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ORIGINAL ARTICLE

Effects of hazelnut-enriched diet on plasma cholesterol and lipoprotein profiles in hypercholesterolemic adult men

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Objective: Frequent consumption of nuts is associated with favorable plasma lipid profiles and reduced risk of coronary heart disease (CHD). This study was conducted to investigate the effects of hazelnut-enriched diet on plasma cholesterol and lipoprotein profiles in hypercholesterolemic adult men compared with baseline and control diet, and also to measure the anthropometric parameters, habitual physical activities, nutrient intake and endothelial function.
Subjects and design: Fifteen hypercholesterolemic men aged 48 ± 8 years were recruited voluntarily. A well-controlled, 2-period (P_1 and P_2) study design with a total of 8-week was implemented. In the P_1, subjects consumed a control diet (low-fat, low-cholesterol and high-carbohydrate). During the P_2, the control diet was supplemented with MUFA-rich hazelnut (40 g/day), which provided 11.6% of total energy content. Anthropometric parameters and habitual physical activities were recorded. Plasma total and HDL cholesterol, TAG, ApoA-1, Apo B, total homocysteine *and glucose* concentrations were measured. All parameters and measurements were obtained at baseline and end of each 4-week diet period.
Results: Body weights of subjects remained stable throughout the study. Compared with baseline, the hazelnut-enriched diet decreased ($P<0.05$) the concentrations of VLDL cholesterol, triacylglycerol, apolipoprotein B by 29.5, 31.8, and 9.2%, respectively, while increasing HDL cholesterol concentrations by 12.6%. Total/HDL cholesterol and LDL/HDL cholesterol ratios favorably decreased ($P<0.05$). Although insignificant there was a decreasing trend for the rest of parameters, particularly in total (5.2%) and LDL cholesterol (3.3%) in subjects consuming a hazelnut-enriched diet compared to that of the baseline. No changes were found in fasting levels of glucose, Apo A-1 and homocysteine between the control and hazelnut-enriched diets.
Conclusions: This study demonstrated that a high-fat and high-MUFA-rich hazelnut diet was superior to a low-fat control diet because of favorable changes in plasma lipid profiles of hypercholesterolemic adult men and, thereby positively affecting the CHD risk profile.
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Introduction

Traditionally, nuts have been perceived by the general public as being unhealthy because of their high-fat content. However, recent epidemiologic and clinical studies suggest that frequent nut consumption is associated with favorable plasma lipid profiles (Durak *et al*., 1999; Edwards *et al*., 1999; Kris-Etherton *et al*., 1999b; Zambón *et al*., 2000; Rajaram *et al*., 2001; Hyson *et al*., 2002; Garg *et al*., 2003) and reduced risk of coronary heart disease (CHD), cardiovascular disease (CVD), myocardial infarction, atherosclerosis and other chronic ailments (Fraser *et al*., 1992; Hu and Stampfer, 1999; Sabaté *et al*., 2000; Albert *et al*., 2002; Feldman, 2002). The FDA (2003) has recently authorized a health claim about the relationship between the consumption of nuts and reduced risk of CHD. According to the new Healthy Eating

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Pyramid (Willett, 2004), one to three serving of nuts and legumes should be incorporated into the diet each day for lifelong health.

Although current recommended National Cholesterol Education Program /American Heart Association Step I or Step II diets have beneficial effects on lowering total and LDL cholesterol concentrations (NCEP, 1993), they tend to decrease HDL cholesterol and increase triacylglycerol (TAG) concentrations, thereby potentially adversely affecting coronary risk factors (Connor *et al*., 1997; Krauss *et al*., 2000). It is, therefore, imperative to identify alternative diets that can more effectively modify the plasma lipid profiles, and thus reduce CHD risk. In contrast with a Step I or Step II diet, a MUFA diet tends to raise HDL cholesterol and lower TAG concentrations (Grundy, 1986; Mensink and Katan, 1987; Rajaram *et al*., 2001; Hyson *et al*., 2002). In keeping with this evidence, hazelnut that is an excellent source of MUFA (Alasalvar *et al*., 2003b, c), may help in this respect.

Among tree nuts, hazelnut plays a major role in human nutrition and health because of its unique fatty acid composition (predominating MUFA), fat soluble bioactives (tocopherols and phytosterols), vitamins (vitamin E), essential minerals (selenium), essential amino acids, antioxidant phenolics (caffeic acid), dietary fiber (soluble), bioactives and phtytochemicals (Alasalvar *et al*., 2003b, c). In addition to MUFA, some other components found in hazelnut have been reported to reduce plasma total and LDL cholesterol concentrations, including PUFA (Zambón *et al*., 2000; Feldman, 2002), phytosterols (Jones *et al*., 1997; Weststrate and Meijer, 1998) and soluble dietary fiber (Anderson *et al*., 1994; Brown *et al*., 1999). Moreover, hazelnut is an excellent source of vitamin E (Alasalvar *et al*., 2003a), which has been shown to reduce the risk of CHD (Rimm and Stampfer, 1997). This cardioprotective effect appears to be due to vitamin E-induced inhibition of LDL oxidation (Steinberg and Lewis, 1997). Finally, whole nuts including hazelnut may provide a variety of non-fat cardioprotective constituents including arginine (Cooke and Tsao, 1997), copper (Klevay, 1993) and magnesium (Elin and Hosseini, 1993). Besides its nutritional value, the presence of distinctive taste- and aroma-active components of hazelnut (Alasalvar *et al*., 2003a, b) may have positive influence in increasing its consumption.

Few studies have been carried out using hazelnut-enriched diet (Alphan *et al*., 1997; Durak *et al*., 1999). Therefore, little is known about how MUFA-rich hazelnut might affect the plasma lipid response to a cholesterol-lowering diet. A detailed research on the effects of MUFA-rich-hazelnut diet on plasma lipid profiles will enhance our knowledge and appreciation for the use of hazelnut in a variety of food and specialty products. Thus, the objectives of present study were to investigate the effects of hazelnut-enriched diet on plasma cholesterol, lipoprotein, apolipoprotein, homocysteine and glucose concentrations in hypercholesterolemic adult men compared with baseline and control diet, and also to measure the anthropometric parameters, habitual physical activities, nutrient intake and endothelial function.

Subjects and methods

Subjects

Fifteen hypercholesterolemic (>200 mg/dl) adult males aged 33–59 years were recruited voluntarily from the staff of the Hacettepe University. Patients with TAG levels above 300 mg/dl were excluded from the study. All subjects were required not to be obese, be non-smokers, and non-alcoholics, free of dietary restrictions/food allergies and not taking medications known to alter plasma lipids. They were also screened for diabetes, renal, thyroid, hepatic, cancer and other major diseases. However, one subject who had a history of CHD was accepted. All subjects successfully completed the trials as directed in the study protocol.

Study design

As single group study, during the baseline period, all accepted subjects were first admitted to the Cardiovascular Clinic of the Faculty Hospital (Hacettepe University) to have a detailed physical examination and dietary history. Then, a well-controlled, 2-period's study design with a total of 8-week was used to examine the effects of a hazelnut-enriched diet compared with baseline and control diet. All subjects consumed the diet according to the following 2-diet periods: control diet in the first 4 week (P_1) and then hazelnut-enriched diet during the final 4 week (P_2). Compliance with the study protocol was reinforced and assessed using multiple approaches. Anthropometric parameters, habitual physical activities, plasma measurements, nutrient intakes and endothelial functions were obtained at baseline and end of each 4-week diet period. The study protocol was explained to each subject, who signed an informed consent. The study protocol was approved by the Ethical Committee of The University.

Study diets

The planned nutrient profiles of the control and hazelnut-enriched diets are shown in Table 1. In the P_1, subjects consumed control diet (low-fat, low-cholesterol and high-carbohydrate). During the P_2, the control diet was supplemented with MUFA-rich hazelnut (40 g/day), equivalent to 25 g fat. The main difference between the control and hazelnut-enriched diets was that the increase of MUFA and decrease of simple carbohydrate in the hazelnut-enriched diet, by ~5–10%. The energy obtained from fat was 25–30% (<7% SFA, 13–15% MUFA and 7–8% PUFA) and 35–40% (<7% SFA, 17–20% MUFA and 7–8% PUFA) in the control and hazelnut-enriched diets, respectively (Table 1). A control diet was used to see the difference of high-fat and high-MUFA diet from hazelnut against to control diet (low-fat and low-cholesterol). The hazelnut-enriched diet was designed to provide the same amount of protein, SFA, PUFA, dietary fiber and cholesterol as the control diet.

Table 1 Planned composition of the control and hazelnut-enriched diets[a]

Constituent	*Diets*	
	Control	*Hazelnut-enriched*
Carbohydrate (% of energy)	55–60	50–55
Protein (% of energy)	12–15	12–15
Fat (% of energy)	25–30	35–40
SFA (% of energy)	<7	<7
MUFA (% of energy)	13–15	17–20
PUFA (% of energy)	7–8	7–8
Cholesterol (mg/day)	⩽300	⩽300
Dietary fiber (g/day)	25–30	25–30

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, ployunsaturated fatty acid; SFA, saturated fatty acid.
[a]40 g hazelnut provides: 254 kcal energy, 6.7 g carbohydrate, 5.04 g protein, 25 g fat (of which 1.84 g SFA, 19.64 g MUFA, and 2.4 g PUFA).

During the 8-week diet period, there was no washout period between the diets. Giresun quality raw Turkish Tombul hazelnuts (*Corylus avellana* L) were provided in pre-weighed packages of 40 g throughout the 4-week diet period (P_2). Hazelnut was consumed as snacks once (40 g) a day. In addition, subjects were asked to eliminate hazelnut and other tree nuts from their diet other than those provided by the study for the entire 8-week study period. Food intake was assessed by dietary records 3 consecutive days including one weekend for each week of each diet period. Nutrient intake was estimated by using the BeBis computer program (BeBis Beslenme Bilgi Sistemi, Istanbul, Turkey). Subjects were instructed individually on how to complete the food records and how to estimate or measure the food portions at home by experienced dietitian. The food records were reviewed weekly by dietitian during an interview with subjects. In addition, they were also instructed to maintain their level of physical activity.

Anthropometric parameters and habitual physical activities

All anthropometric measurements were carried out by trained personnel according to the method described by Lohman *et al*., 1988. Body weight was measured using a portable electronic scale. Height and waist/hip circumferences were measured using a standard tape measure. BMI was calculated as weight (kg)/height (m^2). Bioelectrical Impedance Analysis (Bodystat 1500, Bodystat Inc., UK) was used to find out the percentage of body fat. Blood pressure was measured using by Erka Perfect Sphygmomanometer. Habitual physical activity levels were ascertained by a seven-day recall questionnaire (Blair *et al*., 1985; James and Schofield, 1990).

Plasma measurements

At the beginning and end of each 4-week diet period, blood samples were drawn into vacutainer tubes containing Na_2EDTA (1 g/l final concentration) from the antecubital vein after an overnight fast. The tubes were then immediately stored into ice water. Within 2 h, plasma was separated by centrifugation at 2500 *g* for 20 min at 4°C. All the measurements excluding total homocysteine were made immediately after the plasma collection. All the plasma for total homocysteine measurement kept at −70°C for 8–10 weeks. Plasma glucose, total and HDL cholesterol, and TAG concentrations were determined with Roche/Hitachi MODULAR ANALYTICS (Tokyo, Japan). Reagents and calibrators from the same manufacturer (Roche Diagnostics, Mannheim, Germany), were used for each assay, and instrument operations and calibrations were performed as the instructions from manufacturer. Glucose concentrations were measured by glucose oxidase and peroxidase reactions. Total cholesterol was measured by cholesterol esterase, cholesterol oxidase and peroxidase reactions. Total TAG was measured by glycerol-phosphate-oxidase and peroxidase reactions. Method for direct determination of HDL-cholesterol uses polyethylene glycol ('PEG') based system in which sulfated a-cyclodextrin, dextran sulfate and MgCl2 form water soluble complexes with the non-HDL lipoproteins present in a sample, after which pegylated cholesterol esterase and cholesterol oxidase are introduced. The non-HDL complexes are not accessible to the PEG-modified enzymes, permitting measurement of the HDL fraction. LDL cholesterol concentrations were calculated using the Friedewald formula: (total cholesterol)−(HDL cholesterol)−(VLDL cholesterol) = LDL cholesterol. VLDL cholesterol concentrations were estimated as TAG divided by 5, when concentrations are expressed in mg/dl (Friedewald *et al*., 1972). Apolipoproteins A-1 and B (Apo A-1 and Apo B) were determined by immunonephelometric methods with the Behring Nephelometer II (Dade Behring, Marburg, Germany) using the original fixed-time method and the original reagent kits of the same manufacturer (Fink *et al*., 1989). The plasma homocysteine was measured by fluorescence polarization immunoassay (Axis-Sheild, Oslo, Norway) with Abbott IMx analyzer (Abbott Laboratories, Abbott Park, IL, USA) (Shipchandler and Moore, 1995). Endothelial function was evaluated by the assessment of vascular reactivity of various peripheral arteries by studying the changes in the diameter of the blood vessels following stimulation of the vascular endothelium with vasoactive substances or the shear stress of post ischemia increase in blood flow. We measured the internal diameter of the branchial artery by doppler ultrasound technique before and after ischemic stress induced by using a sphygmomanometer.

Statistical analysis

Data were analyzed by statistical analytical systems (SAS, USA 1997) software. The normality assumption was tested by using One-Sample Kolmogrow–Smirnov test. The mean ± s.d. were determined, and the differences among baseline, control diet, and hazelnut-enriched diet were compared by analysis of variance (Repeated Measures ANOVA). Nutrient intake (total fat, SFA, MUFA, and PUFA) were also compared

Table 2 Some variables of the subjects

Variables	*Baseline*	*Diet periods*	
		Control (P_1)	*Hazelnut-enriched (P_2)*
Age (years)	48.0±8.0		
Height (cm)	169.8±5.9		
Weight (kg)	74.3±5.0	74.2±5.1	74.0±5.5
BMI (kg/m^2)	26.1±1.6^b	25.9±1.7^c	25.8±1.8^c
Waist/hip	0.96±0.1	0.95±0.1	0.94±0.1
Body fat (%)	26.2±5.2^b	26.3±6.0^{b,c}	23.5±4.3^c
Family CHD history (%)	60.0		
Mother (%)	33.3		
Father (%)	33.3		
Mother + father (%)	11.1		
Sister or brother (%)	22.2		
Blood pressure (mm/Hg)			
Systolic	<140		
Diastolic	<90		
Drug user for hypertension (%)	33.3		

Abbreviation: BMI, body mass index; CHD, coronary heart disease.
Values are means±s.d., $n=15$.
Values on the same row not sharing a common superscript are significantly different, $P<0.05$.

with the changes of blood lipid concentrations. Benferroni Confidence Interval Adjustment was used for pair-wise comparison. The level of significance was $P<0.05$.

Results

Fifteen hypercholesterolemic subjects completed the trial as detailed in the study protocol. Table 2 shows the changes of some variables of the subject characteristics over the 8-week diet period. The mean age for the group was 48±8 years. No significant changes in body weight and waist/hip ratio were observed throughout the 8-week diet period, whereas BMI and the percentage of body fat was reduced ($P<0.05$) when subjects consumed the hazelnut-enriched diet compared with baseline. Subjects (60%) had a family history of CHD, which is known as an irreversible risk factor as far as age, sex and diabetes are concerned. Although none of the subjects had hypertension during the diet periods, 33.3% of them were on a hypertension drug. Daily habitual physical activities of subjects are given in Table 3. The physical activity level (PAL) of subjects was considered equivalent to mild activity (1.66), which was associated with their working status (26.7% were retired).

The nutrient intake of subjects at baseline and end of each diet period are shown in Table 4. As expected, inclusion of hazelnut (40 g/day) into the diet resulted in a significant ($P<0.05$) increase in MUFA, whereas the percentage of energy from SFA was significantly ($P<0.05$) lower in hazelnut-enriched diet (8.3%) compared to that of the baseline

Table 3 Habitual physical activities

Type of activities	*IEI*	*Duration (h/day)*	*Energy cost of activities (kcal/h)*
Sleeping	1.0	8.0±1.0	8.0
Sitting at work (watching TV, reading, office work etc.)	1.7	12.4±1.4	19.9
Standing at work	2.5	3.2±1.1	7.3
Walking	3.5	1.2±0.9	4.6
Total			39.8 kcal/24 h PAL = 1.66

Abbreviations: IEI, integrated energy indices; PAL, physical activity level.
Values are means±s.d., $n=15$.

(9.6%). MUFA provided 11.5% of total energy at baseline and 17.4% in the hazelnut-enriched diet. Dietary fiber was higher ($P<0.05$) in both control and hazelnut-enriched diets compared with baseline. Dietary cholesterol concentrations did not change significantly among diet groups.

Compared with baseline, the hazelnut-enriched diet favorably decreased ($P<0.05$) the concentrations of VLDL cholesterol, TAG and Apo B by 29.5, 31.8 and 9.2%, respectively, while increasing HDL cholesterol concentrations by 12.6% (Figure 1 and Table 5). The ratios of total/HDL cholesterol and LDL/HDL cholesterol favorably decreased ($P<0.05$). Although there were no statistical significant changes in fasting levels of total and LDL cholesterol, glucose, Apo A-1 and homocysteine, it was appeared a decreasing trend for the parameters, particularly in total (5.2%) and LDL cholesterol (3.3%) to be lower in subjects consuming a hazelnut-enriched diet compared to that of the baseline. In addition, the hazelnut-enriched diet favorably altered HDL cholesterol and the ratio of total/HDL cholesterol concentrations ($P<0.05$) compared with that effects of the control diet. Therefore, hazelnut-enriched diet, despite its high-fat content, was superior to that of the control diet (Table 5).

The effects of hazelnut diet on lipid parameters and their overall effects on endothelial function were also examined. In spite of the improvement in lipid parameters, the endothelial functional improvements were negligible and did not reach statistical significance with the hazelnut-enriched diet.

Discussion

Theoretically, hazelnut is a fatty food and its regular consumption may be expected to lead to body weight gain. However, there were no significant changes in the body weight throughout the study period. None of the well-controlled metabolic-type feeding studies show significant changes in body weight compared to the nut and the nut-free control diet (Abbey *et al*., 1994; Spiller *et al*., 1998; Almario *et al*., 2001; Rajaram *et al*., 2001; Sabaté, 2003). An inverse relation between frequent nut consumption and BMI has been reported (Garg *et al*., 2003; Sabaté, 2003).

Table 4 Nutrient intake of subjects at baseline and during feeding periods

Nutrient	*Baseline*	*Diet periods*		F	P
		Control (P_1)	*Hazelnut-Enriched (P_2)*		
Energy (kcal/day)	2016±510[b]	2033±345[b]	2284±411[c]	6.613	0.004
Total protein (g/day)	71±20.1	71±12.8	76±13.4	1.155	0.330
% of energy	14.4±2.4	14.3±1.5	13.8±2.0	0.633	0.488
Vegetable protein (g/day)	36±11.8[b]	39±9.2[b]	44±11.0[c]	6.308	0.005
Carbohydrate (g/day)	270±74.3	274±55.8	284±69.6	0.582	0.566
% of energy	55.1±6.2[b]	55.2±4.6[b]	50.3±4.5[c]	12.571	0.002
Total fat (g/day)	69±21.5[b]	69±13.8[b]	91±12.9[c]	28.472	0.000
% of energy	30.4±5.9[b]	30.5±4.1[b]	36.0±3.9[c]	19.545	0.000
SFA (g/day)	21.5±7.5	19.7±4.1	20.6±3.0	0.851	0.404
% of energy	9.6±2.5[b]	8.8±1.9[b,c]	8.3±1.6[c]	6.004	0.007
MUFA (g/day)	25.4±6.7[b]	25.2±5.0[b]	43.3±5.3[c]	120.908	0.000
% of energy	11.5±2.3[b]	11.3±2.1[b]	17.4±3.0[c]	107.850	0.000
PUFA (g/day)	18.0±8.4[b]	19.8±7.7[b,c]	21.5±8.5[c]	4.562	0.019
% of energy	8.0±2.5	8.6±2.3	8.3±2.1	1.037	0.368
Cholesterol (mg/day)	189±94.9	160±49.0	171±47.0	0.998	0.381
Dietary fiber (g/day)	24.8±8.5[b]	29.8±8.2[c]	31.3±9.1[c]	8.539	0.001

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, ployunsaturated fatty acid.
Values are means±s.d., $n=15$.
Values on the same row not sharing a common superscript are significantly different, $P<0.05$.
F- and *P*-values show the general differences among baseline and diet periods ($P<0.05$; significantly different).

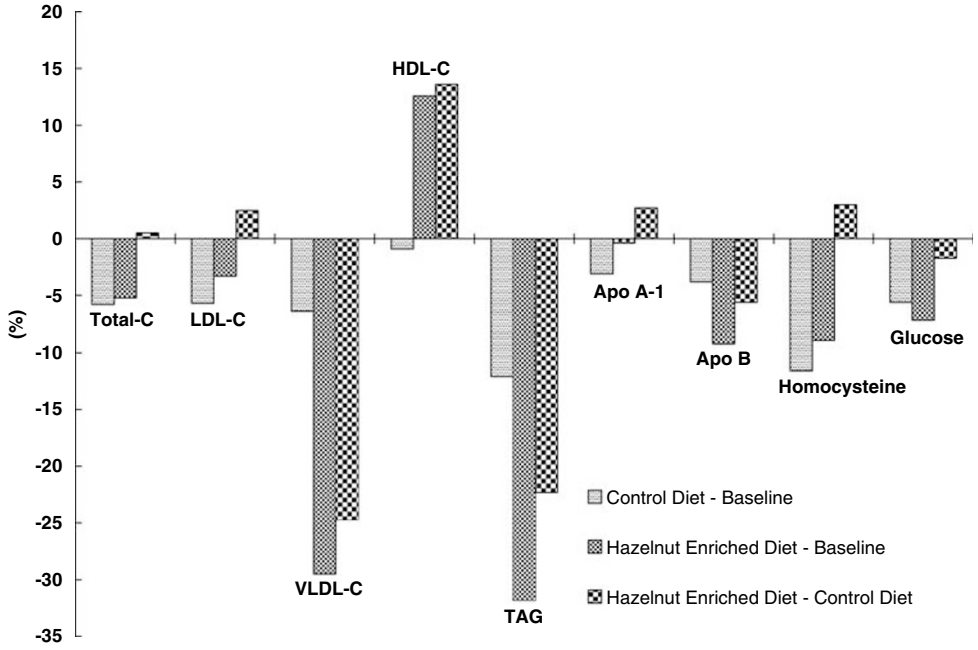

Figure 1 Comparison (%) of plasma cholesterol and lipoprotein profiles among baseline, control, and hazelnut-enriched diet. Abbreviations (TAG, triacylglycerol; Apo A-1, apolipoprotein A-1; Apo B, apolipoprotein B).

Hazelnut, which contains lipid-lowering constituents such as MUFA, PUFA, β-sitosterol and soluble dietary fiber (Jones *et al*., 1997; Brown *et al*., 1999; Zambón *et al*., 2000; Alasalvar *et al*., 2003b, c), offers an opportunity as a potential hypercholesterolemic 'heart-healthy' diet component. Incorporation of hazelnut (40 g/day) into human diet favorably altered multiple plasma lipid variables in hypercholesterolemic adult men (Figure 1 and Table 5). Our results are comparable to those reported for other tree nuts such as almond (Hyson *et al*., 2002), macadamia (Garg *et al*., 2003), pecan (Rajaram *et al*., 2001), pistachio (Edwards *et al*., 1999) and walnut (Zambón *et al*., 2000), despite the fact that inclusion of

Table 5 Serum lipid, lipoprotein, homocysteine and glucose levels at initial and at the end of each diet period

Variables	*Baseline*	*Diet periods*		F	P
		Control (P_1)	*Hazelnut-Enriched (P_2)*		
		mg/dl (mmol/l)			
Total cholesterol	240.1±21.0	226.3±23.6	227.6±25.7	3.033	0.064
	(6.22±0.54)	(5.86±0.61)	(5.89±0.67)	3.033	0.064
LDL cholesterol	155.5±23.4	146.7±20.4	150.4±21.0	0.827	0.448
	(4.03±0.61)	(3.80±0.53)	(3.90±0.55)	0.827	0.448
VLDL cholesterol	40.7±26.2^{b}	38.1±29.4^{b,c}	28.7±13.1^{c}	4.983	0.030
	(1.05±0.68)^{b}	(0.99±0.76)^{b,c}	(0.74±0.34)^{c}	4.983	0.030
HDL cholesterol	43.9±9.6^{b}	43.5±11.3^{b}	49.4±12.6^{c}	10.094	0.001
	(1.14±0.25)^{b}	(1.13±0.29)^{b}	(1.28±0.33)^{c}	10.094	0.001
TAG	203.3±130.9^{b}	178.7±134.0^{b,c}	138.7±65.9^{c}	6.073	0.006
	(2.30±1.48)^{b}	(2.02±1.51)^{b,c}	(1.57±0.75)^{c}	6.073	0.006
Glucose (fasting)	93.5±9.7	88.3±11.9	86.8±8.6	3.058	0.063
	(5.19±0.54)	(4.90±0.66)	(4.82±0.48)	3.058	0.063
Total/HDL cholesterol	5.66±1.03^{b}	5.53±1.48^{b}	4.85±1.21^{c}	12.943	0.000
	(0.146±0.027)^{b}	(0.143±0.038)^{b}	(0.126±0.031)^{c}	12.943	0.000
LDL/HDL cholesterol	3.64±0.67^{b}	3.60±1.07^{b,c}	3.23±0.93^{c}	3.395	0.048
	(0.094±0.017)^{b}	(0.093±0.028)^{b,c}	(0.084±0.024)^{c}	3.395	0.048
		mg/dl (g/l)			
Apolipoprotein A-1	136.3±19.0	132.1±15.5	135.7±19.9	0.598	0.557
	(1.36±0.19)	(1.32±0.15)	(1.36±0.20)	0.598	0.557
Apolipoprotein B	133.1±22.2^{b}	128.0±22.3^{b,c}	120.8±17.2^{c}	5.417	0.010
	(1.33±0.22)^{b}	(1.28±0.22)^{b,c}	(1.21±0.17)^{c}	5.417	0.010
		mg/l (μmol/l)			
Homocysteine	1.97±0.48^{c}	1.74±0.48^{b}	1.80±0.47^{b,c}	4.523	0.020
	(14.6±3.5)^{c}	(12.9±3.5)^{b}	(13.3±3.5)^{b,c}	4.523	0.020

Abbreviation: HDL, high-density lipoprotien; LDL, low-density lipoprotien; TAG, triacylglycerol.
Values are means±s.d., $n=15$.
Values on the same row not sharing a common superscript are significantly different, $P<0.05$.
F and *P*-values show the general differences among baseline and diet periods, ($P<0.05$; significantly different).

hazelnut into the human diet in our study was less than half of those reported for other nuts.

The intake of SFA generally increases the risk of CHD by increasing the concentrations of total and LDL cholesterol, and Apo B, and increasing the ratios of total/HDL cholesterol and LDL/HDL cholesterol (NRC, 1989). The results presented in Table 5 suggest that consumption of hazelnut, despite its high fat content, has a beneficial effect on total cholesterol. Hazelnut-rich MUFA diet demonstrated a 5.2% reduction in total cholesterol compared with baseline (Figure 1). Durak *et al* (1999), provided about 68–69 g/day of hazelnut (1 g/day/kg body weight) in the diet of 30 healthy students and found a significant reduction ($P<0.005$) in their total plasma cholesterol concentrations. However, the amount of hazelnut consumed was about 30 g/day more than that used in the present study.

The significance of both LDL and HDL cholesterol concentrations in affecting CHD risk is well established. Controlled clinical trials have estimated that for every 1% reduction in total and LDL cholesterol concentrations, there is a approximately 1.5% reduction in the incidence of CHD (Davis *et al*., 1990; NCEP, 1993). Moreover, coronary events are reduced by 2–3% for every 1 mg/dl increase in HLD cholesterol (Gordon *et al*., 1989). Thus, an increase of 89 mg/dl in TAG concentrations is associated with a 14% increase in the incidence of CHD in men and a 37% increase in women (Austin *et al*., 1998). In this respect, the average of 8.5% decrease in both total and LDL cholesterol, 5.5 mg/dl increase in HDL cholesterol, and 64.4 mg/dl decrease in TAG concentrations after consumption of hazelnut-enriched diet would be expected to decrease the risk of CHD by approximately 13, 11–17 and 10%, respectively. Overall, hazelnut-enriched diet compared with baseline is expected to decrease the risk of CHD. Epidemiologic studies (Fraser *et al*., 1992; Hu *et al*., 1998) have estimated that the percentage decrease in CHD risk with frequent consumption of nuts is up to 30–50%.

Much attention has been focused on LDL cholesterol, since blood concentrations have frequently been found to correlate with CHD and likewise with the levels of dietary saturated fat intake. The intake of SFA should be reduced to 7% of daily energy intake in order to maintain normal LDL cholesterol (Davis *et al*., 1990). In this study, the percentage of total fat of hazelnut-enriched diet was significantly ($P<0.05$) increased by 5.6% as compared to the control diet. The percentage of total energy obtained from SFA at baseline was 9.6, as this was significantly ($P<0.05$) reduced to 8.3 in the hazelnut-enriched diet (Table 4).

Although not significant, a 3.3% decrease in LDL cholesterol concentrations by the hazelnut-enriched diet may play a very important role in the reduction of incidence of CHD, as stated above. It is possible that significant reduction in total and LDL cholesterol concentrations in our study may be attained by increased supplementation of hazelnut in the human diet. Spiller *et al*. (1998) reported that adding 100 g of almonds, as MUFA-rich foods, to the diet of hypercholesterolemic volunteers reduced total and LDL cholesterol from 9 to 12% compared with baseline values, respectively.

Hazelnut-enriched diet significantly ($P<0.05$) increased the concentrations of HDL cholesterol by 12.6 and 13.6% compared both with baseline and control diet, respectively. In contrast, there was no difference between the control diet and the baseline. The significant increase in HDL cholesterol concentrations has been reported after consumption of tree nuts such as hazelnut (Durak *et al*., 1999), almond (Hyson *et al*., 2002), pistachio (Edwards *et al*., 1999) and macadamia (Garg *et al*., 2003). It can be speculated that the increase in HDL cholesterol might be most likely due to decreased triglycerides, where HDL becomes less of a substrate for catabolism (Tokgözoglu, 1999). However, it is still hard to explain the absence of a significant increase in Apo A-1 in the present study. Genetic or metabolic variation of apoproteins, especially Apo A-1, $ApoC_3$, Apo E and Apo B can have a profound effect on the metabolism of these lipoproteins (Tokgözoglu, 1999). A recent study by Kinosian *et al*. (1995) observed that changes in the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations were better predictors of CHD than the changes in LDL cholesterol alone. The risk of CHD is increased when the ratios of total/HDL cholesterol and LDL/HDL cholesterol concentrations exceed >4.5 and >3.5, respectively (Mahley *et al*., 1995). Hazelnut-enriched diet compared with baseline favorably altered ($P<0.05$) these important parameters in cardioprotective direction. Edwards *et al* (1999) reported that substituting 20% of the daily caloric intake in the form of pistachio nuts after 3-week period significantly reduced both total/HDL cholesterol and LDL/HDL cholesterol ratios in subjects with moderate hypercholesterolemia compared to initial values.

Unlike the study by Durak *et al*. (1999) in which there was a increase in TAG in the hazelnut supplemented diet, we found a significant ($P<0.05$) reduction in TAG (31.8%) after supplementation with a hazelnut-enriched diet compared with baseline (Figure 1). Our results are in agreement with those previously published for other tree nuts (Rajaram *et al*., 2001; Hyson *et al*., 2002) and other MUFA-rich foods (Grundy, 1986; Kris-Etherton *et al*., 1999a). The present study also demonstrated that a high-MUFA-rich diet had the added benefit of not increasing TAG, as the low-cholesterol diets recommended by the American Heart Association tend to do (Ginsberg *et al*., 1990). In addition, there was a significant ($P<0.05$) decrease in VLDL cholesterol concentrations (29.5%) after supplementation with a hazelnut-enriched diet compared with that of the control diet. VLDL cholesterol, which is the primary carrier of hepatic TAG, may be responsible for causing a decrease in TAG (Roche *et al*., 1998).

Studies give suggestive evidence that both the dietary fat content and the dietary fatty acid composition affects glucose metabolism in healthy subjects. High-fat diets have been associated with the worsening of insulin sensitivity in many clinical studies of nondiabetic persons and in several epidemiologic studies (Lovejoy *et al*., 2002). In patients with established diabetes, high-fat diets increased fasting and postprandial glucose and insulin concentrations. However, it was reported by Garg *et al*. (1988) that the high-MUFA diets resulted in improvements in glycemia. In the present study, no significant differences were observed in fasting glucose concentration in the group of hazelnut-enriched diet compared to others.

An elevated homocysteine concentrations is a risk factor for CVD (Feldman, 2002). No significant changes were observed between the hazelnut-enriched diet and the baseline diet, whereas this decrease was significant ($P<0.05$) when control diet was compared with baseline. Homocysteine concentration increases in relation to insufficient intake or metabolism of folate and vitamins B_6 and B_{12} (Scott, 2000). However, our study was not designed and does not provide any clues regarding the interaction between total homocysteine level and B vitamins. The relative risk of CVD is increased significantly when homocysteine concentrations exceed 15.8 μmol l (Robinson *et al*., 1998). The homocysteine values throughout this study were lower (<14.6 μmol/l) than that reported for increased CVD risk.

Hazelnut has many beneficial health attributes (Alasalvar *et al*., 2003b, c). The benefits of inclusion of hazelnut into the human diet is partly related to the fatty acids of hazelnut oil, which is rich in MUFA (83.2%) and PUFA (9%) (Alasalvar *et al*., 2003c). In addition to this, as stated, there may be a number of fat- and non-fat constituents in hazelnut that may elicit additional cholesterol-lowering and cardioprotective effects (Kris-Etherton *et al*., 1999b). In contrast, Hyson *et al*. (2002) observed that the effects of whole almond and almond oil on plasma lipids were not different from one another in healthy men and women. Thus, these researchers concluded that the lipid-lowering effect of almond was mediated primarily by the almond oil fraction. Although our study was not aimed at examining the independent effects of non-fat constituents, further studies are needed to identify these non-fat constituents and establish their relative cholesterol-lowering potency in tree nuts. At the same time, it should be born in mind that the alteration in carbohydrates when the hazelnut-enriched diet were introduced, may have an effect on the differences in lipid levels and could explain some of the findings in the present study. To distinct this, a randomized cross-over and at least two fats keeping the same carbohydrate level and with the difference in MUFA due to introduction of hazelnuts controlled for other differences in fatty acids that would be introduced

simultaneously. However, this type of study is admittedly difficult to design. The present study, however, suggests the same probable cardio-protection that other nut feeding studies have shown.

In summary, the hazelnut-enriched diet favorably altered the plasma cholesterol and lipoprotein profiles despite an increase in the dietary fat content. On the basis of the results of the present study, although the limited number of subjects were included, high-MUFA-rich hazelnut diet is preferred to a low-fat control diet because of more favorable effects on the CHD risk profile. Further research can be extended to both men and women subjects with normal and hypercholesterolemia for comparison.

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