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Hazelnut-enriched diet improves cardiovascular risk biomarkers beyond a lipid-lowering effect in hypercholesterolemic subjects

Asım Orem, MD*, Fulya Balaban Yucesan, PhD, Cihan Orem, MD, Buket Akcan, MSc, Birgül Vanizor Kural, PhD, Cesarettin Alasalvar, PhD, FIFT, Fereidoon Shahidi, PhD, FACS, FAGFD-ACS, FAOCS, FCIC, FCIFST, FIAFoST, FIFT, FRSC

Department of Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey (Drs. A. Orem, Yucesan, and Kural; and B. Akcan); Department of Cardiology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey (Dr. C. Orem); TUBITAK Marmara Research Center, Food Institute, Gebze–Kocaeli, Turkey (Dr. Alasalvar); and Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL, Canada (Dr. Shahidi)

KEYWORDS:
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Inflammation;
LDL oxidation;
Vitamin E

BACKGROUND: Tree nuts, particularly almonds, walnuts, and pistachios, have been shown to possess cardioprotective effects. However, there is little information on the effects of hazelnut consumption on cardiovascular risk markers.

METHODS: The antiatherogenic effect of hazelnut before and after consumption in hypercholesterolemic subjects was investigated. Twenty-one hypercholesterolemic volunteers (18 men and 3 women) were recruited in a double control sandwich model intervention study with a single group and three isoenergetic diet periods. These were control diet I (4 weeks), hazelnut-enriched diet (4 weeks; hazelnut contributing 18%–20% of the total daily energy intake), and control diet period II (4 weeks). The cardiovascular risk biomarkers such as endothelial function, using flow-mediated dilation (FMD) technique, low-density lipoprotein (LDL) oxidation products and inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 (sVCAM-1) as well as lipids and lipoprotein levels were monitored.

RESULTS: Consumption of a hazelnut-enriched diet significantly improved FMD (56.6%), total cholesterol (−7.8%), triacylglycerol (−7.3%), LDL-cholesterol (−6.17%), and high-density lipoprotein cholesterol (6.07%) compared with the control diet I. Oxidized-LDL, hs-CRP, and sVCAM-1 levels were significantly lower in the group ingesting a hazelnut-enriched diet compared with the control diets I and II. Modest correlations between sVCAM-1 and FMD and between sVCAM-1 and hs-CRP were observed ($r = -0.49$, $P < .025$; $r = 0.66$, $P < .001$, respectively).

CONCLUSION: Hazelnut-enriched diets may exert antiatherogenic effect by improving endothelial function, preventing LDL oxidation, and inflammatory markers, in addition to their lipid and lipoprotein-lowering effects. These beneficial effects appeared to be reversible after 4 weeks on a hazelnut-free diet. Therefore, hazelnut may be incorporated into daily diet without change in total caloric intake for sustained health benefit.

* Corresponding author.
E-mail address: aorem64@yahoo.com
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Cardiovascular disease is a leading cause of death in many countries around the globe. The relationship between the consumption of nuts, particularly almonds, walnuts, and

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pistachios, and coronary heart disease (CHD) has been a major focus of health research. There is a large body of epidemiologic and controlled clinical studies related to nuts demonstrating their multiple beneficial effects on CHD.[1–3] Tree nuts are highly nutritious and provide macronutrients, micronutrients, and lipophilic bioactive compounds (or phytochemicals).[4–6] Although cardioprotective effects are generally attributed to all kinds of nuts, each nut has its own specific nutrition and bioactive compounds and may render different kind and degrees of benefit. In this regard, hazelnuts have the highest ratio of unsaturated to saturated fatty acids and a high level of monounsaturated fatty acids (MUFA), which play an important role in improving plasma lipid and lipoprotein levels. A high level of vitamin E, which protects low-density lipoprotein (LDL) against oxidation and a high level of L-arginine, which is precursor of nitric oxide and other bioactives, may contribute to its antiatherogenic effect of hazelnut. However, there are limited number of studies related to hazelnut consumption, and these are generally focused on lipid-lowering effects.[7–9] In a recent study, improving effects of hazelnut consumption on lipid and lipoprotein levels, LDL subfraction, and susceptibility of LDL to oxidation were reported in normolipidemic healthy volunteers.[9] Endothelial dysfunction plays a key role in the development and progression of CHD and is an independent predictor of future cardiovascular events.[10] Ros et al[11] showed that walnut intake improves endothelial function in hypercholesterolemic subjects by using a flow-mediated dilation (FMD) technique.

According to prospective epidemiologic studies, regular consumption of nuts is quite important to take advantage of their beneficial effect against cardiovascular events. However, in many case–control studies related to nut consumption, researchers have not focused on postconsumption on a nut-free diet. This study design, which is a double control sandwich model intervention study, is expected to shed light on diet changes resulting from hazelnut consumption after 4 weeks on a hazelnut-free diet. Here, we investigated the antiatherogenic effect of hazelnut consumption by evaluating cardiovascular risk biomarkers, namely endothelial function using FMD technique, LDL oxidation products, inflammatory markers such as high-sensitivity C-reactive protein (hs-CRP), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1), as well as lipid and lipoprotein levels in hypercholesterolemic subjects.

Subjects and methods

Subjects

Twenty-one hypercholesterolemic volunteers (18 men and 3 women) with a mean age of 44.6 ± 10.4 years were recruited. The eligibility criteria were as follows: serum cholesterol level greater than 200 mg/dL with or without triacylglycerol greater than 150 mg/dL, not on a medication or supplementation known to alter lipid metabolism. Four of the hypercholesterolemic subjects (N = 21) had triacylglycerol levels lower than 150 mg/dL. Individuals were excluded if they had any systemic illness (diabetes mellitus, liver or kidney disease, or hypertension) or history of allergy to hazelnuts. Before starting the study, all participants were trained on the importance of maintaining their routine daily diet, physical activity, and other lifestyle habits. The study protocol was explained to each subject who signed an informed consent, approved by the Ethics Committee of the University.

Study design

It is well known that randomized controlled crossover study is a gold standard in the evaluation of dietary intervention studies. However, to evaluate the changes resulting from supplement consumption during the post-supplement period is complex and difficult. Therefore, this study was designed as a double control sandwich model intervention with a single group, isoenergetic three periods for a total of 12 weeks. All subjects consumed the diet according to three diet periods of control diet I in the first 4 weeks, followed by a hazelnut-enriched diet for the second 4 weeks, and finally control diet II during the last 4 weeks. With this study design we evaluated the effects of a hazelnut-enriched diet by comparing it with control diet I and control diet II. Caloric and nutrient composition of the study periods are given in Table 1.

Before starting the study, a 1-week pre-experiment training period was contemplated for all participants. Control diet I, National Cholesterol Education Program adult treatment panel (ATP) III step 2 diet (<7 % energy from SFA and <200 mg/d dietary cholesterol) was equivalent to control diet II. During the hazelnut-enriched diet period, hazelnut contributed 18%–20% to the total daily energy intake without increasing total daily energy intake. Subjects were instructed on reducing total food intake by approximately 18%–20%, which was the caloric value of the supplemented hazelnut, including reduction in starchy food intake (such as breads ext) by 8%–10%. Approximately 40–70 g/day of nuts were used in a previous clinical trial.[12] In the present study, 49–86 g/day natural or raw hazelnuts (Giresun quality Turkish Tombul hazelnut) were used.[13] The daily hazelnut supplement was provided in preweighed packages to each study participant. Subjects consumed hazelnuts as provided. Total daily amount of hazelnut was divided in two portions. One portion was consumed between breakfast and lunch and the other portion was taken between lunch and dinner. Only water was allowed with the hazelnut consumption. Participants were asked to eliminate hazelnut and other tree nuts from their diet other than those provided throughout the study. Anthropometric and biochemical parameters as well as endothelial functions were obtained at baseline and at the end of each diet period. Study participants were instructed by a dietitian to record their food intake for three consecutive days (two weekdays and one weekend day) at end of each period. Caloric and nutrient

Table 1 Caloric and nutrient composition of hazelnut-enriched diet and control diet I and II

Variables	Control diet I	Hazelnut-enriched diet	Control diet II	F	*P*
Calories, kcal/day	2406 ± 369	2386 ± 378	2369 ± 381	0.176	.683
Carbohydrates, % energy	54 ± 2.6	46 ± 4.3*	53 ± 1.7†	39.8	.000
Protein, % energy	14.6 ± 2.68	13.4 ± 2.50*	13.7 ± 2.00	7.24	.021
Fat, % energy	31.4 ± 1.5	40.2 ± 3.4*	32.7 ± 1.3*,†	14.1	.003
SFA	7.3 ± 0.70	6.4 ± 0.67*	7.2 ± 0.48†	203	.000
MUFA	13.2 ± 0.54	22.5 ± 2.57*	14.4 ± 1.36†	110	.000
PUFA	10.7 ± 0.87	13.1 ± 1.50*	12.0 ± 1.20*	50	.000
MUFA/SFA	1.7 ± 0.15	3.5 ± 0.44*	2.0 ± 0.29*,†	226	.000
MUFA/PUFA	1.24 ± 0.12	1.73 ± 0.24*	1.21 ± 0.18†	96	.000
SFA/UNSFA	0.33 ± 0.02	0.18 ± 0.01*	0.27 ± 0.03*,†	133	.000
Total fiber, g/day	19.4 ± 3.9	25.5 ± 3.4*	18.7 ± 3.2†	228	.000
Cholesterol, mg/day	<200	<200	<200		

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
F and *P* values are from repeated measures ANOVA.
*Significantly different from control diet I.
†Significantly different from hazelnut-enriched diet.

intake was estimated by using the BeBis computer program (BeBis; Beslenme Bilgi Sistemi, Istanbul, Turkey).

Determination of blood biochemical parameters

Blood samples were drawn after a 12-hour overnight fasting at baseline and days 30, 60, and 90. After subjects rested for 15 minutes, blood samples were collected into tubes with and without ethylenediaminetetraacetic acid anticoagulant to obtain plasma and serum, respectively. Samples were obtained by low-speed centrifugation at 1500*g* for 15 minute at 4°C. To reduce interassay variation, samples were stored at −80°C and analyzed at the end of the study. The levels of serum total cholesterol (TC), triacylglycerol (TAG), LDL-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and glucose were determined by enzymatic methods using a ROCHE autoanalyzer (Modular System, GmbH, Mannheim, Germany). Apolipoprotein AI (apo AI), apolipoprotein B (apo B), lipoprotein (a), and hs-CRP were assessed by immunonephelometric method (DADE BEHRING, BN II, GmbH, Marburg, Germany). A quantitative determination of total homocysteine was performed with an IMMULITE 2000 analyzer (DPC, Deerfield, IL). Insulin, vitamin B12, and folic acid levels also were measured with the ROCHE Modular System (modular E170; Gmbh, Mannheim, Germany). These parameters were analyzed by the use of routine laboratory quality control studies. The manufacturer's supplied reagents were used in each of the analysis. Insulin resistance was measured by homeostasis model assessment method-insulin resistance (HOMA-IR) and calculated by using the appropriate formula.[14]

Determination of vitamin E (α-tocopherol) in serum and isolated LDL

Serum vitamin E levels were determined by high-performance liquid chromatographic system (Agilent 1100, Waldbronn, Germany) with Immuchrom assay kit according to the manufacturer's protocols (cat. no. IC1600; Immuchrom, GmbH, Hepenheim, Germany). The results were expressed as mg/L. LDL was isolated by discontinuous density gradient ultracentrifugation according to the method of Sclavons et al.[15] The samples were extracted by the use of a modification of the technique developed by Pussinen et al.[16] The operating conditions for high- performance liquid chromatography were as follows: colon: Zorbax Eclipse XDB-C18 (15 cm × 4.6 mm × 5 μm), mobile phase: methanol/acetonitrile ratio (99:1, v/v), flow rate: 1 mL/min, injection volume: 20 μL, and assay time: 11 minutes. An UV detector at 292 nm was chosen for the analysis. The vitamin E concentration in isolated LDL was reported as μg/mg LDL protein. The protein content in isolated LDL was determined by the method of Lowry et al. [17]

Determination of fatty acids in isolated LDL

Preparation of fatty acid methyl esters in isolated LDL was performed by using the method of Paiker et al[18] and Esterbauer et al[19] with some modifications. Fatty acid composition in isolated LDL was determined by a gas chromatographic method (Agilent Technologies 6890N, Waldbronn, Germany) using a flame ionization detector. Experimental conditions for gas chromatographic–flame ionization detection were as follows: column: DB-23 60 m × 0.25 mm ID, 0.15 μm, inlet temperature: 250°C, injection volume: 1 μL, split ratio: 1:50, carrier gas: hydrogen, head pressure: 230 kPa constant pressure (33 cm/s at 50°C), oven temperature: 50°C for 1 minute, 25°C/min to 175°C, 4°C/min to 230°C, hold at 230°C for 5 minutes, detector gas: hydrogen 40 mL/min. Analytical standard (Supelco 37-component fatty acid methyl esters mixture, 47885-U; Sigma-Aldrich Co., Dorset, UK) was used for the verification of retention times. Fatty acids in isolated

LDL were reported as weight percentages of total fatty acids analyzed.

Determination of endothelial-derived peptides, adiponectin, and oxidized LDL

Human sICAM-1 (R&D Systems, DCD540, Minneapolis, MN), sVCAM-1 (R&D Systems, DVC00, Minneapolis, MN), adiponectin (R&D Systems, DRP300, Minneapolis, MN), oxidized LDL (ox-LDL; Mercodia, 10–1143–01, Uppsala, Sweden), and endothelin-1 (ET-1; Biomedica, BI-20052, Vienna, Austria) levels were determined by commercial enzyme linked immunosorbent assay kits. Interassay coefficient of variations of aforementioned tests were 7.0%, 4.3%, 5.0%, 6.0%, and 4.6%, respectively.

Determination of endothelial FMD

Endothelial function was assessed by FMD of the brachial artery by use of the vascular ultrasound (14 MHz echo Doppler probe, Vivid 7 System; GE-Vingmed Ultrasound AS, Horten, Norway) according to the current guidelines.[20] For the FMD of brachial artery, patients fasted ≥8 hours before the study. Because of circadian variations of peripheral tone, FMD procedure was performed at the same time in each individual in the morning (between 9 and 11:00 am). Patients were studied in a quiet, temperature-controlled room. Caffeine intake and cigarette smoking were prohibited for at least 4–6 hours before the study. The right arm was immobilized using two cushions supporting the elbow and the wrist. A sphygmomanometric cuff was placed on the forearm. After 10–15 minutes of rest, the brachial artery was visualized longitudinally with the ultrasonic scanner operating B mode. After an optimal image of the artery was obtained, the ultrasonic transducer was fixed in this position with a custom-built probe holder. Brachial artery diameter was determined in end-diastole, indicated by the R wave of the electrocardiogram. After three baseline measurements were obtained, ischemia was induced by the inflation of the cuff to 100 mm Hg greater than the systolic arterial pressure to occlude arterial flow for 5 minutes. After the deflation of the cuff, diameter measurements were performed 30 seconds, 1 minute, 2 minutes, 3 minutes, and 4 minutes, consecutively. Because the arterial dilation most likely related to nitric oxide release occurs at 1 minute after ischemia, we used FMD at 1 minute after ischemia to represent the spontaneous endothelial function. Maximal obtained diameter during ischemia-induced hyperemia was used for the calculation of the percentage of FMD (maximum diameter-baseline diameter)/baseline diameter × 100. The intra- and inter-observer reproducibility of resting arterial diameter were 0.01 ± 0.01 and 0.01 ± 0.02 mm, respectively. The intra- and inter-observer variations were less than 10%.

Statistical analysis

Data are given as mean and SD for normally distributed variables and median (interquartile range) for skewed variables. The distribution of variables was assessed by the Kolmogorov-Smirnov test. Parameters that followed the normal distribution were analyzed with repeated measures analysis of variance test. Bonferroni adjustment was used for pair-wise posthoc comparison. Skewed variables were evaluated by Friedman test; Wilcoxon test was performed for pair-wise comparison for variables with $P < .05$. Pearson's or Spearman correlation analysis was used to assess the relationships between the parameters considering the skewness of data distribution. $P < .05$ was accepted as statistically significant. Hazelnut percent change was calculated according to control diet I. Control Δ% was calculated according to the end use of hazelnut-enriched diet. Analyses were performed using SPSS software for Windows (version 15.0, Chicago, IL).

Results

Daily hazelnut consumption was well tolerated by all subjects. The addition of hazelnut to the usual diet, 18%–20% of the total daily energy intake, of the study subjects changed the dietary macronutrient distribution (Table 1). Body weight and body mass index in hazelnut-enriched diet showed a significant change when compared with control diet I (−2.3% and −2.02%, respectively; Table 2). TC, LDL-C, TAG, and apo B/apo AI ratio in hazelnut-enriched diet period were significantly lower than control diet I and control diet II, while HDL-C and apo AI were greater. A significant decrease in ox-LDL level during hazelnut-enriched diet period was observed. Ox-LDL showed positive correlations with LDL-cholesterol and apo B ($r = 0.65$, $P < .001$ $r = 0.60$, $P < .004$, respectively). No changes were observed in lipoprotein (a) levels among the three periods. Glucose, insulin, and HOMA-IR index showed slight improvement, but these changes did not reach statistical significance during consumption period of hazelnut-enriched diet (Table 2).

Hazelnut-enriched diet significantly improved FMD compared with control diet I. However, the level of FMD decreased to the initial level at end of control diet II (Fig. 1 and Table 3). FMD showed a negative correlation with sVCAM-1 ($r = -0.49$, $P < .025$; Fig. 2A). The level of hs-CRP was significantly lower during hazelnut-enriched diet consumption period than both control diets I and II (Table 3). The level of sVCAM-1 in the hazelnut-enriched diet period was significantly lower than both control diets I and II. sVCAM-1 also showed a positive correlation with hs-CRP ($r = 0.66$, $P < .001$; Fig. 2B). No changes were observed in ET-1 levels among different periods (Table 3). sICAM-1 and adiponectin levels in hazelnut-enriched diet period were significantly different from control diet I period. Adiponectin showed correlations with HDL-cholesterol, apo

Table 2 Lipids, lipoproteins, glucose, and insulin levels and theirs percent changes at the end of each diet period

Variables	Control diet I	Hazelnut-enriched diet	Control diet II	Hazelnut Δ (%)	Control Δ (%)	F	χ^2	*P*
Weight, kg	81.0 ± 14.1	79.1 ± 13.5*	79.5 ± 13.9*	−2.30 ± 1.90	0.40 ± 1.28	15.5		.001
BMI, kg/m^2	27.4 ± 3.11	26.9 ± 3.03*	26.9 ± 3.03*	−2.02 ± 1.69	0.07 ± 1.53	15.6		.001
TC, mg/dL	223 ± 22.3	205 ± 24.7*	225 ± 23.1†	−7.82 ± 4.87	9.78 ± 9.16	52.3		.000
TAG, mg/dL	146 (99–193)	122 (92–165)*	144 (90–180)†	−7.3 ± 14.4	13.7 ± 25.7	–	10.4	.006
HDL-C, mg/dL	43.2 ± 6.6	45.9 ± 7.3*	40.0 ± 6.5†	6.07 ± 4.27	−3.67 ± 4.88	35.2		.000
LDL-C, mg/dL	155 ± 26.2	145 ± 25.0*	158 ± 22.7†	−6.17 ± 7.17	9.37 ± 10.09	22.3		.000
Apo AI, mg/dL	131 ± 16	146 ± 13*	138 ± 16†	12 ± 9.1	−5.61 ± 5.71	77.4		.000
Apo B, mg/dL	115 ± 14	112 ± 18	120 ± 16†	−1.90 ± 12.8	8.10 ± 1.1	4.75		.041
Apo B/Apo AI	0.89 ± 0.15	0.77 ± 0.13*	0.88 ± 0.16†	−12.1 ± 12.1	15.2 ± 18.0	28.3		.000
Lp(a), mg/dL	20.5 (13.8–22.6)	20.3 (12.2–24.7)	20.7 (14.1–29.6)	−0.21 ± 19.41	14.4 ± 30.8	–	4.6	.097
Glucose, mg/dL	94 ± 10.4	92 ± 11.2	88 ± 10.2	−1.52 ± 10.3	−3.51 ± 10.6	0.13		.724
Insulin, IU/mL	7.1 ± 4.4	7.6 ± 5.4	6.3 ± 5.4	14.7 ± 53.8	−11.9 ± 53.3	1.75		.203
HOMA-IR	1.69 ± 1.09	1.78 ± 1.35	1.39 ± 1.26	13.1 ± 54.6	−12.7 ± 58.0	1.13		.301

Apo AI, apolipoprotein AI; Apo B, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment method-insulin resistance; LDL-C, low-density lipoprotein-cholesterol; Lp (a), lipoprotein (a); TAG, triacylglycerol; TC, total cholesterol.

Mean ± SD is shown for variables with normal distribution and evaluated by repeated-measures analysis of variance test with post hoc Bonferroni adjustment for pair-wise comparison.

Median (interquartile ranges) is shown for variables with skewed distribution and evaluated by Friedman test. Wilcoxon test was used for pair-wise comparison for variables with *P* value lower than .05.

Hazelnut Δ (%): Percent change between control diet I and hazelnut-enriched diet.

Control Δ (%): Percent change between hazelnut-enriched diet and control diet II.

*Significantly different from control diet I.

†Significantly different from hazelnut-enriched diet.

AI, and folic acid ($r = 0.60$, $P < .007$; $r = 0.57$, $P < .011$; $r = 0.50$, $P < .037$, respectively).

Hazelnut-enriched diet significantly increased the vitamin E level (Table 3) and changed the fatty acid profiles (such as oleic acid, 18:1n-9; Table 4) of LDL particles and decreased plasma ox-LDL level. Hazelnut-enriched diet significantly increased the levels of vitamin B12 and folic acid as compared to control diet II period, while no change was observed in homocysteine level (Table 3). Folic acid also exhibited negative correlations with homocysteine, insulin, and HOMA-IR ($r = -0.54$, $P < 0.014$; $r = -0.53$, $P < 0.014$, $r = -0.52$, $P < 0.019$, respectively).

Discussion

This study demonstrated that substitution of hazelnuts for 18%–20% of the total daily energy intake showed multiple, potent antiatherogenic effects. A slightly weight loss was observed in the hazelnut intervention period with isocaloric diets (Table 1). This result is consistent with other nut-related epidemiologic and clinical studies.

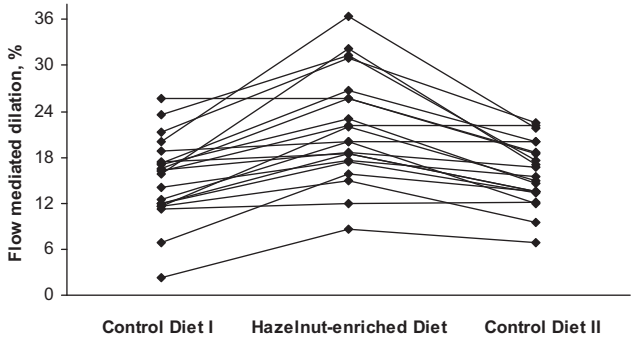

Figure 1 FMD values of subjects at the end of each diet period.

Plasma lipid, lipoprotein, and apolipoprotein levels were improved as a result of hazelnut-enriched diet (Table 2). According to an extensive pooled analysis described by Sabate et al,[21] the mean estimated reduction of TC and LDL-C were 11 mg/dL (5%) and 10 mg/dL (7%), respectively. Most of these previous studies are related to almond, walnut, peanut, and pistachio. Observed changes in lipids and lipoproteins in the present study are in line with earlier studies related to other nuts. There are a few studies related to hazelnut consumption in humans.[7–9] Similar changes in lipids and lipoproteins were observed in our previous study with hazelnuts performed in healthy volunteers.[9]

Mercanligil et al[8] investigated the effect of hazelnut consumption on plasma lipids and lipoproteins by using a similar study design. They did not find any significant change for TC and LDL-C between control diet and hazelnut-enriched diet. However, a decrease in TAG and an increase in HDL-C level were evident. There are convincing evidences that the fatty acid profiles of nuts have favorable effects on blood lipid profiles.[2,21] Hazelnut has the greatest ratio of unsaturated to saturated fatty acids (11.6) and the second richest MUFA (79% of total fat) content among nuts.[22] Nonetheless, other bioactive components in hazelnuts, such as soluble dietary fiber and phytosterols, may contribute to this observed lipid-lowering effect. In the present study, approximately 4.75–8.34 g fiber (15.8–27.8%

Table 3 The levels of parameters related with endothelial function, inflammation, and vitamin E and theirs percent changes at the end of each diet period

Variables	Control diet I	Hazelnut-enriched diet	Control diet II	Hazelnut Δ (%)	Control Δ (%)	F	χ^2	*P*
Flow-mediated dilation, %	15.2 ± 5.37	21.8 ± 6.99*	15.9 ± 4.19†	56.6 ± 60.3	−24.6 ± 14.3	46.1		.000
Parameters in plasma								
hs-CRP, mg/L	1.30 (0.75–2.10)	0.70 (0.35–1.05)*	0.90 (0.65–1.35)†	−35.9 ± 31.6	71.1 ± 111		15.4	.000
ox-LDL, U/L	106 ± 30	93 ± 23*	102 ± 26†	−9.25 ± 18.1	9.77 ± 15.8	12.1		.002
sICAM-1, ng/mL	236 ± 82.6	216 ± 80*	234 ± 100	−8.08 ± 9.57	6.80 ± 12.9	18.2		.000
sVCAM-1, ng/mL	981 ± 393	864 ± 315*	1025 ± 435†	−10.6 ± 10.1	18.4 ± 27.5	18.6		.000
ET-1, fmol/mL	0.21 (0.12–0.47)	0.19 (0.13–0.47)	0.20 (0.12–0.49)	17.0 ± 59	21.3 ± 93	–	0.857	.651
Adiponectin, ng/mL	4598 ± 3312	5615 ± 3481*	5057 ± 3117	29.1 ± 41.9	−5.13 ± 37.1	5.6	–	.029
Vitamin E, mg/L	11.7 ± 2.20	13.7 ± 3.35*	13.1 ± 2.4*	16.9 ± 20.6	−2.24 ± 12.7	11.3		.003
Vitamin B12, pg/mL	375 ± 150	386 ± 156	334 ± 145*,†	2.94 ± 7.62	−13.8 ± 8.19	30.8		.000
Folic acid, ng/mL	8.58 ± 2.27	9.08 ± 2.52	8.04 ± 2.28†	6.24 ± 11	−11.3 ± 11.7	15.2		.001
Homocysteine, μmol/L	15.6 ± 5.33	15.9 ± 5.07	15.3 ± 4.7	5.41 ± 21.7	−0.31 ± 22.3	0.508		.484
Parameter in LDL								
Vitamin E, μg/mg LDL protein	4.71 ± 1.01	5.76 ± 1.69*	4.41 ± 1.35†	24.5 ± 34.5	−22.3 ± 19.7	22.4		.000

ET-1, endothelin-1; hs-CRP, high-sensitivity C-reactive protein; ox-LDL, oxidized-low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1.

Mean ± SD is shown for variables with normal distribution and evaluated by repeated measures analysis of variance with post hoc Bonferroni adjustment for pair-wise comparison.

Median (interquartile ranges) is shown for variables with skewed distribution and evaluated by Friedman test. Wilcoxon test was used for pair-wise comparison for variables with *P* value lower than .05.

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*Significantly different from control diet I.

†Significantly different from hazelnut-enriched diet.

of the recommended daily fiber intake) was estimated to increase by hazelnut-enriched diet. The change of glucose and insulin levels, and HOMA-IR index were slightly improved during hazelnut enriched diet.

Vascular endothelial function was improved upon hazelnut consumption, but this change was reversible when hazelnut consumption was stopped (Fig. 1). Multiple factors may play important roles in improving endothelial function during the consumption period of hazelnut-enriched diet. Decreased bioavailability of nitric oxide is one of the factors associated with endothelial dysfunction.[23] Nuts are a good source of dietary L-arginine, ranging from 1.18 to 3.1 g/100 g.[3] In the present study, intake of L-arginine was estimated to increase by 1.0–1.9 g/d. According to previous studies,[24,25] L-arginine supplementation of the diet by 2–2.5 g/d improved the lipemia-induced endothelial dysfunction in healthy volunteers and in subjects with hypercholesterolemia associated with endothelial dysfunction. It is well known that correction of dyslipidemia is associated with decrease of vascular oxidant stress and lipid peroxidation. It also plays an important role in improving the bioavailability of nitric oxide.[26] On the other hand, it has been demonstrated that vitamin E preserves bioavailability of nitric oxide in some animal studies.[26]

Hazelnut, in particular, is a rich source of tocopherols (particularly α-tocopherol, the most abundant form of vitamin E), with potential antioxidant activity in LDL.[9,27–29] In the present study, vitamin E levels in plasma and LDL particles were increased during hazelnut-enriched diet period (Table 3). Therefore, the two beneficial effects observed in the present study, which improved endothelial function and decreased LDL oxidation, may be attributed to vitamin E.[30,31]

The other important factor affecting LDL oxidation is fatty acid composition of LDL. Hazelnuts contain approximately 82%–83% MUFA, mainly 18:1n-9. A predominant change of fatty acids composition in LDL was observed in 18:1n-9 (Table 4). It can be suggested that increased MUFA contents of LDL particle together with increased vitamin E during hazelnut-enriched diet period could be more effective in decreasing susceptibility of LDL to oxidation. Decreased plasma ox-LDL level may be a consequence of the above mentioned changes in LDL during consumption period of hazelnut-enriched diet and it plays an important role for anti-atherogenic effect by improving endothelial dysfunction and decreasing foam-cell formation in atherosclerotic lesion.

Increased plasma levels of markers expressed on the surface of vascular endothelial cells, such as sVCAM-1 and sICAM-1, were associated with endothelial dysfunction. In this respect, seven clinical trials assessed the effect of nut consumption on endothelial function by determining

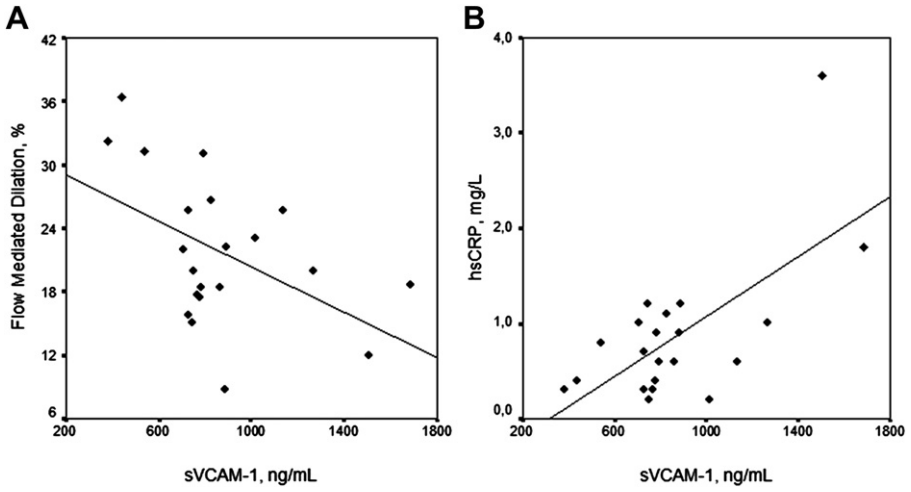

Figure 2 Correlation between (A) sVCAM-1 and FMD and (B) sVCAM-1 and hs-CRP at hazelnut-enriched diet.

peripheral changes in ICAM-1 and VCAM-1 concentrations by Casas-Agustench et al[32]; they found that four of the studies reflected beneficial effects upon nut consumption, but this was not observed in the other three studies. In addition, in two studies, subjects with continued almonds or walnuts consumption, authors showed no effects of nut consumption on VCAM-1 and ICAM-1 levels compared with the control diets. In the present study, it was demonstrated that the hazelnut-enriched diet decreased plasma sVCAM-1 and sICAM-1 levels. These changes may be associated with improved FMD and are consistent with endothelial dysfunction (Fig. 2).

In a limited number of studies on nuts, especially walnut, authors have investigated endothelial dysfunction by using FMD in subjects with hypercholeserolemia.[11,33] It has been shown that the ingestion of walnuts improves endothelial function in patients with hypercholesterolemia. Walnuts differ from other nuts because they contain a high content of alpha-linolenic acid, which might confer additional antiatherogenic properties by increasing membrane fluidity of endothelial cells promoting enhanced synthesis and/or release of nitric oxide.[11] However, walnuts did not improve LDL oxidation and inflammation marker by using CRP.[11] High MUFA and vitamin E content of hazelnut, which are main protective factors for LDL oxidation, make it different from walnut and hence its anti-inflammatory effect and improving endothelial dysfunction, as observed in the present study. Mercangil et al[8] found negligible improvement in endothelial function by consumption of a hazelnut-enriched diet. Study population and design may be important factors for different results obtained in these two hazelnut consumption studies.

Plasma adiponectin level showed an increase in hazelnut enriched diet compared with control diet I (Table 3). Adipocytes abundantly secrete adiponectin into plasma and have multiple, potent antiatherogenic functions by improving endothelial dysfunction and suppressing foam-cell formation.[34]

Chronic low-grade inflammation is closely related to the pathogenesis of atherosclerosis.[35,36] hs-CRP showed a decrease during the hazelnut-enriched diet period. It has been suggested that oxidized LDL plays a key role in the generation of inflammatory processes in atherosclerotic lesions at all stages.[37] Improved LDL oxidation in the present study may be associated with decreasing hs-CRP, sVCAM-1, and sICAM-1 levels. In this respect, observed correlation between hs-CRP and sVCAM-1 may be explained by relationship between inflammation and endothelial dysfunction (Fig. 2). The effect of nut consumption on inflammation was discussed by Casas-Agustench et al,[38] and their findings are consistent with the observed results in the present study.

Folic acid plays an important role in determining homocysteine levels and potentially in regulating endothelial function.[36] A significant increase was observed in plasma folic acid level in hazelnut-enriched diet period, but not in homocysteine level. It may also exert beneficial effects on endothelium through a mechanism independent of lowering homocysteine.[39]

Observed beneficial effects of hazelnut-enriched diet on cardiovascular risk biomarkers such as endothelial dysfunction, oxidizability of LDL particle, and inflammatory process may be due to bioactive compounds including MUFA, vitamin E, L-arginine, and folic acid, among others, by supplemented hazelnut.

It was demonstrated that observed improvement in biochemical parameters and endothelial dysfunction resulting from hazelnut consumption nearly returned to their basal levels during the subsequent 4 weeks on a hazelnut-free control diet II period. Therefore, hazelnuts consumption should be in part of daily eating habit without changing total caloric intake. As described by Kelly and Sabate,[1] across the studies, an 8.3% reduction in risk of CHD death was observed for each week of being on a nut-included diet. Clinically and statistically significant cardioprotective effects from nut consumption were observed and the average risk reduction from CHD mortality was 37% (risk ratio 0.63, 95% confidence interval 0.51–0.83).[1] When considering the observed results of the present study, we believe that incorporation of hazelnuts into the diet of patients with hypercholesterolemia provides cardiovascular benefits.

Table 4 Fatty acid composition of LDL at the end of each diet period

Fatty acids	Control diet I	Hazelnut-enriched diet	Control diet II	Hazelnut Δ (%)	Control Δ (%)	F	*P*
14:0	0.68 ± 0.25	0.60 ± 0.20	0.64 ± 0.21	−6.38 ± 26	10.4 ± 32	3.9	.061
16:0	19.5 ± 1.0	18.8 ± 0.94*	19.3 ± 1.18	−3.45 ± 4.38	2.53 ± 6.47	8.0	.010
16:1n7	1.28 ± 0.41	1.07 ± 0.34*	1.34 ± 0.55	−13.6 ± 23.3	29.3 ± 48	12.5	.002
18:0	6.07 ± 0.37	6.07 ± 0.48	6.24 ± 0.31	0.05 ± 5.7	3.34 ± 8.4	1.2	.289
18:1n9	16.1 ± 2.83	18.9 ± 2.48*	15.9 ± 2.34†	18.6 ± 14.2	−15.4 ± 8.9	63	.000
18:2n6	40.0 ± 3.72	39.1 ± 3.87	40.9 ± 4.30†	−2.10 ± 6.3	4.86 ± 6.9	13.3	.002
18:3n6	0.58 ± 0.25	0.62 ± 0.31	0.67 ± 0.31	8.2 ± 28	13.4 ± 40	0.04	.843
18:3n3	0.35 ± 0.13	0.31 ± 0.15	0.36 ± 0.11	−5.07 ± 72	36.6 ± 47	1.5	.239
20:3n6	2.01 ± 0.46	2.06 ± 0.60	2.11 ± 0.50	3.23 ± 22	7.51 ± 28	0.006	.938
20:4n6	6.55 ± 1.26	6.60 ± 1.52	7.19 ± 1.43	0.56 ± 9.2	10.2 ± 14.3	3.5	.079
22:0	1.18 ± 0.21	1.16 ± 0.35	1.19 ± 0.20	−0.58 ± 29	11.8 ± 33.4	0.29	.595
20:5n3	1.29 ± 0.97	0.93 ± 0.78	0.81 ± 0.57	−24 ± 37	7.88 ± 76	0.36	.554
22:6n3	4.40 ± 1.14	3.99 ± 0.87	3.74 ± 0.97	−6.65 ± 17	−4.16 ± 20	0.195	.664
SFA	27.5 ± 1.26	26.7 ± 1.26*	27.3 ± 1.37	−2.81 ± 3.67	2.21 ± 5.0	8.5	.009
MUFA	17.4 ± 3.09	20.0 ± 2.66*	17.3 ± 2.70†	16.1 ± 13.5	−13.2 ± 9.84	50	.000
PUFA	54.5 ± 3.87	52.9 ± 3.22	54.9 ± 4.01	−3.55 ± 5.6	4.20 ± 5.41	0.013	.910
MUFA/SFA	0.63 ± 0.09	0.75 ± 0.09*	0.63 ± 0.08†	19.6 ± 14.9	−15.4 ± 8.87	51	.000
MUFA/PUFA	0.33 ± 0.08	0.39 ± 0.07*	0.32 ± 0.07†	24.1 ± 19.8	−16.7 ± 14.8	20.5	.001
SFA/UNSFA	0.38 ± 0.02	0.36 ± 0.02*	0.37 ± 0.03	−4.36 ± 5.01	4.59 ± 7.06	12.1	.005

LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
F and *P* values are from repeated measures ANOVA.
Hazelnut Δ (%): Percent change between control diet I and hazelnut-enriched diet.
Control Δ (%): Percent change between hazelnut-enriched diet and control diet II.
*Significantly different from control diet I.
†Significantly different from hazelnut-enriched diet.

Limitations of current study

One of the limitations of current study is study design. Ideally, a randomized control study is recommended because it controls for any environmental factor(s) influence that could arise during any of the feeding periods that could affect the test diet results. Nevertheless, the current diet design provides information about what happens when subjects go off the high hazelnut diet. The other limitation of the study may arise from slightly differences of PUFA levels observed during control diet I and II. Seventeen subjects of the study group had hypercholesterolemia and hypertriglyceridemia. The rest of the study group had only hypercholesterolemia. Different response to hazelnut-enriched diet of these subjects may bring about some degree limitation of current study.

Conclusions

Hazelnut-enriched diets may render antiatherogenic effect by improving endothelial function, LDL oxidation, and inflammatory markers beyond the lipids and lipoprotein-lowering effects. These beneficial effects seem to be reversible after 4 weeks on a hazelnut-free diet; therefore, hazelnut may be incorporated into daily diet without change total calories intake for sustained health benefit.

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