Hazelnut Oil Administration Reduces Aortic Cholesterol Accumulation and Lipid Peroxides in the Plasma, Liver, and Aorta of Rabbits Fed a High-cholesterol Diet

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Hazelnut oil (HO) is rich in monounsaturated fatty acids and antioxidants. We wanted to investigate the effect of HO on lipid levels and prooxidant–antioxidant status in rabbits fed a high-cholesterol (HC) diet. An HC diet caused significant increases in lipids and lipid peroxide levels in the plasma, liver, and aorta together with histopathological atherosclerotic changes in the aorta. Glutathione levels, glutathione peroxidase, and glutathione transferase activities decreased significantly, but superoxide dismutase activity and vitamin E and C levels remained unchanged in the livers of rabbits following HC diet. HO supplementation reduced plasma, liver, and aorta lipid peroxide levels and aorta cholesterol levels together with amelioration in atherosclerotic lesions in the aortas of rabbits fed an HC diet, without any decreasing effect on cholesterol levels in the plasma or liver. HO did not alter the antioxidant system in the liver in the HC group. Our findings indicate that HO reduced oxidative stress and cholesterol accumulation in the aortas of rabbits fed an HC diet.

Key words: hazelnut oil; atherosclerosis; lipid peroxidation; antioxidants; rabbit

The composition and amount of lipids in the diet are effective factors in the prooxidant–antioxidant balance in the organism.1) Although saturated fatty acids (SFA) increase the tendency to atherosclerosis by increasing serum cholesterol levels,1,2) polyunsaturated fatty acids (PUFAs) have a reducing effect on serum lipid levels.3) But PUFAs increase the susceptibility of the organism to lipid peroxidation and disturb the prooxidant–antioxidant balance in favor of prooxidation.4,6) Hence some investigators have suggested that PUFAs might accelerate the development of atherosclerosis.7,8) On the other hand, monounsaturated fatty acids (MUFAs), like PUFAs, have been reported to have antilipidemic effects.9,10) Since MUFAs are resistant to oxidation, they may lower the susceptibility of the organism to lipid peroxidation9–12) and LDL oxidation.13,14) Olive oil, which is rich in MUFAs, and is known as an antiatherogenic oil, has been gaining importance in nutrition.

Hazelnut oil (HO), like olive oil, is rich in MUFAs.15,16) Seventy-five percent of HO production in the world is in the Black Sea region of Turkey. The use of HO has increased in recent years. HO contains high amounts of vitamin E and flavonoids and luteolin,15–17) and has higher total radical scavenger capacity and resistance to sunlight than olive oil.18,19) Durak et al.20) have shown that hazelnut administration itself caused decreases in serum cholesterol and malondialdehyde (MDA) levels and an increase in serum antioxidant activity in healthy humans. But data concerning the effects of HO on serum lipids, lipoproteins, atherosclerosis, and the prooxidant–antioxidant balance are extremely limited.21,22) For the first time we determined that HO supplementation reduces lipid peroxide levels in plasma and apolipoprotein B 100-containing lipoproteins and ameliorates aortic atherosclerotic lesions, but does not alter plasma lipid levels in rabbits fed an high cholesterol (HC) diet, as shown in our recent study.23) In the current study, we wanted to investigate the effects of HO on cholesterol accumulation and lipid peroxidation in the liver and aorta, as well as on the hepatic antioxidant system in rabbits fed an HC diet.

Abbreviations: HC, high cholesterol; HO, hazelnut oil; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acid; MDA, malondialdehyde; DC, diene conjugate; GSH, glutathione; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione transferase; LDL, low density lipoprotein

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Material and Methods

Animals and diets. Male New Zealand white rabbits weighing 2.0–2.5 kg were used in all the experiments. Animals were obtained from Eczacıbaşı Pharmaceutical Company (Istanbul, Turkey). The animals were fed diets containing different amounts of cholesterol and HO in different periods to produce more stable atherosclerotic lesions and to eliminate hepatic, gastrointestinal, and allergic complications. HO and cholesterol were supplied by Ordu Sanayii A.S. (Turkey) and Sigma (U.S.A.), respectively. 100 g of HO contains 7.1 g SFA, 82.1 g MUFA, 10.7 g PUFA, and 25 mg vitamin E. The iodine number of HO was 85 (required g of iodine per 100 g of oil), and the peroxide value was less than 10 (meq O$_2$/kg of oil). The percent fatty acid composition of HO is 5.2% palmitic acid, 0.18% palmitoleic acid, 2.36% stearic acid, 81.7% oleic acid, 10.1% linoleic acid, 0.12% linolenic acid, and 0.17% gadoleic acid.

The rabbits were divided into four groups: a) A control group (n = 6): The animals were fed commercial rabbit feed containing 11% moisture, 10% crude ash, 15% protein, 3.5% crude fat, 47% carbohydrate, and 7.5% cellulose, 3.5% salt mixture (AIN 76), and 1% vitamin mixture (AIN 76) (w/w). b) An HO group (n = 6): Rabbits were fed the control diet enriched with HO (w/w) (5% for 8 weeks, 7.5% for 4 weeks, and 10% for 2 weeks successively). c) An HC group (n = 8): The animals were fed the control diet enriched with cholesterol (w/w) (0.5% for 8 weeks, 0.75% for 4 weeks, and 1% for 2 weeks successively). d) An HC + HO group (n = 6): The animals were fed the control diet enriched with cholesterol plus HO (0.5 + 5% for 8 weeks, 0.75 + 7.5% for 4 weeks, and 1 + 10% for 2 weeks successively).

The diets were stored at 4°C. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages. The fatty acid composition of the diets was measured in the laboratories of the Unilever Company. According to these measurements, the control and HC (1%) diets contained approximately 0.8 g saturated fatty acids (SFA), 1.0 g MUFA, and 1.8 g PUFA per 100 g diet. HO (10%) and HC + HO (1% + 10%) contained 1.1 g SFA, 10.3 g MUFA, and 2.2 g PUFA per 100 g diet. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of Istanbul.

Sample collection and preparation. At the end of the feeding period of 14 weeks, the animals were fasted overnight and anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Blood was collected in tubes containing EDTA by cardiac puncture. Plasma samples were obtained by centrifugation and stored at −70°C until analysis.

The livers were rapidly removed, washed in 0.9% NaCl, and kept in ice. The aorta, from the aortic valve to the renal artery, was quickly removed, rinsed, and cut into small segments. The livers and aortas were stored at −70°C until analysis.

Biochemical Analyses. Plasma cholesterol and triglyceride levels were measured with the kits from Sigma. The degree of endogenous lipid peroxidation in the plasma was assessed by two different methods: a) Malondialdehyde (MDA) levels were determined according to the method of Buege and Aust. 24) b) Diene conjugate (DC) formation was measured in chloroform:methanol [2:1] extracts of the plasma. Extracted lipids were evaporated and dissolved in cyclohexane and DC was measured at 233 nm. 24)

Liver portions were homogenized in ice-cold 0.1 M KCl (10%, w/w). Lipids were extracted with chloroform:methanol [2:1]. After extraction and evaporation, hepatic lipids were re-dissolved in isopropanol, and hepatic cholesterol and triglyceride levels were assayed by the kits provided from Sigma. The degree of lipid peroxidation in the liver was also assessed by two different methods. First, the level of MDA was measured by the thiobarbituric acid test according to the method of Ohkawa et al. 25) The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Second, DC was determined in hepatic lipid extracts at 233 nm spectrophotometrically and calculated using a molar extinction coefficient of 2.52 × 10$^{4}$M$^{-1}$cm$^{-1}$. 24)

Liver glutathione (GSH) levels were measured with 5,5'-dithiobis-(2-nitrobenzoate) at 412 nm. 26) Vitamin E and vitamin C levels were measured in liver homogenates by the method of Desai 27) and Omaye et al., 28) respectively. Hepatic superoxide dismutase (SOD) activity was assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of orthodianisidine in postmitochondrial fractions. 29) Glutathione peroxidase (GSH-Px) 30) and glutathione transferase (GST) 31) activities were measured using cumene hydroperoxide and 1-chloro-2,4-dinitrobenzene as substrates, respectively, in postmitochondrial fractions. Protein levels were determined using bichinchoninic acid. 32)

Aorta lipids were extracted with a chloroform:methanol mixture. Aortic cholesterol and DC 24) levels were determined in these lipid extracts. Aortic MDA levels 25) were measured in 10% (w/w) homogenates of aorta, as described for the liver.

Histopathological Analysis. Aortas were dissected and fixed in 10% buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological study.

Statistical Analysis. The results were expressed as mean ± SD. Statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post-hoc test.
No statistically significant differences were noted in terms of increased body weight, liver weight/body weight, or consumption of food between the fed HO, HC, and HC + HO diets during the 14 week period (data not shown). In addition, no allergic or gastrointestinal complications or hepatotoxicity were observed during the experimental period.

The results are shown in Fig. 1–5 and Table 1. According to them:

a) Cholesterol, triglyceride, MDA and DC levels in plasma, liver and aorta increased significantly in the HC group as compared to the control group. The HO diet did not have any effect on lipid or lipid peroxide levels in the plasma, liver, or aorta of rabbits as compared to control. HO supplementation in rabbits fed on an HC diet did not alter plasma or liver cholesterol levels, but significantly lowered aorta cholesterol levels as compared to the HC group. Plasma triglyceride levels increased, but liver triglyceride levels remained unchanged under HO supplementation in rabbits fed an HC diet. The MDA and DC levels in plasma, liver, and aorta were found to decrease significantly in the HC + HO group as compared to the HC group (Fig. 1–3).

b) HO supplementation in normal rabbits did not influence hepatic antioxidant system parameters. The GSH level and GSH-Px and GST activities were significantly lower, but the vitamin E and C levels and SOD activity were unchanged in the livers of the rabbits in the HC group. When the results for the HC + HO group were compared with those for the HC group, no differences were observed in the levels of GSH, vitamin E, or vitamin C or the activities of SOD, GSH-Px, or GST in the liver (Table 1).

c) Normal aortic structure was seen in the control and HO groups. Aortic tissue from the HC group exhibited endothelial damage in some subendothelial areas, with foamy cell infiltration. The macrophage content in the cross-sectional lesioned area of the aorta was higher in the HC group, and typical atheromatous plaques were observed in the same area (Fig. 4). Although atheroma plaque formation was observed in rabbits fed the HC diet, no such formation was observed in rabbits fed HC + HO, but a light-medium deposit of histiocytes was observed in the aortic intima in rabbits fed HC + HO (Fig. 5).

**Discussion**

Increased levels of cholesterol, particularly low density lipoprotein (LDL) cholesterol are associated with a higher risk of atherosclerosis. Although the precise mechanism by which cholesterol and LDL-
roles in atherogenesis. Several studies suggest that oxidatively modified LDL plays an important role in the development of atherosclerosis by uptake via the macrophage scavenger receptor and foam cell formation. Cholesterol feeding has often been used to elevate serum and tissue cholesterol levels, and rabbits are the animal most susceptible to the development of atherosclerosis via cholesterol feeding. HC diet results in alterations in prooxidant–antioxidant status in several models. Oxidative stress is now recognized as an important aspect in the pathogenesis of atherosclerosis. Oxidized low-density lipoprotein (LDL) and apolipoprotein (apo) B are the predominant oxidized lipoprotein species in the arterial wall and are implicated in the progression of atherosclerosis. Despite the wealth of information on oxidized LDL and atherosclerosis, the mechanism by which it causes atherosclerosis has not yet been completely elucidated, it has been suggested that hypercholesterolemia and/or hypercholesterolemic atherosclerosis and lipid peroxidation play complementary roles in atherogenesis. Several studies suggest that oxidatively modified LDL plays an important role in the development of atherosclerosis by uptake via the macrophage scavenger receptor and foam cell formation.

### Table 1. Effect of Hazelnut Oil (HO) on Glutathione (GSH), Vitamin E, and Vitamin C Levels and Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px), and Glutathione Transferase (GST) Activities in the Liver of Rabbits Fed a High-cholesterol Diet (HC)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>HO (n = 6)</th>
<th>HC (n = 8)</th>
<th>HC + HO (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (nmol/mg protein)</td>
<td>30.0 ± 3.58a</td>
<td>29.2 ± 3.41a</td>
<td>23.6 ± 4.00b</td>
<td>25.0 ± 4.45ab</td>
</tr>
<tr>
<td>Vitamin E (nmol/g tissue)</td>
<td>59.8 ± 11.3a</td>
<td>59.4 ± 15.5a</td>
<td>63.3 ± 13.0a</td>
<td>65.2 ± 10.5a</td>
</tr>
<tr>
<td>Vitamin C (nmol/g tissue)</td>
<td>420.2 ± 72.6a</td>
<td>381.0 ± 58.5a</td>
<td>297.0 ± 111.3a</td>
<td>338.4 ± 115.3a</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>21.6 ± 2.43a</td>
<td>17.9 ± 3.44a</td>
<td>18.2 ± 3.42a</td>
<td>17.9 ± 2.37a</td>
</tr>
<tr>
<td>GSH-Px (µmol/mg protein/min)</td>
<td>2.04 ± 0.32a</td>
<td>1.97 ± 0.38a</td>
<td>1.49 ± 0.24b</td>
<td>1.65 ± 0.24ab</td>
</tr>
<tr>
<td>GST (µmol/mg protein/min)</td>
<td>4.63 ± 0.58a</td>
<td>4.31 ± 0.61a</td>
<td>1.51 ± 0.39b</td>
<td>1.49 ± 0.43b</td>
</tr>
</tbody>
</table>

1 HO, hazelnut oil group; HC, high cholesterol group; HC + HO, high cholesterol plus hazelnut oil groups. Each value is expressed as the mean ± SD. Differences among groups were investigated by ANOVA. After a significant ANOVA result, a comparison was made using Tukey’s honestly significant difference test. Values not sharing a common superscript letter are significantly different (p < 0.05).
Fig. 3. Aorta Cholesterol, Malondialdehyde (MDA), and Diene Conjugate (DC) Levels in Rabbits Fed a High-cholesterol (HC) Diet with or without Hazelnut Oil (HO).

CON, control group (n = 6); HO, hazelnut oil group (n = 6); HC, high cholesterol group (n = 8); HC + HO, high cholesterol plus hazelnut oil group (n = 6). Each value is expressed as the mean ± SD. Differences among groups were investigated by ANOVA. After a significant ANOVA result, a comparison was made using Tukey’s honestly significant difference test. Bars with different letters are significantly different (p < 0.05).

Fig. 4. Histopathological Findings in the Aorta in Cholesterol-fed Rabbits (Magnification × 310, stained with hematoxylin–eosin).
1, internal elastic lamina; 2, foam cell; 3, macrophage; 4, atheromatous plaque.
tissues as well as typical atherosclerotic changes in the aorta of rabbits.\textsuperscript{12,35–37} In our study, HC diet also caused oxidative stress in rabbits. These findings are in accordance with those of other investigators\textsuperscript{12,35,37} and our earlier study.\textsuperscript{36}

The literature related to atherosclerosis has emphasized the beneficial effects of MUFA containing oils, especially olive oil, on lipids and lipid peroxidation.\textsuperscript{3,9–14} But the effects of HO consumption on serum lipids and lipid peroxides are not clearly known. In our study, an HO diet given alone to normal rabbits did not affect plasma or tissue lipids, or prooxidant–antioxidant status. On the other hand, HO supplementation did not alter cholesterol levels in plasma and liver, but decreased aortic cholesterol levels in hypercholesterolemic rabbits. In addition, HO supplementation reduced atherosclerotic lesions in rabbit fed an HC diet. On the other hand, the HC + HO diet caused significant decreases in MDA and DC levels in plasma, liver, and aorta, but antioxidant system parameters remained unchanged in the livers of rabbits in the HC + HO groups as compared to the HC group. These findings indicate that HO has a reducing effect on lipid peroxidation without any change in antioxidant system parameters. Although it is not clear which mechanism plays a role in the reduction of lipid peroxide levels, the replacement of PUFA by MUFA in plasma and tissues and/or the powerful antioxidant capacity of hazelnut oil itself might be effective.

In conclusion, this study indicates that HO reduces oxidative stress and cholesterol accumulation in the aorta of rabbits fed an HC without any reducing effect on plasma lipids. The results of our current and previous\textsuperscript{23} studies suggest that HO may have an antiatherogenic potential which may be related to its reducing effect on oxidative stress, especially on LDL oxidation, but not to its antilipidemic action.

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References


