Influence of dietary oils on liver and blood lipid peroxidation

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ABSTRACT

Objective: Diets high in unsaturated fatty acids have been recommended to lower the risk of cardiovascular disease. However, these lipids are more susceptible to lipid peroxidation than saturated fatty acids. The aim of the present study described herein was to investigate the effects of dietary oils (differing in their degree of saturated and unsaturated fatty acids) on liver and blood lipid peroxidation in chicks.

Methods: The experiments were conducted at the laboratories of University of Dumlupinar, Kutahya, Turkey and Osmangazi University, Eskisehir, Turkey between November 2002 and December 2003. The animals were randomly divided into 5 groups of 30 and fed dietary butter, margarine, olive oil, sunflower oil or corn oil for 7 weeks. Liver malondialdehyde level, blood superoxide dismutase activity (SOD) and glutathione peroxidase activity (GPx), serum vitamin E, and total antioxidant (AOA) levels were measured to determine the effects of the dietary oils on lipid peroxidation.

Results: No significant differences were observed in SOD and GPx activities, or vitamin E and AOA levels between the experimental groups. However, the results indicated that the corn oil feeding caused significant increases in liver malondialdehyde (a genotoxic byproduct of lipid peroxidation) level as compared with the other oils.

Conclusion: The results demonstrate that corn oil feeding increases lipid peroxidation significantly and thus may raise the susceptibility of tissues to free radical oxidative damage.

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Lipids are an essential part of the diet as they yield large amounts of energy upon oxidation in metabolism and are required for the absorption and transport of lipid-soluble vitamins through the bloodstream. As an important constituent of cell membranes, they also play specific roles in membrane signaling events and thus cell development. Certain lipids are indicators of cellular events, and lipid concentration can represent physiological conditions of cells.[1,2] The incidence of cardiovascular disease is correlated with diets high in saturated fatty acids. Animal fats, which contain higher proportions of saturated fatty acids, increase the risk of vascular system diseases. Numerous studies indicate that dietary butter elevates the level of total cholesterol, low density lipoprotein (the major carrier of cholesterol in plasma) and triglycerides. It has also been claimed that consumption of dietary butter contributes to hypercholesterolemia due to its composition enriched in saturated fatty acids.[3-5] Margarine made from corn or sunflower oils is much lower in saturated fatty acids than butter. Substitution of

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margarine for dietary butter reduces total cholesterol and low-density lipoprotein (LDL) level.[6,7] By reducing serum cholesterol levels without any effect on high-density lipoprotein (HDL) cholesterol level, olive oil rich in monounsaturated fatty acids shows protective effects against arteriosclerosis.[8,9] Sunflower oil and especially corn oil, whose consumption has increased significantly recently, are quite rich in linoleic acid, an essential polyunsaturated fatty acid. Although sunflower oil and corn oil reduce cholesterol synthesis and thus its level, they are considered as risk factors for their sensitivities to free radical formation because of their high contents of polyunsaturated fatty acids (PUFAs). It is well know that PUFAs are more susceptible to lipid peroxidation than saturated fatty acids (SFA).[10,11]

In organisms, endogenous and exogenous free radicals can damage structures of lipids, proteins, carbohydrates and nucleic acids by interacting with them and can subsequently produce new free radicals.[12-14] Among all biomolecules lipids are the most sensitive molecules to free radicals. Double bonds in fatty acids form peroxide products by reacting with free radicals, and lipid radicals can be formed subsequently upon removal of electrons.[15,16] As a result of lipid peroxidation, quite harmful degradative products [namely malondialdehyde (MDA)] can be formed in cell membranes. Malondialdehyde shows both mutagenic and carcinogenic effects by changing membrane properties.[16-18]

Organisms protect themselves from harmful effects of free radicals by antioxidant defense mechanisms. The antioxidant system involves both enzymatic and non-enzymatic antioxidants. The first step in the enzymatic system is superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion ($O\cdot 2^{-}$) to H_2O_2. The conversion of H_2O_2 to H_2O by either glutathione peroxidase (GPx) or catalase forms the second step of enzymatic system. Superoxide dismutase and GPx enzyme activities and the balance between them are very crucial protection against oxidative stress.[19-21] Lipid-soluble vitamin E is a non-enzymatic antioxidant which plays a significant role in the protection of the cell membrane and against LDL cholesterol as well. It can reduce free radicals and most importantly breaks the chain reaction in lipid peroxidation.[22,23] The measure of total antioxidant activity (AOA), which is the cumulative action of all the antioxidants present in plasma, provides an insight into the delicate balance in-vivo between oxidants and antioxidants.[24]

In the present study, our objective was to investigate the effects of dietary butter, margarine, olive oil, sunflower oil and corn oil on liver and blood lipid peroxidation. Superoxide dismutase and GPx activities as well as MDA, vitamin E and AOA levels were measured to shed light on the effects of dietary oils on lipid peroxidation in chicks.

Methods. The study was performed on 150 newly hatched hybrid chicks (Avian farm species). The animals were randomly assigned into 5 groups of 30. Utmost care was taken to provide equal physical and environmental housing conditions (namely size of units, light, temperature and aeration). The temperature was kept at 32°C in the first week and at 30°C in the second week, and subsequently was lowered to 18°C with 3°C per week. The units were illuminated 24 hours a day. All experimental procedures used were in accordance with the published ethical guidelines for the animal use and care.

In the first week, groups were fed a basal diet composed of 89.2% dry matter, 20.93% crude protein, 4.91% crude cellulose, 5.31% ash, 2.83% crude oil, 0.73% methionine-cysteine, 1.01% lysine, 0.9% calcium and 0.68% phosphor with a 2830 kcal/kg metabolic energy. The selected lipids were added to respective diets as 5.4% in the 2nd and 3rd week, 6% in the fourth and fifth week, and 7% in the sixth and seventh week. The diets differed in the nature of lipids (namely dietary butter, margarine olive oil, sunflower oil or corn oil), and were prepared fresh every week. Margarine and butter were added to each diet after solubilization at low temperature. Basal diet was mixed with lipids completely and chicks were provided with this diet ad libitum. No significant differences in food consumption were detected between groups by measuring each day's food intake. Chicks were sacrificed after 16 hours of food deprivation. Bloods from vena axillaries were collected into serological tubes; afterwards, serum or plasma was separated from blood cells by centrifugation. Analyses of plasma samples were carried out immediately. Serum samples were kept at -70°C until analysis. After sacrifices, livers were excised immediately, washed thoroughly with ice-cold 0.9% NaCl, and kept at -70°C in phosphate buffer (pH 7.4) until analysis.

Measurements. Analysis of liver MDA level was carried out according to the previously published procedure[25] on supernatant fractions after homogenization and centrifugation of tissue samples diluted 10-fold with 1% KCI. Superoxide dismutase and GPx activities were spectrophotometrically assayed with commercial kits. Total blood SOD activity was determined by inhibition of formazan dye (505 nm) employing the xanthin-xanthin oxidase enzymatic method to generate superoxide radicals[26] and expressed as U/mg of hemoglobin. Glutathione peroxidase activity was measured by Paglia-Valentina method based on nicotinamide adenine dinucleotide phosphate oxidation at 340 nm

using cumen hydroperoxide as the substrate (Randox Laboratories, Crumlin, UK) and expressed in U/l. Serum vitamin E analysis was carried out with Emmerie-Engel colorimetric method based on an oxidation-reduction reaction,[27] and finally serum total AOA determination with a colorimetric method developed by Koracevic et al.[28]

Statistical analysis. All of the data were expressed as means ± SEM. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Results were considered statistically significant at $p<0.05$. The strength of the association between the parameters (namely MDA and SOD or MDA and GPx) measured was determined by the Pearson correlation coefficient method.

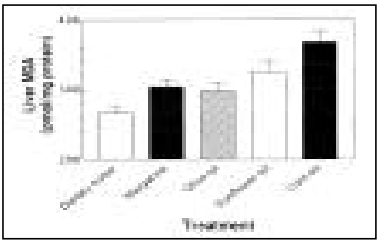

Figure 1 - Effect of dietary oils on liver malondialdehyde (MDA) levels.

Results. To determine the dietary oils most susceptible to lipid peroxidation, we measured the activities of SOD and GPx as well as the levels of MDA, Vit. E and AOA. As seen in **Table 1**, although SOD activity were decreased by 18% and GPx activity by 25%, by corn oil feeding as compared to the dietary butter fed group, no statistically significant differences were found between the 2 experimental groups. Similarly, no significant differences were detected between corn oil fed groups versus sunflower oil, olive oil or margarine fed group. There were no statistical significant differences between Vit. E and AOA levels in chicks fed the oils tested. However, as can be seen in **Figure 1**, liver MDA levels (an indicator of lipid peroxidation) were significantly different between groups (ANOVA F-test value 8.742, $p<0.0001$) and Tukey's post tests showed specific differences between corn oil fed group versus dietary butter fed group ($p<0.001$), corn oil fed groups versus olive oil fed group ($p<0.01$), and corn oil fed group versus margarine fed group ($p<0.01$). The MDA level of sunflower oil fed group was significantly higher than that of the group fed dietary butter ($p<0.05$). Significant differences were not detected in the level of MDA between dietary butter, margarine and olive oil fed groups. Analysis of the MDA, SOD and GPx parameters by the Pearson correlation method indicated a negative correlation between MDA and SOD activity ($r=-0.65$, $p<0.0001$), and between MDA and GPx activity ($r=-0.77$, $p<0.0001$), suggesting that the increased lipid peroxidation is accompanied by a decrease in the activities of the enzymes involved in antioxidant defense mechanism.

Discussion. Plant oils with high PUFA ratio were hitherto thought as the healthiest lipids because of their cholesterol lowering effects. However, recent studies indicate that PUFA are more susceptible to lipid peroxidation than SFA. Lipid peroxidation usually results in decreasing membrane fluidity, cell injury and may cause the

Table 1 - Effect of dietary oils on superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity and on the level of Vitamin E and total antioxidant.

Groups	n	Blood SOD (U/gr Hb)	Blood GPx (U/l)	n	Serum Vitamin E (mg/dl)	Serum AOA (mmol/l)
Dietary butter	20	2627.635 ± 186.33	68.910 ± 9.66	27	1.997 ± 0.016	0.773 ± 0.040
Margarine	17	2217.355 ± 176.51	63.193 ± 9.34	22	1.986 ± 0.013	0.712 ± 0.030
Olive oil	21	2318.788 ± 162.42	77.611 ± 11.89	26	1.974 ± 0.014	0.775 ± 0.037
Sunflower oil	20	2214.006 ± 166.37	54.901 ± 8.41	21	2.01 ± 0.011	0.723 ± 0.036
Corn oil	20	2157.057 ± 147.19	52.025 ± 6.72	22	1.977 ± 0.010	0.711 ± 0.035

The values are expressed as means ± SEM, AOA - antioxidant.

formation of atherosclerotic plaques.[9,29] Antioxidant enzymes SOD and GPx scavenge lipid peroxides and free radicals and detoxify them. No significant differences were detected when the activities of SOD and GPx were compared between the experimental groups. In a similar study, although soybean or olive oil diets modified the liver microsomal fatty acid phospholipid composition, SOD and GPx activities remained unchanged.[30] We also determined the level of the natural antioxidant Vit. E, which is important for the maintenance of good health. Although several groups have reported that Vit. E level can be sensitive to the composition of the diet,[31,32] the results presented here indicate that Vit. E level is not affected by the type of dietary oils investigated in this study. The probability of lipid peroxidation increases with increasing number of double bonds in fatty acids. As a result of the degradation of lipid peroxides, MDA forms and is used as an indicator of lipid peroxidation.[18] Therefore, to better assess the effects of the dietary oils on lipid peroxidation, we measured the level of MDA in chicks fed dietary oils differed in the degree of fatty acid saturation. The results indicated that the highest level of MDA was observed in the group fed with corn oil containing the highest PUFA content among the lipids supplemented to the diets (**Figure 1**). In rats, MDA levels were similarly found to increase with increasing amounts of corn oil supplemented to a semi-synthetic diet.[11] In agreement with our results, Sarraga and Regueiro[33] also indicated that lipid peroxidation was higher in broilers fed sunflower oil (composed of highest amount of PUFA among lipids tested), and it was very low in the group fed olive oil. There are numerous harmful effects of MDA. For example, by crosslinking with the membrane components, MDA causes inactivation of enzymes and receptors in membranes and thus changes membrane properties. Malondialdehyde also causes mutations by reacting with guanine nucleotide in DNA.[16,34]

In the light of evidence presented here, it is suggested that corn oil and, to a lesser degree, sunflower oil feeding increases lipid peroxidation significantly and thus challenge the antioxidant defense system and may increase the susceptibility of tissues to degradation products of lipid peroxides.

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