FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 9(3), 201-211 (2023) • https://doi.org/10.3153/FH23019

Research Article

Hazelnut-enriched diet effectively increases lipoproteins' resistance to oxidation in hypercholesterolemic subjects

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Cite this article as:
Akcan, B., Örem, A., Kural, B., Altınkaynak, Y., Yücesan, F.B., Örem, C. (2023). Hazelnut-enriched diet effectively increases lipoproteins' resistance to oxidation in hypercholesterolemic subjects. *Food and Health,* 9(3), 201-211. https://doi.org/10.3153/FH23019

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Submitted: 23.11.2022
Revision requested: 06.03.2023
Last revision received: 08.03.2023
Accepted: 15.03.2023
Published online: 07.06.2023

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Oxidative modifications of lipoproteins are crucial in the early stages of cardiovascular diseases (CVD) like hypercholesterolemia. The major aim is to determine the effects of hazelnut consumption on the resistance of low- and high-density lipoproteins to oxidation in hypercholesterolemic subjects. Fifteen hypercholesterolemic subjects (13 men, 2 women) who did not require drug treatment according to the National Cholesterol Education Program Adult Treatment Panel III criteria and had serum levels of TC>200 mg/dL were included in the study. This study was designed as a dual control sandwich model intervention with a single group, isoenergetic three periods. The periods were; Control Diet I (30 days), Hazelnut-Enriched Diet (30 days), and Control Diet II (30 days). The susceptibility of LDL and HDL to copper-mediated oxidation was determined spectrophotometrically by following conjugated diene formation. The resistance of lipoproteins, mainly LDL, was increased by hazelnut consumption. It can be concluded that a hazelnut-enriched diet had protective effects against lipoprotein oxidation in hypercholesterolemic subjects. These changes may play important roles in reducing the development of the atherosclerotic process.

Keywords: Hypercholesterolemia, Hazelnut, Lipids, Oxidation, Lipoproteins

Introduction

Cardiovascular diseases (CVD) are among the most important health problems nowadays. The main underlying cause of cardiovascular disease is atherosclerosis (Nag et al., 2013). Atherosclerosis is a chronic inflammatory disease. Genetic susceptibility, endothelial dysfunctions, hyperlipidemia changes in lipoprotein levels, and oxidation that occurs in the structure of low-density lipoproteins (LDL) are very important in the pathogenesis of atherosclerosis (Borén et al., 2020; Steinberg et al., 1989).

Hypercholesterolemia is a pathological condition caused by increased serum TC and LDL cholesterol levels. It has been shown that there is a strong relationship between hypercholesterolemia and the development of atherosclerosis. Hypercholesterolemia leads to the formation of reactive oxygen species as a result of oxidative stress and may cause the oxidation of lipids and lipoproteins (Negre-Salvayre et al., 2006; Singh et al., 2017). In particular, the oxidation of LDL has important pathophysiological roles in initiating and sustaining the complex cascade of events in atherosclerosis. LDL oxidation is considered an important biomarker in studies examining the effects of bioactive foods on protection against cardiovascular diseases (Winklhofer-Roob et al., 2017).

In terms of heart and vascular diseases, increasing the proportion of fruits, vegetables, nuts such as hazelnuts, and whole grains in the daily diet are the main ingredients of healthy nutrition (Renzo et al., 2019; Segovia-Siapco et al., 2018). Daily dietary intake of nuts and hazelnut oil (not to exceed 20% of the energy from the lipids), which is rich in saturated fatty acids, rich in monounsaturated fatty acids (MUFA), especially oleic acid, high in polyunsaturated fatty acids (PUFA), and high in antioxidant vitamin, vitamin E, would be beneficial in protecting against development atherosclerotic CVD related to hypercholesterolemia (Akhlaghi et al., 2020; Segovia-Siapco et al., 2018).

Cohort studies in nutritional epidemiology reported a 30-50% reduction in CVDs risk associated with consuming nuts (Fraser, 2000). According to the research, people who consumed nuts 1 to 4 times a week and those who consumed less than 1 per week showed a 25% reduction in the risk of death from CVDs. Also, when the frequency of consumption is 5 or more per week, this risk decreases by 50% (Fraser, 2000; Mukuddem-Petersen et al., 2005).

Although many studies investigate the effects of hazelnut consumption on health, studies on hypercholesterolemic individuals are very limited (Brown et al., 2022). In addition, a study examining the impact of hazelnut on HDL oxidation has yet to be done.

In this study, we aimed to investigate the effects of 30 days of hazelnut consumption on LDL and HDL in the hypercholesterolemic subjects who did not require drug treatment.

Materials and Methods

Chemicals

Chemicals; Ethylenediamine Tetra Acetic Acid (EDTA, Disodium salt, CARLO ERBA Barcelona, Spain); Folin-Ciocalteu's Phenol Reagent (SIGMA Missouri, ABD); Sodium Hydrogen Phosphate Dihydrate, Sodium-Hydrogen Phosphate Monohydrate, Sodium Hydroxide, Sodium Bicarbonate, Sodium Potassium Tartrate (MERCK, Darmstadt, Germany); Sodium-Chloride, Sodium Dioxide, Sodium Hydrogen Carbonate (SIGMA-ALDRICH, Missouri, ABD).

Subjects

Fifteen hypercholesterolemic subjects (13 men, 2 women) who did not require drug treatment according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria (Grundy et al., 2004) and had serum levels of TC >200 mg/dL with a mean age 43.6 ±9.5 were included in the present study. Exclusion criteria included nut allergy, smoking status, alcohol or drug abuse, acute and chronic inflammatory diseases, chronic kidney disease, obesity, and endocrine disorders related to lipid and lipoprotein metabolism. All participants were warned not to change their diet, physical activity, or other lifestyle.

Study Design

This study was designed as a dual control sandwich model intervention with a single group, isoenergetic three periods. Each period was 30 days. Calories and nutrients taken in the study periods are given in Table 1. Control Diet I (CDI), hazelnut-enriched diet (HED), and Control Diet II (CDII) were applied to all subjects for the first, second, and last 30 days, respectively. CDI was equivalent to CDII: NCEP ATP III step 2 diet (<7% energy from saturated fatty acids and 200 mg/day dietary cholesterol). During the HED period, hazelnut contributed to 18%–20% of the total daily energy intake, which did not change the daily total energy intake significantly. Two equally weighted packages of daily hazelnuts (49–86 g/day Corylus avellana L. varieties “tombul” from Giresun in Black Sea Region of Türkiye, approximately 82.72% palmitic, 8.89% palmitoleic, 4.85% stearic, 2.73% oleic and

linoleic acids.) was provided to all participants to consume as one portion between breakfast and lunch, and other portion between lunch and dinner. Only water was allowed during hazelnut consumption. Participants were instructed not to consume nuts or nut products other than the hazelnut they were given during the HED period. All diet periods were completed smoothly, and all subjects tolerated daily hazelnut consumption well. A dietitian recorded participants' Food intakes for three consecutive days at the end of each period (two weekdays and one weekend). BeBis computer program (BeBis; Nutrition Information System, Istanbul, Türkiye) were used to estimate calorie. After resting for 15 min, 12h (overnight), fasting venous blood of the participants was taken into serum separator tubes and EDTA-tubes, at baseline and the end of each period. Samples were centrifuged (Centrifuge 5810, Beckman Coulter, Allegra 64R, Germany) at 1500xg for 15 min to obtain serum and plasma, respectively, and stored at -80ºC (Thermo Electron Corp. Farma -86ºC ULT Freezer, Waltham, MA USA) until analysis. Anthropometric measurements were taken at baseline and the end of each period. Body weights (kg), body fat percentages, and body mass index (BMI) were obtained using impedance scales (Tanita Body Composition Analyzer, TBF-300, Illinois, USA).

The study protocol was approved by the local research ethics committee of Karadeniz Technical University Farabi Hospital (file number:2007/10-09). All participants gave written informed consent. The study was conducted according to the recommendations of the Declaration of Helsinki.

Biochemical Determinations

The levels of TC, TG, HDL-C, and LDL-C were determined by using the original reagents in the ROCHE / HITACHI Modular System autoanalyzer, and levels of apolipoprotein AI (ApoAI) and apolipoprotein B (Apo B) by immunonephelometric method (Nephelometer, DADE BEHRING, BN II, Germany) based on monoclonal immunoprecipitation method, after doing the daily quality control applications.

Isolation of Lipoproteins

Isolations of lipoproteins from plasma were performed using the multiple discontinuous density gradient methods used by Sclavons *et al.* (Sclavons et al., 1985).

Beckman Optima LE80K Ultracentrifuge was used with Beckman 90 Ti fixed-angle rotor and polycarbonate ultracentrifuge tubes (Beckman, Lot No: 9.30-99, 10.4 mL). The densities of the prepared NaBr solutions were checked by measuring with a densitometer (Anton Paar DMA 35 N, Austria). VLDL, LDL, and HDL-enriched plasma were isolated by centrifugation at 50000 rpm at 10 ° C for 3 h. Detailed isolated protocols were explained by Vanizor Kural *et al.* (Vanizor Kural et al., 2003). The obtained HDL-enriched plasma was subjected to a second centrifugation process at 37000 rpm at 10°C for 17 h to isolate HDL. Then, isolated HDL and LDL were dialyzed with cellulose membrane (Sigma, D 9777, USA) within PBS (pH=7.4) at 4ºC for 24 h to remove EDTA and other impurities that prevent the oxidation process. Total protein concentrations of the lipoproteins were determined by the method of Lowry *et al.* (Lowry, 1951).

Determination of the Susceptibility of LDL and HDL to Oxidation

Lipoprotein oxidations were performed by the chain reactions method based on the principle that Cu^{2+} binds to molecules to cause chemical, physicochemical, and biological changes, developed by Proudfoot *et al.*(Proudfoot et al., 1997). This type of oxidation causes the loss of tryptophan residues on proteins (Gießauf et al., 1995). The first event in LDL oxidation is hydroperoxide formation in LDL PUFAs. These lipid hydroperoxides are conjugated dienes and give a maximum absorbance of 234 nm. Following this wavelength, the conversion of double-bonded PUFAs to conjugated double-bonded hydroperoxides is determined (Gießauf et al., 1995). Diene conjugation in Cu^{2+}-catalyzed oxidation process [(50 µg lipoprotein/mL)/ (1.67 µM $CuSO_4 \cdot H_2O$) in 10 mM PBS (pH 7.4)] in LDL was monitored at 234 nm for 270 min and at 234 nm for 500 min in HDL, spectrophotometrically (UV-1601, UV Visible Spectrophotometer, Shimadzu) (Hasselwander et al., 1999; Puhl et al., 1994). Time of the resistance to lipoprotein oxidation [t-lag (min), time before onset of lipid peroxidation], the rate of diene conjugation [RDC (nmol/min/mg protein), calculated by using the slope of the propagation phase and the molar absorbance coefficient (ε234=29500) for conjugated dienes], the maximum diene conjugation [MDC (nmol/mg protein), calculated by using the time to reach maximum absorbance and again ε234=29,500] and the time to reach maximum absorbance (t-max, min) were evaluated.

Determination of LDL Oxidation in the Presence of HDL

The effects of isolated HDL on LDL oxidation were also examined. For this purpose, the LDL pool was obtained from mixture samples of all subjects. Cu^{2+}-catalyzed oxidations [(100 µg HDL+ 50 µg pool LDL/mL)/ (1.67 µM $CuSO_4.H_2O$) in 10 mM PBS (pH 7.4)] was monitored at 37°C and 234 nm for 500 min spectrophotometrically (UV-1601, UV Visible Spectrophotometer, Shimadzu) (Hasselwander et al., 1999; Puhl et al., 1994). Time of the resistance to lipoprotein oxidation [t-lag (min), time before onset of lipid peroxidation],

the rate of diene conjugation [RDC (nmol/min/mg protein), calculated by using the slope of the propagation phase and the molar absorbance coefficient (ε234=29500) for conjugated dienes], the maximum diene conjugation [MDC (nmol/mg protein), calculated by using the time to reach maximum absorbance and again ε234=29,500] and the time to reach maximum absorbance (t-max, min) were evaluated.

Statistical Analysis

Data were given as mean and SD for normally distributed variables and median (interquartile range) for non-normally distributed variables. The distribution of variables was assessed by the Shapiro-Wilk test. Parameters that followed the normal distribution were analyzed with repeated measures analysis of variance test. Bonferroni adjustment was used for pair-wise post hoc comparison. The Friedman test evaluated Non-normally distributed variables; the Wilcoxon test was performed for pair-wise comparison for variables with $p<0.05$. Pearson's or Spearman correlation analysis assessed the relationships between intra-group parameters and percentage changes. $p<0.05$ was accepted as statistically significant. F values were given for the parameters by repeated-measures ANOVA. Statistical procedures were performed on SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL) software.

Results and Discussion

Caloric and nutrient compositions of Hazelnut- Enriched Diet and Control Diets are given in Table 1.

The values of body weight, body mass index (BMI), and the levels of lipids and lipoproteins are given in Table 2. Weights, BMI, TC, TG, and LDL-C levels were lower, but Apo AI was higher at the end of the HED period than the CDI period ($p<0.05$). Lipid parameters increased in the CDII period compared to the HED period but were not statistically significant, excluding LDL-C. Percentage changes in the levels of lipid parameters between CDI and HED periods and between HED and CDII periods are shown in Figure 1. While changes % in the levels of HDL-C (3.84 ±7 vs. -1.3 ±10) and Apo AI (4.7 ±15 vs. 6.9 ±13) were not statistically significant ($p>0.05$), changes % in the levels of TC (-8.03 ±6 vs. 8.6 ±12), TG (-12.73 ±15 vs. 8.6 ±33), LDL-C (-8.2 ±7 vs. 9.3 ±13) and Apo B (-1.5 ±13 vs. 11 ±18) were significant ($p<0.05$).

Table 1. Caloric and nutrient compositions of HED and Control Diets (n=15).

Variables	CD I	HED	CD II	F*	*p**
Calories, kcal/day	2395 ±413	2345 ±400[a]	2332 ±401[a]	15.8	0.001
Carbohydrates, energy %	54 ±2.3	44 ±4.6[a]	54 ±1.6[b]	103	<0.001
Protein, energy%	15.1 ±2.2	14.0 ±2.1[a]	14.1 ±1.7[a]	24.8	<0.001
Total fiber, g/day	19.3 ±3.8	25.7 ±2.9	18.6 ±2.9	240	<0.001
Fat, energy%	31 ±1.6	41.9 ±3.6[a]	32.2 ±1.7[a,b]	153	<0.001
MUFA, energy%	13 ±0.5	23.1 ±2.9[a]	14.2 ±1.3[b]	338	<0.001
PUFA, energy%	10.6 ±0.8	13.4 ±1.3[a]	11.9 ±1.1[b]	147	<0.001
SFA, energy%	7.4 ±0.6	6.7 ±0.7[a]	7.1 ±0.6[b]	390	<0.001
MUFA/SFA	1.7 ±0.2	3.4 ±0.3[a]	2.0 ±0.2[b]	282	<0.001
MUFA/PUFA	1.2 ±0.13	1.7 ±0.2[a]	1.19 ±0.16[b]	198	<0.001
SFA/UNSFA	0.3 ±0.04	0.2 ±0.03[a]	0.27 ±0.04[b]	373	<0.001
Cholesterol, mg/day	<200	<200	<200		

Values are expressed as (X±SD)

*; P and F values according to repeated-measures ANOVA.

$P<0.05$ was accepted as statistically significant.

[a]; significant with respect to CDI,

[b]; significant with respect to CDII

CDI: Control Diet I; CD II: Control Diet II; HED: Hazelnut-enriched diet; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid.

Table 2. Values of anthropometric and lipid parameters at the end of each diet period (n=15).

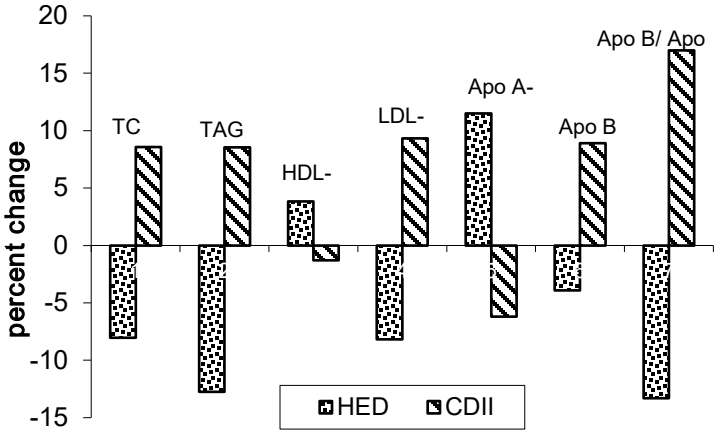
Parameters n= 15	BASELINE (Day 0)	CD I (Day 30)	HED (Day 60)	CD II (Day 90)	F	χ^2	*p*
Age (year)			43.6 ±9.5				
Weight (kg)	82.1 ±14.1	79.5 ±13.7[a]	77.8 ±13.4 [a,b]	77.5 ±13.4[a]	15.3	-	0.001
BMI (kg/m^2)	27.9 ±3.4	27.0 ±3.1[a]	26.4 ±2.9[a,b]	26.3 ±2.9[a]	15.5	-	0.001
TC (mg/dL)	239.1 ±18.4	216.2 ±22.9[a]	198.9 ±24.9[a, b]	214.3 ±22.1[a]	15.2	-	0.002
TG (mg/dL)	170 (115-256)	146 (104-190)	117 [a,b] (95-158)	160 (76-185)	-	4.8	0.091*
HDL-C (mg/dL)	40.6 ±6.9	42.4 ±7.0	44 .1 ±8.5[a,b]	43.2 ±7.5	2.63	-	0.127
LDL-C (mg/dL)	164.9 ±25.1	150.4 ±28.6[a]	137.4 ±24.9[a,b,c]	148.3 ±21.6[a]	13.3	-	0.003
Apo AI (mg/dL)	135.2 ±14.3	128.3 ±16.9	133.2 ±19.3[b]	140.8 ±15.5[b]	6.17	-	0.026
Apo B (mg/dL)	132.3 ±18.5	113.3 ±14.4[a]	111.5 ±20.3[a]	121.9 ±19.8[a]	4.98	-	0.042

P and F values according to repeated-measures ANOVA, posthoc Bonferonni. Data were expressed as mean ± SD.
*; P values according to the Friedman test. Data were expressed as median (interquartile range for 25–75%)
P<0.05 is accepted as statistically significant.
a; significantly different from baseline, b; significantly different from Control diet I, c; significantly different from Control diet II
Apo AI: apolipoprotein AI; Apo B: apolipoprotein B; BMI: body mass index; CDI: Control Diet I; CD II: Control Diet II; HDL-C: high-density lipoprotein-cholesterol; HED: Hazelnut-enriched diet; LDL-C: low-density lipoprotein n-cholesterol; TG: triacylglycerol; TC: total cholesterol.

Table 3. Values of lipoprotein oxidation parameters at the end of each diet period (n=15).

Parameters	CD I	HED	CD II	F *	*p**
LDL oxidation					
t-lag (min)	60.5 ±9.6	70.8 ±12.5[a,b]	58.4 ±12.2	14.1	0.002
RDC (nmol/min/protein)	6.99 ±1.82	6.6 ±2.1	6.9 ±2.6	0.61	0.445
MDC (nmol/mg protein)	420.8 ±132.5	437.8 ±142.4	409.3 ±103.9	0.28	0.601
t-max (min)	135.3 ±12.9	158.6 ±27.8[a]	127.4 ±18.7[b]	18.6	0.010
HDL oxidation					
t-lag (min)	29.1 ±17.5	28.1 ±7.4	24.7 ±7.9	0.77	0.394
RDC (nmol/min/protein)	1.5 ±0.7	1.3 ±0.6	1.2 ±0.6[a]	12.6	0.003
MDC (nmol/mg protein)	128.1 ±19.7	117.9 ±15.4	117.3 ±18.3[a]	5.39	0.036
t-max (min)	154.6 ±45.8	151.6 ±39.1	153.8 ±44.1	0.20	0.661
HDL+ pool LDL oxidation					
t-lag (min)	133.4 ±37.4	163.2 ±48.1[a]	142.5 ±43.8	4.97	0.043
RDC (nmol/min/protein)	3.7 ±1.7	3.6 ±1.6	3.1 ±1.9	1.80	0.200
MDC (nmol/mg protein)	645.1 ±80.1	641.7 ±69.2	614.5 ±114.3	2.01	0.178
t-max (min)	344.2 ±81.3	394.2 ±93.7[a]	379.5 ±105.4	4.22	0.061

a; significantly different from Control diet I, b; statistically different from Control diet II (p<0.05). Values are expressed as (X±SD).
*; P and F values according to one-way ANOVA with repeated measures.
CDI: Control Diet I; CDII: Control Diet II; HED: Hazelnut-enriched diet; MCD: Maximum diene conjugation, RDC: The rate of diene conjugation.



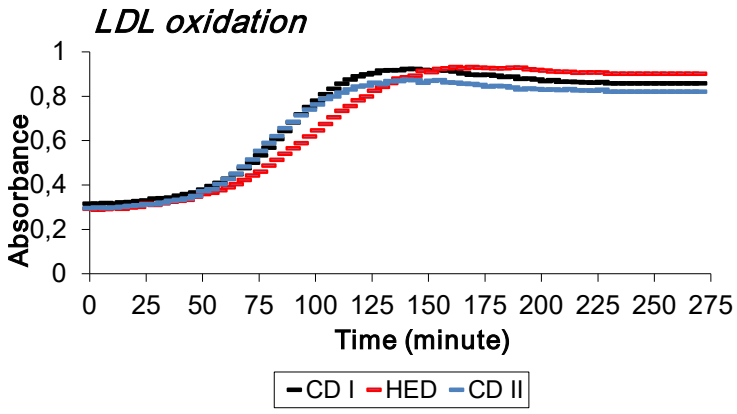
: Percentage changes between CDI and HED periods
: Percentage changes between HED and CDII periods
CDI: Control Diet I, CDII: Control Diet II; HED, Hazelnut-enriched diet

Figure 1. Percentage changes in lipid and lipoprotein levels between diet periods

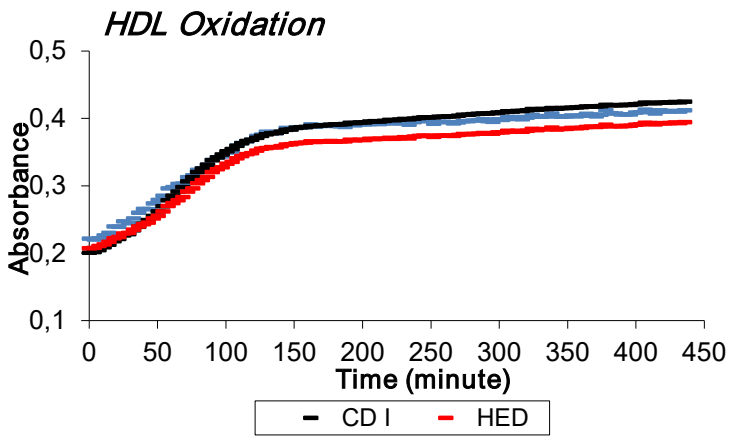
As seen in Table 3, t-lag and t-max for LDL and HDL+pool LDL oxidation stages increased at the end of the HED period compared to CDI ($P<0.05$), but RCD and MDC did not show any significance. On the other hand, HED did not affect HDL oxidation parameters significantly ($p>0.05$). LDL, HDL, and HDL+ pool LDL oxidation kinetics graphs are given in Figure 2.

When the correlations between parameters were analyzed, it was observed that the values of t-lag during the LDL oxidation process were negatively correlated with LDL-C levels at the end of CDI, and the values of t-lag during HDL+ pool LDL oxidation process with TC levels at the end of HED (Figure 3).

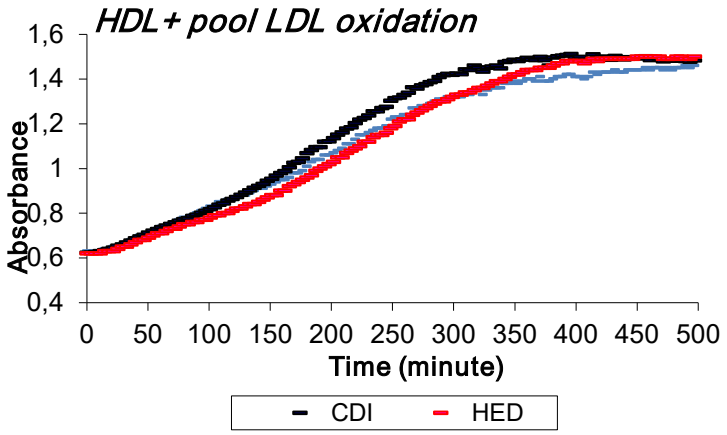
A



B



C



CDI: Control Diet I, CDII: Control Diet II; HED, Hazelnut-enriched diet

Figure 2. Kinetics of lipoprotein oxidations by copper.

Since hazelnut is an oily food, it can be thought that regular consumption may cause an increase in body weight. However, when our study was completed, a significant decrease was observed in body weight and body mass index. Many studies support this result we have obtained. Epidemiological studies show an inverse relationship between the frequency of consumption of nuts and body mass index. In very large cohort studies involving tens of thousands of people, such as the "Adventist Health Study" and the "Nurses' Health Study", a negative correlation was reported between nut consumption and body mass index (Sabate, 2003). Another large cohort study involving tens of thousands of people indicated an inverse relationship between the frequency of the

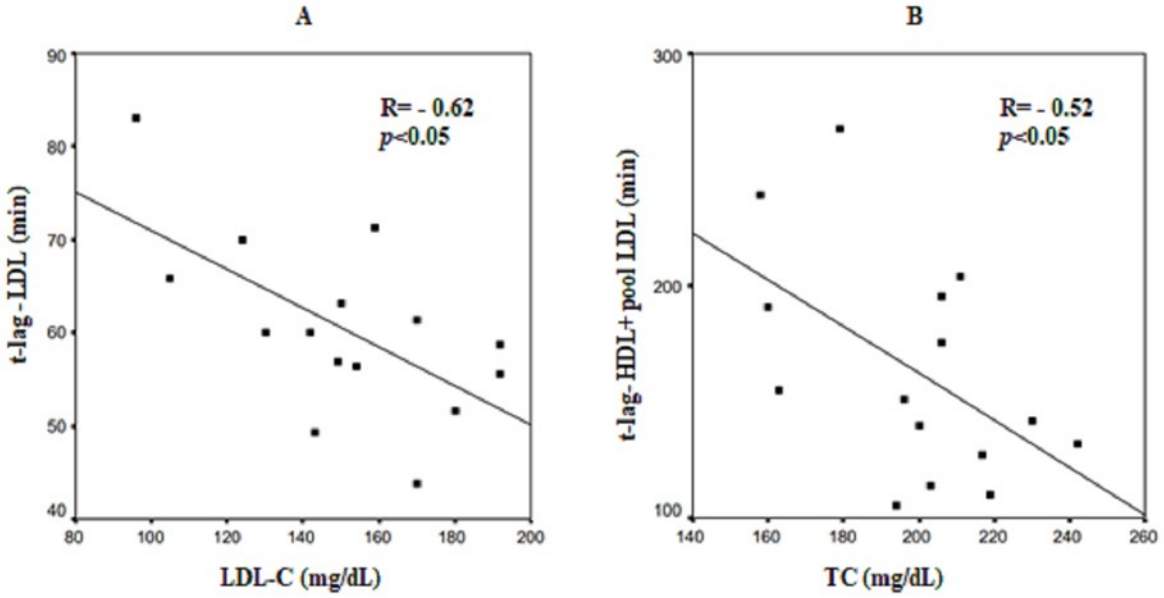
consumption of hard-shelled fruit and body mass index (Segovia-Siapco et al., 2018). Another study involving 6,080 people (Multi-Ethnic Study of Atherosclerosis, MESA) found that nuts consumption caused a reduction in body mass index (Jiang et al., 2006). Studies show that the isocaloric replacement of hazelnuts with other foods in the diet will not increase body weight. In one study, it was reported that there was a significant decrease in body weight in the group that consumed nuts. Frequent consumption of hard-shelled fruits increases the feeling of satiety, increases the metabolic rate at rest due to high protein and unsaturated fatty acid content, and decreases in usable energy due to insufficient digestion of fecal fat are thought to be the causes of weight loss (Jiang et al., 2006; Sabate, 2003). Although the relationship between hazelnut consumption and reduction in body weight has not been fully explained yet, it is attributed to the effect of nuts in delaying gastric emptying, inhibiting anabolic mechanisms, and stimulating catabolic pathways (Akhlaghi et al., 2020).

Nevertheless, studies show no significant change in body mass index with hazelnut consumption. In the study conducted by Mercanlıgil et al., hypercholesterolemic individuals were given a control diet for one month, 40 g of hazelnuts were added to their diets in the second month, and no significant change was observed in their body weights (MercanlIgil et al., 2007). In the study conducted by Durak et al., hazelnuts were added to the regular diets of healthy individuals at 1 g per kilogram per day for 30 days, and it was observed that their body weights did not change (Durak et al., 1999). Koçyiğit et al. investigated the effects of pistachio nut consumption on plasma lipid profile and oxidative status in healthy normolipidemic individuals. Individuals consumed pistachio nuts for 3 weeks, constituting 20% of the daily calorie intake, and it was determined that their body mass index did not change (Kocyigit et al., 2006)

In the present study, serum TC, TG, and LDL-C levels decreased by consuming hazelnuts, and Apo AI levels increased significantly. Some studies, with the consumption of hazelnuts (MercanlIgil et al., 2007), walnuts (Ros et al., 2004), and pistachio nuts (Edwards et al., 1999) in hypercholesterolemic patients, support our findings. Lowering the effects of atherogenic lipid parameters is important to prevent CVDs. It was reported that every 1% reduction in TC and LDL-C concentrations would result in a 1.5% decrease in the incidence of coronary heart disease (Balaban Yucesan et al., 2010). Dietary fatty acids like alpha-linolenic acids and vitamins like γ-tocopherol affect cholesterol levels negatively (Edwards et al., 1999). In addition, diets with a low saturated fat ratio and a high MUFA ratio effectively regulate plasma lipid levels in the protective direction (Fraser, 2000; Renzo et al., 2019).

Hazelnut consumption affected lipoprotein oxidation, such as that t-lag which reflects the resistance of LDL to oxidation, and t-max, which reflects time to reach maximum diene conjugations reduced significantly $P<0.05$ (Table 3). This shows that LDL gained resistance to oxidation through the consumption of hazelnuts. Vitamin E, a chain-breaking antioxidant against lipid peroxidation, finds in high amounts in hazelnut, and consumption of these nuts may lead to an increase in the antioxidant content of LDL. It may affect fatty acid compositions in LDL. In our previous study, Vitamin E levels of serum and LDL particles were higher in hypercholesterolemic individuals in hazelnut-enriched diet periods (Orem et al., 2013). Another study showed that vitamin E levels in plasma and LDL particles were increased by consuming hazelnuts (Puhl et al., 1994). In the same study, when the fatty acid composition of LDL was examined, it was seen that 18:1 (n9) fatty acid increased. This is an expected result considering that 82-83% of hazelnuts contain MUFA and predominantly 18:1 (n9) fatty acid. Increased MUFA contents of LDL particles and increased vitamin E during the HED period could be more effective in decreasing the susceptibility of LDL to oxidation. A decrease in the number of double bonds in the structure of the fatty acids and increased amounts of vitamin E are the factors that cause the elongation of t-lag (Edwards et al., 1999; Spiller et al., 1998). A study on the effects of the peanuts diet on LDL oxidation in healthy individuals showed that the t-lag is increased (Hargrove et al., 2001). Also, it has been reported that polyphenols in walnuts inhibit LDL oxidation in vitro (Hargrove et al., 2001).

HDL of individuals who consume hazelnuts was also effective in reducing LDL oxidation. Alpha-tocopherol affects apolipoproteins, lipids, and antioxidants of HDL positively, and that's way may protect HDL structure and antiatherogenic properties of HDL (Garner et al., 1998; Nicholls et al., 2019; Zabłocka-Słowińska et al., 2019). The enzymes in HDL, mainly paraoxonase, are responsible for HDL's antioxidant properties and, thereby, inhibition effects against LDL oxidation (Negre-Salvayre et al., 2006). We consider that there are changes in the chemical structure of HDL with the hazelnut diet, and thus it can protect LDL against oxidation.



A; Correlation of t-lag in LDL oxidation stage with LDL-C at the end of CDI
B; Correlation of t-lag in HDL + pool LDL oxidation stage with TC at the end of HED period.

Figure 3. Correlations of t-lag with lipid parameters

To support this perspective, according to the findings, while there was no significant change in the amount of HDL-C by the hazelnut consumption, HDL obtained at the HED period leads to a significant increase in LDL oxidation t-lag phase. It has been reported that the quality of HDL contributes to the anti-atherogenic effects (Norata et al., 2006).

According to a systematic review published in 2022 (Brown et al., 2022), although there are many studies on hazelnut consumption and its effects on health, only three of them have been done in hypercholesterolemic individuals (MercanlIgil et al., 2007; Orem et al., 2013; Tey et al., 2011) two of them in hyperlipidemic individuals (Deon et al., 2018; Öngün Yılmaz et al., 2019).

Although the effects of hazelnut consumption on lipid profile and LDL oxidation were shown in these studies, our study is the first to examine the impact of hazelnut on HDL oxidation.

Conclusion

In conclusion, it was thought that the consumption of hazelnut could be led to changes in the structure of the HDL and LDL, can increase the resistance of lipoproteins to oxidation, and have lipid-lowering and weight-loss effects in hypercholesterolemia. Daily consuming certain amounts of hazelnuts (not exceeding 20% of their energy needs) may protect moderately hypercholesterolemic individuals from side effects of hypercholesterolemia, such as atherosclerosis.

The limitation of the study may be the number of subjects. A more significant number of subjects could have been much better regarding results.

Compliance with Ethical Standards

Conflict of interests: The author declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: The study protocol was approved by the local research ethics committee of Karadeniz Technical University Farabi Hospital (File Number: 2007/10-09). All participants gave written informed consent. The study was conducted according to the recommendations of the Declaration of Helsinki.

Funding disclosure: This research was supported in 2007 by the Karadeniz Technical University Scientific Research Projects Coordination Unit. (Project number: 2007.114.01.4.)

Acknowledgments: The authors thank Filiz Kansız, a dietician at the Farabi Hospital, for collecting nutritional data.

Disclosure: -

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