# Hazelnut consumption decreases the susceptibility of LDL to oxidation, plasma oxidized LDL level and increases the ratio of large/small LDL in normolipidemic healthy subjects

## *Fındık tüketimi normolipidemik sağlıklı kişilerde LDL'nin oksidasyona duyarlılığını, plazma okside LDL düzeyini azaltır ve büyük/küçük LDL oranını arttırır*

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## ABSTRACT

**Objective:** Nut consumption has beneficial effects on protection for development of atherosclerotic process.
**Methods:** Single intervention study design was used to determine the effects of hazelnut-enriched diet (1 g/kg/day) during 4 weeks period on atherogenic tendency of low-density lipoprotein (LDL) by evaluating susceptibility of LDL to oxidation, α-tocopherol content of LDL, LDL subfractions, plasma oxidized (ox) LDL, lipid and lipoprotein levels in normolipidemic healthy subjects (n=21). Statistical analysis was performed using paired t test, ANOVA for repeated measurements test, Pearson's and Spearman correlation analyses.
**Results:** Lag time for oxidation (baseline 54.6±12.3 min, 15$^{th}$ day 59.3±13.4 min, 30$^{th}$ day 65.2±17.8 min, p=0.001) and α-tocopherol content of LDL (baseline 4.82±1.2 μg/mg LDL protein, 15$^{th}$ day 4.88±1.4 μg/mg LDL protein, 30$^{th}$ day 5.35±1.7 μg/mg LDL protein, p=0.02) were found to be increased while ox-LDL levels (baseline 57.2±16.2 U/L, 15$^{th}$ day 51.2±13.6 U/L, 30$^{th}$ day 48.2±14.2 U/L, p=0.001) decreased during the study period. Total cholesterol, LDL-cholesterol, apolipoprotein (apo) B and apo B/apo AI ratio were found to be significantly lower while apo AI was higher (p<0.05). In respect to LDL subfraction, ratio of large/small LDL was significantly increased at the end of the study (baseline 3.79±1.35, 15$^{th}$ day 3.41±1.60, 30$^{th}$ day 4.28±2.44, p= 0.046).
**Conclusion:** Hazelnut-enriched diet may play important role in decrease in atherogenic tendency of LDL by lowering the susceptibility of LDL to oxidation and plasma ox-LDL levels, and increasing the ratio of large/small LDL beyond its beneficial effect on lipid and lipoprotein levels.
*(Anadolu Kardiyol Derg 2010; 10: 28-35)*
**Key words:** Hazelnut consumption, oxidized LDL, atherogenic tendency of LDL, α-tocopherol, lipids

## ÖZET

**Amaç:** Sert kabuklu meyvelerin tüketimi aterosklerotik prosesin gelişimini engellemede faydalı etkiye sahiptir.
**Yöntemler:** Normolipidemik sağlıklı şahıslarda (n=21) fındıkla zenginleştirilmiş diyetin düşük yoğunluklu lipoprotein (LDL)'in aterojenik yatkınlığı üzerine etkisi tek müdahale çalışma dizaynı kullanılarak 4 haftalık periyod boyunca (1 g/kg/gün) LDL'nin oksidasyona duyarlılığı, α-tokoferol miktarı, LDL alt grupları ve plazma okside LDL, lipit ve lipoprotein düzeyleri değerlendirilerek belirlendi. İstatistiksel analizde eşleştirilmiş t, tekrarlayan ölçümler ANOVA testleri, Pearson ve Spearman korelasyon analizleri kullanıldı.
**Bulgular:** Çalışma periyodu esnasında LDL'nin α-tokoferol miktarı (başlangıç 4.82±1.2 μg/mg LDL protein, 15. gün 4.88±1.4 μg/mg LDL protein, 30. gün 5.35±1.7 μg/mg LDL protein, p=0.02) ve LDL oksidasyonu için gecikme zamanı artarken (başlangıç 54.6±12.3 dk, 15. gün 59.3±13.4 dk, 30. gün 65.2±17.8 dk, p=0.001) okside LDL düzeyi azaldı (başlangıç 57.2±16.2 U/L, 15. gün 51.2±13.6 U/L, 30. gün 48.2±14.2 U/L, p=0.001). Toplam kolesterol, LDL kolesterol, apolipoprotein (apo) B ve apo B/apo AI oranı önemli derecede düşük iken apo AI yüksek bulundu (p<0.05). LDL alt gruplarına bakıldığında çalışma sonunda büyük/küçük LDL oranı önemli derecede arttı (başlangıç 3.79±1.35, 15. gün 3.41±1.60, 30. gün 4.28±2.44, p=0.046).
**Sonuç:** Fındıkla zenginleştirilmiş diyet lipid ve lipoprotein miktarları üzerindeki faydalı etkisinin yanında LDL'nin oksidasyona duyarlılığını ve plazma okside LDL seviyesini azaltıp, büyük/küçük LDL oranını arttırarak LDL'nin aterojenik yatkınlığını azaltmada önemli role sahip olabilir.
*(Anadolu Kardiyol Derg 2010; 10: 28-35)*
**Anahtar kelimeler:** Fındık tüketimi, okside LDL, LDL'nin aterojenik yatkınlığı, α-tokoferol, lipitler

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## Introduction

Many studies show that nut consumption protects against cardiovascular disease (1, 2). Food and Drug Administration (FDA) has authorized a health claim that the consumption of nuts leads to a reduced risk of coronary artery disease (CAD) (3). Almond, peanut, walnut, macadamia and pecan have been studied extensively. There are few trials investigating the effects of hazelnut consumption on lipid profiles in normolipidemic (4) and hypercholesterolemic subjects (5). Because of their biochemical composition, hazelnut might have antiatherogenic effects in addition to their lipids lowering properties.

Hazelnut has a number of cardioprotective compounds including vitamin E ($\alpha$-tocopherol isomer), phytosterols, vitamin $B_6$, folate, L-arginine, polyphenols and fiber (6). In addition, hazelnut is the second richest source of monounsaturated fatty acids (MUFAs) among nuts. It contains approximately 82-83% MUFAs, mainly oleic acid (18:1) and less than 7.7-8 % saturated fatty acids (SFA) (6). One of the most important features of the hazelnut among tree nuts, it has the highest ratio of unsaturated/saturated fatty acids (10.8-11.9) (7). Monounsaturated fatty acids have been reported to have hypolipidemic effects (5, 8). It is believed that low-density lipoprotein (LDL) is one of the major risk factors for CAD. When LDL is oxidized, it is even more atherogenic and plays an important role in the pathogenesis of atherosclerosis (9). The susceptibility of LDL to oxidation depends chiefly on the fatty acid composition and vitamin E content of the LDL particle (10).

Also, other risk factors for CAD including C-reactive protein, lipid hydroperoxide (LHP), total antioxidant status (TAS), homocysteine, folic acid, vitamin $B_{12}$ and endothelial derived peptides such as vascular cells adhesion molecule-1 (VCAM-1), endothelin-1 (ET-1) and plasminogen activator inhibitor-1 (PAI-1) were evaluated. Apolipoprotein (apo) E plays an important role in lipid metabolism and it has been suggested that apo E genotypes with other genetic and environmental factors affect the individual response to diet (11). Therefore, apo E genotypes were determined to extinguish the difference between dietary responses of individuals.

The aim of the present study was to investigate antiatherogenic effects of hazelnut consumption on LDL particle by evaluating the susceptibility of LDL to oxidation, the vitamin E content of LDL particle, plasma oxidized LDL (ox-LDL), the ratio of large/small LDL, lipid and lipoprotein levels in normolipidemic subjects.

## Methods

### Subjects

Twenty-one healthy individuals (13 women and 8 men) with a mean age 28±5 years were included in this study. All volunteer participants were selected among highly educated university staff members. All subjects were examined by a cardiologist. Individuals were excluded if they had any systemic illness (diabetes mellitus, liver or kidney disease, or hypertension) or history of allergy to hazelnut. Also, individuals who consume alcohol or smoke were excluded. One of the exclusion criteria was the consumption of nuts, nut butters, or nuts oil more than once per week. Before starting the study, all participants were educated on the importance of maintaining their routine daily diet, physical activity and other lifestyle habits. They signed informed consent forms approved by local Ethics Committee.

### Study design

A single-intervention study design was used to examine the effect of hazelnut. The study had 2 phases; a one-week pre-experimental education period and a four-week experimental period. Study participants were instructed by a dietitian to record their normal food intake for 3 consecutive days (2 weekdays and 1 weekend day). Caloric and nutrient intake was estimated by using the BeBis computer program (BeBis, Beslenme Bilgi Sistemi, Istanbul, Turkey). The diet of the subjects was not standardized before the study but none of the subjects changed their dietary habits during this period. During the 4-week period, 1 g/kg/day of hazelnut was added to their usual daily diet (hazelnut-enriched diet). Previous studies used approximately 40-70 g/day of nuts (36). In this study 49-86 g/day Giresun quality unpeeled raw Turkish Tombul (round) hazelnuts (*Corylus avellana L*) were used. The daily hazelnut supplement was provided in preweighed packages to each study participant. Subjects consumed the hazelnuts as they were provided. Total daily amount of hazelnut was divided in two portions. One portion was consumed between breakfast and lunch. 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 by the study for the entire 4-week study period. Anthropometric parameters, caloric and nutritional intakes were obtained at baseline and the end of the 4-week hazelnut-enriched diet period.

Blood samples were drawn at baseline, 15$^{th}$ day and 30$^{th}$ day of the study period. These samples were obtained after a 12 -hour overnight fasting. After the subjects rested for 15 minutes, blood samples were collected into tubes with and without EDTA anticoagulant to obtain serum and plasma. Samples were obtained by low-speed centrifugation at 1500g for 15 min at 4$^{o}$C. To reduce inter-assay variation, samples were stored at - 80$^{o}$C and analyzed at the end of the study.

### Determination of serum lipids, lipoproteins and other biochemical parameters

The levels of serum total cholesterol (TC), triglyceride (TG), 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 and B (apo AI and apo B), lipoprotein (a) [Lp (a)], high sensitive-C-reactive protein (hs-CRP) were assessed by immunonephelometric method (DADE BEHRING, BN II, GmbH, Marburg, Germany). A quantitative determination of total homocysteine was performed using an

IMMULITE 2000 analyzer (DPC LA, USA). Vitamin $B_{12}$ and folic acid levels were also measured using a ROCHE Modular System (modular E170). These parameters were analyzed using routine laboratory quality control studies. The manufacturers supplied reagents were used in each of the analyzers. Lipid hydroperoxide level was measured by a modification of the method described by Ruiz et al. (12). This technique is based on coupled enzymatic reaction and used t-butyl hydroperoxide (37.5-300 µmol/L) to determine the standard curve. Inter-assay coefficient of variance (CV) was 4.4%. Serum total antioxidant status was measured using a new automated method developed by Erel (13). Trolox, a water soluble vitamin E analogue, was used as the standard (0-1 mmol/L). Inter-assay CV was 2.5%.

**Determination of susceptibility of LDL to oxidation and plasma oxidized LDL levels**

The isolation of the three main lipoproteins was performed by discontinuous density gradient ultracentrifugation (155.000 g for 3 h at 4°C) using the method of Sclavons et al. (14) with a Beckman 90 Ti fixed-angle rotor and a Beckman optima LE80K Ultracentrifuge (Beckman Coulter, Inc., CA, USA).

Before $Cu^{2+}$-catalyzed LDL oxidation [(50 µg LDL protein/mL)/(1.67 µmol/L $CuSO^4$) in 10 mmol/L PBS (pH 7.4)], LDL was dialyzed against 10 mmol/L PBS containing EDTA 10 mmol/L (pH 7.4) for 12 h to prevent oxidation and then 10 mmol/L PBS (pH 7.4) for a further 12 h. LDL susceptibility to oxidation was determined by measuring the conjugated diene kinetics. The kinetics of LDL oxidation were determined by monitoring the change in the 234nm absorbance at 37°C for 4.5 h on spectrophotometer (Shimadzu, UV-1601 UV Visible Spectrophotometer, Australia). From the kinetic absorbance profile of each individual LDL preparation, several indexes can be determined which together described the oxidizability of that particular LDL preparation. One of them is lag-time (t-lag), defined as the interval between the intercept of the linear least square slope of the curve with the initial absorbance axis, expressed in minutes (15). Inter-assay CV was 4.2%. Oxidized LDL concentrations in plasma were determined by an enzyme-linked immunoassay kit (Mercodia, Cat No: 10-1143-01, Uppsala, Sweden). Inter-assay CV was 6.2%.

**Determination of alpha-tocopherol (vitamin E) levels in isolated LDL**

The $\alpha$-tocopherol levels were measured by using high performance liquid chromatography (HPLC, Agilent 1100, Germany). The samples were extracted using a modification of technique developed by Pussinen et al (16). The conditions for HPLC: Colon; Zorbax Eclipse XDB-C18 (15 cm x 4.6 mm x 5 µm), mobile phase; Methanol: Acetonitrile (99:1), flow rate; 1mL/min, injection volume; 20 µL, assay time; 11 minutes. An UV detector at 292 nm was chosen for the analysis. The $\alpha$-tocopherol concentration of LDL was reported as µg/mg LDL protein. The protein content of LDL was determined by the method of Lowry et al. Inter-assay CV was 2%.

**Determination of LDL subfractions**

The LDL subfractions were measured with a Lipoprint System (Quantimetrix, Inc., Redondo beach, CA, USA) using the manufacturers LDL subfractions kit (Quantimetrix, Cat No: 48-7002, Redondo beach, CA, USA). This system separates lipoproteins in a non-denaturing gel gradient of polyacrylamide (17). The lipoproteins are separated on the basis of net surface charge and size. The dye used in Lipoprint System binds proportionally to the relative amount of cholesterol in each lipoprotein. In addition to providing seven LDL subfractions (LDL-1-7), we divided LDL particles into "large" and "small" species as described in the study of Vega et al (18). Intermediate-density lipoprotein (IDL) subfractions (IDL-A, -B, -C) and very low-density lipoprotein (VLDL) fraction levels were obtained with the assay system.

**Determination of endothelial-derived peptides**

Human sVCAM-1, PAI-1 and ET-1 were determined using enzyme-linked immunoassay kits (R&D Systems, DVC00, Minneapolis, USA; American Diagnostica Inc., 822, Pfungstadt, Germany; Biomedica, BI-20052, Wien, Austria; respectively). Inter-assay CVs were 7%, 4.3%, and 6% respectively.

**Apolipoprotein E genotype determination.**

Leukocyte DNA was extracted from whole blood and apo E variants were determined by previously described method (19).

**Statistical analysis**

Analyses were performed using SPSS software for Windows (version 15.0) (Chicago, IL, USA).

Data are given as mean and standard deviation (SD). The distribution of variables was assessed by Kolmogorov-Smirnov test. The dietary macronutrient distribution of baseline and hazelnut-enriched diet was compared using paired t test. Parameters that followed the normal distribution were analyzed with repeated measures ANOVA test. Least Significant Difference (LSD) adjustment was used for pair-wise posthoc comparison. A Friedman test was performed for parameters with abnormal distribution. Pearson's or Spearman correlation analyses were used to assess the relationships between the parameters considering the skewness of data distribution. $p<0.05$ was accepted as statistically significant.

## Results

The addition of 1 g/kg/day of hazelnut to the usual diet of the study subjects changed ($p=0.005$) the dietary macronutrient distribution as seen in Table 1. As to be expected main significant increase was observed in the ratio of MUFA/SFA (122%). Fiber content was increased by 25.2%.

In spite of increasing total calorie intake, no significant change was observed in weight and body mass index (Table 2).

Day 15 and day 30 blood samples were obtained in order to observe the effects of hazelnut-enriched diet on the early phase changes in the content and biology of LDL and lipid parameters. The most profound changes of the parameters were seen at the end of the study period. The changes of plasma lipid, lipoprotein and apolipoprotein levels are shown in Table 2 and Figure 1. Total cholesterol, VLDL, IDL-C, LDL-cholesterol, apo B and apo B/apo AI ratio were found to be significantly lower ($p<0.0001$, $p=0.017$,

p=0.01, p=0.008, p=0.005, p=0.006, respectively), while apo AI were higher (p=0.005) as compared with baseline levels. HDL-cholesterol levels showed a tendency to increase, but did not reach a statistically significant level (p=0.086). Triglyceride-rich lipoproteins particles including VLDL and IDL-C particles decreased (p=0.017 and p=0.01). In respect to the LDL subfraction, large and small LDL fractions and total LDL particles were decreased (p=0.009, p=0.04, p=0.038, respectively). The ratio of large/small LDL was significantly increased (p=0.046).

The oxidation parameters of LDL, endothelial derived peptides levels and other biochemical parameters are shown in Table 3. The lag time (t-lag) and $\alpha$-tocopherol content of LDL were found to be significantly increased (p=0.001 and p=0.02, respectively) while the ox-LDL and LHP levels decreased (p=0.001 for both).

Oxidized-LDL levels at baseline and day 15 correlated with t-lag measurements (r=-0.52, p<0.05; r=-0.50, p<0.05; r=-0.35, p=0.12, respectively) (Fig. 2). No change was observed in the TAS level. No significant change was observed in the endothelial derivated peptides. Homocysteine, vitamin $B_{12}$, folic acid, glucose and hs-CRP levels were not changed.

Oxidized LDL was correlated with cholesterol, LDL-cholesterol, apo B and small LDL on 15th day (r=0.55, p<0.01; r=0.65, p<0.003; r=0.75, p<0.001; r=0.50, p<0.05, respectively), and with cholesterol on 30th day (r=0.56, p<0.005). The ratio of large/small LDL were inversely correlated with triglyceride and VLDL cholesterol on 15th day (r=-0.5, p<0.05; r=-0.61, p<0.005).

Nineteen subjects had 3/3 and two subjects had 2/3 and 3/4 apo E genotype.

## Discussion

In this 4-week feeding trial in normolipidemic subjects, it was found that adding 1 g/kg/day of hazelnut to participants' usual diet improved not only lipid profile but also chemical and biologic modifications of LDL including susceptibility of LDL to oxidation, $\alpha$-tocopherol content of LDL, the ratio of large/small LDL and ox-LDL levels in plasma. These changes were more profound on day 30 than on day 15 of the study.

Numerous studies showed that ox-LDL plays a key role in pathogenesis of atherosclerosis (20). The high MUFA and vitamin E content of hazelnut could be the factors responsible

**Table 1. Caloric and nutrient composition of the baseline and a hazelnut-enriched diet**

| Variables | Baseline | Hazelnut-enriched Diet | Percentage of Change | p* |
|---|---|---|---|---|
| Total calories, kcal | 1851±360 | 2222±398 | 20.5±5.6 | 0.005 |
| Protein, % | 13.6±1.8 | 12.5±1.8 | -8.2±2.4 | 0.002 |
| Carbohydrate, % | 54±3.6 | 45.0±3.6 | -16.9±3.3 | 0.005 |
| Fat, % | 32.6±3.4 | 42.7±4.1 | 31.3±6.8 | 0.005 |
| Fiber, g/day | 24.7±9 | 30.2±8.5 | 25.2±11.4 | 0.005 |
| SFA, g/day | 24.9±4.5 | 27.8±4.7 | 12.2±3.1 | 0.005 |
| MUFA, g/day | 21.8±4.2 | 53.6±8.4 | 150±41 | 0.005 |
| PUFA, g/day | 14.8±7.2 | 19.1±7.9 | 34.7±16.7 | 0.005 |
| MUFA/SFA | 0.88±0.1 | 1.94±0.25 | 122±30 | 0.005 |
| MUFA/PUFA | 1.76±0.8 | 3.10±0.98 | 87±33 | 0.005 |

Data are presented as mean±SD
*- paired samples t-test
MUFA - monounsaturated fatty acid, PUFA - polyunsaturated fatty acid, SFA - saturated fatty acid

**Table 2. Anthropometric measurements, lipids, lipoproteins levels at baseline, 15th day and 30th day**

| Variables | Baseline | 15th day | 30th day | F | p |
|---|---|---|---|---|---|
| Age, years | 28±5 | | | | |
| Height, cm | 164.00±0.06 | | | | |
| Weight, kg | 64.52±11.50 | 64.64±11.37 | 64.73±11.35 | 2.063 | 0.166 |
| BMI, kg/m$^2$ | 23.89±3.52 | 23.94±3.49 | 23.90±3.48 | 2.068 | 0.166 |
| TC, mmol/L | 4.21±0.59 | 3.94±0.72[a] | 3.85±0.63[a] | 24.20 | 0.000 |
| TG, mmol/L | 1.01±0.67 | 0.97±0.59 | 0.88±0.48 | 4.07 | 0.131 |
| LDL-C, mmol/L | 2.81±0.66 | 2.58±0.81 | 2.60±0.68[a] | 8.53 | 0.008 |
| HDL-C, mmol/L | 1.38±0.32 | 1.42±0.29 | 1.44±0.31 | 3.27 | 0.086 |
| Apo AI, g/L | 1.35±0.18 | 1.48±0.25[a] | 1.41±0.20 | 9.83 | 0.005 |
| Apo B, g/L | 0.78±0.20 | 0.72±0.17[a] | 0.71±0.18[a] | 9.87 | 0.005 |
| Apo B/ Apo AI | 0.59±0.20 | 0.50±0.15[a] | 0.52±0.17[a] | 9.49 | 0.006 |
| Lp(a), g/L | 0.22±0.18 | 0.21±0.18 | 0.22±0.20 | 4.67 | 0.10 |
| VLDL, mmol/L | 0.56±0.20 | 0.52±0.19 | 0.51±0.17[a] | 6.78 | 0.017 |
| IDL-C, mmol/L | 0.30±0.12 | 0.27±0.11 | 0.27±0.10[a] | 8.00 | 0.010 |
| IDL-B, mmol/L | 0.32±0.09 | 0.33±0.24 | 0.30±0.08 | 1.04 | 0.32 |
| IDL-A, mmol/L | 0.38±0.09 | 0.35±0.21 | 0.39±0.12 | 0.21 | 0.649 |
| Total IDL, mmol/L | 1.00±0.26 | 0.95±0.49 | 0.96±0.26 | 0.83 | 0.373 |
| Large LDL, mmol/L | 1.02±0.32 | 0.86±0.32[a] | 0.91±0.31[a] | 8.33 | 0.009 |
| LDL-1 | 0.87±0.26 | 0.72±0.27[a] | 0.78±0.27[a] | 5.39 | 0.031 |
| LDL-2a | 0.15±0.08 | 0.14±0.08 | 0.13±0.07[a] | 7.76 | 0.011 |
| Small LDL, mmol/L | 0.31±0.15 | 0.29±0.16 | 0.27±0.15[a] | 4.49 | 0.047 |
| LDL-2b | 0.23±0.12 | 0.21±0.12 | 0.20±0.11[a] | 7.76 | 0.011 |
| LDL-3 | 0.07±0.04 | 0.07±0.05 | 0.06±0.04 | 0.46 | 0.506 |
| LDL-4 | 0.01±0.01 | 0.01±0.02 | 0.01±0.016 | 0.6 | 0.74 |
| LDL-5-7 | ND | ND | ND | - | - |
| Large LDL/ Small LDL | 3.79±1.35 | 3.41±1.60 | 4.28±2.44[b] | 4.52 | 0.046 |
| *Total LDL, mmol/L | 2.33±0.67 | 2.14±0.68 | 2.15±0.61[a] | 4.95 | 0.038 |

Data are presented as mean±SD
Repeated measures ANOVA test with posthoc LSD adjustment for pair-wise comparison,; significantly different. a - p<0.05 as compared with baseline, b - p<0.05 as compared with 15th day.
*Total LDL includes LDL and IDL subfractions.
Apo AI - apolipoprotein AI, Apo B - apolipoprotein B, BMI - body mass index, HDL-C - high-density lipoprotein-cholesterol, IDL - intermediate-density lipoprotein, LDL-C - low-density lipoprotein-cholesterol, Lp(a) - lipoprotein small a, ND - non detected, TC - total cholesterol, TG - triglyceride, VLDL - very low-density lipoprotein

**Table 3. The levels of LDL oxidation parameters, endothelial derived peptides and other biochemical parameters at baseline, 15th day and 30th day**

| Variables | Baseline | 15th day | 30th day | F | p |
|---|---|---|---|---|---|
| ox-LDL, U/L | 57.2±16.2 | 51.2±13.6[a] | 48.2±14.2[a] | 32.00 | 0.001 |
| t-lag, minute | 54.6±12.3 | 59.3±13.4[a] | 65.2±17.8[a,b] | 15.61 | 0.001 |
| LHP, µmol/L | 64.9±19.9 | 54.9±11.1[a] | 49.6±11.9[a] | 15.69 | 0.001 |
| TAS, mmol/L | 0.69±0.18 | 0.67±0.22 | 0.63±0.33 | 0.85 | 0.37 |
| α-tocopherol, µg/mg LDL protein | 4.82±1.20 | 4.88±1.40 | 5.35±1.70[a,b] | 6.78 | 0.02 |
| sVCAM-1, ng/mL | 478±168 | 431±135 | 446±131 | 1.84 | 0.191 |
| PAI-1, ng/mL | 44.5±9.6 | 42.2±10.5 | 46.5±11.1 | 1.02 | 0.325 |
| ET-1, fmol/mL | 2.04±0.50 | 1.95±0.52 | 1.99±0.48 | 0.38 | 0.542 |
| Homocysteine, µmol/L | 12.80±4.01 | 12.60±4.10 | 12.20±3.59 | 2.52 | 0.13 |
| Vitamine B12, pg/mL | 264±86 | 259±83 | 252±91 | 4.12 | 0.06 |
| Folate, ng/mL | 5.22±0.85 | 5.42±1.45 | 5.48±1.52 | 1.22 | 0.28 |
| Glucose, mmol/L | 4.54±0.37 | 4.61±0.34 | 4.62±0.34 | 0.608 | 0.445 |
| hs-CRP, mg/dL | 0.13±0.17 | 0.10±0.13 | 0.11±0.11 | 1.83 | 0.40 |

Data are presented as mean±SD
Repeated measures ANOVA test with posthoc LSD adjustment for pair-wise comparison,; significantly different. a - p<0.05 as compared with baseline, b - p<0.05 as compared with 15th day.
ET-1 - endothelin-1, hs-CRP - high sensitive C-reactive protein, LHP - lipid hydroperoxide, Ox-LDL - oxidized-LDL, PAI-1 - plasminogen activator inhibitor-1, sVCAM-1 - soluble vascular cellular adhesion molecule-1, TAS - total antioxidant status

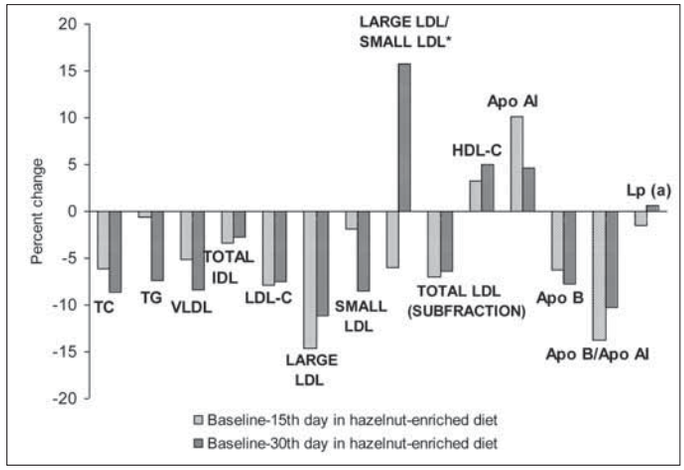


**Figure 1. Percentage changes of plasma lipids and lipoproteins on 15th day and 30th day compared with baseline during consumption of a hazelnut-enriched diet**

*p<0.05

Apo AI - apolipoprotein AI, Apo B - apolipoprotein B, HDL - high-density lipoprotein-cholesterol, IDL - intermediate-density lipoprotein, LDL-C - low-density lipoprotein-cholesterol, Lp(a) - lipoprotein small a, TC - total cholesterol, TG - triglyceride, VLDL - very low-density lipoprotein

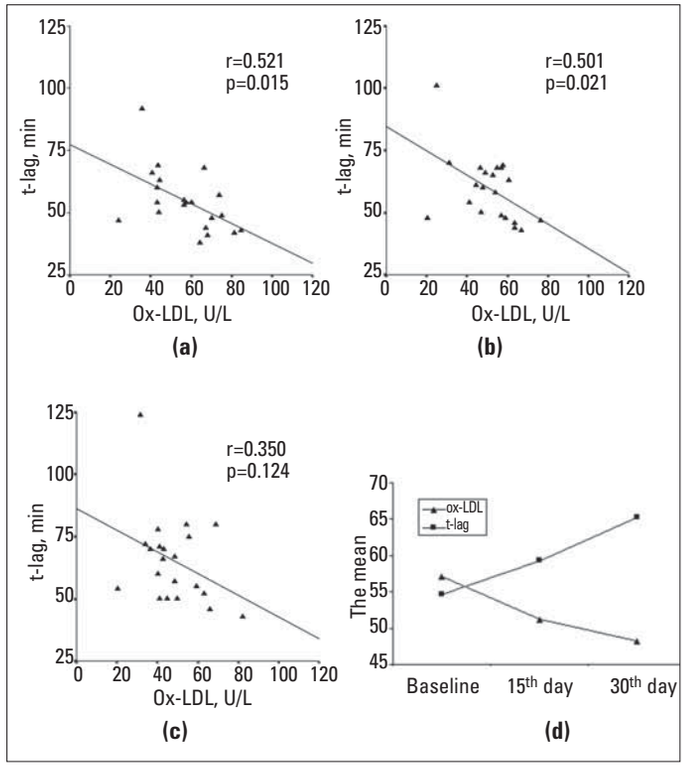


**Figure 2. Correlation between t-lag and plasma ox-LDL levels at baseline (a), 15th day (b) and 30th day (c) in a hazelnut-enriched diet and change of the means of t-lag and ox-LDL levels during the study period (d)**

ox-LDL - oxidized low-density lipoprotein

for decreasing the susceptibility of LDL particle to oxidation. A few studies of other nuts have investigated their effects on LDL oxidation. Ros et al. (21) showed that a walnut diet improves endothelial function in hypercholesterolemic subjects but not ox-LDL levels and susceptibility of LDL to oxidation. This discordance may be because of the different fatty acids and vitamin E content between hazelnut and walnut. Walnut also has a higher PUFA content rather than the higher MUFA content of hazelnut. The same author (22) has reviewed fatty acid composition of a number nuts and he indicated that nuts are high in fat, but most of the fat is MUFA and PUFA. Hazelnut has the highest ratio of unsaturated to saturated fatty acid. High MUFA intake has been associated with reduction of cardiovascular disease risk (23). Lapointe et al. (24) reviewed several studies related to dietary factors affecting the oxidation of LDL particles. These studies suggest that MUFA-rich diets are associated with an inhibition of oxidation of LDL particles. Another reason could be high α-tocopherol levels in hazelnut compared to walnut, 15.03 mg vs. 0.7 mg/per 100 g, respectively. Kornsteiner et al. (25) studied the tocopherol contents of oil extracted from different nuts, and found that vitamin E was highest in hazelnut oil (33.1 mg/100 g), followed by in descending order, almond > peanut > pistachio > pine nut > walnut > Brazil nut > pecan > macadamia. Recently, Alasalvar et al. (6) found α-tocopherol content as 40.4mg/100g hazelnut oil. Unlike most other major antioxidants, α-tocopherol is lipid soluble and therefore, able to travel within the LDL particle and indeed constitutes the main antioxidant species directly coating the LDL particle surface. It can inhibit radical chain propagation by scavenging highly reactive lipid peroxyl and alkoxyl radicals, which promote the propagation of the chain reaction of lipid peroxidation (26, 27). Moreover, it may decrease the assembly of active NADPH-oxidase responsible for reactive oxygen species production (27), which is involved in lipid peroxidation of LDL particles. Alpha-tocopherol

supplementation has been found to decrease the susceptibility of LDL particle to oxidation, which suggests that it could prevent atherosclerotic lesions through the decrease of LDL oxidation (28). According to above- mentioned data, it may be suggested that high MUFA and $\alpha$-tocopherol content of a hazelnut-enriched diet could be responsible for decrease in the susceptibility of LDL to oxidation. Decreased plasma ox-LDL level and its inverse correlation with t-lag at the baseline and 15$^{th}$ day of the study period were observed as possible consequences of decreased susceptibility of LDL to oxidation.

LDL particle circulates in plasma, including a fraction, which reenters the circulation from the subendothelial space (29). The plasma antioxidants provide effective protection against oxidation of LDL (30). This means major site of LDL oxidation is the subendothelial space. The transit of LDL across the subendothelial space may cause a small amount of circulating LDL to be oxidized. Several studies have reported that high plasma ox-LDL concentrations are related to intima media thickness and plaque occurrence in carotid and femoral arteries in men (31) as well as with the progress of atherosclerosis in carotid arteries (31, 32). Also, Toshima et al (33) demonstrated that plasma ox-LDL concentration was a more specific and more sensitive marker for CAD risk than total cholesterol, triglyceride, apo B and HDL-cholesterol concentrations in patients with established CAD. The change in Ox-LDL level was correlated with change of LDL related parameters including plasma cholesterol, LDL-cholesterol, apo B and small-dense LDL on 15$^{th}$ day of this study. Recently, Meisinger et al (34) made concordant observations and identified circulating ox-LDL concentrations as a strong predictor for acute CAD in middle-aged men.

Decreased LHP levels in the present study could be explained by the high MUFA and vitamin E content of a hazelnut-enriched diet. Monounsaturated fatty acids are not only present in LDL but also they are membrane components of the all body cells. Therefore, measured LHP levels may be resulted from decreased oxidation process in all lipid content of the body.

The four-week hazelnut-enriched diet also altered plasma lipid and lipoprotein concentrations in this study. Until now, only two studies investigated the effects of hazelnut consumption on plasma lipid and lipoproteins concentrations. First a preliminary study was performed by Durak et al. (4). They evaluated adding 1 g/kg/day hazelnut to usual diet of healthy volunteers for a 4-week period. They found a significant decrease in plasma total cholesterol and LDL-cholesterol and an increase in HDL-cholesterol and triglyceride levels. Similar changes in lipid and lipoprotein levels were observed in the present study except triglyceride levels. Most recently, Mercanligil et al. (5) evaluated 15 hypercholesterolemic subjects for 8 weeks. The first 4 weeks of the study was a control diet. The next 4 weeks was a hazelnut-enriched diet period (40 g/day). They found a significant decrease in triglyceride and apo B levels and an increase in HDL-cholesterol level. Our plasma lipid and lipoproteins findings are in partial agreement with these studies (4, 5, 35, 36). The differences between these studies could result from the amount of consumed hazelnut, the baseline lipid and lipoprotein levels in the individuals, environmental conditions and subject genetic differences. High beta-sitosterol and fiber content of a hazelnut-enriched diet may contribute to some extent to the observed changes in lipid and lipoprotein concentrations (36). Among the hazelnut related studies, the change in the distribution of LDL subfractions was another new finding in the present study. There was a decrease in triglyceride-rich lipoproteins particles including VLDL and IDL-C. Large and small LDLs were decreased and the ratio of large/small LDL was significantly increased by the 30$^{th}$ day. Almario et al. (37) found a decrease in small-dense LDL in patients with combined hyperlipidemia by using a walnut-enriched diet. The change in the ratio of large /small LDL correlated with a change of VLDL and triglyceride levels by the 15$^{th}$ day. This could be explained by the inverse relationships between triglyceride and small dense LDL levels (36). It is well known that IDL and small dense LDL are the most atherogenic among lipoprotein particles. The observed decreased oxidizability of LDL particles and plasma ox-LDL levels could be associated with decreased levels of these highly atherogenic lipoprotein particles. Two hazelnut-enriched studies mentioned above did not measure t-lag, ox-LDL levels and LDL subfractions.

No changes were observed in endothelial derived peptides including sVCAM-1, PAI-1 and ET-1. These peptides' levels in plasma change during the endothelial cell activation or dysfunction and appear to be closely related to inflammatory process (38). Hs-CRP levels, which are a sensitive marker for inflammation, did not change during the study period. Hazelnut contains additional constituents such as antioxidants including vitamin E and phenolic components, folic acid and L-arginine. These substances appear to have beneficial effects on vascular endothelial function (39). Improved lipid and lipoprotein profile in these subjects may be another factor, which plays an important role in vascular endothelial function (21). Because of the high calorie content of a hazelnut-enriched diet, one may expect the increase in body weight and body mass index. Nevertheless, there was no significant change during the study period. This was similar to the results found by Durak et al (4), who used hazelnuts with same dose and duration in normolipidemic healthy subjects. Sabate (7) also reported an inverse relation between the frequency of nut consumption and BMI. Several potential mechanisms had been proposed why nuts do not cause weight gain (40, 41). They increase satiety because of the high fiber and protein content and low glycemic index. Nuts are high in unsaturated fatty acids and thus may affect diet- induced thermogenesis by increasing resting energy expenditure. Incomplete digestion and/or absorption may cause increase in excretion of dietary fat in stool but decrease in availability of the energy (40, 41). Lovejoy et al. (42) found that high fat diets have been associated with the decreased insulin sensitivity in many clinical studies of nondiabetic subjects. On the other hand, Gary et al. (43) reported that high MUFA diets result in improvement in glycemia. No significant change was observed in fasting glucose concentration during the study period in the present study.

Because apo E genotypes with other genetic and environmental factors affect the individual response to diet (11), apo E genotypes were determined to extinguish the difference between dietary responses of individuals. In this study 19 subjects had 3/3, and 2 subjects had 2/3 and 3/4 apo E genotype. Therefore the standardization between individuals was provided.

**Study limitations**

The fatty acid composition of LDL could have been determined to observe the changes in fatty acids with hazelnut-diet but the volume of LDL was inadequate.

## Conclusion

Hazelnut-enriched diet having high MUFA and vitamin E may play an important role in reduction of the atherogenic tendency of LDL by lowering the susceptibility of LDL to oxidation, plasma oxidized-LDL levels and increasing the ratio of large/small LDL beyond its beneficial effects on lipid and lipoprotein levels. Hazelnut is densely packed nutrient having widely different cardiovascular benefits. Therefore, hazelnut can be readily incorporated into the healthy diets. Further studies are required to show their effects on these parameters in dyslipidemic subjects.

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**Conflict of interest:** None declared

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