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# EFFECT OF FISH OIL AND COCONUT OIL ON ANTIOXIDANT DEFENCE SYSTEM AND LIPID PEROXIDATION IN RAT LIVER

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Diets high in fish oil containing polyunsaturated fatty acids of the n-3 family, have been suggested to decrease the risk of cardiovascular disease. However these lipids are highly susceptible to oxidative deterioration. In order to investigate the influence of n-3 fatty acids on oxidative status, the effect of feeding rats with fish oil or cocunut oil diets was studied by measuring different parameters related to an oxidative free radical challenge. Synthetic diets containing 15% (w/v) fish oil or coconut oil were used to feed growing rats for 4 weeks. As compared to control diet, the fish oil containing diet produced a significant decrease of cholesterol and triglyceride concentration in serum, however there was a significant increase in lipid peroxidation products. In addition, in fish oil fed animals, there was also a decrease in vitamin E and A concentration. Furthermore, the rate of lipid peroxidation in isolated microsomes was three fold higher in rats fed fish oil as compared to rats with coconut oil diet. No significant differences between the two experimental groups were observed in superoxide dismutase (SOD) and phospholipid hydroperoxide glutathione peroxidase (PHGPX) activities. However, there was a decrease in glutathione peroxidase (GPX) activity. These results suggest that fish oil feeding at an amount compatible with human diet, although decreasing plasma lipids, actually challenge the antioxidant defence system, thus increasing the susceptibility of tissues to free radical oxidative damage.

KEY WORDS: Antioxidant enzymes, lipid peroxidation, fish oil.

## INTRODUCTION

Epidemiological evidence suggests that diets rich in fish might protect from the risk of cardiovascular disease, by lowering plasma lipids, and by affecting eicosanoid biosynthesis and endothelial cell and platelet function.[1-3]

These effects have been attributed to the relatively high concentration in fish lipids of polyunsaturated fatty acids (n-3), particularly eicosapentanoic and docosahexaenoic.

On the other hand, due to the high level of unsaturation, these lipids are highly susceptible to free radical oxidative reactions. In fact, increased rates of lipid peroxidation have been observed in liver microsomes of rats fed with relatively high amounts of fish oil.[4] In agreement with this observation, but using diets compatible with different human habits, we showed that polyunsaturated fatty acid and vitamin E can affect the susceptibility of isolated perfused rat heart to an oxidative stress.[5]

However, if and how the physiological antioxidant defense system counteracts the



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oxidative challenge induced by dietary polyunsaturated fatty acids remains to be explored.

The present work was designed to investigate the effects of polyunsaturated fatty acid contained in fish oil, on oxidative stability and antioxidative capacity of rat liver. For comparison the effect of highly saturated coconut oil was measured.

## MATERIALS AND METHODS

### *Diets and animals*

Semisynthetic diets, containing fish oil or coconut oil were prepared weekly and stored at 4°C under nitrogen. The composition of these diets was: 20% casein, 0.3% dl - methionine, 40% rice starch, 17% sucrose, 15% oil, 3% fiber, 3.5% salt mixture (AIN-76), 0.2% choline chloride. Male albino rats, weighing 100 ± 5 g, were individually housed in wire bottom stainless cages. Lighting was regulated to provide equal hours of light dark. The animals were divided in two groups of six and fed with the experimental diets for 6 weeks. At the end of this period, after an overnight fasting, rats were sacrificed.

Serum samples were prepared and stored at −80°C until analysis.

### *Diet analysis*

Samples of diets were analyzed for fatty acid composition by GLC[6] (Table I). Tocopherol content in the diet was measured according to McMurray,[7] on the same day of preparation and after one week. The content of vitamin E (38 mg/kg) was made equal in both diets. Dietary oils were analyzed for peroxide by iodimetric titration,[8]

TABLE I
Fatty acid composition of fish oil and coconut oil containing diets

| Fatty acid | Fish-oil % | Coconut-oil % |
|---|---|---|
| 8:0 | 0.2 | 1.3 |
| 10:0 | | 4.0 |
| 12:0 | 0.1 | 43.7 |
| 14:0 | 6.3 | 19.4 |
| 16:0 | 17.0 | 11.3 |
| 16:1 | 9.8 | |
| 18:0 | 3.8 | 4.4 |
| 18:1 | 14.3 | 12.3 |
| 18:2 | 1.6 | 3.3 |
| 18:3 n-3 | 0.3 | |
| 18:3 n-6 | 1.0 | |
| 18:4 n-3 | 3.9 | |
| 20:1 | 2.3 | |
| 20:4 | 1.1 | |
| 20:5 n-3 | 18.5 | |
| 22:1 | 1.6 | |
| 22:5 n-3 | 2.3 | |
| 22:6 n-3 | 11.7 | |
| 24:1 | 1.0 | |

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and showed very low values of autoxidation (fish oil: 0.25 mEq/g and coconut oil: 0.20 mEq/g).

### *Serum analysis*

Glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GTP) activities and triglyceride, total cholesterol, glucose, urea and uric acid concentration were determined using reagent kits from Boehringer Mannheim.

Vitamin A and vitamin E were determined according to Bieri. Thiobarbituric-Reactive Substance (TBA-RS), expressed as malondialdehyde (MDA), was measured according to Yagi.[10]

### *Liver analysis*

Livers were homogenized in three volumes of 0.25 M sucrose, 10 mM Tris-HCl pH 7.4 and differentially centrifuged. Superoxide-dismutase (SOD, E.C.1.15.9.1) activity[11] and TBA-RS were measured on the 900 × g supernatant.[10] Glutathione Peroxidase (GPX) and Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPX) were measured according to Ursini *et al*[12] on the 15.000 × g supernatant. Liver microsomes samples were extracted with chloroform/methanol[13] (2/1; v/v) in the presence of 0.01% butylated hydroxytoluene (BHT) and fatty acid composition analyzed by GLC. On microsomal samples, vitamin E and vitamin A were measured by HPLC with fluorescence detection according to Diplock.[14] Lipid peroxidation was induced

TABLE II
Serum chemistry in rats fed with fish oil or coconut oil containing diets

| Parameter | Fish oil diet | Coconut oil diet |
|---|---|---|
| Triglycerides (mg/100 ml) | 46.9 ± 11.3 | 85.7 ± 14.7 |
| Total cholesterol (mg/100 ml) | 44.7 ± 8.0 | 64.6 ± 8.2 |
| Glucose (mg/100 ml) | 134.7 ± 20.3 | 136.8 ± 16.7 |
| Urea (mg/100 ml) | 26.9 ± 1.9 | 22.5 ± 2.9 |
| Uric acid (mg/100 ml) | 1.0 ± 0.1 | 1.3 ±0.2 |
| GOT (U/100 ml) | 34.2 ± 13.2 | 37.3 ± 6.9 |
| GPT (U/100 ml) | 30.1 ± 17.9 | 23.4 ± 13.5 |

TABLE III
Fatty acid composition of liver microsomes of rats fed with fish oil or coconut oil containing diets

| Fatty acid | Fish oil % | Coconut oil % |
|---|---|---|
| 16:0 | 20.04 ± 0.29 | 18.43 ± 0.35 |
| 16:1 | 1.80 ± 0.25 | 1.40 ± 0.42 |
| 18:0 | 23.70 ± 1.12 | 30.62 ± 0.48 |
| 18:1 n-9 | 7.87 ± 0.29 | 12.05 ± 0.38 |
| 18:2 n-6 | 2.64 ± 0.65 | 4.17 ± 0.85 |
| 18:3 n-9 | 0.35 ± 0.10 | 0.28 ± 0.14 |
| 20:4 n-6 | 14.43 ± 0.60 | 23.19 ± 1.05 |
| 20:5 n-3 | 6.15 ± 0.23 | 0.41 ± 0.03 |
| 22:5 n-3 | 2.32 ± 0.25 | 0.51 ± 0.28 |
| 22:6 n-3 | 15.54 ± 0.74 | 3.70 ± 0.30 |

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in liver microsomes (0.5 mg protein/ml) incubated at 30°C in 0.1 M Tris-HCl, pH 7.2 in the presence of 5 $\mu$M $FeCl_3$, 0.5 mM ADP, and 0.2 mM acòrbate. TBA-RS was measured on samples, withdrawn at different time according to Slater.[15] BHT (0.01%) was added to the TBA reagent to prevent free radical reactions during the boling step.

## RESULTS

Serum values of biochemical parameters in two rat groups are reported in Table II. As expected fish oil fed group showed lower levels of both triglycerides and total cholesterol than coconut oil fed group.

In fish oil treated animals a large proportion of n-3 polyunsaturated fatty acids, was incorporated into liver microsomes (mainly eicosapentanoic and docosahexaenoic acid), whilst saturated and (n-6) unsaturated fatty acids were incorporated in lipid microsomes of coconut oil diet group (Table III).

The fish oil diet feeding was associated to a dramatic decrease of vitamin E, in serum and liver microsomes, (76.4 ± 5.4 v.s 37.0 ± 2.7 ng/mg protein in serum, and 110.3 ± 11.2 vs. 38.0 ± 8.1 ng/mg protein in liver microsomes). Also vitamin A content was lower in liver microsomes after feeding fish oil diet (17.5 ± 4.4 vs. 7.0 ± 2.4 ng/mg protein).

In both, liver and serum, TBA-RS, measured as MDA, was higher in the fish oil fed group (41 ± 3 vs. 14 ± 1 nmol/gr of liver, and 3.2 ± 1.1 vs. 1.1 ± 0.4 nmol/ml of serum).

The rate of microsomal lipid peroxidation *in vitro* was three times higher in fish oil fed rats than in coconut oil fed rats (Figure 1).

No significant differences between two dietary groups were found in the activity of SOD and PHGPX, whilst GPX was lower in the liver of animals fed with the fish oil diet (Table IV).

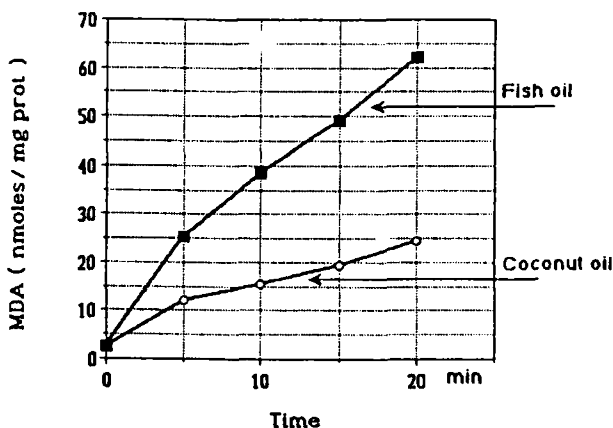


FIGURE 1 Lipid peroxidation rate in liver microsomes of rats fed with fish oil and coconut oil containing diets. Liver microsomes (0.5 mg protein/ml) were incubated at 30°C, in 0.1 M Tris-HCl buffer, pH 7.2, containing 5 $\mu$m $FeCl_3$, 0.5 mM ADP and 0.2 mM ascorbate. MDA was measured on aliquots withdrawn at different times.

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TABLE IV
Superoxide dismutase (SOD), glutathione peroxidase (GPX) and phospholipid hydroperoxide glutathione peroxidase (PHGPX) activities in liver of rats fed with fish oil or coconut oil containing diets.

| DIET | SOD | GPX (mU/mg prot) | PHGPX |
|---|---|---|---|
| Fish oil | 73 ± 9 | 43 ± 11 | 2.15 ± 0.10 |
| Coconut oil | 65 ± 6 | 75 ± 10 | 1.95 ± 0.10 |

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## DISCUSSION

The cholesterol and triglyceride lowering effect of n-3 polyunsaturated fatty acids containing fish oil was confirmed in this study.

However the high unsaturation level of these fatty acids, that were actually inserted in liver microsomal lipids, induced an oxidative challenge, as shown by the higher level of both MDA and lipid peroxidation rate.

This oxidative stress in plasma and tissues is very likely the cause of the observed lower concentration of vitamin E and vitamin A in fish oil fed animals. Among antioxidant enzymes, SOD and PHGPX were not affected by different diets, whereas GPX was lower when animals were fed with fish diet. However, the ratio between the two Se-dependent peroxidases was actually modified suggesting that either GPX is more sensitive to an oxidative damage or that a shift in seleno-enzymes biosynthesis might take place.

In conclusion these results indicate that a diet containing high amounts of unsaturated n-3 lipids, although lowering plasma lipids, induces an oxidative challenge that leads to a faster consumption of antioxidant vitamins. A modification of the enzymatic antioxidant system does not seem to be relevant, except for the different ratio between the two Se-peroxidases. Under oxidative conditions induced by dietary fatty acids, indeed, the activity of the enzyme active on membranes (PHGPX) seems to be spared more efficiently that the activity of the enzyme active in the water soluble compartment (GPX).

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