

Effects of dietary fats (fish, olive and high-oleic-acid sunflower oils) on lipid composition and antioxidant enzymes in rat liver

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The effects of two oleic-acid-rich diets (containing olive oil, OO, and high-oleic-acid sunflower oil, HOSO) on plasma and liver lipid composition detoxification enzyme activities, were compared with those of a fish-oil (FO) diet and a control diet. Compared with the control diet, plasma and hepatic total triacylglycerol concentrations were increased in the animals fed on the HOSO and OO diets and decreased in those fed on the FO diet. The animals fed on FO showed the highest level of cholesterol in the liver and had lower plasma cholesterol concentrations when compared with those fed on the two oleic-acid-rich diets. In comparison with the animals fed on the diets enriched in oleic acid, the FO group showed higher hepatic levels of polyunsaturated fatty acids of the *n*-3 series and lower levels of fatty acids of the *n*-6 series. Livers of FO-fed rats, compared with those of OO- and HOSO-fed rats showed: (1) significantly higher activities of catalase (EC 1.11.1.6) glutathione peroxidase (EC 1.11.1.9) and Cu/Zn superoxide dismutase (EC 1.15.1.1); (2) no differences in the NADPH-cytochrome *c* reductase (EC 1.6.99.3) activity. The HOSO diet had a similar effect on liver antioxidant enzyme activities as the OO diet. In conclusion, it appears that changes in the liver fatty acid composition due mainly to *n*-3 lipids may enhance the efficiency of the antioxidant defence system. The two monounsaturated fatty acids oils studied (OO and HOSO), with the same high content of oleic acid but different contents of natural antioxidants, had similar effects on the antioxidant enzyme activities measured.

Fatty acids: Antioxidant enzymes: Oleic acid

Many clinical studies have indicated that diets rich in fish are associated with cardiovascular health (Kromhout *et al.* 1985; Herold & Kinsella, 1986). The responsible component of fish appears to be the high content of polyunsaturated fatty acids (PUFA) of the *n*-3 series (Bang *et al.* 1986). However, there is growing concern that habitual intake of large quantities of PUFA may induce carcinogenesis, probably because they are very susceptible to peroxidation and production of free radicals (Kok *et al.* 1994; Fang *et al.* 1996). The production of free radicals has been associated with ageing (Harman, 1992); however, some studies indicate that fish oil (FO) seems to extend the life span in animal models of autoimmune disease (Jeng & Fernandes, 1989).

The role of the antioxidant defence system, which includes Cu/Zn superoxide dismutase (EC 1.15.1.1; Cu/Zn SOD), catalase (EC 1.11.1.6; CAT), and glutathione peroxidase (EC 1.11.1.9; GSH-Px), in protection against

oxidative insults is well characterized in the liver, and it has been suggested that this antioxidant defence system may be influenced by nutrition (Huang *et al.* 1994).

Besides FO, olive oil (OO), an oil rich in monounsaturated fatty acids (MUFA), is also related to cardiovascular health. OO is beneficial in lowering LDL-cholesterol but not HDL-cholesterol, and decreasing the susceptibility of the LDL to oxidation, which in turn reduces the atherogenicity of the LDL and the development of CHD (Mattson & Grundy, 1985). It has been reported that oleic acid is not necessarily the only component responsible for this effect and that other antioxidant compounds contained in the non-glyceride fraction of OO, such as sterols and polyphenols, may contribute to these beneficial results (Papadopoulos & Boskou, 1991). Although the traditional source of dietary MUFA is OO, other sources are now becoming available such as the new high-oleic-acid variety of sunflower oil

Abbreviations: CAT, catalase; FO, fish oil; GSH-Px, glutathione peroxidase; HOSO, high-oleic-acid sunflower oil; MUFA, monounsaturated fatty acids; OO, olive oil; PUFA, polyunsaturated fatty acids; SOD, superoxide dismutase.

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(HOSO), which has a similar fatty acid composition but different antioxidant content (Perez-Jiménez *et al.* 1995). Compared with FO, the rate of peroxidation and production of free radicals in the MUFA oils is lower.

The aim of the present study was to compare the effects of dietary FO, OO and the HOSO on plasma and hepatic lipid composition and on some hepatic antioxidant enzyme activities. The results show that the behaviour of CAT, Cu/Zn SOD and GSH-Px seems to be related to the *n*-3 content of lipid in the liver. The FO diet provided the greatest antioxidant capability whilst the OO and HOSO diets behaved similarly with regard to the antioxidant enzyme activities.

Methods

Animals and diets

Male Wistar rats (Letica, Barcelona, Spain), weighing about 80 g at the beginning of the experiments, were used. The animals were housed in a well-ventilated room maintained at $22 \pm 2^\circ$ on a 12 h light–dark cycle. The rats were randomly divided into four groups of ten animals. Each group was fed on one of the following diets for 12 weeks: a semipurified diet (basal diet) containing 20 g unspecified lipid/kg, purchased from Panlab SRL (Barcelona, Spain) (control group) or the basal diet supplemented with 100 g/kg OO (OO group) or HOSO (HOSO group) or FO (FO group). The composition of the experimental diets is shown in Table 1. To minimize oxidation, all diets were prepared once weekly and stored at 4° under an atmosphere of N_2 until needed. Changes in composition during storage were not detected.

The fatty acid compositions of the oils were determined and are shown in Table 2. The non-fatty-acid components of the oils are presented in Table 3.

Table 1. Composition of experimental diets (g/kg)

Ingredients	Control	OO	HOSO	FO
Casein	209	203	203	203
Sucrose	450	374	374	374
Maize starch	202	180	180	180
Lipids	20	20	20	20
OO	–	100	–	–
HOSO	–	–	100	–
FO	–	–	–	100
Cellulose powder	52	56	56	56
Mineral mix*	57	57	57	57
Vitamin mix†	10	10	10	10
Total energy (MJ)	15.2	17.1	17.1	17.1
Protein (% energy)	23.1	19.7	19.7	19.7
Lipid (% energy)	4.9	26.3	26.3	26.3
Carbohydrate (% energy)	71.9	53.9	53.9	53.9

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

* Mineral mix contained (mg/g): NaCl 139.3, K_2HPO_4 389.1, $CaCO_3$ 381.4, $MgSO_4 \cdot 7H_2O$ 57.3, $FeSO_4 \cdot 7H_2O$ 27.0, $MnSO_4 \cdot H_2O$ 4.0, $ZnSO_4 \cdot 7H_2O$ 1.25, KI 0.8, $CuSO_4 \cdot 5H_2O$ 0.5, $CoCl_2 \cdot 6H_2O$ 0.02.

† Vitamin mix provided (/kg diet): retinol 5.9 mg, calciferols 0.15 mg, thiamin 20 mg, riboflavin 15 mg, niacin 70 mg, pyridoxine 10 mg, inositol 150 mg, cobalamin 50 μ g, tocopherols 170 mg, vitamin K 40 mg, choline 1.36 g, peroylmonoglutamic acid 5 mg, *p*-aminobenzoic acid 50 mg, biotin 0.3 mg.

Table 2. Fatty acid composition of dietary fats (g/100 g total fatty acids)

Fatty acids	Control	OO	HOSO	FO
14:0	–	–	–	3.8
16:0	13.2	11.8	4.3	13.9
16:1 <i>n</i> -7	1.2	0.9	0.1	15.1
17:0	–	0.4	0.1	–
18:0	3.1	2.8	4.7	0.9
18:1 <i>n</i> -9	36.8	79.2	80.2	26.2
18:2 <i>n</i> -6	41.7	3.5	9.4	5.1
18:3 <i>n</i> -3	4.1	0.6	0.1	0.2
18:4 <i>n</i> -3	–	–	–	2.5
20:0	–	0.3	0.4	–
20:1 <i>n</i> -9	–	0.2	0.2	2.4
20:2 <i>n</i> -6	–	–	–	1.4
20:3 <i>n</i> -6	–	–	–	0.4
20:4 <i>n</i> -6	–	–	–	0.6
20:5 <i>n</i> -3	–	–	–	13.8
22:4 <i>n</i> -6	–	–	–	0.3
22:6 <i>n</i> -3	–	–	–	12.7
24:0	–	0.4	0.4	–

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

Blood sampling and tissue preparations

At completion of the study the animals were killed by cervical dislocation. To minimize diurnal variations the rats were routinely killed between 09.00 and 10.00 hours. Blood samples were removed from the heart and collected into tubes containing EDTA (1 g/l). Plasma was separated by low-speed centrifugation at 1500 g at 4° for 30 min and was immediately analysed. Cholesterol, phospholipids and triacylglycerols were determined using an autoanalyser and conventional enzymic methods (Nelson, 1972; Bucolo & David, 1973; Allain *et al.* 1974).

The livers were immediately rinsed in ice-cold 0.145 M-NaCl, trimmed and quickly weighed. A 2 g portion of liver was used for lipid extraction and the rest was used to determine enzyme activities. All subsequent processing procedures were carried out at $0-4^\circ$. Homogenates (100 g/l)

Table 3. Composition of the non-fatty-acid components of the experimental oils

	OO	HOSO	FO
Total unsaponifiable fraction (g/kg)	15	10	11
Total sterols (mg/kg)	1696	1754	1500
Cholesterol (%)	0.15	0.01	100
Brassicasterol (%)	–	0.32	–
Campesterol (%)	3.80	10.25	–
Stigmasterol (%)	0.81	11.59	–
β -Sitosterol (%)	93.99	59.35	–
Δ -5-Avenasterol (%)	–	2.88	–
Δ -7-Stigmasterol (%)	0.22	11.84	–
Δ -7-Avenasterol (%)	0.15	3.27	–
Squalene (mg/kg)	3000	90	7400
Tocopherols (mg/kg)	47	10	300
α -Tocopherols	34	10	300
γ -Tocopherols	13	–	–
Fraction of polyphenols (mg/kg)	470	–	–
Total polyphenols (mg/kg)	430	–	–
Ortodiphenol (mg/kg)	40	–	–

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

were prepared in 0.25 M-sucrose, 1 mM-EDTA, 1 mM-DL-dithiothreitol and 15 mM-Tris HCl (pH 7.4), using an all-glass Potter Elvehjem homogenizer (Selecta, Barcelona, Spain). Each homogenate was centrifuged for 20 min at 800 g. The resulting supernatant fraction was used to determine enzyme activities.

Extraction and separation of lipids

Quantitative extraction of total lipids from 2 g liver was carried out following the method of Folch *et al.* (1957) in the presence of butylated hydroxytoluene as antioxidant. Tissue dissociation was achieved by homogenization in ice-cold chloroform-methanol (2:1, v/v) containing 0.1 g BHT/l using an Ultra-Turrax model Type TP-18-1 (Ultra-Turrax, Vineland, NJ, USA).

The lipid extract was quantified gravimetrically and kept in stoppered tubes under N₂ at -30° until the assays. Lipid composition was determined by means of the Iatroscan TLC technique with flame ionization detection (De Schrijver & Vermeulen, 1991). The Iatroscan MK-5 (Iatron Laboratories Inc., Tokyo, Japan) was used in combination with Chromarods S, which have a precoated active silica thin layer. Samples of total lipids (3 µl) were spotted onto each rod, using a 10 µl Hamilton syringe. To separate total lipids, rods were developed in hexane-diethyl ether-formic acid (90:10:2, by vol.). Rods were scanned under the following conditions: H₂ flow, 150 ml/min; air flow, 1750 ml/min; scanning speed, 47 mm/s; chart speed, 42 mm/min. An Iatroscorder TC-11 integrator was used for recording and area integration.

Fatty acid analysis

Fatty acids (g/100 g total fatty acids) were determined by GC, as previously described (Molina *et al.* 1989). The samples were saponified by heating for 25 min with 5 ml 0.2 M-sodium methylate and heated again at 80° for 25 min with H₂SO₄ in anhydrous methanol (60 g/l). The fatty acid methyl esters thus formed were eluted with hexane and analysed in a Hewlett Packard 5890 series II GC equipped with flame ionization detector and using an Omegawak 320 fused silica capillary column (30 m × 0.32 mm i.d., 0.25 mm film). The initial column temperature was 200°, which was held for 10 min, then programmed from 200–230° at 2°/min.

Non-fatty-acid components

For the extraction of the unsaponifiable matter, 20 g oil was saponified for 30 min with 75 ml of ethanolic KOH (100 g/l). The solution was transferred to a 5000 ml decanting funnel, 100 ml distilled water was added, and the mixture was extracted with 100 ml portions of hexane. The hexane solution was evaporated to dryness in a rotary evaporator at 30° under reduced pressure. The sterol fraction was analysed by capillary GLC (Garcia Regueiro *et al.* 1994). Tocopherols were analysed by HPLC (Kramer *et al.* 1997). For the assay of squalene, the hydrocarbon fraction was separated from the oil by column chromatography on silica gel and analysed by capillary GLC (Sulpice & Ferezou,

1984). The composition of the polyphenols fraction was determined by capillary GLC (Arce *et al.* 1998).

Enzyme activities

CAT activity was assayed according to the method of Beers & Sizer (1952). The final concentrations in the cuvettes were 0.5 M-KH₂PO₄ (pH 7), 100 mM H₂O₂ and 0.05–0.1 mg tissue sample. The decrease in the absorbance at 240 nm after the addition of the substrate was followed spectrophotometrically.

GSH-Px activity was assayed with a coupled enzyme system in which GSSG reduction was coupled to NADPH oxidation by glutathione reductase (EC 1.6.4.1; Lawrence & Burk, 1976). The assay mixture contained 0.1 M-KH₂PO₄ (pH 7.5), 5 mM-EDTA, 2 mM-Na₃N, 1 mM-GSH, 0.2 mM-NADPH, 1 U glutathione reductase and tissue sample (0.05–0.2 mg). After 5 min pre-incubation (20–25°), the reaction was initiated by the addition of 0.05 ml 5 mM-H₂O₂ (final volume 1.0 ml). The decrease in the absorbance at 365 nm was followed spectrophotometrically.

SOD activity was measured using the xanthine oxidase (EC 1.2.3.2)-cytochrome *c* method as described by McCord & Fridovich (1969). The final concentrations in the cuvettes were 50 mM-KH₂PO₄ (pH 7.8), 0.1 mM-EDTA, 10 mM-cytochrome *c*, 50 mM-xanthine, 50 mM or 2 mM-cyanide, 1 U CAT and 0.05–1.0 mg tissue sample. The reaction was initiated by the addition of 1 U xanthine oxidase. The inhibition of xanthine oxidase was followed spectrophotometrically at 550 nm. One unit of SOD activity is defined as the amount of enzyme required to inhibit the rate of cytochrome *c* reduction by 50 %.

NADPH-cytochrome *c* reductase (EC 1.6.99.3) activity was measured as described by Vermilion & Coon (1978). The 1.0 ml assay mixture contained the following components: 300 mM-phosphate buffer (pH 7.7), 0.04 mM-cytochrome *c*, 0.1 mM-EDTA, 0.2 mM-NADPH and tissue (0.05–0.2 mg). The reaction was initiated by the addition of the NADPH, and the reduction of cytochrome *c* was followed spectrophotometrically at 550 nm.

All spectrophotometric measurements were carried out in a Shimadzu 160 A ultraviolet spectrophotometer (Shimadzu Corporation, Kyoto, Japan) with 1.0 ml quartz cuvettes with a light path of 1.0 cm. All enzyme assays were performed at 25°. Specific activities were expressed as nmol/min per mg protein. Protein concentrations were determined by the method of Lowry *et al.* (1951).

Statistical methods

All results were subjected to one-way ANOVA, and represent means, with their standard errors, of ten animals per group. Differences in mean values between groups were assessed by the two-tailed Student's *t* test and were considered statistically different at *P* < 0.05.

Results

In the present study, rats in the four experimental groups consumed similar amounts of food (Table 4). Animals fed on diets containing OO or HOSO had similar body weights,

Table 4. Effect of different dietary fats, fed for 12 weeks, on body weight, food intake, liver weight and liver lipid content in rats*
(Mean values with their standard errors for ten rats per group)

Diet...	Control		OO		HOSO		FO		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Food intake (g/d)	20.8	1.1	21.0	0.9	22.8	1.9	19.1	1.2	
Body wt (g)	at entry	85.1	2.3	77.6	3.2	78.3	3.1	86.8	2.4
	at 12 weeks	302.4 ^a	7.8	325.9 ^b	6.1	331.1 ^b	13.2	260.4 ^c	6.6
Liver wt (g)	9.3 ^a	0.8	10.4 ^a	0.8	9.4 ^a	1.2	7.5 ^b	0.6	
Liver wt (g/kg body weight)	31	2	30	3	31	2	29	4	
Liver lipid content (g/kg)	21 ^a	5	45 ^b	4	44 ^b	3	32 ^c	2	

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different: $P < 0.05$.

* For details of diets and procedures, see Tables 1–3 and pp. 234–235.

but these were higher than those of animals fed on the control or FO diets. Animals fed on the diet containing FO had significantly lower body and liver weights when compared with the other groups; however, the liver:body weight values were similar in all the groups. Liver lipid content was higher in animals fed on the OO and HOSO diets than in those fed on the control or FO diets.

Table 5 shows the effect of dietary fat treatment on plasma lipid content. When compared with the control diet, the HOSO and OO diets led to significant increases and the FO diet led to a significant decrease in plasma triacylglycerol level. Consumption of the FO diet resulted in a decrease in the total cholesterol and phospholipid concentrations in plasma in comparison with the high-oleic-acid diets. The HOSO and OO groups did not differ in plasma lipid concentrations, but these were higher than those in the control group.

Total triacylglycerol concentrations in liver are shown in Table 6. The OO-fed group showed the highest values and the FO-fed group the lowest. Total hepatic cholesterol was lowest in the control group and highest in the FO group. OO- and HOSO-fed groups showed intermediate values, and no difference was found between these two high-oleic-acid diets. The hepatic phospholipid content was lowest in the animals fed on the control diet. The animals fed on the oil-enriched diets showed similar hepatic phospholipid contents.

Table 7 shows the proportions of fatty acids (g/100 g total fatty acids) in liver lipids in rats fed on the different diets. The highest proportions of the two major saturated fatty acids, palmitic (16:0) and stearic (18:0) acids, were found in

rats fed on the FO and control diets as compared with the high-oleic-acid oil groups. Thus, the levels of total saturated fatty acids in liver were higher in the control and the FO-fed groups in comparison with the animals fed on the diets enriched in oleic acid. The proportions of 18:1 n -9 were similar in the OO- and HOSO-fed groups and higher than in the control- or FO-fed animals. The MUFA:saturated fatty acids ratio was consequently increased in animals fed on the two oleic-acid-rich diets compared with the other groups. The FO group had a higher proportion of long-chain PUFA of the n -3 series (20:5, 22:5 and 22:6) than the control, OO, or HOSO groups. The FO group also had lower proportions of long-chain PUFA of the n -6 series (20:4, 22:4 and 22:5) with respect to the other groups. Therefore the n -6: n -3 ratio and the 20:4/18:2 ratio were markedly lower in the FO group.

The animals fed on the FO diet expressed higher CAT activity in the liver (5.06 (SE 0.27) U/mg protein) when compared with the other groups (Fig. 1(a)). The CAT activity was similar in the OO and HOSO groups (approximately 3.70 U/mg protein). The GSH-Px activity in animals fed on the control diet was 0.63 (SE 0.01) U/mg protein. Feeding the FO diet increased this activity to 0.74 (SE 0.05) U/mg protein and feeding OO or HOSO decreased the activity to 0.39 (SE 0.02) U/mg protein and 0.47 (SE 0.08) U/mg protein respectively (Fig. 1(b)).

The Cu/Zn SOD activity was highest in the FO-fed group (3.51 (SE 1.12) U/mg protein) and was significantly higher than in the other groups. The oleic-acid-enriched oils had no effect on Cu/Zn SOD activity compared with the control diet (Fig. 2(a)). The activities of NADPH-cytochrome *c*

Table 5. Plasma lipid concentrations in rats fed for 12 weeks on diets containing different fats*
(Mean values with their standard errors for ten rats per group)

Diet ...	Control		OO		HOSO		FO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Triacylglycerols (mmol/l)	1.25 ^a	0.23	1.72 ^b	0.12	1.68 ^b	0.17	0.89 ^c	0.14
Cholesterol (mmol/l)	2.02 ^a	0.41	2.55 ^b	0.22	2.44 ^b	0.23	2.20 ^a	0.21
Phospholipids (mmol/l)	1.29 ^a	0.12	1.44 ^b	0.15	1.46 ^b	0.21	1.23 ^a	0.15

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different: $P < 0.05$.

* For details of diets and procedures, see Tables 1–3 and pp. 234–235.

Table 6. Liver lipid concentrations in rats fed for 12 weeks on diets containing different fats*
(Mean values with their standard errors for ten rats per group)

Diet ...	Control		OO		HOSO		FO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Triacylglycerols (mg/g)	5.5 ^a	0.8	12.3 ^b	1.7	8.3 ^c	0.9	1.9 ^d	0.6
Cholesterol (mg/g)	3.1 ^a	0.3	4.4 ^b	0.6	4.7 ^b	0.5	6.7 ^c	0.6
Phospholipids (mg/g)	13.4 ^a	1.3	29.6 ^b	3.7	32.3 ^b	3.8	26.5 ^b	3.2

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil.

^{a,b,c,d} Mean values within a row not sharing a common superscript letter were significantly different: $P < 0.05$.

* For details of diets and procedures, see Tables 1–3 and pp. 234–235.

reductase were similar in livers of rats fed on the oil-enriched diets, and were higher than that for the control animals (Fig. 2(b)).

Discussion

The present study was designed to determine whether feeding a PUFA oil (FO) or two MUFA oils, prepared from two different sources (OO and HOSO), affects the liver lipid composition and the antioxidant defence system; the plasma lipid composition was also studied.

Liver lipid triacylglycerol levels were lowest in FO-fed rats (Table 6). This may have been due to inhibition of hepatic triacylglycerol synthesis (Wong *et al.* 1984; Froyland *et al.* 1997), and stimulation of hepatic peroxisomal

β -oxidation (Yamazaki *et al.* 1987) produced by the FO diet. Decreases in lipoprotein lipase (*EC* 3.1.1.34) and triacylglycerol lipase (*EC* 3.1.1.3) activities in the liver after FO consumption have also been described (Huff *et al.* 1993), and this may be an adaptive response to the low concentrations of substrates (triacylglycerols) for these enzymes. The low levels of triacylglycerols in liver after FO consumption may also be related to the reduced levels of plasma triacylglycerols found in our study (Table 5) and also described by others in man (Phillipson *et al.* 1985) and rats (Yamazaki *et al.* 1987).

The OO diet resulted in a higher liver triacylglycerol content than the HOSO diet. This fact has been recently corroborated by other studies from our group (Perona & Ruiz-Gutierrez, 1998), in which it has been reported that the

Table 7. Fatty acid composition (g/100 g total fatty acids) of the liver lipids of rats fed for 12 weeks on diets containing different fats*

(Mean values with their standard errors for ten rats per group)

Diet ...	Control		OO		HOSO		FO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
14:0	0.4	0.1	0.3	0.1	0.4	0.1	0.4	0.1
16:0	21.1 ^a	0.4	15.4 ^b	2.0	17.3 ^b	0.7	22.3 ^a	1.0
16:1 n -7	1.8	0.3	1.1	0.1	1.5	0.1	1.1	0.1
18:0	18.5 ^a	3.3	13.9 ^b	0.7	13.3 ^b	2.1	19.7 ^a	1.8
18:1 n -9	13.1 ^a	1.3	27.7 ^b	0.5	24.6 ^b	2.7	8.4 ^c	0.2
18:1 n -7	1.4	0.1	1.5	0.4	1.7	0.1	1.0	0.1
18:2 n -6	13.1	1.7	11.9	0.5	13.3	1.1	13.9	1.6
18:3 n -3	0.3 ^a	0.0	0.2 ^b	0.0	0.2 ^b	0.0	0.3 ^a	0.0
20:0	0.5 ^a	0.1	0.2 ^b	0.1	0.3 ^b	0.0	0.3 ^b	0.0
20:1 n -9	0.1 ^a	0.0	0.4 ^b	0.1	0.3 ^b	0.0	0.1 ^a	0.0
20:4 n -6	23.2 ^a	1.6	21.9 ^a	1.5	21.6 ^a	1.6	15.6 ^b	0.7
20:5 n -3	0.2 ^a	0.0	0.1 ^a	0.0	0.1 ^a	0.0	3.0 ^b	0.9
22:0	0.6 ^a	0.1	0.5 ^a	0.1	0.7 ^a	0.1	0.9 ^b	0.0
22:4 n -6	0.5 ^a	0.0	0.4 ^a	0.1	0.3 ^a	0.1	0.6 ^b	0.0
22:5 n -6	0.6 ^a	0.2	0.4 ^a	0.1	0.4 ^a	0.1	0.2 ^b	0.0
22:5 n -3	0.7 ^a	0.1	0.3 ^b	0.1	0.4 ^b	0.1	1.7 ^c	0.4
22:6 n -3	3.8 ^a	0.6	3.6 ^a	0.8	3.3 ^a	0.4	11.1 ^c	1.8
SFA	41.1 ^a	3.4	30.3 ^b	2.8	31.9 ^b	2.9	43.5 ^a	2.8
MUFA	16.5 ^a	1.7	30.8 ^b	1.1	28.1 ^b	2.4	10.6 ^c	0.5
PUFA	42.4 ^a	3.4	39.0 ^b	2.3	39.7 ^b	1.3	45.9 ^a	1.7
Total n -6	37.4 ^a	3.9	34.7 ^a	2.2	35.7 ^a	3.7	29.9 ^b	2.2
Total n -3	5.0 ^a	0.4	4.3 ^a	0.4	4.0 ^a	0.4	16.1 ^b	2.9
n -6 : n -3	7.5 ^a	1.9	8.1 ^a	1.7	8.9 ^a	2.3	1.9 ^b	0.6
MUFA : SFA	0.4 ^a	0.3	1.0 ^b	0.3	0.9 ^b	0.2	0.2 ^a	0.1
20:4/18:2	1.7 ^a	0.4	1.8 ^a	0.5	1.6 ^a	0.3	1.1 ^b	0.2

OO, olive oil; HOSO, high-oleic-acid sunflower oil; FO, fish oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different: $P < 0.05$.

* For details of diets and procedures, see Tables 1–3 and pp. 234–235.

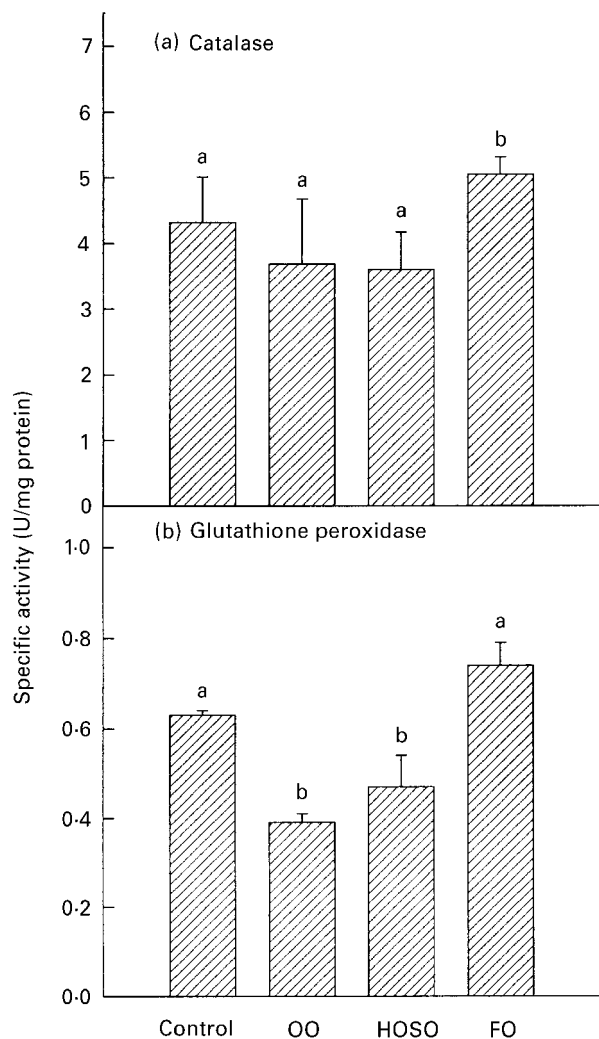


Fig. 1. Specific activities of (a) catalase and (b) glutathione peroxidase in the livers of rats fed for 12 weeks on a control diet or diets containing 100 g/kg of olive oil (OO), high-oleic-acid sunflower oil (HOSO) or fish oil (FO). Values are means for ten rats per group, with standard deviations represented by vertical bars. ^{a,b}Mean values not sharing a common letter were significantly different: $P < 0.05$.

consumption of OO results in a greater amount of liver triacylglycerol when compared with consumption of HOSO. These results suggest that factors other than the oleic acid content of the oils may be responsible for enhancing triacylglycerol synthesis in the liver of the animals fed on the OO diet. Furthermore, the same authors in the present study observed different distributions of triacylglycerol molecular species in rat liver; OO led to a higher content of dioleoylglycerol species while the HOSO diet led to an increase in saturated triacylglycerol species, suggesting that these effects may be due to an enhancement in the synthesis of such molecular species. In plasma we did not observe differences in triacylglycerol content for the two high-oleic-acid diets studied.

Cholesterol levels in liver were increased in animals fed on the lipid-supplemented diets compared with the control group (Table 6). The FO diet led to higher values than the HOSO and OO diets. The increased liver cholesterol content

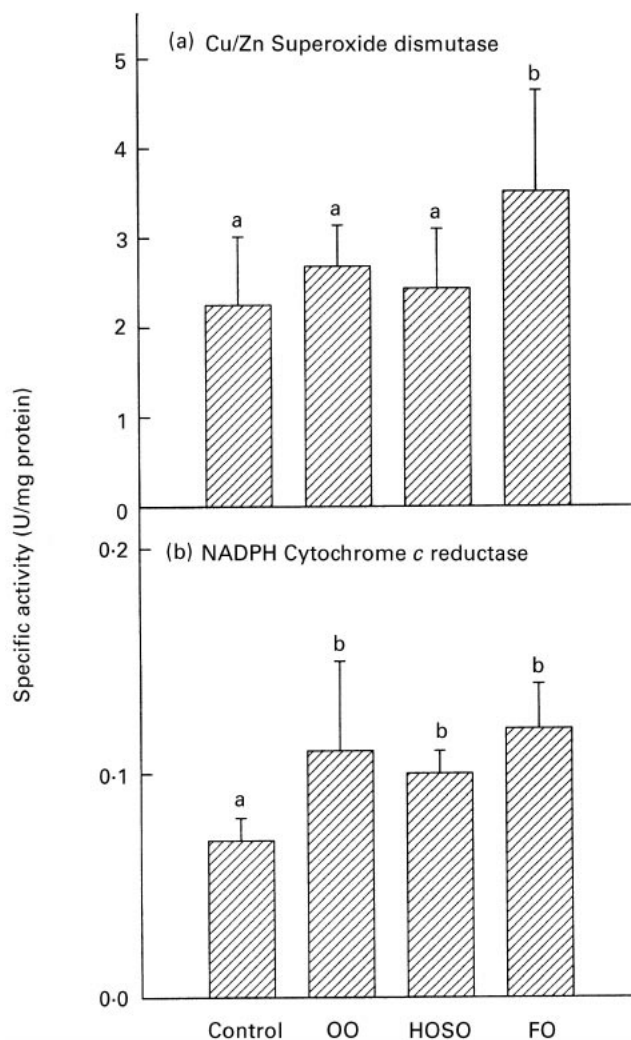


Fig. 2. Specific activities of (a) Cu/Zn superoxide dismutase and (b) NADPH-cytochrome *c* reductase in the livers of rats fed for 12 weeks on a control diet or diets containing 100 g/kg of olive oil (OO), high-oleic-acid sunflower oil (HOSO) or fish oil (FO). Values are means for ten rats per group, with standard deviations represented by vertical bars. ^{a,b}Mean values not sharing a common letter were significantly different: $P < 0.05$.

in the FO-fed rats might have been due to the increased squalene content in this diet compared with the others (Table 3). The dietary effect of this precursor of cholesterol biosynthesis is not clear, but it has been shown to increase biliary cholesterol secretion (Ulloa & Nervi, 1985). In plasma we found a lower cholesterol level in the FO group than in the OO and HOSO groups. It has also been described that FO consumption reduces cholesterol levels in blood, and that it is more hypocholesterolaemic than OO (Masi *et al.* 1986). Garg *et al.* (1988) reported that PUFA lower cholesterol levels in man and animals due to a redistribution of cholesterol from blood to tissues.

We found high cholesterol levels in both liver and plasma following oleic acid feeding compared with the control diet. This hypercholesterolaemic effect of a diet high in OO in serum and liver of rats has been reported previously by Yaqoob *et al.* (1995). Jeffery *et al.* (1996) confirmed the

effect on serum total cholesterol concentrations in rats fed on diets containing OO or HOSO. In man, however, hypocholesterolaemic effect of MUFA is well established (Mattson & Grundy, 1985) although the mechanism by which it is brought about remains unclear.

Analysis of the fatty acid composition in rat liver homogenates showed a significant increase of 18:1 in liver lipids obtained from OO- and HOSO-fed rats. We also found lower saturated fatty acid levels in both groups. This increase in oleic acid content was probably related to the higher content of oleic acid in the diet of these animals. It has been reported that when endothelial cell cultures are directly supplemented with oleic acid, an increase in its content is found, accompanied by a decrease in the saturated acid content (Spector & Yorek, 1985).

With respect to the FO diet, we found a marked increase in the proportion of total *n*-3 fatty acids (mainly 20:5 and 22:6), and a concomitant decrease in *n*-6 (20:4, 22:4 and 22:5) fatty acids in the liver. As a consequence, a significant reduction in the ratio *n*-6:*n*-3 was found in these animals. Other authors have reported a decrease in arachidonic acid in liver (Venkatraman *et al.* 1994) and lung (Archer *et al.* 1987) in animals fed on FO.

Animals fed on the FO diet had higher activities of GSH-Px, CAT and Cu/Zn SOD compared with those fed on the control diet and the oleic-acid-enriched diets. Because the lipid composition of the liver of rats reflected the lipid composition of the diet, the liver lipids of the rats fed on the FO diet contained higher levels of PUFA. This fact might render the livers of rats fed on the FO diet more susceptible to lipid peroxidation and the activity of antioxidant enzymes might be induced. A potential mechanism for the induction of hepatic antioxidants following FO feeding might be an increase of the expression of their genes; induction of the expression of antioxidant enzymes has been reported in circumstances where an increase in free radicals is produced, such as ageing (De Haan *et al.* 1992) or several pathologies (Ceriello *et al.* 1996; Larrea *et al.* 1998). It is also well known that free radicals may regulate the transcription of many other genes (Cramer *et al.* 1995; Roche & Romero-Alvira, 1995).

This greater activity of antioxidant enzymes may contribute to the hypothesis that consumption of FO extends lifespan. This fact has been described in animal models of autoimmune disease (Jeng & Fernandes, 1989). Antioxidants have been closely linked with the preservation of health and longevity in both mice and rats (Semsei *et al.* 1989; Rao *et al.* 1990).

Yamazaki *et al.* (1987) found that feeding FO to animals increased the activities of some liver enzymes (fatty acyl-CoA synthetase (EC 2.3.1.85), CAT and GSH-Px) when compared with safflower-oil-fed animals. Venkatraman *et al.* (1994) described an increase in the activities and mRNA expression of CAT, GSH-Px and SOD in mice fed on a diet rich in FO compared with other diets rich in *n*-6 lipids. Recently, the same group has reported increases in CAT and GSH-Px activities in sedentary and exercised Fischer-344 rats respectively, after consumption of FO, and non-significant changes in membrane lipid peroxidation (Venkatraman *et al.* 1998). In contrast, L'Abbe *et al.* (1991) reported that diets high in *n*-3 fatty acids elevate the lipid peroxidation in

heart and liver, due in part to decreased SOD and GSH-Px activities. Nalbhone *et al.* (1980) reported that the GSH-Px activity of rats fed on FO diets remained unchanged in liver. Berge & Thomassen (1985) showed that CAT activity was affected only slightly by a FO diet, and Van Noorden (1995) described a moderate increase in CAT activity.

Less attention has been paid to the effect of oleic-acid-enriched diets on antioxidant enzymes. In our studies we have not found differences in the activities of the antioxidant enzymes between the two high-oleic-acid diets tested. The non-glyceride fractions of these oils are different (Table 3); polyphenols, which have free-radical-scavenging properties, are only present in OO, and tocopherols, which also have an antioxidant effect, are higher in OO. These differences in the natural antioxidant content in the two oils seems not to affect the activity of the hepatic antioxidant enzymes studied.

NADPH-cytochrome *c* reductase participates in the detoxification of drugs and xenobiotics. Similar activities of NADPH-cytochrome *c* reductase were observed in rats fed on the OO, HOSO and FO diets in the present study, and each oil diet supported higher activity than the control diet. Hepatic NADPH-cytochrome *c* reductase activity was not significantly affected by the FO diet in comparison with a low-fat diet or diets enriched in *n*-6 PUFA (Van Noorden, 1995). The concentration of cytochrome P450 and rates of oxidative drug metabolism were greater when polyunsaturated fats were incorporated into the diet than when fat-free diets or saturated fats were given (Smith & Willis, 1981).

In summary, it appears that changes in the liver fatty acid composition, due mainly to *n*-3 lipids, may increase the activity of some antioxidant enzymes, compensating the risk of carcinogenesis due to the peroxidation of PUFA and free-radical production. On the other hand, the two MUFA oils studied (OO and HOSO), in spite of their different contents of natural antioxidants, have similar effects on the antioxidant enzyme activities studied.

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