Effects of Dietary Linseed Oil and Marine Oil on Lipid Peroxidation in Monkey Liver *in vivo* and *in vitro*

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Diets rich in linoleic acid (CO) from corn oil, or in linoleic acid and either α-linolenic acid (LO) based on linseed oil or n-3 fatty acids (MO) from menhaden oil were fed to male and female Cynomolgus monkeys for 15 wk. In the liver a 40% reduction of α-tocopherol occurred in the MO group relative to the CO and LO groups followed by increased formation of lipofuscin *in vivo*. A four-fold increase of α-tocopherol in the MO diet (MO + E) brought the level in the liver to that found with CO and LO. The increased peroxidation in the MO group in the liver phospholipids was associated with the replacement of 60% of the n-6 fatty acids by n-3 fatty acids from menhaden oil. Similar fatty acid profiles were found in groups fed MO and MO + E, respectively. Compared to the CO fed group, feeding α-linolenic acid only resulted in a slight incorporation of n-3 fatty acids in the liver membranes mainly due to a direct incorporation of α-linolenic acid. However, in monkeys fed menhaden oil more than 30% of the total fatty acids in the liver phospholipids were n-3 fatty acids. The various diets did not influence the activity of liver catalase (EC 1.11.1.6) nor superoxide dismutase (EC 1.15.1.1), but glutathione-peroxidase activity (EC 1.11.1.9) was higher in monkeys fed the MO diet. The catalase activity in females was 20% higher than in males. In an *in vitro* assay, liver microsomes from monkeys fed the MO diet or the MO diet supplemented with tocopherol produced similar amounts of thiobarbituric acid reactive substances and at a much higher rate than microsomes from the CO and LO groups. It appeared that α-tocopherol did not protect long-chain n-3 C_{20} and C_{22} fatty acids as well as n-6 fatty acids against peroxidation. The present data showed that monkeys were not fully able to compensate for increased peroxidative stress but a four-fold supplement of vitamin E to the diets reduced the oxidation.

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Diets rich in n-3 fatty acids have been shown to increase unsaturation in the membrane phospholipids of rat heart, liver and kidney (1,2) and of erythrocytes of cynomonkeys (3). Yet, little is known how the cells respond to the resulting increase in peroxidative stress. There is also only little information on the effects of dietary, highly unsaturated fatty acids on tissue lipid composition in primates in general.

The present study was undertaken to examine the effects of highly unsaturated dietary fats such as linseed oil and menhaden oil on the fatty acid profiles of liver phospholipids in monkeys. We further examined whether the monkey compensates for the increased oxidative stress by an induction of the three enzymes: catalase, superoxide dismutase (SOD) and glutathione-peroxidase (GSH-Px), which are known to remove the oxygen species believed to initiate lipid peroxidation (4). Furthermore, the ability of α-tocopherol to inhibit lipid peroxidation was examined.

MATERIALS AND METHODS

Four groups of Cynomolgus monkeys *(Macaca fascicularis)*, five males and five females in each group, were fed diets containing 15% (w/w) fat for 15 wk as previously described (3). The diets were: Diet 1, fat consisting of corn oil (Mazola Oil, Ottawa, Canada) and lard (Canada Packers, Toronto, Canada) designated corn oil (CO) diet; Diet 2, fat consisting of lard and linseed oil (Maple Leaf Monarch, Toronto, Canada) designated linseed oil (LO) diet; Diet 3, fat consisting of corn oil, lard and menhaden oil (Zapata Haynie Corp., Reedville, VA) designated menhaden oil (MO) diet; Diet 4, same as MO diet, supplemented with *all-rac* α-tocopheryl acetate and designated MO + E diet (Table 1). According to the manufacturer the menhaden oil contained 50 ppm free α-tocopherol and only 0.20% free fatty acids. In order to reduce the autoxidation to a minimum, all dietary oils were stored at −18°C under nitrogen, and the diets were freshly prepared every week and kept at 4°C as a gel. The monkeys were 6.5 ± 0.3 years old at the start of the feeding experiment.

After being fed the experimental diets for 15 wk, the monkeys were fasted for 18 h and anaesthetized with ketamin·HCl (Rogarsetic, Bristol Laboratories, Syracuse, NY). Blood was quickly drained from the *vena cava* by syringe. Liquid containing 10% (wt/vol) sodium citrate and 0.9% (wt/vol) NaCl was perfused through the body from a syringe in the left ventricle. The still-pumping heart caused an efficient perfusion of the whole body in a few minutes after which the liver was quickly removed. Portions of approximately 4 g were immediately frozen in liquid nitrogen and stored at −78°C for subsequent assays. Liver tissue (7 g) was immediately cooled in ice-cold 0.25 M sucrose, homogenized by a Potter-Elvehjem glass/Teflon homogenizer and adjusted with 0.25 M sucrose to a 10% (wt/vol) crude homogenate. Whole cells and cell debris were removed by centrifugation at 600 × *g* for 10 min. The supernatant (named liver homogenate) was kept on ice and enzyme, lipofuscin and protein assays were performed the day the animals were killed.

For the tocopherol analysis, 2 g of frozen liver was homogenized for 10 s in 4 mL of 0.25 M sucrose with a Polytron homogenizer (Kinematika AG, Littau, Switzerland) and adjusted to a 25% (wt/vol) homogenate. Following extraction, α- and γ-tocopherol was assayed by the high-performance liquid chromatography (HPLC) method of Thompson and Hatina (5) as described by Behrens and Madère (6).

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Abbreviations: CO, corn oil diet; GLC, gas-liquid chromatography; GSH-Px, glutathione peroxidase; HPLC, high-performance liquid chromatography; LO, linseed oil diet; MO, menhaden oil diet; MO + E, menhaden oil diet supplemented with *all-rac* α-tocopheryl acetate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TLC, thin-layer chromatography.

TABLE 1

Fatty Acid Compositions of Dietary Fats and Content of α- and γ-Tocopherol[a]

Fatty acids	Dietary group			
	CO	LO	MO	MO + E
	(area percent)			
Saturated	39.7	39.7	43.9	43.1
Monoenoic	35.2	35.5	32.8	35.5
18:2n-6	24.7	11.8	10.6	10.8
18:3n-3	0.4	13.0	0.9	0.9
18:4n-3	—	—	1.8	1.9
20:5n-3	—	—	4.7	4.6
22:6n-3	—	—	2.8	2.4
Σ (n-6)	24.8	11.8	10.8	11.2
Σ (n-3)	0.4	13.0	10.9	10.2
	(μg/g diet)			
α-tocopherol	70.2	59.3	61.6	270.2
γ-tocopherol	59.0	9.4	8.5	6.5

[a]The dietary fats were the following: CO diet (36% corn oil and 64% lard); LO diet (34% linseed oil and 66% lard); MO diet (68% menhaden oil, 16% lard, and 16% corn oil); MO + E diet (68% menhaden oil, 16% lard, and 16% corn oil + an additional four-fold supplement of *all-rac* α-tocopheryl acetate). Fatty acid composition was determined by GLC after extraction and transmethylation of the lipids from the diets, and the tocopherols in the diets were measured by HPLC after saponification using external standards.

Lipofuscin was extracted from the liver homogenate and measured by fluorescence spectroscopy as described by Koster and Slee (7), and protein was assayed by the method of Lowry *et al.* (8).

The activity of the selenium containing GSH-Px (EC 1.11.1.9) was determined by the method of Flohé and Günzler (9). The activity of Cu,Zn-SOD (EC 1.15.1.1) was determined in the liver homogenate, diluted 10 times with 1% Tween-80 in 50 mM phosphate buffer, pH 7.0, using the procedure given by Prohaska (10). The assay for catalase (EC 1.11.1.6) was performed as previously described (11).

The lipids were extracted from the liver by the method of Bligh and Dyer (12), and the phospholipids were separated from the neutral lipids by thin-layer chromatography (TLC) (13), saponified, and methylated (14). The relative contents of the methyl esters were determined by gas-liquid chromatography (GLC) (15).

Microsomes for measurement of nonenzymatic peroxidation were prepared from a 25% (wt/vol) liver homogenate in 0.25 M sucrose by centrifugation at 20,000 × *g* for 15 min, followed by ultracentrifugation of the supernatant at 105,000 × *g* for 1 h. The pellet was resuspended in 50 mM Tris/HCl, 140 mM NaCl buffer pH 7.5 and kept on ice.

The microsomes were incubated with $FeCl_3$ and ascorbate to induce lipid peroxidation by the modified method of Hill and Burk (16). The final concentrations during the incubations were as follows: 12 μM $FeCl_3$, 2 mM ADP, 0.5 mM ascorbic acid, 50 mM Tris/HCl (pH 7.5), 140 mM NaCl, and microsomes corresponding to 0.5 mg protein/mL; the total volume was 1.5 mL. The mixture was incubated for 50 min at 37°C with vigorous shaking. After the incubation, aliquots were taken for tocopherol and TBA assays (17). For basal levels, Tris/HCl buffer replaced the ascorbic acid. Three incubations were carried out for each animal. Data were statistically analyzed by Student's *t*-test.

RESULTS

α- and γ-Tocopherol in monkey liver. Three of the diets contained similar levels of α-tocopherol; no differences in α-tocopherol contents were found in livers from monkeys fed either a diet rich in 18:2n-6 (CO diet) or rich in both 18:3n-3 and 18:2n-6 (LO diet), whereas feeding menhaden oil as a source of n-3 fatty acids (MO diet) reduced the level of α-tocopherol in the liver by 40% (Table 2). A four-fold higher dietary supplement of α-tocopherol given in the MO + E diet resulted in a liver content similar to the level found in the CO and LO groups.

The diets contained various amounts of γ-tocopherol of which corn oil, in particular, is a rich source (18). The level of γ-tocopherol in the liver reflected the level in the diets, the content in the CO group being approximately five times higher than in the three other groups. The levels of γ-tocopherol in the liver were in general lower in the MO and MO + E groups than in the LO group although these three groups received comparable dietary levels. No significant difference in either α- or γ-tocopherol between males and females given the same diets was observed. The presence of significant amounts of γ-tocopherol in the liver indicated that sufficient antioxidative capacity was present in the diets considering the superior antioxidant activity of γ-tocopherol compared to α-tocopherol in an oil (19). In addition the menhaden oil had a natural content of free α-tocopherol of 50 μg/g.

Lipofuscin. The content of extractable lipofuscin in liver homogenate was used as an indicator of *in vivo* lipid peroxidation. The level in the male MO group was three-fold higher than in the CO and LO groups (Table 3), whereas the level in monkeys fed the fish oil diet enriched in vitamin E, MO + E group, was neither significantly different from the MO nor the CO and LO groups. The same tendencies were found in the corresponding female groups, but the differences were not significant. No sex-related difference was observed in monkeys fed the same diets.

Catalase, superoxide dismutase and GSH-peroxidase activity in liver homogenate. The activities of catalase, SOD and GSH-Px were measured in a liver homogenate. The female monkeys had a 20% higher catalase activity than the males (significant only for CO and MO groups). The catalase activity was influenced neither by the dietary fat nor the vitamin E levels used in this experiment (Table 4).

The SOD activity seemed to be independent of the diets fed to the monkeys; no sex-related differences were observed.

The activity of the Se-containing GSH-Px was slightly increased in the groups fed the fish oil diets (MO and MO + E groups) but the increase was only significant in the female MO and MO + E groups, when compared with the CO group. The activity with LO fell between that obtained with CO and MO, without being significantly different from either of them. The activity found in the monkeys was of the same order of magnitude as found in human liver (36–62 nmol NADPH/min/mg protein (20)).

Fatty acid composition of phospholipids from liver. The fatty acid composition of liver phospholipids generally

TABLE 2

The α- and γ-Tocopherol Contents of Monkey Liver After Fifteen Weeks on the Experimental Diets[a]

	Dietary group			
	CO	LO	MO	MO + E
α-Tocopherol (μg/g liver)				
Males	21.0 ± 1.5[b]	20.0 ± 1.9[b]	12.4 ± 2.5[c]	26.7 ± 3.7[b]
Females	20.2 ± 1.7[b]	20.9 ± 3.9[b,c]	11.7 ± 1.0[c]	23.7 ± 2.9[b]
γ-Tocopherol (μg/g liver)				
Males	9.1 ± 0.7[b]	1.9 ± 0.3[c]	1.2 ± 0.3[c,d]	0.8 ± 0.1[d]
Females	8.4 ± 1.1[b]	2.1 ± 0.4[c]	0.9 ± 0.1[d]	0.8 ± 0.2[d]

[a]Contents of α- and γ-tocopherol determined in a 25% liver homogenate as measured by HPLC relative to external standards. Values represent mean ± SEM (n = 5). Values within each row not sharing a common superscript letter (b,c,d) are statistically different at $P < 0.05$. The dietary fats are as in Table 1.

TABLE 3

Content of Extractable Lipofuscin in Monkey Liver Homogenate[a]

	Dietary group			
	CO	LO	MO	MO + E
	(% T per mg protein)			
Males	7.7 ± 1.3[b]	7.4 ± 1.9[b]	23.4 ± 6.3[c]	13.5 ± 3.3[b,c]
Females	5.4 ± 0.4	10.0 ± 5.0	16.9 ± 5.0	8.0 ± 2.3

[a]Lipofuscin was measured by fluorescence spectroscopy; 50 mM sulfuric acid and 84.4 nM quinine hydrobromide was used for calibration at 0% and 100% transmittance, respectively. Excitation maximum: 344 nm; emission maximum: 540 nm. Values represent mean ± SEM (n = 5). Means in the same row not sharing a common superscript (b,c) are significantly different at $P < 0.05$. Dietary fats are as in Table 1.

TABLE 4

Activity of Catalase, Cu, Zn-Superoxide Dismutase (SOD) and Se-GSH-Peroxidase (GSH-Px) in Monkey Liver[a]

		Dietary group			
Enzyme	Sex	CO	LO	MO	MO + E
Catalase	M	32.5 ± 1.4[b]	33.4 ± 2.6	32.6 ± 2.1[b]	28.6 ± 2.7
	F	39.5 ± 2.1	39.6 ± 2.2	38.2 ± 0.9	34.9 ± 1.6
SOD	M	113 ± 6	115 ± 8	115 ± 6	104 ± 9
	F	118 ± 9[b,c]	132 ± 4[b]	120 ± 5[b,c]	112 ± 6[c]
GSH-Px	M	74 ± 8	78 ± 3	96 ± 8	89 ± 11
	F	71 ± 7[b]	92 ± 7[b,c]	99 ± 7[c]	89 ± 3[c]

[a]The activities were determined in a 10% liver homogenate after removal of cell debris by centrifugation at 600 × *g*. The units are: SOD (U/mg protein); GSH-Px (nmol NADPH/min/mg protein); catalase ($\Delta OD_{240\ nm}$/min/mg protein). The values represent mean ± SEM (n = 5). Means in a row not sharing a common superscript letter (b,c) are statistically different at $P < 0.05$. Dietary fats are as in Table 1.

[b]The activity in males (M) is significantly different ($P < 0.05$) from the activity in females (F) belonging to the same dietary group.

reflected the dietary fatty acids, the LO group being a noteworthy exception, as can be seen from Figure 1. The LO group fed the diet rich in both 18:3n-3 and 18:2n-6 differed only little in fatty acid composition from the CO group fed the high 18:2n-6 diet. The relative content of n-3 fatty acids was doubled in the LO group, but quantitatively the changes were small. The increase was mainly caused by incorporation of 18:3n-3, whereas the levels of some of its desaturation products, 20:5n-3, and 22:5n-3, were only slightly increased when compared to the CO group. No changes, however, were seen in the relative content of 22:6n-3.

The increase in n-3 fatty acids in the LO group was counterbalanced by a general decrease in the content of all the n-6 fatty acids. As a result the percentage of polyunsaturated fatty acids was similar in the CO and LO groups.

Feeding a diet rich in both 18:2n-6 and n-3 fatty acids

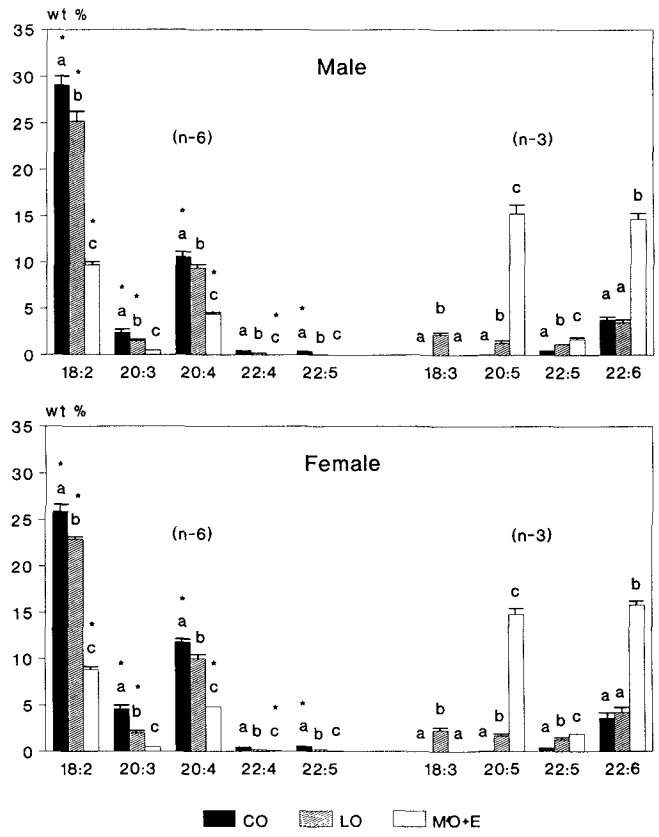

FIG. 1. Distribution of the major polyunsaturated fatty acids in liver phospholipids from monkeys fed the CO, LO and MO + E diets (area percentages determined by GLC). Each bar represents the mean ± SEM (n = 5), and bars for one fatty acid not sharing the same label are significantly different ($P < 0.05$); *, indicates a sex-related difference ($P < 0.05$).

from menhaden oil led to much more pronounced changes in the composition of the polyunsaturated fatty acids in the liver phospholipids. Compared to the CO group, the relative content of all the n-6 fatty acids was reduced by more than 60%, and was replaced particularly by 20:5n-3 and 22:6n-3. These highly unsaturated n-3 fatty acids were the most abundant polyunsaturated fatty acids in the liver phospholipids of monkeys fed the MO and MO + E diets. The addition of four times more α-tocopherol to the MO diet did not influence the fatty acid composition; therefore only the fatty acid composition for the CO, LO and MO + E groups are shown in Figure 1.

In general, similar tendencies were found in males and females, but a comparison between sexes fed the same diets showed that the females were lower in 18:2n-6 and higher in its desaturation products, 20:3n-6 and 20:4n-6. This sex-related difference was observed in all the dietary groups.

In vitro *lipid peroxidation in liver microsomes.* The microsomal contents of α-tocopherol in the CO, LO and MO + E groups were similar, whereas the content in the MO group was 10 times lower (Table 5).

After 50 min of incubation, all tocopherol levels decreased, with the CO group having a higher amount of α-tocopherol than the MO group; the levels in the LO and MO + E groups were not significantly different from the levels found in the CO or the MO groups. γ-Tocopherol levels in the microsomes were below the detection limit.

The basal TBARS level of the MO group was four times higher than that of the CO and LO groups, and twice as high as the MO + E group (difference not significant in the female MO + E group). Microsomes prepared from the MO + E group contained twice as much TBARS as the CO and LO groups (Table 5).

The monkeys fed the diets containing fish oil (MO and

TABLE 5

α-Tocopherol and TBARS in Monkey Liver Microsomes Before and After Fifty Minutes of Incubation with Fe^{3+} and Ascorbic Acid[a]

		Dietary group			
		CO	LO	MO	MO + E
α-Tocopherol (ng/mg protein)					
t_0	M	109 ± 15^{c}	89 ± 12^{c}	8 ± 2^{d}	120 ± 37^{c}
	F	130 ± 24^{c}	105 ± 24^{c}	13 ± 5^{d}	123 ± 29^{c}
t_{50}	M	56 ± 20^{c}	54 ± 26^{c,d}	4 ± 2^{d}	17 ± 15^{c,d}
	F	96 ± 18^{c}	35 ± 24^{c,d}	4 ± 3^{d}	54 ± 48^{c,d}
TBARS (nmol MDA/mg protein)[b]					
t_0	M	1.2 ± 0.2^{c}	1.0 ± 0.2^{c}	4.8 ± 0.8^{d}	2.6 ± 0.6^{e}
	F	1.2 ± 0.2^{c}	1.4 ± 0.2^{c}	5.2 ± 0.8^{d}	3.4 ± 0.6^{d}
t_{50}	M	22 ± 9^{c}	24 ± 10^{c}	106 ± 5^{d}	92 ± 9^{d}
	F	13 ± 7^{c}	39 ± 10^{d}	98 ± 4^{e}	72 ± 17^{d,e}

[a]The final incubation mixture contained 12 μM $FeCl_3$, 2 mM ADP, 0.5 mM ascorbic acid, 50 mM Tris/HCl (pH 7.5), 140 mM NaCl, and microsomes corresponding to 0.5 mg protein/mL. α-Tocopherol was determined by HPLC, and TBARS were measured in a TBA-assay; t_0 indicates the basal level in the microsomes, t_{50} indicates the level after 50 min incubation. Values represent mean ± SEM (n = 5). Means in a row not sharing a common superscript (c,d,e) are significantly different at $P < 0.05$. M, males, F, females. Dietary fats as in Table 1.

[b]TBARS relate to a MDA standard.

MO + E) produced more TBARS than the CO group (males four-fold higher and females eight-fold). TBARS production in the microsomes in the female LO group was significantly higher than in the CO group (three-fold) and lower than in the MO group (two-fold), although the CO, LO and MO diets contained the same amount of α-tocopherol. Compared to the MO + E group, the microsomes from the LO group produced 2-3 times less TBARS; however, the difference was only significant in the male groups.

It should be noted that the intra group variation for lipofuscin, α-tocopherol and TBARS contents was very large even though the experimental error was less than 5%. This reflected uniformly scattered data caused by biological variations between individuals.

DISCUSSION

Superoxide dismutase, catalase and GSH-Px participate in the cellular protection against lipid peroxidation *in vivo* by removing the potential initiators of the peroxidative processes, $O_2^{\cdot -}$, H_2O_2 and possibly other peroxides as well (4,21,22). In the present experiment only selenium containing GSH-Px seemed to be induced in the liver by the higher peroxidative stress associated with the intake of menhaden oil. However, the increase in activity could not fully compensate for the decrease in vitamin E in preventing the high accumulation of lipofuscin when fish oil was fed.

The very long-chain fatty acids, 20:1 and 22:1, are metabolized in the peroxisomes and induce higher peroxisomal β-oxidation and catalase activity in liver when fed to rats (23,24). Similarly, it was shown in a short term study that 20:5n-3 increased the peroxisomal β-oxidation in rat liver (25) and together with 22:6n-3 appears to be metabolized mainly by the peroxisomes (26). However, menhaden oil, rich in 20:5n-3 and 22:6n-3, did not seem to induce increased peroxisomal activity in the monkey liver when catalase activity was used as an indicator for the peroxisomal β-oxidation, even though the two n-3 fatty acids are likely to be better substrates for peroxisomal β-oxidation than 20:4n-6 as was shown in rats (27).

The higher catalase activity in females may indicate increased β-oxidation activity in the peroxisomes in females compared to males. A similar effect was observed in rats fed partially hydrogenated marine oil, where the increased peroxisomal β-oxidation in the female rats was accompanied by a lower accumulation of triglycerides in the heart compared to male rats fed the same diet (24). The increase in the content of 20:3n-6 and 20:4n-6 relative to 18:2n-6 seen in females did not affect the peroxidative status. Alternatively, the higher catalase activity prevented increased peroxidation.

The amount of α-tocopherol fed to the monkeys is considered to be adequate to fulfill the nutritional needs for vitamin E under normal conditions (28). In monkeys fed a diet similar to a typical Western European diet (the CO diet), the liver contents corresponded well to those observed in humans: 18.3 μg/g tissue and 9.2 μg/g tissue for α- and γ-tocopherol, respectively (29).

From the γ- to α-tocopherol ratio, it seemed that the absorption or retention of γ-tocopherol in the liver was less efficient than of α-tocopherol. It was the actual content of γ-tocopherol in the diet that influenced the γ-tocopherol content in the liver rather than the ratio of the two tocopherols. These results are in good accordance with the findings of Bieri and Evarts (30) and Peake and Bieri (31), who found that α- and γ-tocopherol were similarly absorbed but that γ-tocopherol was excreted more rapidly. Behrens and Madère (32), however, observed that a high amount of α-tocopherol affected the amount of γ-tocopherol absorbed, transported and taken up by tissues. The present data obtained on primates did not support the idea that additional dietary α-tocopherol affected the level of γ-tocopherol *in vivo*.

The levels of both antioxidants in the liver were reduced equally by the intake of the highly unsaturated menhaden oil as compared to monkeys fed diets rich in 18:3n-3. This suggests that although the biological antioxidant activity of γ-tocopherol is only about 30% of the activity of α-tocopherol (33,34), both tocopherols may play an important role as antioxidants *in vivo*. The reduced levels of tocopherols observed when menhaden oil was fed without vitamin E supplementation (MO group) were associated with increased lipid peroxidation, as indicated by the increased levels of lipofuscin and TBARS. This is in accordance with Mouri *et al.* (35), who demonstrated an increase in liver TBARS and a decrease in liver α-tocopherol when rats were fed increased amounts of cod liver oil. A four-fold increase in dietary α-tocopherol normalized the liver level when menhaden oil was fed, which is in good agreement with the findings of Meydani *et al.* (36) in mice. This, however, did not fully restore the antioxidative capacity of the liver *in vivo* (when lipofuscin is used as an indicator for peroxidation) and *in vitro* as measured by TBARS formation.

Feeding 18:3n-3 to the monkeys did not affect the peroxidative status of the liver *in vivo* compared with monkeys fed 18:2n-6. This can be explained by the moderate influence of the dietary 18:3n-3 on the fatty acid composition in liver phospholipids. Replacing the 18:3n-3 in the diets with the more unsaturated n-3 fatty acids of marine origin led to an extensive replacement of the less unsaturated n-6 fatty acids with the more unsaturated n-3 fatty acids, 20:5n-3 and 22:6n-3, followed by increased lipid peroxidation. This peroxidation could not be fully compensated for by a four-fold increase in dietary α-tocopherol. The liver phospholipid fatty acid pattern of monkeys fed menhaden oil with normal or high vitamin E content was similar, despite the increased level of one of the fatty acid peroxidation products, lipofuscin, found in the MO group. This could indicate that the damaged fatty acids were rapidly replaced by fatty acids from the diets or that the actual amount of polyunsaturated fatty acids lost due to lipid peroxidation is low compared to the total amount present in the membranes. However, it should be pointed out that the implication of lipofuscin formation is not clear.

In the *in vitro* experiment, even a small increase in the relative content of n-3 fatty acids after feeding linseed oil resulted in a higher risk of peroxidation under stressed conditions. Risk of peroxidation was further increased when the linseed oil was replaced by menhaden oil and a ten-fold increase of microsomal α-tocopherol could not reduce the peroxidation rate. It thus seems that α-tocopherol cannot protect membranes rich in n-3 fatty acids, especially with five or six double bonds, as efficient-

ly as membranes rich in n-6 fatty acids. This has also been observed in rat tissues (37-40).

The results from the Fe(III)/ascorbate incubation assay were in accordance with the results for lipofuscin formation; only the differences between the groups were much more pronounced in the *in vitro* assay. The non-enzymatic *in vitro* peroxidation in microsomes could as such be used as a measure of the peroxidation *in vivo* due to the dietary treatment.

Previous experiments have established that a four-fold supplement of α-tocopherol normalized the lipofuscin in rats fed marine oil (41). The present study indicated that such an additional supplement of α-tocopherol to primates did not fully prevent the peroxidative changes when menhaden oil was fed. This is probably caused by a higher level of polyunsaturated n-3 fatty acids incorporated in the liver from primates (32%) compared to rats (21%) after intake of marine oil, mainly due to a high incorporation of 20:5n-3 in the monkeys. This indicates that in primates a supplement of α-tocopherol beyond the levels used in this study is necessary in order to prevent peroxidation *in vivo*.

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