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The Effect of Walnut Consumption on Small Dense Low-Density Lipoprotein and Other Serum Lipid Parameters in Healthy Volunteers

Sağlıklı Gönüllülerde Ceviz Tüketiminin Küçük Yoğun Düşük Yoğunluklu Lipoprotein ve Diğer Serum Lipid Faktörleri Üzerine Etkisi

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ABSTRACT Objective: This study has investigated the effects of walnut consumption on the serum lipids and especially small dense low-density lipoprotein (sdLDL) values of healthy volunteers. **Material and Methods:** Volunteers participating in this study were asked to consume 30 grams of walnuts per day. Walnuts were presented to individuals in packages and were asked to consume at breakfast every morning for 21 days. An short message service (SMS) reminder was made every day so that individuals would not forget to intake walnuts. The blood values of subjects were measured at the beginning and the end of the study. Serums were obtained from the collected blood, and blood lipid parameters were analyzed routinely in the biochemistry laboratory. The sdLDL parameter was analyzed by the ELISA method. **Results:** The effect of walnut consumption on the lipid parameters was investigated. It was found that regular walnut consumption has significantly decreased the LDL ($p<0.001$), total cholesterol ($p<0.001$), sdLDL ($p<0.001$), and HDL ($p=0.008$) parameters. A significant change was not observed for the triglyceride levels. Besides, there is a statistically significant difference in carbohydrate, linoleic acid, and a-linolenic acid intake compared to Turkey Nutrition and Health Research. There was no significant difference in the individuals' energy, cholesterol, monounsaturated fatty acid, and saturated fatty acid intakes Also, food consumption record form was obtained from individuals. **Conclusion:** In this study, supplementation walnut to the healthy volunteers' diet has reduced LDL, sdLDL, and total cholesterol levels. We think that walnut consumption has a protective effect especially on cardiovascular risk factors.

Keywords: Serum lipids; nutritional habits; small dense low-density lipoprotein; walnut consumption

ÖZET Amaç: Bu çalışmada, sağlıklı gönüllülerin ceviz tüketiminin serum lipid ve özellikle küçük yoğun düşük yoğunluklu lipoprotein [small dense low-density lipoprotein (sdLDL)] değerleri üzerine etkileri araştırılmıştır. **Gereç ve Yöntemler:** Bu çalışmaya katılan gönüllülere günde 30 gram ceviz ikram edilmiştir. Cevizler paketler hâlinde kişilere takdim edilmiş ve 21 gün boyunca her sabah kahvaltıda tüketilmesi istenmiştir. Bireylerin ceviz alımını unutmamaları için her gün kısa mesaj hizmeti (SMS) ile hatırlatma yapılmıştır. Bireylerin kan değerleri çalışmanın başında ve sonunda ölçülmüştür. Toplanan kandan serumlar elde edilmiştir ve kan lipid parametreleri rutin olarak biyokimya laboratuvarında analiz edilmiştir. Küçük yoğun LDL parametresi ise ELISA yöntemi ile analiz edilmiştir. Ayrıca kişilerden besin tüketim kaydı formu alınmıştır. **Bulgular:** Ceviz tüketiminin lipid parametreleri üzerindeki etkisi incelenmiştir. Düzenli ceviz tüketiminin LDL ($p<0.001$), toplam kolesterol ($p<0.001$), sdLDL ($p<0.001$) ve HDL ($p=0.008$) parametrelerinde anlamlı bir düşüş yarattığı bulunmuştur. Trigliserid seviyelerinde önemli bir değişiklik gözlenmemiştir. Ayrıca karbonhidrat, linoleik asit ve a-linolenik asit alımında Türkiye Beslenme ve Sağlık Araştırmasına göre istatistiksel olarak anlamlı farklılık vardır. Bireylerin enerji, kolesterol, tekli doymamış yağ asidi ve doymuş yağ asidi alımlarında önemli bir fark bulunmamıştır. **Sonuç:** Bu çalışmada, sağlıklı gönüllülerin diyetine ceviz eklenmesi LDL, sdLDL ve toplam kolesterol seviyelerini düşürmüştür. Ceviz tüketiminin özellikle kardiyovasküler risk faktörleri üzerinde koruyucu bir etkisinin olduğunu düşünüyoruz.

Anahtar Kelimeler: Serum lipidleri; beslenme alışkanlıkları; küçük yoğun düşük yoğunluklu lipoprotein; ceviz tüketimi

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There is a strong relationship between eating habits and diseases of the cardiovascular system. There is a relationship between coronary artery disease (CAD) and elevated serum cholesterol levels. Efforts were focused on lowering cholesterol levels. CAD is the primary cause of death worldwide. Studies conducted in recent years have proven the positive effect of nut consumption on blood lipids; various nuts lower cholesterol levels and prevent cardiovascular diseases.[1,2]

Many factors affect total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol levels in serum. These factors, like heredity, nutrition, obesity, and stress, affect TC, and LDL cholesterol levels. Respectively, the optimal TC and LDL cholesterol levels for healthy individuals are ≤200 mg/dL and ≤100 mg/dL. Elevated TC and LDL cholesterol levels increase the risk of CAD. Many studies have emphasized that a decrease in serum TC and LDL cholesterol levels may decrease CAD risk.[3]

However, in 30% of patients taking cholesterol-lowering drugs, cholesterol parameters may drop to the desired levels, and severe vascular lesions still may occur. Also, individuals with normal LDL values may have cardiovascular diseases. Findings are suggesting that in these individuals, the prevalence of CAD is higher due to sdLDL positivity. sdLDL passes through the endothelial barrier more easily and exposed to oxidation more than LDL, more massive and lower in density. Therefore, sdLDL is atherogenic.[4-6]

According to recent research findings, it is indisputable that diet and dietary habits applied to cardiovascular diseases affect coronary risk factors. While investigating bioactive substances in foods that reduce the risk of cardiovascular disease, walnut started to attract attention.[7,8]

In epidemiological studies, it has been shown that people who eat walnuts have a 25% reduction in death risk from CAD. The American Food and Drug Institute advises 42.8 g of daily walnut intake and has added it to healthy eating recommendations. It reduces the risk of CAD.[9-11]

MATERIAL AND METHODS

PARTICIPANTS

The study was conducted with 30 volunteers [18 females and 12 males with mean age; 23.6±3.6 years, mean body mass index (BMI); 22.2±3.1 kg/m^2], who were under the research criteria.

This study was conducted on healthy individuals between December 2014 and April 2015 after obtaining the local ethics committee permissions. Subjects who do not smoke, do not consume any products such as nuts and walnuts (the frequency of consumption should not be more than once a week), have no allergies to foods such as nuts and walnuts, do not use any medication regularly (including vitamins), have no cardiovascular problems, do not take drugs on a daily and continuous basis were included in the study. Besides, individuals with average body values without obesity, diabetes, alcohol, and chronic diseases such as kidney, thyroid, hepatitis, cancer were also included in the study. Patients with inflammatory diseases, those who have had infectious diseases, and recent surgical interventions have been excluded.

INTERVENTION

Participants were asked to consume 30 g of walnuts daily for 21 days. Additionally, walnut belongs to the origin of the Aegean Region in Turkey and have been purchased from "Can Kardeşler" dried nuts. Individuals have consumed walnuts on an empty stomach in the morning or with breakfast. The daily walnut consumption was recorded, and daily reminders were sent. The study started with 41 subjects and completed with 30 people due to several issues (forgetfulness, nausea, illness, and quitting).

During the study, 30 g of walnuts (197.9 kcal) were added to participants' usual dietary habits without interfering with regular nutritional habits. Participants who agreed to the study were provided with 21 packages with 30 grams of walnuts, each weighed and prepared by the researcher during the research period (3 weeks).

CALCULATION OF THE NUTRITIONAL HABITS

Food consumption record forms were requested from the participants for two days (one day on weekdays

and one day on the weekend), and then their mean was taken. These food consumption records taken from individuals were calculated by BEBİS (Nutrition Information System) program 8.1 version.[12]

MEASURING OF THE CHEMICAL CHARACTERIZATION OF WALNUTS

The fatty acids content of walnuts was analyzed with the gas chromatography (GC) method. Firstly, the walnut extract (Soxhlet Extraction method) was obtained, and then fatty acids were analyzed by GC. Therefore, the fatty acid ratios of walnuts consumed at breakfast were determined.

BIOCHEMICAL MEASUREMENTS

Blood samples were taken from fasting participants on the morning of the 1st day of the study. The serum content of taken blood was separated utilizing centrifugation (4000 rpm for 10 min). TC, HDL, TG, and LDL values have been measured immediately. The remaining serum samples were preserved at -80 °C. After 21 days, blood was taken, parameters were calculated, and samples were stored again at -80 °C. The stored serum samples were analyzed with Randox branded sdLDL kit at the end of the study period.

SMALL DENSE LDL MEASUREMENTS (SDLDL)

Small dense LDL measurements were studied using the sdLDL kit manufactured by Randox. sdLDL concentration at a local hospital was measured on machines branded Roch Cobas 6000 C501. Randox brand kit was first applied, and Calibrations were completed using the calibrator. Control groups were created. 1st group 18-15.9, 2nd group 38.4-41.3, 3rd group 47.7-47.3 were tested at these intervals. Control serum results were within the required ranges. Then, sample measurements started. The results were determined in mg/dL, and calculations are valid for TC<300 mg/dL. For higher levels of TC, re-measurement should be done by diluting the sample. This process was not required since there was no sample of TC level exceeding 300 mg/dL.

The reference range for men aged 21-44 years and women aged 21-54 years was 9.5-42.5 mg/dL.

The reference range for men aged 45-75 years and women aged 55-75 years was 10.7-48.7 mg/dL.

ETHICAL STATEMENT

All participants were informed about the study and completed a consent form. The Clinical Research Ethics Committee of Şifa University approved all human subjects procedures (10.12.2014/63). This trial is registered under award number 227-63. The study was conducted per the ethical standards in the Declaration of Helsinki.

STATISTICAL ANALYSIS

Statistical analysis was done in Rstudio version 0.98.501 software with R language. The suitability of variables to normal distribution was examined using analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk tests) in all individuals (before and after). Descriptive statistics have been represented with mean±standard deviation for normally distributed variables and with median (minimum-maximum) for non-normally distributed variables. The dependent groups (comparison between the walnut consuming group's measurement values before and after 21 days of study) between continuous variables (demographic information and biochemical analysis table) were analyzed by paired t-test. When all individuals' (before and after) biochemistry values did not show a normal distribution, the Wilcoxon Signed Ranks Test was used for dependent groups. When comparison serum lipid levels of females and males did not show normal distribution, the Mann-Whitney U test was used for independent groups. One-sample Wilcoxon sign rank test was applied for nutritional habits variables. Values, where the p value was below 0.05, were considered statistically significant, and G-Power v. 3.1.7 software was used for to determine the number of samples and then generalize to the population [Standard Intakes of Turkey Nutrition and Health Research (TBSA) etc.].

RESULTS

Eighteen (60%) female and 12 (40%) male individuals participated in the study. The total number of individuals who participated in the study is 30. The parameters of the male and female individuals stated in the study were analyzed as before-after variables. In general, descriptive statistics of the before-after measurement results were calculated. Before comparing these variables, their distributions were exam-

ined. Those with normal distribution and those without normal distribution were determined.

The used walnuts' oil content was 62.39%; the free fatty acid level (in terms of oleic acid) was measured as 0.84%. Fatty acid amounts; oleic acid was calculated as 18.32%, linoleic acid 60.03%, and linolenic acid 11.94% (Table 1).

In this study, TBSA values were compared with the values of healthy individuals' nutritional habits.[13] As a result, there is a statistically significant difference in the carbohydrate, polyunsaturated fatty acid, linoleic acid, and a-linolenic acid intake. A significant difference was found only in the dietary fiber values of male individuals (Table 2).

The effect of consumed walnuts on blood lipid levels has been determined. A significant decrease was observed between the before and the after blood lipid levels, except for TG. Especially a significant difference between the before and the after sdLDL was found (Figure 1, Table 3).

When blood lipids were compared between women groups before and after, there was a significant difference between them except for HDL and TG, while men had a significant difference in blood parameters except for TG. In comparison between male and female groups, a significant difference was found in HDL Before, HDL After, sdLDL After, TG Before, and TG After parameters (Table 4).

TABLE 1: Chemical properties of walnut consumed at breakfast.

Walnut composition	Content (30 g)
Energy (kcal)	197.9
Total fat (%)	62.39
Oleic acid (%)	18.32
Linoleic acid (%)	60.03
a-linolenic acid (%)	11.94
Free fatty acid (%)	0.84

There was a strong positive correlation between LDL Before and LDL After, a strong positive correlation between sdLDL Before and LDL Before, and a strong positive correlation between sdLDL After and LDL After.

DISCUSSION

In this study, we investigated the effect of walnut consumption on the serum lipid levels, especially on small, dense LDL values of healthy volunteers.

During the study, blood lipid levels showed a significant decrease in TC, sdLDL, HDL, and LDL cholesterol values with walnuts' addition to the diet. There was no statistically significant difference in TG levels.

In another study, the effect of walnut diet on hypercholesterolemia patients was investigated: It was

TABLE 2: The nutrition habits of healthy individuals to mean daily intake of Turkish people were compared.

	Female (n=17)			Male (n=11)		
Nutrient Intake	TBSA mean	Mean±SD	p value	TBSA mean	Mean±SD	p value
Energy (kcal)	1649.4	1574.7±314.2	0.309	2241.8	1991.4±392.6	0.091
Carbohydrate (g)	203.6	171.2±53.7	0.039*	281.8	216.8±74.2	0.026*
Protein (g)	51.9	56.6±17.0	0.266	71.3	73.5±13.9	0.423
Total fat (g)	66.6	72.6±12.9	0.093	86.0	91.2±18.8	0.182
SFA (g)	21.7	22.3±7.4	0.758	28.3	27.3±10.6	0.594
MUFA (g)	23.1	21.6±4.5	0.136	30.0	26.9±7.2	0.248
PUFA (g)	17.40	24.7±5.9	<0.001*	21.9	32.5±8.8	0.008*
Linoleic acid (g)	16.1	20.8±5.9	0.005*	20.2	27.5±9.0	0.026*
a-linolenic acid (g)	1.2	3.0±0.3	<0.001*	1.6	2.9±0.4	0.003*
Cholesterol (mg)	191.8	207.6±103.5	0.653	266.0	286.8±210.6	0.656
Dietary fiber (g)	19.0	18.0±7.6	0.619	22.4	18.4±4.0	0.006*

TBSA: Standard Intakes of Turkey Nutrition and Health Research (2010)[13]; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; SD: Standard deviation; *One-Sample Wilcoxon Signed Ranks Test; p values <0.05 were considered statistically significant.

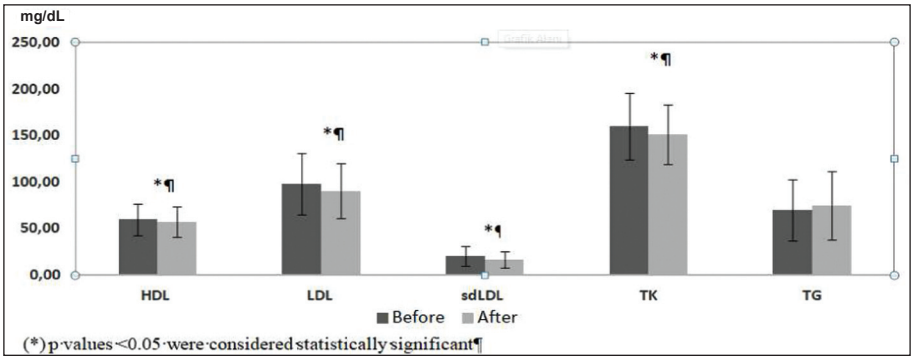


FIGURE 1: The before and after difference of blood lipids.
HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SD: Standard deviation; TC: Total cholesterol; TG: Triglycerides.

TABLE 3: Descriptive statistics and comparison of variables.

	Before (n=30)		After (n=30)			
Blood lipid parameters (mg/dL)	**Mean±SD**	**Minimum-maximum**	**Mean±SD**	**Minimum-maximum**	**Mean difference (%)**	**p value**
HDL	59.8±17.0	28.4-95.0	56.4±15.7	28.6-93.0	4.4	0.008*
LDL	98.0±32.8	58.7-203.7	91.1±29.5	48.6-186.5	7.4	<0.001*
TC	160.4±35.3	105.7-270.1	151.5±31.9	88.2-243.8	5.3	<0.001*
Parameters (mg/dL)	**Median**	**Minimum-maximum**	**Median**	**Minimum-maximum**	**Mean difference (%)**	**p value**
sdLDL	17.7	5.3-46.2	15.1	7.2-45.0	18.3	<0.001**
TG	55.0	34.0-180.2	75.8	35.6-183.4	-6.8	0.136**

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SD: Standard deviation; TC: Total cholesterol; TG: Triglycerides; *Paired- samples t-test; **Wilcoxon Signed Ranks Test; p values <0.05 were considered statistically significant.

shown that there was a 7.4% decrease in TC and a 10.0% decrease in LDL.[14] In our study, similar results were obtained. It was observed that there was a decrease of 5.25% in TC and 7.42% in LDL.

In a study published by Bamberger et al., walnut consumption on 194 healthy individuals was investigated. These individuals were divided into two groups. While the Before group received 43 g of walnuts a day, the second group received no walnuts. Subsequently, the second group was given walnuts, and the Before group received no walnuts. The LDL, TC, TG, and HDL parameters were measured at the beginning, 4th, and eighth weeks. It has been found that walnut consumption has a positive effect on blood lipid levels except for HDL.[15] Unlikely, our study has not shown a significant difference in TG levels, and a 4.37% decrease was observed in HDL levels. Many studies have revealed that the walnut diet does not affect HDL cholesterol concentrations compared to the control diet. Also, our study showed no significant difference in HDL levels in female groups. For studies investigating the effect of walnut consumption on HDL levels, the larger groups and longer walnut diet duration are required.

Minihane and Harland have investigated the effects of vegetable-derived ω-3 fatty acids and fish-derived ω-3 fatty acids on HDL levels. They showed that ω-3 fatty acids of plant origin lower HDL cholesterol levels, whereas ω-3 fatty acids from fish increase the HDL levels.[16] If walnuts are of vegetable origin, our study's finding that walnut consumption decreases the HDL cholesterol levels overlaps with that study. As seen in many studies, different HDL value results can be explained by the walnut composition or other factors (environmental, number of persons, duration).[17,18]

For obese subjects (n=100) to lose weight, diets with limited energy and low energy diets enriched with walnuts (15% of energy) were prepared and distributed randomly. The evaluations were made at the

TABLE 4: Inter-group comparison of the before-after variables in female and male groups.

	Female (n=18)			Male (n=12)					
	Before	After		Before	After				
Blood lipid parameter (mg/dL)	Median (minimum-maximum)	Median (minimum-maximum	p^a	Median (minimum-maximum)	Median (minimum-maximum)	p^b	p^c	p^d
HDL	65.2 (41.4-95.0)	63.5 (42.2-93.0)	0.112	49.3 (28.4-69.9)	43.6 (28.6-68.4)	0.011*	0.001**	<0.001**
LDL	81.8 (61.0-146.6)	82.1 (57.0-132.7)	0.048*	109.2 (58.7-203.7)	98.9 (48.6-186.5)	0.010*	0.150	0.310
sdLDL	14.1 (10.5-31.8)	13.8 (8.9-20.7)	<0.001*	22.7 (5.3-46.2)	18.6 (7.2-45.0)	0.019*	0.083	0.042**
TC	156.5 (109.4-220.9)	145.7 (114.6-203.5)	0.031*	161.4 (105.7-270.1)	145.7 (88.2-243.8)	0.012*	0.641	0.899
TG	50.5 (34.0-102.2)	56.2 (35.6-82.8)	0.647	87.1 (39.7-180.2)	100.9 (40.1-183.4)	0.099	0.031**	0.002**

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SD: Standard deviation; TC: Total cholesterol; TG: Triglycerides; [a]Comparison between female (Before) and female (After) groups; [b]Comparison between male (Before) and male (After) groups; [c]Comparison between female (Before) and male (Before) groups; [d]Comparison between female (After) and male (After) groups; *Wilcoxon Signed Ranks Test; **Mann-Whitney U Test; p values <0.05 were considered statistically significant.

beginning, at 3-month and 6-month periods during the clinical visits. Participants were evaluated for fasting, satiety, and expected consumption rates at three-time points. Repeated measurements of body measurements, blood pressure, physical activity, lipid levels, tocopherols, and fatty acid levels were analyzed using mixed models. Walnut enriched diet group demonstrated a decrease in TC and LDL cholesterol in 6 months. Although both diets caused weight loss and improvements in cardiovascular disease risk factors, the walnut-enriched diet has been shown to have more positive effects on LDL levels and systolic blood pressure.[19] We can say that long term studies and our study both showed a similar effect of walnut consumption on TC and LDL levels.

There is much evidence that different amounts of fatty acids in walnuts affect the blood lipid profile. Other reasons were also revealed because walnuts contain many bioactive structures. Studies conducted on soy have shown beneficial effects of both soy protein and phytoestrogens. So, it is known that walnut contains vegetable protein and many phytochemical contents. One can estimate that these chemical contents may cause biological effects.[20]

It can be said that walnut consumption reduces the risk of CAD. Many clinical studies have shown that various walnuts and peanuts cause a decrease in blood lipid concentrations. The fact that walnuts contain fatty acids can be shown to have a beneficial effect on blood lipid levels. We can attribute this to the fact that walnuts contain high levels of unsaturated and low levels of saturated fatty acids. In various studies, the walnut-enriched diet has been reported to reduce TC, LDL and TG.[21,22] In our study, we think that no significant change in TG may be related to the fatty acid content or duration of the walnut consumption.

In a study, ten obese individuals consumed a walnut enriched diet. The diet included 48 g walnuts a day for five days. Individuals who have consumed walnuts showed a significant reduction in sdLDL levels and a significant increase in large HDL particles. As a result, it provides a mechanical perspective on the effect of walnut consumption on cardiometabolic variables and shows the benefits of long-term walnut consumption on cardiovascular risk.[11] In this study, the walnut consumption of sdLDL is similar to the result found in our study. However, there is no similarity in other blood lipids. Besides, compared to our study, this study has a more limited subject group and application time.

Our study showed that dietary walnut supplementation significantly reduces sdLDL concentration. Diseases like dyslipidemia and hypertension are among the risk factors for cardiovascular disease. In recent years, sdLDL has been included among these risk factors.[6,23]

In a study on small dense LDL, plant steroids were added to the diet of hypercholesterolemic children, and the effect of sterols on sdLDL was examined. Two g per day was added to their diets for 6-12 months. As a result, plant sterols' effects on lipids were investigated, and significant results were obtained.[24]

sdLDL parameters were investigated in people with CAD whose pharmacological treatment was increased four times and compared with sdLDL analyzes of a control group. The sdLDL concentration was high among female groups. This difference was not seen among male groups.[4] In our study, a significant difference in sdLDL levels was found among female groups consuming walnuts but was not observed in male groups. Also, while HDL was not significantly different in female groups, a significant difference was found in male groups. On the other hand, no significant difference in TG levels was found in both groups.

In another study, the effect of soybean, rice, and palm oil added to hypercholesterolemic women's diet on sdLDL levels was investigated. As a result, a significant decrease in sdLDL levels was observed.[25]

The effect of nutrients other than walnuts on sdLDL concentration was mostly studied in hypercholesterolemic individuals. In our study, a significant difference in sdLDL levels was demonstrated in healthy individuals. A significant reduction of 18.3% was observed between sdLDL Before and LDL After. For this reason, the effect of walnut consumption on people is considered to be beneficial.

In the correlation analysis, a very strong positive correlation between sdLDL Before and LDL Before, and a strong positive correlation between sdLDL After and LDL After were found. For this reason, it is recommended to evaluate the sdLDL parameters as well as LDL in CAD risk analysis.

It has been observed that due to the macronutrients and fatty acid characteristics of walnuts the walnut diet reduces the use of saturated fat by providing energy from unsaturated fatty acids without changing the energy coming from the lipids. In this meantime, it lowers total and LDL cholesterol levels while preventing the increase of triacylglycerol from low-fat high-carb diets.[20] Sabete et al. emphasized that walnut intake increases energy but does not negatively affect.[26] However, in this study, it has been found that the intake of saturated fatty acids, energy, monounsaturated fatty acids, and cholesterol according to the dietary habits of the Turkish people is the same. Also, walnut consumption has decreased carbohydrate intake and has increased the intake of polyunsaturated fatty acid, linoleic acid, and linolenic acid. Accordingly, the direct effect of linoleic and linolenic acids from walnuts may have been seen in this study. Therefore, walnut consumption is thought to have a positive effect on CAD risk parameters in this study.

CONCLUSION

This study was done with the idea that products like almonds, hazelnuts, and especially walnuts have a beneficial effect on lipids. Our findings are consistent with previous studies on other types of nuts. Therefore, walnuts may be the most effective diet (in) for those at risk of cardiovascular system disease. We found that walnuts can improve serum lipid values in healthy people living under controlled conditions. Also, carbohydrate intake was reduced in the positive sense. There was no change in the energy required to be taken. However, the mechanism responsible for this effect is still unclear. Studies regarding this effect should be continued in the future. Subsequent studies should uncover the mechanism causing these effects.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Fatih Cesur, Hüseyin Vural;* ***Design:*** *Fatih Cesur;* ***Control/Supervision:*** *Fatih Cesur;* ***Data Collection and/or Processing:*** *Fatih Cesur;* ***Analysis and/or Interpretation:*** *Fatih Cesur, Hakan Cengiz;* ***Literature Review:*** *Fatih Cesur, Hüseyin Vural;* ***Writing the Article:*** *Fatih Cesur, Hüseyin Vural;* ***Critical Review:*** *Fatih Cesur, Hakan Cengiz, Hüseyin Vural;* ***References and Fundings:*** *Fatih Cesur, Hüseyin Vural;* ***Materials:*** *Fatih Cesur, Hüseyin Vural.*

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