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High cholesterol diet supplemented with sunflower seed oil but not olive oil stimulates lipid peroxidation in plasma, liver, and aorta of rats

Hüseyin Bulur,* Gül Özdemirler,† Büge Öz,‡ Gülçin Toker,† Muzaffer Öztürk,* and Müjdat Uysal†

**Cardiology Institute of Istanbul University, Istanbul, Turkey; †Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; and ‡Department of Pathology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey*

To determine the effect of a high cholesterol diet supplemented with sunflower seed oil or olive oil on plasma, liver, and aorta lipid peroxidation, rats were fed a basal diet, a high cholesterol diet (basal diet containing 2% cholesterol and 0.5% cholic acid), or a high cholesterol diet supplemented with 10% (wt/wt) sunflower seed oil or 10% (wt/wt) olive oil for 4 months. In rats fed the high cholesterol diet supplemented with sunflower seed oil, plasma, liver, and aorta lipid peroxide levels and the aorta cholesterol to phospholipid ratio were greater than in rats fed the high cholesterol diet. In contrast, no change was observed in plasma, liver, and aorta lipid peroxidation and the cholesterol to phospholipid ratio in rats fed the high cholesterol diet containing olive oil as compared with the high cholesterol diet. In addition, atherosclerotic lesions were not detected in the aorta of all groups. We concluded that a high cholesterol diet supplemented with polyunsaturated fats, but not with monounsaturated fats, seems to have a tendency to exaggerate lipid peroxidation. (J. Nutr. Biochem. 6:547–550 1995.)

Keywords: lipid peroxidation; lipids; high cholesterol diet; sunflower seed oil; olive oil; rats

Introduction

Free radical–induced lipid peroxidation has been proposed as an etiological factor in atherosclerosis.[1–3] A direct correlation between lipid peroxidation in the aorta and the severity of atherosclerotic lesions has been detected in humans[4] and experimental animals.[5,6]

Polyunsaturated fats have been widely used to lower serum cholesterol levels and thus to prevent the development of atherosclerosis.[7] However, polyunsaturated fatty acids (PUFAs) are easily susceptible to peroxidation, and a high intake of dietary PUFA might overwhelm the normal antioxidant defences of the organism.[8–11] On the other hand, monounsaturated fatty acids (MUFAs) have been recently reported to lower serum cholesterol levels.[12,13] In addition, it has been suggested that MUFAs have a protective effect against lipid peroxidation.[9,10,14]

In this study we have examined the effects of a high-cholesterol diet supplemented with sunflower seed oil (rich in PUFAs) or olive oil (rich in MUFAs) on lipid peroxidation and cholesterol deposition in rats' liver and aorta as well as histopathology.

Methods and materials

Male Wistar rats (160 to 180 g) were obtained from the Experimental and Medical Research Center, University of Istanbul, Turkey. The animals were maintained on a basal diet (control), a high cholesterol diet (HC), a high cholesterol diet supplemented with sunflower seed oil (HC + SO), and a high cholesterol diet supplemented with olive oil (HC + OO). The basal diet contained 11% moisture, 10% crude ash, 15% protein, 3.5% crude fat, 47% carbohydrate, and 7.5% cellulose (wt/wt). The high cholesterol diet consisted of the basal diet supplemented with 2% (wt/wt)

Address reprint requests to Dr. Müjdat Uysal at the Department of Biochemistry, Istanbul Medical Faculty, Çapa, 34390, Istanbul, Turkey.
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Table 1 Fatty acid and vitamin E amounts

Substance	Sunflower seed oil (g/100 g)	Olive oil (g/100 g)
*SFA	8.5	14.3
MUFA	10.5	78.5
PUFA	81.0	7.1
Vitamin E	0.042	0.008

*Saturated fatty acid.

cholesterol and 0.5% (wt/wt) cholic acid. The HC + SO and HC + OO groups were fed by a high cholesterol diet supplemented with 10% (wt/wt) sunflower seed oil or 10% (wt/wt) olive oil, respectively, for 4 months. Sunflower seed oil and olive oil were supplied by Komili Co. (Ayvalik, Turkey), and their fatty acid and vitamin E contents are given in *Table 1*.

The diets were prepared weekly and stored at 4°C. The animals were allowed free access to food and sterile water and were kept in wire-bottemed stainless steel cages. At the end of the feeding period, the animals were anesthetized with diethyl ether, and blood was collected in heparinized syringes. Plasma lipid peroxide levels were determined according to the method of Yagi et al.[15] Plasma cholesterol, phospholipid, and triglyceride levels were measured with the kits from Biotrol Diagnostic (France).

The livers were rapidly removed, washed in 9 g/L of NaCl and kept in ice. Liver portions were homogenized in ice-cold 0.15 mol/L (10%, wt/vol). Lipids were extracted with chloroform: methanol (2:1)[16] for determination of hepatic cholesterol, phospholipid, and triglyceride levels. Hepatic cholesterol and triglyceride levels were measured again with the Biotrol Diagnostic Kits, and phospholipid levels were determined according to the Gomori procedure.[17] The degree of lipid peroxidation was assessed by two different methods in the liver. First, the level of malondialdehyde (MDA) was measured by the thiobarbituric acid test according to the method of Ohkawa et al.[18] The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Second, diene conjugate formation was determined spectrophotometrically at 233 nm by the method of Buege and Aust[19] using the original 10% homogenate. The approximate amounts of hydroperoxides were calculated using a molar extinction coefficient of 2.52×10^4 $(mol/L)^{-1}$ cm^{-1}.

Liver glutathione levels were measured with 5,5′-dithiobis-(2-nitrobenzoate) at 412 nm according to Ellman's method.[20] The protein concentration of liver homogenates was determined by the method of Lowry et al.[21]

The aorta, from the aortic valve to the renal artery, was quickly removed, rinsed, cut into small segments, and aorta lipids were extracted with chloroform:methanol (2:1). Aortic cholesterol, phospholipid, and diene conjugate levels were determined in this lipid extract as described for the liver. Histopathologic examinations of liver and aortic segments were also performed.

The results were expressed as Mean ± SD. Statistical analysis was performed using one-factor analysis of variance, and the Tukey test for multiple comparison. Differences with $p < 0.05$ were considered statistically significant.

Results

Plasma, liver, and aorta lipids are shown in *Table 2*. Plasma and liver cholesterol levels were found to increase in the HC, HC + SO, and HC + OO groups. The highest increases in cholesterol levels were observed in the HC + OO group. Liver phospholipids remained unchanged but liver triglyceride levels significantly increased in all groups compared with controls.

When aorta cholesterol levels were compared by one-factor analysis of variance, the differences among groups were found to be significant ($P < 0.05$). However, when they were analyzed by a multiple comparison method, the differences were not significant. Aorta phospholipids did not differ among all groups. The cholesterol to phospholipid ratio in aorta was elevated in the HC + SO and HC + OO groups compared with controls. On the other hand, this ratio was greater only in the HC + SO group when the comparison was done with the HC group.

Table 3 shows the plasma, liver, and aorta lipid peroxide levels. Plasma and liver malondialdehyde levels increased in the HC + SO group compared with the control, HC, and HC + OO groups. Liver diene conjugate levels were also elevated in the HC + SO group compared with the HC and HC + OO groups. Aorta diene conjugates were also increased in the HC + SO group compared with controls.

Liver glutathione levels were decreased only in the HC + SO group when compared with all other groups (*Table 3*).

Table 2 Plasma, liver, and aorta lipid concentrations in rats fed high cholesterol diets supplemented with sunflower seed oil or olive oil

Parameter	Control	HC	HC + SO	HC + OO
Plasma				
Cholesterol (mmol/L)	1.93 ± 0.30^a	3.54 ± 0.87^b	4.92 ± 0.89^c	6.38 ± 1.14^d
Phospholipid (mmol/L)	1.49 ± 0.10^a	2.03 ± 0.36^b	$1.72 \pm 0.18^{a,b}$	1.97 ± 0.38^b
Triglyceride (mmol/L)	0.78 ± 0.11^a	$1.00 \pm 0.22^{a,b}$	$0.94 \pm 0.20^{a,b}$	1.15 ± 0.20^b
Liver				
Cholesterol (μmol/g)	8.71 ± 1.90^a	86.37 ± 20.63^b	180.27 ± 47.51^c	271.61 ± 70.16^d
Phospholipid (μmol/g)	41.13 ± 5.64	42.22 ± 1.54	39.89 ± 3.72	39.92 ± 4.09
Triglyceride (μmol/g)	8.81 ± 2.70^a	21.37 ± 4.41^b	42.51 ± 10.82^c	38.39 ± 5.38^c
Aorta				
Cholesterol (μmol/g)	4.19 ± 0.92	4.92 ± 0.86	5.56 ± 1.19	5.58 ± 1.19
Phospholipid (μmol/g)	21.93 ± 4.22	21.91 ± 2.88	19.60 ± 3.15	22.75 ± 3.22
Cholesterol:phospholipid	0.19 ± 0.03^a	$0.22 \pm 0.03^{a,b}$	0.28 ± 0.03^c	$0.24 \pm 0.04^{b,c}$

The results are expressed as the means ± SD of eight rats in each dietary group.
^{a,b,c,d}Values not sharing a common superscript letter are significantly different by ANOVA (Tukey test); $P < .05$.
HC, high cholesterol diet; HC + SO, high cholesterol diet supplemented with sunflower seed oil; HC + OO, high cholesterol diet supplemented with olive oil.

Table 3 Plasma, liver, and aorta lipid peroxide and liver glutathione concentrations in rats fed high cholesterol diets supplemented with sunflower seed oil or olive oil

Parameter	Control	HC	HC + SO	HC + OO
Plasma				
Malondialdehyde (μmol/L)	3.09 ± 0.44[a]	3.64 ± 0.42[a]	4.61 ± 0.79[b]	3.62 ± 0.50[a]
Liver				
Malondialdehyde (pmol/mg of protein)	533.3 ± 65.3[a]	691.1 ± 117.9[a]	958.0 ± 206.4[b]	693.7 ± 151.7[a]
Diene conjugate (nmol hydroperoxide/mg of protein)	10.2 ± 1.98[a]	11.6 ± 2.14[a,b]	14.1 ± 1.70[b]	11.5 ± 1.42[a]
Glutathione (nmol/mg of protein)	30.1 ± 2.84[b]	30.4 ± 4.08[b]	23.4 ± 2.55[a]	29.9 ± 2.58[b]
Aorta				
Diene conjugate (nmol hydroperoxide/mg tissue)	1.37 ± 0.34[a]	1.73 ± 0.39[a,b]	2.26 ± 0.51[b]	1.74 ± 0.40[a,b]

The results are expressed as the mean ± SD of eight rats in each dietary group.
[a,b]Values not sharing a common superscript letter are significantly different by ANOVA (Tukey test); $P < 0.05$.
HC, high cholesterol diet; HC + SO, High cholesterol diet supplemented with sunflower seed oil; HC + OO, high cholesterol diet supplemented with olive oil.

Histopathologic examinations showed a slightly fatty infiltration in the liver, and no atherosclerotic changes were detected in the aorta of the HC, HC + SO, and HC + OO groups.

Discussion

Cholesterol feeding has often been used to elevate serum or tissue cholesterol levels to study the etiology of hypercholesterolemia-related metabolic disturbances.[22] The rabbit is susceptible to the development of atherosclerosis, whereas rats and mice are considered to be resistant to induction of hypercholesterolemia by cholesterol feeding.[22] However, hypercholesterolemia can be produced in rats by feeding cholic acid along with a cholesterol-rich diet, but this treatment does not result in atherosclerotic lesions in the rat.[23]

Studies in animals[24–29] and humans[30,31] have shown that there is a close relationship between lipid peroxidation and hypercholesterolemia and/or hypercholesterolemic atherosclerosis. On the other hand, Yamaguchi et al.[32] have reported the presence of atherosclerotic features and cholesterol deposition in the aorta, as well as elevated serum lipid peroxides, in mice fed a high cholesterol diet supplemented with linoleic acid but not without linoleic acid. In addition it has been reported that fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits.[26] In the present study, plasma, liver, and aorta lipid peroxidation were exaggerated, and an increased cholesterol to phospholipid ratio in the aorta was observed in rats fed the high cholesterol diet supplemented with sunflower seed oil when compared with a high cholesterol group (*Tables 2* and *3*).

However, atherosclerotic lesions were not detected in the aorta. We have also investigated the effects of a high cholesterol diet supplemented with olive oil on plasma, liver, and aorta lipid peroxidation and lipid levels in rats. We found that this diet did not alter plasma, liver, and aorta lipid peroxidation and the aorta cholesterol to phospholipid ratio in comparison to a high cholesterol diet.

Thus, high cholesterol diets supplemented with polyunsaturated fats, in contrast to monounsaturated fats, seem to have a tendency to exaggerate lipid peroxidation in the organism, as well as to disturb the balance between cholesterol and phospholipid in the aorta.

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