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Relationship between red blood cell membrane fatty acid composition and dietary fatty acids level in obese adolescents with/without metabolic syndrome

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Summary. *Background:* Dietary lipids may lead to significant changes in the composition of cell membrane structure. *Objective:* The association between red blood cell (RBC) membrane fatty acid composition and dietary fatty acids intake in obese adolescents with and without metabolic syndrome was investigated**.** *Methods:* Ninety-six adolescents (59 obese and 37 lean) were included in the study. The obese subjects were divided into two groups with (n=26, aged 12.3 years) and without (n=33, aged 12.7 years) metabolic syndrome. The mean age of lean participants was 11.8. The criteria for metabolic syndrome were: 10 <16 age group; BMI ≥ 95th percentile, triglycerides ≥ 150 mg/dL, HDL- C< 40 mg/dL, blood pressure systolic ≥ 130, diastolic ≥ 85 mm Hg, glucose ≥ 100 mg/dL. Subjects with any 3 of these 6 items were accepted with metabolic syndrome. Nutrient intakes were recorded for 3 days by a dietitian. Anthropometric and blood chemistry variables and RBC membrane fatty acid levels were measured. *Results:* BMI, BMI-SDS, body fat percentage, blood pressure, TG, ALT, insulin, HOMA-IR levels ($p<0.001$) of obese were significantly higher than lean adolescents whereas HDL-C levels were lower ($p<0.001$) in obese than lean. Fiber, C22:1 ω-9 MUFA, C20:4 ω-6 PUFA consumption were higher ($p<0.05$) in obese with metabolic syndrome. Consumption of total ω-3 PUFAs were higher ($p<0.05$) in obese with metabolic syndrome and in leans than those without the metabolic syndrome. The RBC membrane C16:1 ω-7 fatty acid levels were higher but C20:5 ω-3 levels were lower ($p<0.001$) in obese. Diet fiber was positively associated with RBC C16:1 ω-7 fatty acid ($r=0.239$, $p<0.05$). A positive slight correlation was determined between diet and RBC membrane C20:5 ω-3 ($r= 0.200$, $p= 0.051$). *Conclusion:* Dietary fatty acids affected some anthropometric measurements and blood chemistry. The RBC membrane C20:5 ω-3 slightly correlated with diet C20:5 ω-3.

Key words: adolescent, dietary fatty acids, metabolic syndrome, obesity, red blood cell membrane fatty acids

Introduction

Obesity, a metabolic disorder, is the final results of fat deposition through lipogenesis and fatty acid esterification (1). Childhood obesity has been associated with type 2 diabetes mellitus, the early-onset of metabolic syndrome **(MS),** subclinical inflammation, dyslipidemia, coronary artery diseases and adulthood obesity (1, 2). Consumption of excess fat is one of the main factors that contribute to the development of obesity (1). Not only the amount taken, but also the fatty acid composition of the diet has great importance in the development of obesity (3). Metabolic syndrome in childhood is defines as the presence existing of three or more of the

syndromes such as obesity, increased triglycerides, decreased high density lipoprotein cholesterol, high blood pressure and/or glucose intolerance (4).

Essential fatty acids (EFAs) play an important role in cell membrane composition (5).The nature of the fat taken with diet affects the blood lipid concentrations in children as in adults (6). In inflammatory diseases, increases in the consumption of saturated fatty acids (SFAs) and trans FAs are positively correlated with disease development (7, 8). Saturated fatty acids trigger the production of precursor of prostaglandins (PG), and proinflammatory leukotrienes (LT) (9). High proportion of the saturated and trans-unsaturated fatty acid intake increases plasma concentrations of total and LDL cholesterol and, even at a young age, may increase vascular lipid storage and the early vascular lesion formation (10).

Since the mature erythrocytes cannot synthesize lipids spontaneously, any loss of lipids must be compensated by exchange of the plasma lipids (11). Lipids taken with diet may lead to significant changes in the composition of the cell membrane structure (6). Morgado et al. (12) who compared the effects of ω-6, ω-9 and SFAs in rats determined changes in ω-3/ω-6 ratio in hepatic membranes and a decrease in HDL-C level with ω-3 fatty acid**.** In a recent review, Simopoulos (13) has reported that the risk of obesity increases with the increasing level of ω-6 fatty acids and the ω-6/ ω-3 ratio in red blood cell (RBC) membrane phospholipids, whereas high ω-3 RBC membrane phospholipids decreases the risk of obesity. In pre-school children (2-6 years old) receiving low ω -3 and ω-6 FAs showed high RBC docosahexaenoicacid (DHA) and high arachidonicacid (AA) profiles (6).

Although plasma FAs is a good indicator of intake of FAs in the last few days/weeks (14), they can be easily affected by the metabolic changes and physical activity. Because the FAs distribution in red blood cells is more stable throughout their life (15), measurement of the levels of FAs in cell membrane is recommended to evaluate the long term lipid intake statuses of the individuals.

Early recognition of obesity and implementing necessary measures for the prevention of childhood obesity and associated complications possibly encountered in adulthood is of great importance. In addition, determination of the ratio of fats in total energy intake and types of FAs in the diet are also important because of their role in the etiology of obesity and in the development insulin resistance (16). Therefore, the presented study was performed to investigate the fatty acid composition of the diet and RBC membrane fatty acid levels as well as to determine whether an association exists between diet and RBC membrane fatty acids in obese adolescents with and without metabolic syndrome.

Methods

This clinical and controlled study was initiated with the permission of Ethics Committee of Gülhane Military Medical Academy (GATA), Approval no: 1491-1395-11/1539.

A total of 59 outpatients consisting of 33 male **(**16 with MS aged 12.3years and 17 without MS aged 12.7years) and 26 female (10 with MS aged 11.5 years and 16 without MS aged 13.0 years) who were admitted to GATA Department of Child Health and Diseases, Polyclinics of Pediatric Endocrinology and received obesity diagnosis between June 2011 and November 2012 were included in the study. The patients at pubertal stage (Tanner stages 2-5) (17) with BMI 95th percentile or above according to the Centers for Disease Control and Prevention (CDC) (18) percentile curves with no history of familial dyslipidemia and/or type 2 diabetes mellitus, no endocrinological and chronic disease and having no obesity medication, fish oil and ω-3 FA supplements were included in the study. A total of 37 (18 male aged 12years and 19 female aged 11.9 years) healthy, age matched lean participants having BMI less than 85th percentile were also included as control group. The inclusion criteria for the controls were the same except BMI.

Metabolic syndrome in adult was accepted as a cluster of risk factors for cardiovascular disease and type 2 diabetes mellitus. The risk factors include raised blood pressure, dyslipidemia (raised triglycerides and lowered high-density lipoprotein cholesterol), raised fasting glucose, and central obesity (19). In the present study, the criteria for metabolic syndrome for adolescents were: 10 <16 age group; BMI ≥ 95th percentile, triglycerides ≥ 150 mg/dL, HDL- C< 40 mg/dL, blood pressure sys-

tolic ≥ 130, diastolic ≥ 85 mm Hg, glucose ≥ 100 mg/dL. Subjects with any 3 of these 6 items were accepted as having metabolic syndrome (4).

Participants underwent routine physical examinations. Arterial blood pressure was measured by a mercury sphygmomanometer using an appropriate brachial pressure cuff from right arm following at least 10 minutes rest. The first Korotkoff sound was recorded as systolic blood pressure (SBP), phase V sound was recorded as diastolic blood pressure (DBP).

Measurement of height was performed with a fixed stadiometer with 0.1 cm sensitivity (Harpenden, UK) and weight was measured with a digital scale with 0.1 kg sensitivity (SECA, Deutschland) and body mass index (BMI, kg/m^2) was calculated through dividing the weight (kg) by height (m^2). BMI values to sex- and age-specific standard deviation scores (BMI-SDS) was calculated. The height of individuals was measured while the person was standing without shoes, feet were together, and head was maintained in the Frankfort Horizontal Plane position (triangle of eyes in alignment with the upper side of auricle). The participants with the BMI 95th percentile were considered obese according to the CDC (18). Body fat was measured with Segmental Body Composition Analyzer (TANİTA BC-418). Measurements were performed in the morning following overnight fasting.

Nutrients and food items consumed by the subjects were recorded during face to face interviews by a dietitian for consecutive 3 days and the average amounts of daily consumptions were determined. The daily energy, nutrients and dietary FAs intakes of the participants were determined using the software program of Nutrition Information Systems (BEBIS).

Blood samples were collected from the antecubital vein after an overnight fasting into tubes (Vacutter® greiner bio-one, Austria) with (K3-EDTA) and without anticoagulant. Serum samples were separated, and immediately used for biochemical analysis. Whole blood samples were immediately centrifuged at 4000 rpm for 15 minutes and plasmas were separated. The plasma fraction was allocated into cryovials and stored at -80 °C. After separation of plasma, remaining pellet was washed three times with an equal amount of 0.9% sodium chloride by centrifugation at 4000 rpm for 15 minutes and then the RBCs were resuspended with saline for raising the pellet hematocrit to 45%. The RBC aliquoted to the cryovials previously treated with butyl hydroxy toluene (BHT) (2,6 di-tert-butyl-p-cresol) and stored at -80 ° C until the analysis (20).

Plasma glucose, serum triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels were measured with an Olympus AU2700 (Beckman Coulter, USA) auto analyzer using commercially available kits (Olympus, USA). Low density lipoprotein cholesterol (LDL-C) was calculated by the Fridewald formula [LDL-C = TC - (TG/5 + HDL-C)]. Serum insulin levels were measured by an immunoassay analyzer (Roche Modular Analytics E170, Roche Diagnostics, USA) with electro-chemiluminescence immunoassay (ECLIA) kit (Roche Diagnostics, USA). The homeostasis model of assessment-insulin resistance (HOMA-IR) was calculated using the formula of fasting glucose (mg/dL) x fasting insulin (mU/mL)/405, and HOMA-IR> 4 was considered as insulin resistance.

Red blood cell suspensions (50 µl) were thawed at 4 °C. Fatty acids glycerol esters and plasmalogens were transmethylated by the addition of 3 N methanolic HCl and heating for 4 h at 90 °C. After chilling the samples, fatty acid methyl esters were extracted with hexane by vortex mixing for 10 seconds and evaporated under nitrogen and resuspended with 80 µl of hexane (20). Thermo-Finnigan Trace GC Ultra (Thermo Scientific TM, USA) with SPTM-2560 capillary column (100m x 0.25 mm x 0.2 µm, Catalog no. 48333-07) was used for the measurement of FAs. The peaks of FAs were observed between 17.955-41.825 minutes. Linearity of investigated FAs (r^2) was between 0.9579-0.9973. Recoveries were determined within the range of 87 to 107% and 94 to103% for 50 µg/mL and 100 µg/mL concentrations respectively. LOD was found between 0.3-4.2 µg/mL concentrations. The reproducibilities were determined within 5-10.4% for low concentrations and within 4.2-10% for high concentrations.

Statistical Analysis

Data were analyzed by the Statistical Package for Social Science version 15.0 software (SPSS, Chicago, IL., USA). One Way Anova and Kruskal–Wallis variance analyses were used for comparison of variables.

When the differences were significant, Bonferroni correction was performed to determine which subgroups are different. The relationship between variables was evaluated with Spearman correlation analysis. P values less than 0.05 were considered as significant.

Results

No statistically significant difference was determined between obese adolescents with and without metabolic syndrome concerning the age and gender. Body Mass Index and body fat percentage of obese adolescents were significantly higher (p<0.001) than their age matched lean controls. The BMI-SDS, SBP and DBP levels were the highest in obese with metabolic syndrome, which is followed by obese without metabolic syndrome and controls. Serum triglycerides, ALT, insulin and HOMA-IR levels of obese with metabolic syndrome were significantly higher (p<0.001) than the obese without metabolic syndrome and lean adolescents. The TC and LDL-C levels were also higher in obese with metabolic syndrome but could not reach to statistically significance. The HDL-C levels were lower in both obese groups (p<0.001) compare to controls. There was no significant difference between groups concerning fasting blood glucose level and AST activity (Table 1). Consumption of fiber, C22:1 ω-9 MUFA, C20:4 ω-6

Table1. Anthropometric and laboratory parameters of adolescents

	Patients with obesity (n=59)		**Controls (n=37)**	p
	MS (+) (n=26)	MS (-) (n=33)		
Gender, male; n,%	16(61.5)	17(51.5)	18(48.6)	0.585
Age, years	12.3(10.8-13.3)	12.7(10.6-14.6)	11.8(11-12.8)	0.464
male	12.3(11-13.3) n=16	12.7(10.6-13.9) n=17	12(11-12.8) n=18	0.490
female	11.5(10.8-12.3) n=10	13(11-14.6) n=16	11.9(11.5-12.6) n=19	0.383
BMI, kg/m^2	30.6(28.7-33.6)[a]	27.7(24.9-30.8)[a]	18.1(16.7-20.3)[b]	p<0.001
BMI-SDS	2.14(1.9-2.4)[a]	1.83(1.7-2.2)[b]	-0.23(-0.9-0.3)[c]	p<0.001
SBP	125±16.3[a]	111.67±8.35[b]	103.92±10.41[c]	p<0.001
DBP	83.92±1.07[a]	72.41±8.72[b]	63.95±8.46[c]	p<0.001
Body fat percentage,%	41.6(34.8-50.7)[a]	39.9(34.3-45.2)[a]	16.9(13.9-19.4)[b]	p<0.001
FPG, mg/dL	89±5.4	86±7	87.81±6.6	0.198
TC, mg/dL	178(163-202)	162(152-185)	159(143-180)	0.214
TG, mg/dL	211(123-283)[a]	90(76-148)[b]	81.5(60.5-106.5)[b]	p<0.001
LDL-C, mg/dL	109(84-135)	99(84-116.5)	87(78.8-101.8)	0.92
HDL-C, mg/dL	36(34-39)[a]	46(41-51.5)[b]	53(45.5-62.5)[c]	p<0.001
ALT (IU/mL)	24.5(10-54)[a]	20(10-89)[a]	15(9-44)[b]	p<0.001
AST (IU/mL)	26(15-80)	27(12-87)	29(16-51)	0.718
Insulin, U/mL	21.4(16.9-25.4)[a]	11.5(9.8-13.5)[b]	7.9(4.9-10)[c]	p<0.001
HOMA-IR	4.71(3.2-6.4)[a]	2.57(1.9-3)[b]	1.65(1-2.3)[c]	p<0.001

Data are expressed as the means, median (25th–75th interquartile range) or as number of cases (%) when appropriate.

BMI: body mass index, BMI-SDS: BMI values to sex- and age-specific standard deviation scores, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, FPG: fasting plasma glucose, TC: total cholesterol, TG: triglyceride, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, HOMA-IR: homeostasis model of assessment-insulin resistance; [a-c]: Values with different superscripts differ significantly.

PUFA with the diet were higher (p<0.05) in obese with metabolic syndrome than obese and control. Total ω -3 PUFAs consumed with the diet were higher (p <0.05) in obese with metabolic syndrome and in leans than obese without the metabolic syndrome (Table 2). Red blood cell membrane C16:1 ω-7 fatty acid levels of obese with and without metabolic syndrome were higher whereas C 20: 5 ω-3 levels were lower than controls (p<0.001) (Table 3). Diet cholesterol positively correlated with serum ALT (r= 0.232, p<0.05). Diet total SFAs positively associated with fasting blood glucose (r= 0.206, p<0.05). Diet total MUFAs inversely associated with LDL (r= -0.200, p<0.05), and diet total PUFAs also inversely correlated with TC (r= - 0.256, p<0.05) and LDL-C (r= -0.274, p<0.01). A positive association was determined between diet total ω-3 FAs and fasting blood glucose (r= 0228, p<0.05) Diet total ω-6 FAs inversely affected serum TC (r= -0.295, p<0.01) and LDL-C (r= -0.311,

Table 2. Macronutrient and diet fatty acids intake of adolescents

Diet FAs	**Patients with obesity (n=59)**		**Controls (n=37)**	**p**
	MS (+) (n=26)	MS (-) (n=33)		
Energy	1786(161-220)	1827(142-209)	1883(152-215)	0.484
Protein (%)	13.5(11.8-15)	13(12.5-15)	14(12-15.5)	0.643
Fat (%)	37.5(33.8-41)	38(35-39.5)	39(32.5-43)	0.652
Carbohydrate (%)	50(46-54)	49(47-52)	47(44-53)	0.505
Cholesterol (mg)	289(164-363)	276(184-336)	227(179-331)	0.214
Fiber	20.5(17-26)[a]	17.70(12-22)[b]	16.6(12-22)[b]	0.032
Total SFAs	24.9(22.4-34.9)	29.8(21.8-33)	27.8(21.2-32.7)	0.845
C14:0	3.0(2.2-4.3)	2.8(2.6-4.7)	2.5(2.2-3.7)	0.210
C16:0	13.0(10.6-18.3)	14.2(10.9-16.2)	14.4(10.7-16.3)	0.996
C18:0	5.4(4.6-7.6)	6.2(4.6-8.1)	5.9(4.7-7.9)	0.926
C20:0	0.4(0.3-0.5)	0.3(0.25-0.4)	0.3(0.25-0.4)	0.272
C22:0	0.2(0.1-0.3)	0.2(0.1-0.25)	0.2(0.1-0.2)	0.552
C24:0	0.0(0-0)	0.0(0-0)	0.0(0-0)	0.983
Total MUFAs	26.8(19.6-33.9)	25.7(20-30)	24.6(20.4-28.8)	0.656
C16:1 ω 7	1.7(1.3-2.3)	1.6(1.2-2)	1.7(1.3-2)	0.682
C18:1 ω 9	23.8(15.3-30.1)	22.8(16.6-26.1)	21.2(17.8-25.1)	0.811
C22:1 ω 9	0.5(0.3-0.7)[a]	0.3(0.2-0.5)[b]	0.4(0.3-0.6)	0.05
Total ω -6 PUFAs	14.2±9.7	13.2±7.7	15.8±6.1	0.823
C 18:2 ω 6	15.3±9.3	14.7±7.7	15.6±6.1	0.882
C20:3 ω 6	1.4(0.9-2.1)	1.2(1-1.7)	1.7(1.2-2.5)	0.081
C20:4 ω 6	0.5(0.3-0.6)[a]	0.3(0.2-0.4)[b]	0.4(0.2-0.5)	0.036
Total ω -3 PUFAs	2.35(1.8-3.9)[a]	1.8(1.4-2.5)[b]	2.6(1.6-3.3)[a]	0.017
C20:5 ω 3	0.0(0-0.6)	0.0(0-0.2)	0.0(0-0.1)	0.856
C22:6 ω 3	0.3(0.2-0.4)	0.2(0.1-0.3)	0.2-(0.1-0.3)	0.068

Data are expressed as the means or median (25th–75th interquartile range) when appropriate[a-c] Values with different superscripts differ significantly.

Table 3. Red blood cell membrane fatty acid concentrations of adolescents

RBC membrane FAs (µg/mL)	Patients with obesity (n=59)		Controls (n=37)	p
	MS (+) (n=26)	MS (-) (n=33)		
SFAs	264.5±31.6	276.1±36.7	262.2±37.6	0.241
C14:0	32.9(24-38)	28.2(23-44)	24.5(21-33)	0.148
C16:0	105.7(97-126)	125.4(102-130)	113.3(98-123)	0.228
C18:0	87±2.1	87±1.9	90±1.97	0.753
C20:0	3.4(3-4)	3.6(2-5)	3.3(3-4)	0.390
C22:0	7.2(6-8)	7.5(6-9)	7(6-8)	0.607
C24:0	13.8(11.6-16.7)	14.9(12.8-19.6)	13(11.4-15.5)	0.142
MUFAs	85.3±17.2	94.6±17.4[a]	84.5±14[b]	0.024
C16:1 ω 7	7.2(4-12)[a]	7.3(4-11[a])	3.7(2-7)[b]	0.001
C18:1 ω 9	60±1.32	67.17±1.47	62.5±1.37	0.135
C22:1 ω 9	2.6(2.3-2.9)	2.6(1.9-5.4)	2.2(1.7-2.8)	0.110
C24:1 ω 9	13.84±3.31	15.88±3.83	14.94±3.84	0.117
ω -6 PUFAs	108.8±17.2	108.8±12.6	110.5±14.9	0.924
C 18:2 ω 6	41.8(39-50)	40.3(3849)	42.7(37-47)	0.934
C20:3 ω 6	6.6(5-8)	6.2(5-8)	6.1(5-8)	0.832
C20:4 ω 6	62.2(44-68)	59.6(51-66)	61.2(52-68)	0.785
ω -3 PUFAs	14.6(6.6-31.5)[a]	15.3(7.3-30.3)[a]	17.1(6.1-29.2)[b]	0.047
C20:5 ω 3	3.1(3-4)[a]	3.6(3-4)[a]	4.7(4-6)[b]	0.00
C22:6 ω 3	11.6(10-13)	11.5(10-14)	12.3(11-14)	0.277

Data are expressed as the means or median (25th–75th interquartile range) when appropriate; [a-c]: Values with different superscripts differ significantly.

p<0.01). Diet ω-6/ ω-3 ratio also adversely associated with TC (r= -0.236, p<0.05) and LDL-C (r= -0.223, p<0.05).

Diet fiber positively associated with RBC membrane C16:1 ω-7 fatty acid (r=0.239, p<0.05). A positive but weak association was determined between diet C20:5 ω-3 and RBC membrane C20:5 ω-3 (r= 0.200, p= 0.051). Red blood cell membrane total ω-3 inversely correlated with BMI (r= -0.244, p<0.05).

Discussion

Childhood obesity is becoming a major public health concern in Turkey (21) as in developing and developed countries (22-24). Childhood obesity is of great importance because of the potential consequences associated with adulthood obesity. Onset of obesity has shifted to younger ages. According to WHO report 2012 (25), 170 million children (aged less than 18 years) have been estimated to be overweight.

Determination of higher body fat percentages in obese with and without metabolic syndrome (p<0.001) (Table 1) has suggested that body fat percentage may be used in routine practice to determine the fat distribution in adolescents because of its superiority over BMI for the evaluation of the quantity and the distribution of the fat in the body.

In the presented study, hepatosteatosis, one of gastrointestinal complications of obesity due to excess

free FA uptake of the liver was evaluated by measuring the serum ALT activity, which is a biochemical marker of hepatosteatosis development (26). Determination of the increased ALT activities in obese revealed that screening obese adolescents for hepatosteatosis may be essential in our country. The presence of visceral obesity and the decrease in insulin sensitivity are the main effective mechanisms for the development of metabolic syndrome (27). In the presented study, although no difference was determined between obese and lean adolescents concerning the fasting glucose, TC and LDL-C, which are the constituents of dyslipidemia, the TG and insulin levels were quite high ($p<0.001$) in obese adolescents (Table 1). The HDL-C level, which is another component of dyslipidemia, was significantly lower in obese than the lean control suggesting the development of dyslipidemia in obese adolescents. Determination of high insulin and HOMA-IR levels ($p<0.001$) in obese particularly in those with metabolic syndrome indicated that insulin resistance should be monitored in adolescents. Elevated blood pressure is one of the main components of the metabolic syndrome in association with obesity. Simons-Morton et al. (28) who investigated the relationship between nutrients and blood pressure in children have suggested a direct association between total fats and both SBP and DBP, but an inverse association between fiber and DBP and claimed the potential role of dietary fats, and possibly fiber, on BP levels in children. In our study, systolic and diastolic blood pressure values were significantly higher in obesity groups compare to control but no association was determined between blood pressure and fiber consumption, which was high in obese with metabolic syndrome. The high blood pressure reveals the need for screening obese adolescents in terms of the risk of hypertension.

Consumption of carbohydrate (50%), protein (13.5%) and cholesterol (289 mg) were within the recommended levels whereas the fat consumption, even in lean controls (39%) (Table 2) was higher than the daily recommended levels, which are 45–65% for carbohydrate, 10–30% for protein, 25–35% for fat and 200–300 mg/day dietary cholesterol for children and adolescents (4–18 years) (29). However, no statistically significant difference was observed between obese and lean adolescents concerning the daily intake of energy, protein, fat, carbohydrate and cholesterol except fiber, which was higher in obese with metabolic syndrome ($p<0.05$) (Table 2). And also diet fiber positively associated with RBC membrane C16:1 ω-7 fatty acid ($r=0.239$, $p<0.05$**).** High-fiber diets have been associated with benefits on glucose and lipid metabolism and may lower risk of cardiovascular events (30). These authors speculate that soluble fibers and MUFA-rich foods in the meals could enhance satiety minimizing energy intake and facilitating body weight control. An association between excessive fat consumption habits of children and adolescents and increased prevalence of obesity has been indicated (31).

In our study, a 3-day food consumption record demonstrated rather high total SFA intakes in obese adolescents with and without metabolic syndrome as well as in lean controls compare to recommended levels for adolescents (29, 32). Although it has been shown that SFAs can cause an increase in adipose tissue (33), in the presented study, the SFA consumptions determined in obese and leans were very close to each other (Table 2). However, determination of a significant increase in TG, slight but not significant increases in TC and LDL-C levels and significantly low HDL-C level in obese groups, particularly in obese with metabolic syndrome, were consistent with the previous literatures (34) indicating higher intake of most of dietary SFAs are associated with higher levels of blood TC and LDL-C, which are risk factors for cardiovascular disease.

It has been reported that oleic acid (18:1 ω-9) has no effects on LDL-C but raises HDL-C modestly whereas linoleic acid moderately reduces serum cholesterol and LDL-C levels (34). In the presented study slightly higher intake of oleic acid and significantly higher intake of erucic acid (22:1 ω-9) and arachidonic acid ($p<0.05$) of the obese adolescents with metabolic syndrome (Table 2) as well as significantly lower serum HDL-C levels and slightly but not significantly higher LDL-C levels may suggests an association between serum lipids and these fatty acids.

Fasting TG is the most responsive to changes in blood and tissue levels of ω-3 PUFAs. Both C18 ω-3 and C20–22 ω-3 PUFAs reduce triglycerides. When tested alone, DHA supplements increased HDL-C and LDL particle size, whereas EPA decreased HDL

(35). Cespedes et al. (36) observed lower metabolic syndrome prevalence and lower fasting glucose levels among individuals with higher adipose tissue ALA and the ALA:LA ratio levels in adipose tissue whereas DPA was associated with higher triglyceride levels. These authors suggested that the positive associations for DPA and MS could reflect higher ω-6 desaturase activity caused by increased adiposity.

In the presented study, intake of total ω-3 PUFAs was lower in obese ($p<0.05$) than lean controls, and the HDL-C significantly lower ($p<0.01$) whereas the TG significantly ($p<0.01$) and LDL-C slightly higher in obese in consistent with the findings of a meta-analysis of randomized placebo-controlled trials that shows EPA and DHA supplementations reduce triglycerides (37). Our finding concerning ω-3 PUFAs was also consistent with the results of Morgado et al. (12) who found a decreased HDL-C level with ω-3 PUFAs in rat. On the other hand, according to the findings of Wei et al. (37), DHA raised LDL-C and HDL-C whereas EPA non-significantly reduced LDL-C. Plasma FAs are good indicators of FAs consumed during a short period (14) as evidenced that plasma SFA and MUFA levels were higher than those in the control group, but the ω-3 FAs were low in obese children (38). However, plasma FAs can be influenced by the condition such as current rapid metabolic changes and physical activity. Determination of RBC membrane FA composition is the most commonly used indicator to assess the fat intake status for the long-term in clinical practice (27). In a rat study, Zhou et al. (39) reported that the fatty acid compositions of RBC, plasma, muscle and visceral adipose tissues reflect the dietary fat sources with no differences in food intake, body weight and growth rate or body fat composition among the groups on a diet causing fatty liver in rodents. A study conducted on human from Italy, Finland and the USA investigating the long-term effects of dietary fats on the lipids of plasma, RBCs and platelets showed that the fatty acid compositions of the glycerolphospholipids of plasma, RBCs and platelets reflect the major dietary fatty acids (40).

In dyslipidemic adults with systemic inflammation, palmitoleic acid (C16:1 ω-7) supplementation caused reductions in serum CRP, TG and LDL-C whereas increases in HDL-C levels, which indicates potent anti-inflammatory and lipid-modulating effects (41). In a recent study of Alonso-Vale et al. (42), the palmitoleic acid stimulated lipolysis, mitochondrial FA oxidation and oxygen consumption. In the presented study, palmitoleic acid was high in both obese groups compare to the lean controls. However, determination of high TG in both obese groups, which were also high palmitoleic acid consumed groups, was an unexpected finding because of the lack of significant difference between obese and control. On the other hand, existence of no relation between diet palmitoleic acid and RBC membrane levels may indicate that diet unsaturated fatty acids had no influence on membrane unsaturated fatty acid content in consistent with the results of Abbott et. al. (43) who suggested that extensive changes in diet SFA, MUFA and PUFA have minimal effect on membranes, but have considerable influence on adipose tissue and plasma triglycerides. In the presented study, diet SFA affected fasting blood glucose ($r= 0.206$, $p<0.05$) but not correlated with blood lipids whereas an inverse association occurred between LDL-C ($r= -0.200$, $p<0.05$) and diet total MUFAs, and also diet total MUFAs adversely correlated with LDL-C ($r= -0.274$, $p<0.01$) and TC ($r=- 0.256$, $p<0.05$). Diet ω-3 FUFAs positively associated with fasting blood glucose ($r=0.228$, $p<0.05$) and an inverse association was observed between diet ω-6 FUFAs and TC ($r= -0.295$, $p<0.01$) and LDL-C ($r= -0.311$, $p<0.01$). These findings confirmed the results of the previous studies suggesting that dietary FAs influence blood chemistry (41, 43).

The membrane composition is responsive to ω-3 and ω-6 PUFA levels in the diet (15, 43), and more sensitive to ω-3 PUFA and ω-6/ ω-3 ratio (15). When ω-3 PUFA is <10% of total PUFA, membrane composition conforms to diet whereas when the diet PUFA balance is >10%, the influence on membrane lipids is little (43). Integration of ω-3 PUFA into plasma membrane phospholipids plays an important role on cell signaling by altering membrane fluidity, lipid structure and substrate availability for the synthesis of bioactive oxidized fatty acids (35). DHA plays a key structural role in membrane. ALA and LA are precursors to ω-3 and ω-6 PUFAs.

An imbalance between dietary n-3 and n-6 PUFA associated with a number of diseases such as

dyslipidemia, hypertension, inflammation, depression, abdominal obesity, type 2 diabetes and cardiovascular diseases, which have become prevalent in today's society (43, 44). In the presented study, the ω-6/ω-3 ratios taken by diet in obese with and without metabolic syndrome and control groups were close to the recommended ratio of 5/1. Determination of similar ω-6/ω-3 ratios of RBC membrane in both obese groups were consistent with the results of Gabriel et al. (8) who also found no difference between adolescents with and without metabolic syndrome.

The influences of dietary FAs on RBC membrane ω3-PUFAs and ω-6 PUFAs have been investigated in animal models (39, 43,45) and adult human (40, 41, 46, 47) but to the authors' knowledge there are limited studies in children (6) and adolescents (8). Total lipid contents in RBC membrane of rabbits fed cholesterol were higher than the control after the feeding trial (44). Remesar et al. (45) have investigated the attachment of the extra lipids originated from lipoproteins to the cell surface and effect of diet on composition of this lipid pool. They detected ω-3 PUFA in plasma but could not detect any ω-3 PUFA in RBC extracts suggesting a marked depletion of PUFA in extracts of cells and, in general, of unsaturation. Takkunen et al. (47) have shown that RBC FA composition reflects particularly well the intakes of ω-3 PUFA in adult Finnish men, Ford et al. (6) reported the highest RBC membrane DHA and AA in 2-6 years old children who intake the lowest ω-3 and ω-6 FAs in different geographical areas in South Africa. In the presented study, the EPA levels taken by diet were lower than the range of recommended level of 0.6–1.2% of total energy (Table 2). Gabriel et al. (8) found no significant difference concerning total SFAs, total MUFAs and total PUFAs in RBC membrane between adolescents with and without metabolic syndrome. The RBC membrane EPA concentration was significantly lower in both obese groups ($p<0.001$) compare to lean controls but there was no significant difference between obese with and without metabolic syndrome in the presented study. No significant difference was determined between obese and control subjects concerning RBC membrane ω-6 PUFAs (Table 3) in contrast to the findings of Kabagamle et al. (46) who determined an inverse association between RBC membrane PUFAs and metabolic syndrome, and no significant associations between total or individual ω-3 fatty acids and the metabolic syndrome whereas ω-6 PUFAs, linoleic acid, inversely correlated with metabolic syndrome in adults. In the presented study, no association was determined between diet and RBC membrane total ω-3 and ω-6 PUFAs or ω-6/ω-3 ratio which may result from the lower proportion of ω-3 PUFAs in the diet as indicated by Abbott et al. (43). However, there was a positive association between RBC membrane and diet EPA (C20:5ω-3).

The results of the presented study have shown that BMI-SDS, serum TG and insulin, HOMA-IR and C16:1 ω-7 FA levels in RBC membrane of obese with metabolic syndrome were high but the serum HDL-C level and red blood cell membrane C 20: 5 ω-3 level were low in obese with metabolic syndrome. Cholesterol and FAs affected some anthropometric measurements and blood chemistry, but there was no correlation between nutritional FA consumption and RBC membrane FA composition except a positive correlation between diet C 20:5 ω-3 and RBC membrane C 20:5ω-3, and diet fiber and RBC membrane C16:1 ω-7.

Limitation of the study

Obese adolescents may avoid to record some of food items they consumed to dissemble their excessive food consumption. The short duration of the registration period of 3 days may not fully reflect overall nutritional status. In addition, lifestyle and physical activities, which were not evaluated in detail, may influence the study results.

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