutase, cytochrome oxidase, lysyl oxidase, dopamine hydroxylase and many other oxidase enzymes that diminish molecular oxygen. The protein is transported by ceruloplasmin in the organism (34). Iron (Fe) is required for electron transfer reactions, , binding and transport of oxygen, gene regulation and cell differentiation and arrangement of cell growth. Iron is a crucial component of enzymes that produce peroxide and nitrous oxide (35). In our study we evaluated *Laserpitium carduchorum* Hedge & Lamond. The mineral levels of the plant were found as K (potassium) 868.83±72.92 μg g^{-1}, Mg (magnesium) 180.77±18.47 μg g^{-1} respectively and trace element levels were found as Cu (copper) 9.59±1.1 μg g^{-1}, Fe (iron) 11.74±2.37 μg g^{-1}, Mn (manganese) 34.45±5.35 μg g^{-1}, Zn (Zinc) 23.69±2.46 μg g^{-1} respectively. It was found that the plant was rich in potassium and magnesium and was also very good in manganese and zinc content. Literature records show that studies related to the *Laserpitium carduchorum* Hedge & Lamond which is endemic to Lake Van basin are very limited. In some studies the essential fatty acid components of the plant was reported to be in good level. It has been reported that the phenol ingredient of the plant and its antioxidant capacity are high. Again *Laserpitium carduchorum* Hedge & Lamond essential oil content and extract of the plant was reported to have antimicrobial activity (14). There is no study on the vitamin and mineral content of the *Laserpitium carduchorum* Hedge & Lamond. In this sense, the study is very important. When the studies about different types of *Laserpitium* are evaluated; two different types of endemic *Laserpitium* have a good chemical content and have a cytotoxic effect. Again, two different types of

Balkan endemic *Laserpitium* species have been found to have good essential oil content and have antinociceptive and anti-dementia effects (36, 37). *Laserpitium garganicum* has been reported to show strong antifungal activity (38).

As a result; The endemic species *Laserpitium carduchorum* Hedge & Lamond plant vitamin E content is higher than vitamin A content, the plant is examined in the mineral (K, Mg) and trace element (Fe, Cu, Zn, Mn) levels and is found to have good K (potassium), Mg (magnesium), Mn (manganese), Zn (zinc) content. These results are thought to be a reference to future studies.

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References

1. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food chemistry 2006; 97: 654-660.
2. Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research 2009; 3: 981-996.
3. Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Science and Technology 2008; *41*:385-390.
4. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. Journal of ethnopharmacology 2002; 81: 155-160.
5. Shasany AK, Shukla AK, Khanuja SP. Medicinal and aromatic plants. In *Technical crops,* 2007;175-196. Springer, Berlin, Heidelberg
6. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food chemistry 2004; 85: 231-237.
7. Surveswaran S, Cai YZ, Cork H, Sun M. Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry 2007; 102: 938-953.
8. Downie SR, KatzŞDownie DS, Watson MF. A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA rpl16 and rpoC1 intron sequences: towards a suprageneric classification of subfamily Apioideae. American Journal of Botany 2000; 87: 273-292.
9. Lee SB, Rasmussen SK. Molecular markers in some medicinal plants of the Apiaceae family. *Euphytica*, 2000; 114: 87-91.
10. Christensen LP, Brandt K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. Journal of pharmaceutical and biomedical analysis, 2006; 41(3): 683-693.
11. Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MR. Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. Food chemistry 2010; 120: 765-770.
12. Popovic VB, Petrovic SD, Milenkovic MT, Drobac MM, Couladis MA, Niketic MS. Composition and antimicrobial activity of the essential oils of Laserpitium latifolium L. and L. ochridanum Micevski (Apiaceae). Chemistry & biodiversity 2015; 12: 170-177.
13. Demirkuş N, Koyuncu M, Gül M. Van Havzası Endemik Bitkileri. *Anatolian Journal of Botany* 2018;*2*: 70-83.
14. Dastan D, Salehi P, Maroofi H. Chemical Composition, Antioxidant, and Antimicrobial Activities on Laserpitium carduchorum Hedge & Lamond Essential Oil and Extracts During Various Growing Stages. Chemistry & biodiversity 2016; 13:1397-1403.
15. Bruni R, Muzzoli M, Ballero M, Loi MC, Fantinc A, Polid F, Sacchettia G. Tocopherols, fatty acids and sterols in seed of four Sordinian Willd. Europhorbia species. Fitoteraphia 2004; 75: 50-61.
16. Saleh IA, Billed G, El-Doush I. Levels of Selenium DL-α-tocopherol all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. Journal of Food Composition and Analiysis 2006; 19:167-175.
17. Şahin A, Kıran Y, Karataş F, Sonmez S. Vitamins A, C and E and β-Carotene Content in Sedds of Seven Species of *Vicia L.* Journal of Integrative Plant Biology, 2005; 47: 487-493.
18. Zurera G, Estrada B, Rineon F, Pozo R. Lead and cadmium contamination levels in ediple vegatables. Bulltein of Environmental Contamination and Toxicology 1987; 38: 805-81.
19. Bello MO, Ibrahim AO, Ogunwande IA, Olawore NO. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 2004; 85: 67-71.
20. Başgel S, Erdemoğlu SB. Determination of mineral and trace elements in some medicinal herbs and their infusions consumed in Turkey. Science of the total environment 2006; 359: 82-89.
21. Al-Mustafa AH, Al-Thunibat OY. Antioxidant activity of some Jordanian medicinal plants used traditionally for treatment of diabetes. Pak J Biol Sci 2008; 11: 351-358.
22. Yang CS, Landau JM, Huang MT, Newmark HL. Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual review of nutrition 2001: 21: 381-406.
23. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. Int. J. Mol. Med. Adv. Sci 2005; 1: 375-381.
24. Vasco C, Ruales J, Kamal-Eldin A. Total phenolic compounds and antioxidant capacities of major fruits from Ec-

uador. Food chemistry 2008; 111: 816-823.
25. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. Journal of physiology and biochemistry 2001; 57: 43-56.
26. Villamor E, Fawzi WW. Effects of vitamin A supplementation on immune responses and correlation with clinical outcomes. Clinical microbiology reviews 2005;18: 446-464.
27. Sirimanne SR, Patterson DG, Ma L, Justice JB. Application of cloud-point extraction–reversed-phase high-performance liquid chromatography: A preliminary study of the extraction and quantification of vitamins A and E in human serum and whole blood. Journal of Chromatography B: Biomedical Sciences and Applications 1998; 716: 129-137.
28. Speich M, Pineau A, Ballereau F. Minerals, trace elements and related biological variables in athletes and during physical activity. *Clinica Chimica Acta*, 2001; 312: 1-11.
29. He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. Journal of trace elements in medicine and biology 2005; 19: 125-140.
30. Akkoyun HT. Effect of boric acid on some elemental levels on rats liver and kidney tissues during mercury chloride exposure. Cellular and Molecular Biology Doi:http//dx.doi.org/10.14715/cmb/2018.64.13.16.
31. Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants-A review. African journal of food science 2010; 4: 200-222
32. Sales CH, Pedrosa LDFC. Magnesium and diabetes mellitus: their relation. Clinical nutrition 2006; 25: 554-562.
33. Frossard E, Bucher M, Mächler F, Mozafar A, Hurrell R. Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. Journal of the Science of Food and Agriculture 2000; 80: 861-879.
34. Fraga CG. Relevance, essentiality and toxicity of trace elements in human health. Molecular aspects of medicine 2005; 26: 235-244.
35. Maggini , Wintergerst, ES, Beveridge S, Hornig DH. Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. British Journal of Nutrition 2007; *98*: 29-35.
36. Popovic V, Petrovic S, Tomic M, Stepanovic-Petrovi R, Micov A, Pavlovic-Drobac M, Niketic M. Antinociceptive and anti-edematous activities of the essential oils of two Balkan endemic Laserpitium species. Natural product communications 2014; *9*: 125-128.
37. Popovic V, Heyerick A, Petrovic S, Van Calenbergh S, Karalic I, Niketic M, Deforce D. Sesquiterpene lactones from the extracts of two Balkan endemic Laserpitium species and their cytotoxic activity. Phytochemistry 2013; 87: 102-111.
38. Tirillini B, Pagiotti R, Angelini P, Pintore G, Chessa M, Menghini L. Chemical composition and fungicidal activity of the essential oil of Laserpitium garganicum from Italy. Chemistry of natural compounds, 2009; *45*: 103-105.

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