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Dietary fish oil enhances plasma and LDL oxidative modification in rats

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Incorporation of long-chain polyunsaturated fatty acids (PUFA) in low density lipoprotein (LDL) might induce a decrease in its resistance against oxidative modification because of their high degree of unsaturation. To investigate the in vivo and in vitro influence of dietary ω-3 fatty acids on plasma and LDL susceptibility to oxidative modification, a diet containing 15% wt/wt fish oil was fed to rats together with diets containing 15% wt/wt soybean oil or coconut oil for 6 weeks. The plasma lipid concentration was significantly lower after fish oil feeding compared with the two control diets. Fish oil fed rats exhibited significantly lower total (peroxyl) radical-trapping antioxidant activity (TRAP) than both soybean oil and coconut oil fed animals ($P < 0.01$). The levels of thiobarbituric acid–reactive substance in the native LDL were 0.66 nmol/mg, 0.51 nmol/mg, and undetectable, respectively, in fish oil, soybean oil, and coconut oil rats. Metal-catalyzed oxidative modification of LDL was monitored following the time course of conjugated dienes and lipid hydroperoxides formation and thiobarbituric acid–reactive substance activity. The effect of the two unsaturated diets on the extent of LDL modification was similar, but the lag phase was shorter in fish oil LDL than in soybean oil LDL, despite the same concentration of vitamin E. Also the oxidative modification of native apoB-100, measured by the emission fluorescence spectra at 430 nm (excitation 360 nm), was markedly higher in LDL of fish oil fed rats than in the other two groups. The results indicate that dietary ω-3 fatty acids induce a decrease in the plasma antioxidant potential and an increase in the ex vivo susceptibility of LDL to oxidative modification. (J. Nutr. Biochem. 6: 474–480, 1995.)

Keywords: fish oil; ω-3 fatty acids; atherosclerosis; oxidation; plasma; low-density lipoprotein; rats

Introduction

The role of long-chain ω-3 fatty acids in protecting against cardiovascular diseases is still unsettled. Fish oil has been shown to have an antiaggregatory effect,[1,2] and its supplementation is effective in lowering plasma triglycerides in humans.[3,4] Animal studies have shown the effect of ω-3 fatty acids both in reducing the hepatic secretion of very low density lipoprotein (VLDL)[5] and in accelerating VLDL clearance.[6] However, the reported effects on total cholesterol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) are ambiguous.[7]

Compelling evidence suggests that lipoprotein oxidative modification is involved in the pathogenesis of atherosclerosis.[8] Although the precise mechanism(s) involved in its modification in vivo and its locality are still unclear, several lines of evidence support the in vivo existence of oxidatively modified LDL.[9]

Oxidative modification of low density lipoprotein (LDL) can be mimicked in vitro by incubation with transition metal ions or cells (endothelial cells, smooth muscle cells, monocytes, and macrophages).[10]

The susceptibility of LDL to oxidative modification is modulated by the circulating level of antioxidants and its fatty acid composition.[11–14] Incorporation of monounsaturated fatty acids into LDL has been shown to protect against oxidative modification of LDL, in contrast with highly unsaturated fatty acids.[15–18] Therefore, LDL particles rich in long-chain polyunsaturated fatty acids should be more readily oxidatively modified and, in principle, more atherogenic. Also, plasma enrichment with ω-3 fatty acids

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could impair the antioxidant defense system increasing the requirement for antioxidants.

The aim of this study was to test how much a fish oil–enriched diet would alter the distribution of ω-3 fatty acids in plasma and in LDL and how much a different proportion of ω-3 fatty acids would affect the susceptibility of LDL to in vitro oxidative modification. Therefore high fat diets containing fish oil (high ω-3) or soybean oil (high ω-6) and coconut oil (high saturated) have been fed to rats. The plasma antioxidant potential was determined. The oxidative modification of LDL was measured determining intermediate and final products of Cu^{2+}-catalyzed lipid peroxidation. The fluorescence, due to the reaction of aldehydic lipid peroxidation products with free amino groups of apoB, was also measured in native LDL.

Methods and materials

Diet and animal

Thirty male Wistar rats (initial weight 68 ± 6 g) were individually housed in wire bottom stainless cages under controlled lighting. The animals were randomly divided into three groups of 10 and fed for 6 weeks experimental diets containing 15% (wt/wt) either soybean oil (SO), fish oil (FO), or coconut oil (CO). SO and CO were commercially available oils. FO was kindly provided by Seven Seas Ltd. (Hull, UK).

The high fat level in the diets was chosen to stress the differences in the response. The composition of these diets (wt/wt) was 20% casein, 40% rice starch, 15% fat, 0.3% dL-methionine, 17% sucrose, 3% fiber, 3.5% salt mixture (AIN 76), 1% vitamin mixture (AIN 76), and 0.2% choline chloride. Diets were prepared weekly and stored at 4°C under nitrogen. After 1 week of storage, diets were analyzed for peroxides by iodimetric titration[19] and showed very low values (SO and FO diets, <1 mEq/kg; CO, undetectable). Samples of diets were analyzed for fatty acids composition by gas liquid chromatography (GLC).[20] *Table 1* reports the fatty acid percent composition of the three experimental diets. Oils were analyzed for vitamin E content according to Carpenter.[21] The dietary vitamin E was equalized to 57 IU/kg of diet by adding a suitable amount of vitamin E as tocopherol (supplied by Fluka, cat no. 95240, 1.1 U/mg), and the total tocopherol content was measured according to McMurray[22] on the same day of the preparation of the diet.

Preparation of LDL

LDL fraction (1.019 to 1.055 g/mL) was isolated from plasma collected in ethylenediamine-tetraacetic acid (EDTA) (1 mg/mL) after an overnight fast by sequential ultracentrifugation in salt solutions (NaCl, KBr, containing 1 mg/mL of EDTA Na_2) according to Havel et al.,[23] using a Beckman T-100 bench top Ultracentrifuge (T-100.3 rotor). LDL was flushed with N_2, stored at 4°C, and used within 1 week of the preparation.

Plasma and LDL analysis

Cholesterol, phospholipids, triglycerides, and uric acid concentrations were determined in plasma and LDL using reagent kits from Boerhinger Mannheim (Mannheim, Germany). Vitamin E was determined according to Bieri et al.[24] Plasma ascorbic acid was measured by high performance liquid chromatography (HPLC), following the method of Farber et al.[25] Thiobarbituric-reactive substance (TBA-RS) was measured in lipoprotein fraction according to Maseki et al.[26] The plasma sulfhydryl concentration was measured by the spectrophotometric method described by Ellman.[27] Plasma fatty acids composition was determined by GLC according to Mueller[28] with minor modification. Fatty acid composition was determined in lipoprotein fractions by GLC according to Mueller[28] and Knapp[29] after pooling five samples for each dietary group. Lipoprotein protein was determined by the method of Lowry et al.[30] using bovine albumin as a reference standard. The lipoprotein concentration was calculated from the mass of protein + individual lipids (total cholesterol + triglycerides + phospholipids) according to Sattler et al.[31]

Experimental total (peroxyl) radical-trapping antioxidant activity (TRAP) was measured on plasma by subjecting it to controlled peroxidation using the thermal decomposition of azobis (2-amidinopropane hydrochloride) at 41°C, as described by Wayner et al.[32] The oxygen consumption was measured by using a Clark oxygen electrode (YSI) with a Gilson 5/6 oxygraph.

Table 1 Fatty acid composition of the experimental diets (% of total fatty acids)

Fatty acid	Soybean oil	Fish oil	Coconut oil
10:0	—	—	2.9
12:0	0.1	0.2	46.7
14:0	0.1	7.9	17.5
16:0	9.6	19.8	11.0
16:1 ω-7	—	10.2	0.2
18:0	4.3	3.7	10.6
18:1 ω-7	0.3	4.1	—
18:1 ω-9	26.3	9.5	8.1
18:2 ω-6	52.1	2.1	1.7
18:3 ω-3	6.4	0.9	—
18:3 ω-6	—	0.3	—
18:4 ω-3	—	4.2	—
20:0	0.3	0.4	0.3
20:1 ω-9	—	0.1	—
20:2 ω-6	—	0.2	—
20:3 ω-6	—	—	0.1
20:4 ω-6	0.2	1.0	—
20:5 ω-3	—	19.5	0.3
22:0	0.4	0.2	0.1
22:1 ω-11	—	0.5	—
22:4 ω-6	—	0.2	—
22:5 ω-3	—	1.9	—
22:6 ω-3	—	12.6	—
24:0	—	—	—
24:1 ω-9	—	0.6	—
SFA	14.8	32.2	89.1
MUFA	26.6	25.0	8.2
PUFA	58.7	42.8	2.0
ω-3	6.4	39.1	0.3
ω-6	52.3	3.9	1.9

Fluorescence measurements

ApoB fluorescence was measured on pools of five LDL samples for each dietary group, according to Esterbauer.[33] In brief, 0.8 mg of native LDL was extracted four times with a mixture of chloroform-methanol 2:1 (vol/vol). The aqueous phase of the chloroform-methanol extract was separated by centrifugation and removed. ApoB was washed twice with 1 mL of water, dried under nitrogen, and redissolved in 1 mL of 3% aqueous sodium dodecyl sulfate (SDS) solution. This solution of apoB was then used for fluorescence spectroscopy. Spectra were recorded at 430 nm with excitation at 360 nm.

Oxidation of LDL

Two pools of five LDL samples for each dietary group were dialyzed against a 200-fold volume of 0.01 M phosphate-buffered saline (PBS) 0.15 M NaCl, pH 7.4., for 24 hr at 4°C in the dark. From the dialyzed solutions, a volume was diluted with PBS to obtain a final concentration of 0.5 mg/mL of LDL. Oxidation was initiated by the addition of freshly prepared $CuCl_2$ (5 μM final concentration) at 37°C.[34] The kinetic of conjugated dienes formation was followed by continuous monitoring the 234 nm absorption using Beckman DU 70 Spectrophotometer. Lipid hydroperoxide were measured iodometrically at different time points, according to El Saadani et al.[35] The vitamin E concentration at time zero was measured according to Bieri et al.[24]

Statistical analysis

Statistical analysis was performed using one-factor analysis of variance and the Scheffe F-test method for multiple comparison. In the case of the kinetic of Cu^{2+}- stimulated oxidation, 2-factor ANOVA for repeated measurements was used.

Results

After 6 weeks of feeding the experimental diets, there was no significant difference in food intake, weight gain, final weight, and relative liver weight among rats fed the different diets (data not shown). The plasma fatty acid composition in the three groups is reported in *Table 2*. PUFA account for 50% of FA in SO fed rats, mainly as ω-6 (45.3% of total FA). Plasma from FO and CO fed rats are characterized by a similar proportion of SFA, MUFA, and PUFA, but ω-3 FAs are 17.8% in FO plasma against 2% in CO fed animals.

Table 2 Plasma fatty acid composition (% of total fatty acids)

	Soybean oil		Fish oil		Coconut oil	
Fatty acid	m	SD	m	SD	m	SD
12:0	0.29	0.05	0.37	0.03	1.38	0.14
14:0	0.70	0.03	5.20	0.38	2.41	0.28
16:0	18.70	1.00	28.82	1.19	24.22	1.22
16:1 ω-7	0.95	0.15	8.07	0.22	3.51	0.36
18:0	16.42	0.26	10.68	0.40	13.71	0.58
18:1 ω-7	1.95	0.12	3.65	0.85	2.91	0.08
18:1 ω-9	7.65	0.38	10.49	0.67	19.87	0.60
18:2 ω-6	17.03	0.32	2.58	0.13	5.44	0.27
18:3 ω-3	0.93	0.06	0.44	0.06	0.12	0.04
18:3 ω-6	1.18	0.05	0.31	0.25	0.27	0.18
18:4 ω-3	0.20	0.02	1.53	0.08	—	—
20:0	0.36	0.04	0.42	0.01	0.28	0.01
20:1 ω-9	0.38	0.04	0.49	0.01	1.21	0.71
20:2 ω-6	0.26	0.02	0.29	0.19	5.06	0.15
20:3 ω-6	0.50	0.03	—	—	—	—
20:4 ω-6	25.98	0.63	7.67	0.44	16.17	0.70
20:5 ω-3	0.31	0.12	6.77	0.47	0.24	0.03
22:0	0.64	0.16	0.23	0.02	0.74	0.08
22:1 ω-11	—	—	0.62	0.38	—	—
22:4 ω-6	0.32	0.02	0.17	0.02	—	—
22:5 ω-3	0.57	0.06	1.96	0.16	0.28	0.03
24:0	1.01	0.03	0.51	0.02	0.78	0.05
22:6 ω-3	2.71	0.12	7.07	0.78	1.32	0.13
24:1 ω-9	1.13	0.14	1.87	0.66	—	—
SFA	38.12	1.57	46.23	2.05	43.52	2.36
MUFA	12.06	0.71	25.19	1.94	27.50	1.67
PUFA	49.99	1.39	28.79	2.52	28.90	1.49
ω-3	4.71	0.39	17.77	1.55	1.96	0.35
ω-6	45.27	2.20	11.02	0.71	26.94	1.12

Values are given as mean and SD of six rats for each dietary group

The concentration of various plasma components in rats fed the three experimental diets are reported in *Table 3*. The plasma lipid concentration was lower after fish oil feeding compared with the two control diets. In particular, FO fed rats had a total cholesterol concentration significantly lower than SO fed animals (44.7 ± 3.8 and 72.6 ± 12.4 mg/dL, respectively, $P < 0.0022$) and triglycerides significantly lower than CO fed animals (33.1 ± 11.6 and 78.5 ± 15.0 mg/mL, respectively, $P < 0.015$). As regards phospholipids, FO fed rats had a plasma concentration lower than that of both control groups (67.1 ± 5.8 mg/dL; FO; 113.5 ± 26.5 mg/dL; SO; 147.6 ± 8.8 mg/dL, CO; $P < 0.0002$). Plasma levels of vitamin E (2.8 ± 0.5, SO, and 2.5 ± 0.2 μg/mL, FO versus 7.3 ± 0.4 μg/mL, CO) and uric acid (0.9 ± 0.2, SO, and 1.1 ± 0.1 mg/dL, FO, versus 1.5 ± 0.2 mg/dL, CO) were significantly lower in the high-unsaturated diets.

Vitamin C and sulfhydril group levels were similar in the three diets, while vitamin A was significantly lower in the FO group as compared with the two control diets (274.7 ± 48.6 ng/mL, FO, versus 468.3 ± 14.5, SO, and 466.4 ± 43.8 ng/mL, CO). Plasma levels of carotenoids (α- and β-carotene, cryptoxanthine, lutein, zeaxanthine, and lycopene) were not detectable (<1 nmol/mL of plasma).

As shown in *Figure 1*, TRAP activity was significantly lower in FO fed animals than in both CO and SO groups ($P < 0.01$). Interestingly, there was no significant difference in TRAP between CO and SO groups.

The fatty acid composition of LDL fractions is reported in *Table 4*. Data represent the mean of two pools of five rats for each dietary group. In FO treated animals a large proportion of ω-3 PUFA was incorporated in the LDL lipids (mainly eicosapentaenoic and docosahexaenoic acid), while ω-6 unsaturated and saturated fatty acids were incorporated in the LDL of the soybean oil and coconut oil group, respectively.

Table 5 shows the concentration of vitamin E and TBA-RS in the native LDL, expressed per milligram of lipoprotein. Vitamin E (equalized in the three diets) was not significantly different among the dietary groups. TBA-RS was significantly higher ($P = 0.004$) in the FO group (0.66 nmol/mg of LDL) than in the SO group (0.51 nmol/mg of LDL), although the difference is not biologically relevant. Plasma TBA-RS was under the limit of detection in CO rats.

Figures 2 and *3* show the in vitro susceptibility of the LDL fraction to metal catalyzed lipid peroxidation. The time-course curves of conjugated dienes (CD) (*Figure 2*) give two indications. First, in FO-LDL the absorbance at 234 nm rapidly increases without any detectable lag phase. The other two groups of LDL have a higher protection, corresponding to a lag phase of 18 and 30 min for SO-LDL and CO-LDL, respectively, calculated by extrapolating the propagation phase. Second, the overall CD formation is

Table 3 Effect of dietary oils on various plasma parameters

	Soybean oil	Fish oil	Coconut oil	*P*
Total cholesterol (mg/dL)	72.6 12.4[a]	44.7 3.8[b]	57.6 2.9[a,b]	0.002
Triglycerides (mg/dL)	52.8 21.4[a,b]	33.1 11.6[a]	78.5 15.0[b]	0.012
Phospholipids (mg/dL)	113.5 26.0[a]	67.1 5.8[b]	147.6 8.8[c]	<0.001
Vitamin E (μg/mL)	2.8 0.5[a]	2.5 0.2[a]	7.3 0.4[b]	<0.001
Vitamin C (μg/mL)	8.4 3.6	6.1 1.5	9.3 1.8	NS
−SH groups (nmol/mL)	192.0 33.1	184.6 25.9	176.8 6.2	NS
Uric acid (mg/dL)	0.9 0.2[a]	1.1 0.1[a]	1.5 0.2[b]	0.004

Data are mean ± SD of 10 rats for each dietary group.
Values with different superscript are significantly different by ANOVA (Scheffe *F*-test).

higher in SO-LDL than FO-LDL ($P < 0.05$ by 1-factor ANOVA at 4 h).

Lipid hydroperoxide production (*Figure 3a*) had a shape similar to CD formation. LDL from the SO fed rats presented a peak value for LPO (at time 3 hr) slightly higher than the FO group (peaking at 2 hr, with a value 15% lower), although not statistically significant ($P = 0.393$ by 2-factor ANOVA repeated measurements).

As regards TBA-RS production (*Figure 3b*), the higher increase was observed in LDL of fish oil fed rats, SO having an intermediate behavior) (FO versus SO and CO $P < 0.001$; SO versus CO, $P < 0.003$ by 2-factor ANOVA for repeated measurements).

The reaction of aldehydic lipid peroxidation products with the amino-free group of the lysine can alter the apoB properties. In particular, this process is associated with the increase of the generation of fluorescent chromophores with a strong emission maximum at 430 nm when excitation is performed at 360 nm. *Figure 4* presents the characteristic fluorescence spectra of protein moieties obtained from freshly prepared LDL (native LDL). The three protein subfractions showed a diffuse emission between 400 and 500 nm, but the relative fluorescence intensity of the sample of fish oil fed rats was higher than those of both coconut oil and soybean oil fed rats ($P < 0.001$).

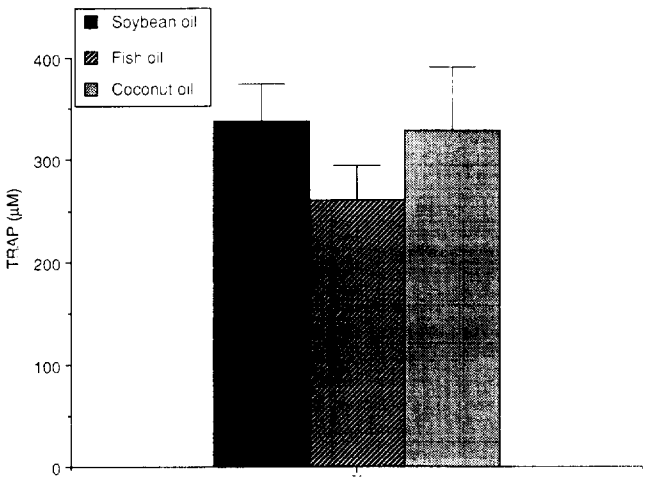

Figure 1 TRAP in the three experimental groups. TRAP (μM peroxyl radical trapped) was measured by the oxygen electrode method on 0.1 mL plasma in 5 mM Na-phosphate buffer, pH 8.0, containing 0.9% NaCl and 10 mM ABAP at 41°C. Data are mean ± SD of six rats for each dietary group. FO vs. SO and CO, $P < 0.01$ by 1-factor analysis of variance

Discussion

Fish oil may induce an increase in the sensitivity to oxidative modification of biological systems because of its high degree of unsaturation.[36] FO supplementation causes the modification of the fatty acid pattern in plasma and leukocyte phospholipids[37] and in rat liver and heart.[38,39] This increase of the unsaturation degree probably increases the requirement for antioxidant nutrients, as supported by the fall of plasma α-tocopherol reported in healthy volunteers after fish oil supplementation.[37]

In a previous experiment, an increase in lipid peroxidation products has been observed in liver microsomes of rats fed fish oil, as compared with a control diet.[38] The rate of Fe^{2+}/ascorbate-induced lipid peroxidation was 3 fold higher in rats fed fish oil, as compared with rats eating

Table 4 Fatty acid composition of LDL (% of total fatty acids)

Fatty acid	Soybean oil	Fish oil	Coconut oil
12:0	—	—	1.0
14:0	1.1	0.6	2.1
16:0	12.8	14.6	19.7
16:1 ω-7	4.0	7.5	4.2
18:0	7.9	8.0	18.9
18:1 ω-7	0.9	6.0	0.9
18:1 ω-9	17.9	14.8	21.4
18:2 ω-6	19.1	3.2	3.5
18:3 ω-3	7.2	3.7	—
18:3 ω-6	4.3	4.0	5.0
18:4 ω-3	—	2.4	2.0
20:4 ω-6	22.2	6.3	21.1
20:5 ω-3	1.0	14.6	—
22:5 ω-3	—	3.1	—
22:6 ω-3	1.9	11.3	0.2
SFA	21.8	23.2	41.7
MUFA	22.9	28.3	26.5
PUFA	55.6	48.6	31.9
ω-3	10.1	35.0	2.2
ω-6	45.6	13.6	29.6

Values are means of two determinations on two different LDL pools for each dietary group.

Table 5 Composition of native LDL

	Soybean oil	Fish oil	Coconut oil	*P*
Vitamin E µg/mg	0.79 ± 0.23	1.01 ± 0.08	1.36 ± 0.58	NS
TBA-RS nmol/mg	0.51 ± 0.05[a]	0.66 ± 0.05[b]	ND	0.0042

Results are expressed per milligram of lipoprotein and represent mean ± SD of six rats for each dietary group.
Values with different superscripts are significantly different by ANOVA (Scheffe *F*-test)

coconut diet. Also cumene hydroperoxide-induced oxidative stress led to increased fatty acid peroxidation in red blood cells from rabbits on a fish oil-enriched diet, compared with RBCs from animals on a conventional diet.[40] Moreover, contrasting results have been presented on the effect of fish oil supplementation on the development of atherosclerosis in Watanabe heritable hyperlipidemic rabbits.[41–43]

The effect of fish oil ingestion on the susceptibility of LDL to peroxidation has been tested in smokers and nonsmokers, using TBARS and degradation of LDL by murine peritoneal macrophages, as indices of oxidative modification. FO supplementation induced a significant rise of the two parameters in both groups of subjects, even if baseline values were lower in nonsmokers than smokers.[44]

In our study, we tested the effect in rats of fish oil supplementation on the in vivo and in vitro susceptiblity of plasma and LDL to oxidative modification using soybean and coconut oil as high unsaturated and low unsaturated control, respectively.

The dietary oil supplementation profoundly affected plasma and LDL fatty acids composition; in particular, in fish oil fed rats, ω-3 fatty acids reached 18% and 35% of the total FA in plasma and LDL, respectively. At the plasma level, fish oil fed rats showed the lower protection against oxidative risk not only when compared with CO fed animals but also with the high-PUFA SO fed rats, despite the absence in the diets of oxidized products. This effect is evidenced by plasma antioxidants level, as well as by the TRAP activity. The differences in the oxidative status of native LDL are less evident.

This suggests that, despite the same vitamin E concentration and the lower total polyunsaturated fatty acids content (SO, 56%; FO, 49%), the LDL of fish oil fed animals is very susceptible to metal-catalyzed oxidative modification, due to its enrichment with ω-3 PUFA.

The oxidation of PUFA leads to the cleavage of the fatty acid carbon chain, resulting in a great number of medium short chain aldehydes.[45] It is currently believed that these medium short chain aldehydes react with the lysine amino group of apoB.[45–47] This oxidative process generates fluorescent chromophores in the lipoprotein. The fluorescence spectra of apoB of native LDL clearly shows that native LDL from FO fed animals has the strongest fluorescence at 430 nm. This result is in agreement with the higher TBA-RS level in native LDL of FO fed rats.

To further explore the susceptibility to oxidative modification induced by the three different diets, LDL were exposed to copper-catalyzed oxidation. LDL of both fish oil and soybean oil treated rats showed a rapid increase of conjugated diene absorbance and LPO and TBARS production. Conjugated dienes formation, higher in the SO than in FO fed animals, could be explained by the very high content of linoleic acid in SO-LDL (19% and 3% of the total FA in SO- and FO-LDL, respectively). The higher TBA-RS in FO-LDL than in SO-LDL can be accounted for by the high content in FO-LDL of long chain ω-3 PUFA with ⩾5 double bounds (336 nmol/mg of protein in FO-LDL and 41 nmol/mg of protein in SO-LDL). These long chain polyunsaturated FAs are rich sources of TBA-RS because they easily form cyclic peroxides, precursors of malondialdehyde which is one of the components of TBA-RS.[48] TBA-RS in SO and CO-LDL is mainly yielded by arachidonic acid (477 nmol/mg of protein, SO-LDL; 74 nmol/mg of protein, FO-LDL; 315 nmol/mg of protein, CO-LDL), the response of linoleic hydroperoxide to the TBA-RS test being very small.[49] On the other hand, in auto-oxidation experiments, long-chain FAs (22:6) have been shown to react faster and with higher yield than arachidonic acid in producing TBA-RS.[45] This behavior also explains the time

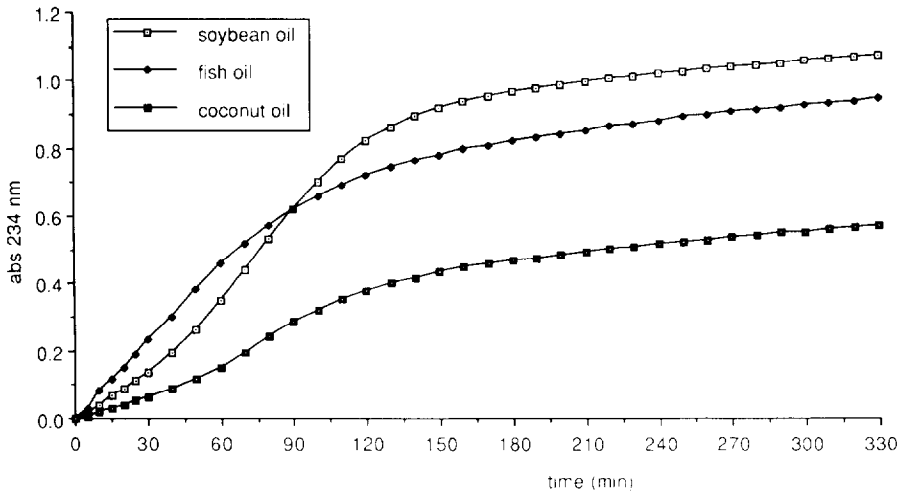

Figure 2 Formation of conjugated dienes during Cu^{2+}-stimulated oxidation of LDL. Two pools of LDL samples (0.250 mg/mL) for each dietary group were oxidized in PBS containing 5 µM Cu^{2+} at 37°C; absorbance was measured continuously at 234 nm. The zero time levels were subtracted from the values shown, and each point represents the mean of two experiments. Difference by 1-factor ANOVA at 4 hr: SO vs. FO, $P < 0.05$; SO and FO vs. CO, $P < 0.0001$.

course of LPO; in fact LPO peaks early and faster in FO-LDL than in SO-LDL.

In conclusion, our results suggest that fish oil enriched diet weakens plasma antioxidant potential and enhances the susceptibility of LDL to in vivo and in vitro oxidative modification in rats. Hence, this study supports the idea that ω-3 fatty acids supplementation in human diets should be associated with the increase of the requirement for antioxidant nutrients.

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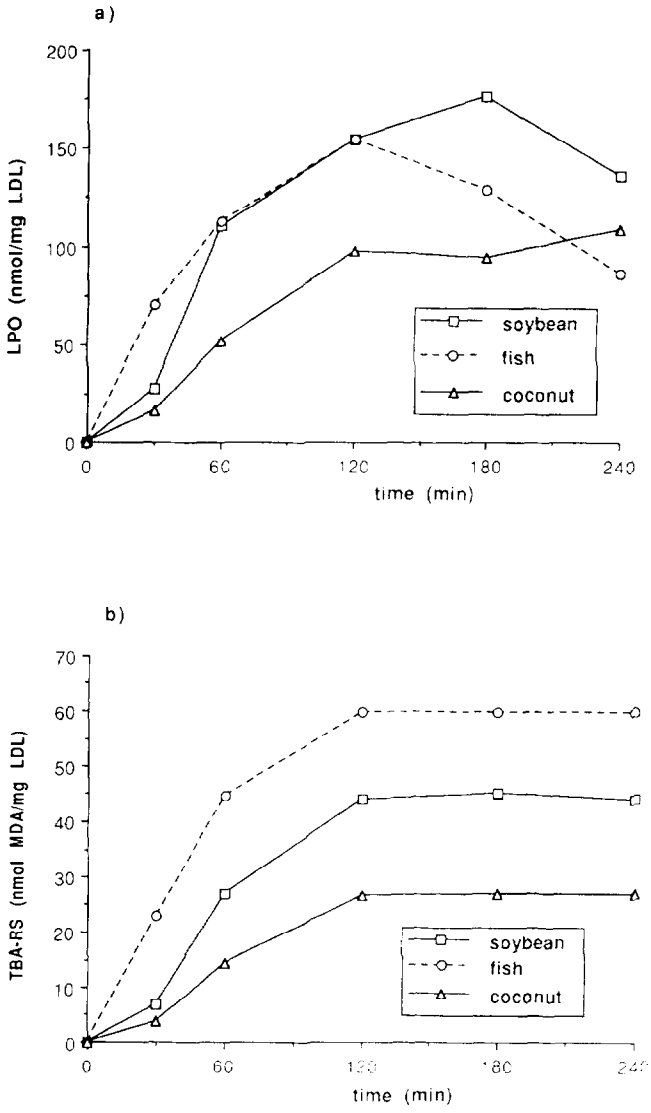

Figure 3 Production of LPO (a) and TBA-RS (b) during Cu^{2+}-stimulated oxidation of LDL. Two pools of LDL samples (0.250 mg/mL) for each dietary group were oxidized in PBS containing 5 μM Cu^{2+} at 37°C. The zero time levels were subtracted from the values shown, and each point represents the mean of two experiments (a) FO and SO vs. CO, $P < 0.03$; (b) FO vs. SO and CO, $P < 0.001$. SO vs. CO, $P < 0.003$ by 2-factor analysis of variance for repeated measurements.

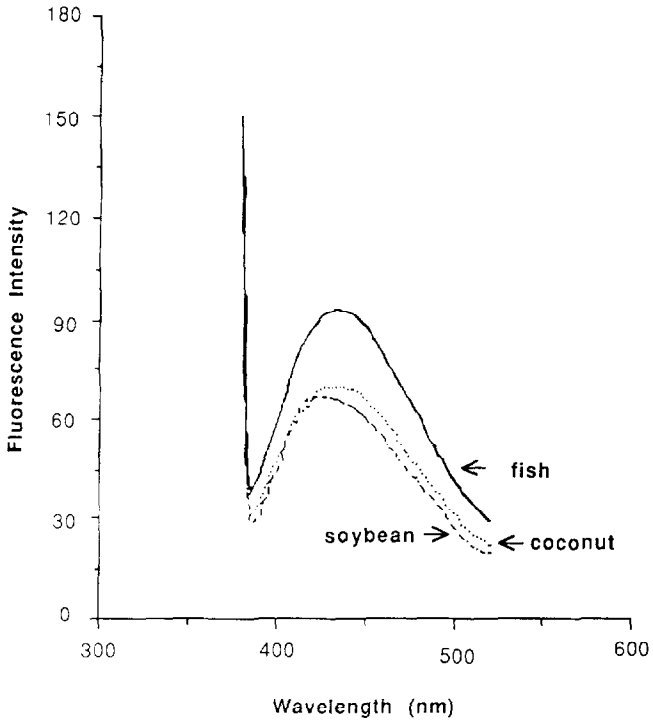

Figure 4 A characteristic emission fluorescence spectra at 360 nm excitation of apoB (corrected for blank value) from 0.8 mg of native LDL, redissolved in 1 mL of 3% aqueous SDS solution.

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