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Effects of walnut-enriched diet on blood lipids and glucose profiles in hyperlipidemic subjects: a randomized-controlled trial

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Summary. *Background and Objectives:* Walnuts have been shown to reduce serum lipids in hyperlipidemic individuals with a well-controlled feeding trials. Current study have been determined the effects of daily walnut consumption on serum lipids, fasting glucose and insulin levels in hyperlipidemic subjects. *Subjects and Methods:* In this, randomized controlled trial, mild to moderate hyperlipidemic subjects were randomly divided into 2 groups as walnut-enriched (n=20) and control (n=17) groups for 6 weeks. All subjects adhered to a medical nutrition therapy as low-fat and low-cholesterol diet. The walnut-enriched group was supplemented with 40 g/day of walnuts added to their diets. In order to follow nutritional and physical activity status of all subjects, they were visited every 15 days (in total 4 times). Anthropometric measurements of the subjects were taken and were monitored at each visit during the study. Blood samples were measured at the beginning and at the end of the study. *Results:* Our study showed that enriching a well controlled diet with walnuts (40g/day) improves the plasma lipid as well as serum glucose levels after the 6-week. Both groups showed a decrease in serum lipids with adaptation to the AHA (Americen Heart Association) diet, but statistically significant reductions ($p<0.05$) in serum glucose, insulin and HOMA-IR levels were found especially walnut-enriched group showed significant decrease in their total cholesterol and low-density lipoprotein cholesterol (LDL-C) by %5.3 ($p= 0.02$) and %8.8 ($p= 0.0$)respectively. Also LDL:HDL ratio and total cholesterol:HDL cholesterol ratio was significantly decreased in walnut-enriched group ($p<0.05$). Fasting glucose and fasting insulin levels decreased by %15.7 and %15.4 in walnut-enriched group, respectively. Walnut consumption did not show any significant changes in either high-density lipoprotein (HDL) cholesterol and triglyceride (TG) levels. *Conclusions:* This study indicated that, walnut-enriched diet improves serum glucose and serum lipids in hyperlipidemic subjetcs, suggesting a potential reduction in overall cardiac risk.

Key words: Walnut, Hyperlipidemia, Serum Cholesterol, Serum Glucose, Serum Insulin, HOMA-IR

Introduction

There has been an increase in the incidence of cardiovascular disease worldwide since 1900, including in developed and developing countries (1). In both developed and developing countries, the prevalence of cardiovascular disease has emerged with changes in dietary habits, lifestyle and environmental factors. Especially in many developed countries, consumption of energy-dense foods with a physical inactivity are the primary factors which cause an increase in obesity prevelance. The epidemic proportions of obesity leads to a significant increase in the number of cardiovascular diseases (1,2).

Hyperlipidemia is a medical condition characterized by the elevation of any or all of the lipid profile and/or lipoproteins. American Heart Association (AHA) defines hyperlipidaemia as the presence of a high lipid ratio in serum (3). Reducing intake of saturated fats (SFA) and refined carbohydrates, increasing consumption of monounsaturated fats (MUFA) and high fiber foods are among the basic targets in order to improve hyperlipidemia risk factors (4). Consumption of saturated fats cause an increase in LDL-C and tryglycerides, while monounsaturated and polyunsaturated fat (PUFA) consumption decrease serum LDL-C (5). At the same time, it appears that mortality due to coronary diseases has decreased as a result of regular consumption of omega 3 polyunsaturated fatty acids (6). It was observed that not only LDL cholesterol but also other plasma lipoprotein levels were positively affected as a result of balanced diet and consequently decreased CVD risk (7).

Large prospective studies have consistently indicated that, increased nut consumption cause a reduction in CVD risk and mortality risk associated with CVD (8,9). Clinical trials also have shown effects on CVD risk factors such as lipid profiles, vascular inflammation and blood pressure after various interventions that have included nuts, such as a Mediterranean diet (10-12). Nuts are a complex food composed of a number of nutrients and phytochemicals that may lower CVD risk. Many nuts are rich in monounstaurated fatty acids, while walnuts are composed mainly of polyunsaturated fatty acids (47.2g/100g) (13). Not many foods are rich in alpha linolenic acid (ALA), which is a type of omega-3 fatty acid found in plant foods. Walnut in particular have a unique profile; which has a quite high amount of both αlinolenic and linoleic acid, studies indicated that while walnut improves serum lipid levels positively and it also cause a reduction in plasma cholesterol levels and in particular improve CVD risk (14, 15, 24).

Although consumption of MUFA and PUFA appear to have similar lowering effect on total cholesterol and LDL cholesterol, it have meaningless to minimal effect on HDL cholesterol (16). Studies indicated that increased consumption of nuts with a controlled diet, especially in CHD patients tends to favorably decrease LDL cholesterol by %9 - %16 (16-18). Previous studies determined that, diets which are low in saturated fats appear to cause a reduction in LDL-cholesterol levels and overall cardiovascular risk (14,17). Furthermore, some studies supporting that, replacing saturated and trans fatty acids with unsaturated fats, including nuts in the diet, may help to prevent from diabetes and other CVD (18).

Walnuts containing components like fiber, potassium, magnesium, vitamin E and magnesium; all those components synergistically have a potential to decrease blood pressure, serum gluocse and serum lipid levels (20-22). Walnut also contain substantial amounts of L-arginine. L-arginine is the precursor amino acid of the endogenous vasodilator nitric oxide (NO) (23). Walnut consumption increases the levels of L-arginine in the body by 0.9 to 1.4 g/d, which that factor appears to reduce the blood pressure of individuals (23). EFSA-2011 indicated a relationship between the consumption of walnuts and improvement of endothelium-dependent vasodilation. The Panel considers that in order to obtain the claimed effect, at least 30 g of walnuts should be consumed daily; these amounts can be consumed in the context of a balanced diet (25). Aim of the current study was to investigate the effects of daily walnut consumption on serum lipids, fasting glucose and insulin levels in hyperlipidemic subjects.

Subjects and Methods

Subjects

In the current study**,** 37 moderate hyperlipidemic subjects (43,3±6.2 years for control group, 47.1±5.44 years for walnut-enriched group) were randomly divided into 2 groups as walnut-enriched (n=20 [10 women and 10 men]) and control group (n=17 [6 women and 11 men]). They were asked to participate from the local government hospital in Turkish Republic of Northern Cyprus. Patients with triglycerides above 300mg/dl and patients with total cholesterol above 500 mg/dl were excluded from the study. All subjects were required not to be obese, be non-smokers, and non frequent alcohol users, free of dietary restriction/food allergies and not taking medications known to alter plasma lipids. They were also screened for diabetes, renal disorders, thyroid diseases, hepatic diseases, cancer and other major diseases. Patients who have other health problem rather than CHD did not include in the study (Figure 1).

Study Design

During the baseline period, all accepted subjects (control and walnut-enriched group), were first admitted to Famagusta Hospital, Northern Cyprus to have a detailed physical examination. Then, a well controlled, 2 armed randomized controlled study was designed. In total, 6 weeks was used to examine the effects of a walnut-enriched diet compared with the baseline and control diet. All subjects in both groups were informed about American Heart Association (AHA) low-fat and low-cholesterol diet (26) advises by an experienced dietitian. Only difference between the groups were; walnut-enriched group have incorporated 40g of walnut to their diet for 6 weeks while control group only adopt to AHA diet and asked not to consume any nut during study period. During the 6-week diet period, participants were visited once in every 15 days and in order to follow nutritional status of participants during each visit '3-Day consecutive Food Records' were taken (including one day weekend each week). Beside, in every visit, body composition analysis and physical activities of participants were recorded. Subjects were instructed individually on how to complete the food records and how to estimate or measure the food portions at home by an experienced dietitian. At the same day of each visit the packed and pre-weighed packages of walnut (40g) were delivered to participants. Walnut was consumed as snacks (40g) once a day. Nutrient intake of participants was estimated by using the BEBİS-Beslenme Bilgi Sistemi (nutrition information system) computer program. Plasma measurements were obtained at baseline and at the end of the study. The study protocol was clearly explained to each subject, who signed an informed consent. The study protocol was approved by the Ethical Committee of the Eastern Mediterranean University and also approved by the Protocol Registration and Results System. Clinical tiral ID number for the current study is; NCT03680027.

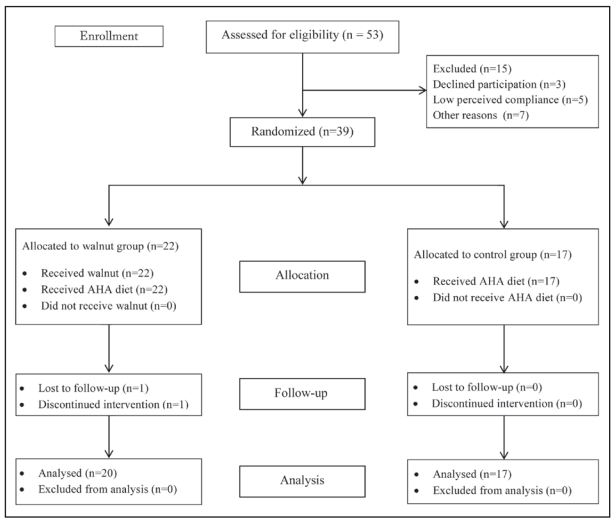

Figure 1. Flowchart of study subjects. In total, 37 subjects were randomized. Three participants droped out since they declined participation (n=3). Five of the participants dropped with a reason of low perceived compliance (n=5) and seven of them were droped out because of other reasons (n=7). A total of 37 subjects were included in statistical evaluation.

Anthropometric parameteres and habitual physical activities

All anthropometric measurements were carried out by dietitian according to the method described by Lohman et al. (27). Body weight and percentage of body fat were measured using a body composition analyser (tanita-BC 420s). Body height, waist and hip circumferences were measured using a standard tape measure. Body mass index (BMI) and waist/hip ratio were calculated (27).

Plasma Measurements

At the beginning and end of the study, blood samples were drawn into vacutainer tubes containing Na2EDTA (1 g/l final concentration) from the antecubital vein after an overnight fast. The tubes were then immediately stored into ice water. Within 2h, plasma was seperated by centrifugation at 2500g for 20 min at 4°C**.** All the measurments were made immediately after the plasma collection. Glucose concentrations were measured by glucose oxidase and peroxidase reactions. Total cholesterol was measured by cholesterol esterase, cholesterol oxidase and peroxidase reactions. Total TAG was measured by glycerol-phosphate-oxidase and peroxidase reactions. Method for direct determination of HDL-cholesterol uses polyethylene glycol ('PEG') based system in which sulfated a-cyclodextrin, dextran sulfate and MgCl2 form water soluble complexes with the non-HDL lipoproteins present in a sample, after which pegylated cholesterol esterase and cholesterol oxidase are introduced. LDL cholesterol concentration were calculated using the Friedewald formula: (total cholesterol)-(HDL cholesterol)-(VLDL cholesterol)=LDL cholesterol. VLDL cholesterol concentrations were estimated as TAG divided by 5, when concentrations are expressed in mg/dl (28).

Statistical analysis

Data were analyzed by statistical analytical systems software (package 20.0). The normality assumption was tested by using One-Sample Kolmogrow–Smirnov test. The mean ± SD were determined, and the differences among baseline, control diet, and walnut-enriched diet were compared by analysis of paired sample t-test. Pearson correlation test was used because of the normal distribution of the data set from the relationships between the parameters.

Nutrient intake (total fat, SFA, MUFA, and PUFA) were also compared with the changes of blood lipid concentrations. Also, Chi Square was used in order to make an assumption and compare the two qualitative (categorical) variables. The level of significance was P<0.05.

Results

In total, 37 hypercholesterolemic subjects completed the trial as detailed in the study protocol. Table 1 shows the changes of some variables of the subject characteristics over the 6-week study period. The mean age for the control group was 43,3±6.2 years, while walnut-enriched diet group mean age was 47.1±5.4 years. In terms of body composition measurment results, there were no significant difference were

Table 1. Initial and final body composition measurements of all subjects.

Anthropometric Measurements	Grup	n	Before			After			B-A
			mean	Sd	p1	mean	s	p1	p2
Body weight (kg)	Control	17	73,59	14,45	0,95	73,70	14,23	0,99	0,69
	WE-D	20	73,32	11,07		73,66	11,20		0,15
Body fat (%)	Control	17	25,89	6,76	0,33	26,56	6,44	0,38	0,14
	WE-D	20	28,20	7,42		28,59	7,23		0,07
Height (cm)	Control	17	169,29	10,17	0,86	169,29	10,17	0,86	-
	WE-D	20	168,70	9,93		168,70	9,93		-
BMI (kg/m2)	Control	17	25,47	3,16	0,84	25,55	3,10	0,75	0,39
	WE-D	20	25,64	2,04		25,84	2,22		0,09
Waist circumference (cm)	Control	17	84,06	14,64	0,89	84,18	14,60	0,86	0,16
	WE-D	20	83,50	8,70		83,50	8,82		
Hip cirfumference (cm)	Control	17	99,35	9,69	0,34	99,35	9,69	0,34	-
	WE-D	20	102,05	7,21		102,05	7,21		-
Waist/hip ratio	Control	17	0,84	0,08	0,32	0,84	0,08	0,32	-
	WE-D	20	0,82	0,06		0,82	0,06		-
Body fat (kg)	Control	17	19,14	6,17	0,51	19,69	5,92	0,53	0,15
	WE-D	20	20,42	5,53		20,91	5,80		0,06
FFM (kg) (fat free mass)	Control	17	54,55	11,57	0,65	54,09	11,14	0,70	0,12
	WE-D	20	52,91	10,58		52,67	10,83		0,13
TBW (kg)	Control	17	38,04	8,01	0,57	37,74	7,79	0,64	0,05
	WE-D	20	36,62	7,14		36,58	7,24		0,66
BMH (kcal)	Control	17	1709,76	340,36	**0,02***	1698,59	343,20	**0,03***	0,14
	WE-D	20	1478,45	249,60		1482,75	244,53		0,46

Abbreviation: BMI, body mass index, WE-D, walnut enriched diet. B-A stands for Before-After. Values are means ± s.d., n = 37. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1; independent t-test and for p2; paired sample t-test was used. The level of significance was P<0.05.

observed in both group throughout the study period (p<0.05). Daily habitual physical activities, energy intake and expenditure of subjects are given in Table 2. The physical activity level (PAL) of both control and walnut-enriched diet subjects was considered equivalent to mild activity (around 1.6).

The nutrient intake of subjects at baseline and the end of diet period are shown in Table 3. As it was expected inclusion of walnut (40 g/day) into the diet resulted in a significant (p<0.05) increase in MUFA. Whereas, unexpectedly the percentage of energy which comes from SFA was significantly (p<0.05) increased in walnut-enriched diet (13.6g) compared to that of the baseline (15.6g). Although AHA diet advises were given to participants walnut-enriched group also increased their dietary cholesterol intake significantly (p<0,05) while control group have decreased their dietary cholesterol intake. In fact, despite an AHA diet was advised to all participants, an increase in saturated fat intake was observed in both groups. As seen in Table 3, saturated fat intake have been increased in control group almost as much as walnut group but there was no statistically significant difference was found in control group. Dietary fiber consumption was increased in walnut-enriched diets compared with baseline however the difference was not determined as significantly different. On the contrary, control group have decreased their fiber consumption throughout the study period.

Compared with baseline, the walnut-enriched diet favorably decreased the concentrations of total serum cholesterol (p<0.05), LDL cholesterol, blood glucose and fasting insulin by 5.3%, 8.8%, 15.7% and 15.4%, respectively, while there were no significant differences found on HDL cholesterol concentrations and triglyceride levels in both groups (Table 4).

Compared to that of the baseline, although there was no statistical significant difference found in Total/HDLcholesterol in walnut-enriched group, it was appeared a decreasing trend for the parameter (%2). In addition, the walnut-enriched diet favorably altered the ratio of LDL/HDL cholesterol (6.2%) compared with the control diet (p<0.05) (Table 4). Therefore, walnut-enriched diet, despite its high fat content, in terms of serum lipid concentrations as well as blood glucose and insulin concentrations was superior to that of the control diet (Table 4).

Table 2: Measurments of BMH, PAL, energy intake and expenditure of subjects

	MEN				WOMEN				TOTAL			
	Control x ± s		**Walnut enriched** x ± s		**Control** x ± s		**Walnut enriched** x ± s		**Control** x ± s		**Walnut enriched** x ± s	
	Before- After		**Before-After**		**Before-After**		**Before-After**		**Before-After**		**Before- After**	
BMR (kcal)	1797, 6± 152	1819,6±146	1761, 2±175	1792,4±171	1388,2±117	1397,3±115	1371,7±121	1385,1±128	1495,8±168	1505,1±141	1489,4±141	1508,7±146
PAL	1,64± 0,2	1,62±0,1	1,51±0,1	1,53±0,1	1,57±0,2	1,57±0,1	1,67± 0,1	1,68±0,2	1,62±0,2	1,60± 0,1	1,59±0,1	1,61± 0,2
Energy expenditure (kcal)	2947,1± 560	2946,8±585	2659,1±530	2741,8±535	2179,2±335	2193,3±345	2289,4±375	2326,1±389	2421,9±447	2408,0± 465	2367,5±452	2427,9±462
Energy intake (kcal)	2166,9±1193	2167,0±121	2085,4±952	2076,7±916	1308,3±116	1341,8±145	1333,5±143	1348,5±128	1898,6±1059	1911,2±1070	1691,6±752	1695,2±724

Abbreviation: PAL, physical activity level. Values are means ± s.d., n = 37. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Table 3. Nutrient composition of the two group (control vs walnut-enriched) during the study period.

Nutrients	Group	n	Before			After			B-A
				s	p1		s	p1	p2
Energy (kcal)	Control	17	1897,79	1059,43	0,49	1910,135	1070,20	0,47	0,32
	WE-D	20	1697,59	752,90		1692,98	724,88		0,74
Prot. (g)	Control	17	93,47	47,66	0,25	96,88	47,94	0,34	0,30
	WE-D	20	78,09	32,01		83,24	38,54		0,13
Prot. (%)	Control	17	19,69	3,48	0,20	20,27	2,42	0,36	0,60
	WE-D	20	18,45	2,78		19,68	3,21		0,21
Fat (g)	Control	17	62,35	30,62	0,74	61,43	33,71	0,82	0,93
	WE-D	20	64,68	30,64		69,78	26,60		0,54
Fat (%)	Control	17	29,57	2,94	0,00*	28,93	2,38	0,00*	0,57
	WE-D	20	34,42	3,51		37,01	31,21		0,46
CHO (g)	Control	17	240,69	132,90	0,33	242,49	134,31	0,27	0,49
	WE-D	20	200,43	88,60		183,55	82,93		0,61
CHO (%)	Control	17	50,74	3,68	0,07	50,80	4,25	0,04	0,89
	WE-D	20	47,13	5,05		43,31	4,54		0,69
Fiber (g)	Control	17	31,41	19,89	0,68	29,27	11,10	0,44	0,19
	WE-D	20	33,15	16,02		34,16	17,21		0,71
Vit.E (mg)	Control	17	14,20	11,66	0,68	10,68	4,41	0,51	0,15
	WE-D	20	13,04	4,96		12,00	6,83		0,37
Saturated fat (g)	Control	17	17,29	7,15	0,11	19,76	13,56	0,23	0,25
	WE-D	20	15,66	6,25		17,61	6,64		0,01*
Monounsaturated fat (g)	Control	17	31,62	9,27	0,19	30,63	10,17	0,52	0,11
	WE-D	20	34,93	5,79		36,95	6,69		0,04*
Polyunsaturated fat (g)	Control	17	12,14	5,61	0,00*	11,04	3,64	0,00*	0,04*
	WE-D	20	14,09	10,65		15,22	12,25		0,10
EPA (g)	Control	17	0,34	0,22	0,39	0,37	0,31	0,74	0,37
	Walnut-	20	0,39	0,27		0,41	0,15		0,70
DHA (g)	Control	17	0,13	0,17	0,20	0,11	0,11	0,07	0,00*
	WE-D	20	0,26	0,37		0,30	0,38		0,00*
Cholesterol (mg)	Control	17	279,86	82,64	<0,01*	271,42	188,83	0,29	0,84
	WE-D	20	166,44	91,66		212,68	142,39		0,01*
Omega 3 (g)	Control	17	1,22	0,44	0,00*	1,24	0,71	0,00*	0,85
	WE-D	20	2,53	2,05		4,12	1,84		0,03*
Omega 6 (g)	Control	17	10,65	5,45	0,00*	8,38	3,12	0,00*	0,03*
	WE-D	20	10,21	8,72		13,08	10,56		0,10

Abbreviation: WE-D, walnut enriched diet. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Table 4. Serum lipid, glucose and insulin concentrations of all subjects.

Serum biochemical parameters	Grup	n	Before			After			B-A	Differences among pre-post study (%)
				S	p1		S	p1	p2	
Total cholesterol	Control	17	237,00	37,93	0,47	231,76	36,54	0,83	0,00*	% - 3.5
	WE-D	20	247,45	41,12		234,35	35,54		0,02*	% - 5.3
LDL-C	Control	17	168,59	29,18	0,52	156,76	27,59	0,98	0,00*	% - 7.1
	WE-D	20	171,45	36,01		156,50	33,02		0,00*	% - 8.7
HDL-C	Control	17	48,47	7,95	0,12	45,18	7,24	0,03*	0,05	% - 6.2
	WE-D	20	53,80	11,73		52,10	10,34		0,31	% - 3.3
Triglyceride	Control	17	143,82	83,59	0,17	151,59	105,26	0,15	0,51	-
	WE-D	20	110,25	61,38		112,10	53,42		0,73	-
LDL-C: HDL-C.	Control	17	3,48	1,01	0,18	3,47	0,99	0,23	0,05	% - 0.3
	WE-D	20	3,22	1,19		3,00	1,12		0,01*	% -6.8
Total Cholesterol: HDL-C	Control	17	4,89	1,37	0,18	5,13	1,33	0,31	0,06	-
	WE-D	20	4,60	1,46		4,50	1,43		0,39	-
Fasting glucose	Control	17	88,41	11,15	0,69	92,24	7,30	0,43	0,04*	% + 4.5
	WE-D	20	98,65	7,31		83,20	159,51		0,03*	% -15.7
Fasting insulin	Control	17	9,61	3,22	0,89	10,49	3,94	0,22	0,22	% +10
	WE-D	20	9,43	4,54		7,98	3,39		0,04*	% -15.4
HOMA-IR	Control	17	2,09	0,6	0,45	2,4	0,9	0,15	0,22	% + 14.8
	WE-D	20	2,29	0,8		1,63	0,7		0,03*	% - 28
VLDL-C	Control	17	28,76	16,72	0,98	30,32	21,05	0,31	0,50	-
	WE-D	20	22,05	12,28		22,42	10,68		0,73	-

Abbreviation: WE-D, walnut enriched diet. p1=Differences among control and walnut enrcihed-diet group, p2=Differences among pre and post study . For p1-independent t-test and for p2-paired sample t-test was used. The level of significance was P<0.05.

Discussion

In terms of nut family, walnuts have been found to be one of the most vulnerable source of plant based, omega 3 fatty acid by which having a cardioprotective benefit. Current well controlled, randomized study results have shown similar benefit as Sabaté *et al* study (17,50) and that daily consumpiton of 40g of walnut for 6 weeks significantly lowered the serum total cholesterol and LDL concentrations respectively by %5.2 and 8.7%. There is strong epidemiologic and clinical evidence that diets rich in omega-3 (n-3) fatty acids are protective and may reduce cardiovascular and overall mortality (29,30). As firstly shown by Sabaté et al.(17) there is an inverse relation between the daily consumption of walnut and serum cholesterol levels.

Nuts, especially walnuts play a key role due to its unique fatty acid composition with high content of unsaturated fatty acids, specifically polyunsaturated fatty acids (PUFA). Furthermore, the high levels of antioxidants found in walnuts conferred an improvement in antioxidant status as noted by increased enzyme activity and stable oxidation of LDL cholesterol. Some inflammatory markers tends to be improved with walnut consumption compared to other diets (31). It has been shown that walnut consumption can affect clinically relevant endpoints (such as cardiac death or endothelial dysfunction), and that this may be mediated through effects on oxidative stress, inflammation, and altered lipid profiles (31,32).

Most of the studies investigated the effect of nuts as part of a diet compared with nut-free control diets, which were either low in total fat (33,34), high in fat

(35), as part of a Mediterranean diet (36,37), or on a habitual diet (38). Although dietary controls have been variable, the overall results of these clinical trials have consistently shown a cholesterol-lowering effect of regular nut consumption.

Our results showed a significant reduction in LDL-C (–14.95 mg/dL), which represents a reduction of 8.8%. These findings resonate with the results of Wu et al. study (2014), in which a similar pattern was observed (non-HDL-C: –5.8%, TC: –3.9%, apoB: –6.2%, VLDL-C: –13.2%, TG: –5.4%, VLDL-TG: –4.0%) (38). Our results suggest that the increased n-3-PUFA consumption was principally responsible for the cholesterol-lowering effect of walnuts. There is evidence that a high n-3-PUFA intake provides cholesterol-lowering effects through several potential mechanisms (30); however, the exact underlying mechanisms are still not fully understood (40).

Although it was invested considerable time and effort (frequent visits with dietetian; detailed analysis of food records), especially subjects in the walnut-enriched group did not fully comply with the recommended diet. During the study period, it was found that compared to the baseline, walnut-enriched subjects, have been increased saturated fat intake in a statistically significant amount. It is estimated that, an increase in saturated fat intake in the walnut group decreased the power of the hypolipidemic effect that will be obtained from the walnut. In fact, despite an AHA diet was advised to all participants, an increase in saturated fat intake was observed in both groups. As seen in Table 3, saturated fat intake have been increased in control group almost as much as walnut group but there was no statistically significant difference was found in control group. A similar increase in both groups suggests that it did not affect the overall outcome of the study. Many studies investigated that, dietary saturated fat intake has been shown to increase LDL cholesterol, and the replacement of saturated fat with monounsaturated fat has been associated with decreased TC, LDL-C, and HDL-C (41). According to those data, increasing consumption of saturated fat in walnut-enriched group have been one of the most key point which was predicted to affect the result of the current study. Despite a significant decrease of LDL-cholesterol and total cholesterol of walnut-enriched subjects, hypolipidemic effect of walnut expected to be more powerful compared to the control group.

Studies give suggestive evidence that both the dietary fat and the dietary fatty acid composition affects glucose metabolism (42,43). Imamura et al., 2016 (43) indicated that, most consistent favorable effects were seen with PUFA, which was linked to improved glycaemia, insulin resistance, and insulin secretion capacity. In our study, both fasting serum glucose concentrations and fasting serum insulin concentrations of walnut-enriched diet have favourably reduced. Results of the current study concluded that, compared to control group, incorporation of walnut to a controlled diet have reduced fasting serum glucose and fasting serum insulin level respectively by 15.7% and 15.4% ($p<0,05$). In addition to those results, HOMA-IR which is a method used to quantify insulin resistance and beta-cell function (44), HOMA-IR concentrations were significantly decreased in walnut-enriched group compared to the control group. Similar and consistent results were determined in The Nurses Health Study which they have found an inverse association between the consumption of nuts and the risk of type 2 diabetes (45).

The intake of SFA generally increases the risk of CHD by increasing the concentrations of total and LDL cholesterol and by increasing the ratios of total/HDL cholesterol and LDL/HDL cholesterol ratio (46). The results presented in table 3 suggests that, in the current study, in spite of walnuts' high fat content and despite to an increase in saturated fat consumpiton of the walnut-enriched group, incorporation of walnut to a controlled diet seems to have a beneficial effect on total cholesterol and LDL cholesterol which is supported by several studies (12,17,31,32). As it demonstrated in table 3, inclusion of walnut into a control diet caused a reduction in total cholesterol by 5.3% and %8.8 in LDL-cholesterol compared with baseline. Ros et al. (2004), in order to determine the effect of walnut consumption carried out a study with 21 hypercholesterolemic men and women. During study period, subjects were asked to have a controlled Mediterranean diet, and they have arranged subjects diet by providing them walnuts which have accounted 18% (40–65 g/d) of their daily energy needs. Similarly to current study results, Ros et al. (37) found a significant reduction ($P<0.05$) in subjects' total plasma cholesterol as

well as LDL-cholesterol concentrations. However, the amount of walnut consumed was more than that used in the present study.

Some studies observed that changes in the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations were better predictors of CHD than the changes in LDL cholesterol alone (48,49). In women, the risk of CHD is increased when the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations exceed > 4.5 and >3.0 respectively. For men, the risk of CHD is increased when the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations exceed >5 and >3.5 respectively (48). In the current study, compared with the baseline, those subjects who were recruited in walnut-enriched diet favorably altered LDL-C/HDL-C ratio (p=0.01) in cardioprotective direction.

Theoretically, walnut is a fatty food and its regular consumption may be expected to lead to body weight gain. However, as seen in table 1, in both group there were no significant changes in the body weight throughout the study period (p> 0.05). None of the well-controlled metabolic-type feeding studies show signficant changes in body weight compared to the nut and the nut-free control diet (50,51). Our study showed a consistent result and indicated that a walnut-enriched diet did not cause any change in body weight or BMI. This finding is consistent with previous clinical studies (50,51).

Interpretation is limited by the fact that the study relied on self-reported food records completed by the participants. These data may be highly susceptible to recall bias. Furthermore, dietary intake was not monitored daily, but rather recorded for three consecutive days in each 15 days. In addition to that, current study may have another limitation about the amount of walnut that the subjects have consumed. Since some of the studies are focusing on dose-response cholesterol lowering effect and indicating that, an average daily intake of 67 g of nuts (roughly equivalent to 20% of energy) is better as a therapeutic effect for CVD (13, 52).

In conclusion, according to epidemiological data, a 10 percent reduction of LDL cholesterol leads to a 20 percent decrease in the coronary heart disease risk throughout life (52). On the basis of the results of the present study, although the limited number of subjects were included, high-PUFA-rich walnut diet is preferred with a low-fat control diet since it showed a favorable effect on the CHD risk profile. Current results indicated that, walnut enriched diet decreased total and LDL concentrations by %5.2, %8.8 respectively. This means adding of walnut to a controlled diet may have a potential to decrease the risk of CHD by 10-15%.

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