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Polyunsaturated n-3 and n-6 fatty acids at a low level in the diet alter mitochondrial outer membrane parameters in Wistar rat liver

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This study was designed to examine whether n-3 and n-6 polyunsaturated fatty acids (PUFA) at a very low level in the diet (about 0.2%) may alter the fatty acid composition of mitochondrial outer membranes and the characteristics of carnitine palmitoyltransferase I (CPT I) activity in the liver of normal Wistar rats. The animals were fed diets containing different oil mixtures (5% wt/wt) with the same ratio of n-6/n-3 fatty acids supplied either as fish oil or arachidonic acid concentrate. The cholesterol content of the mitochondrial outer membranes from liver was similar for all diets, while the percentage of 22:6n-3 and 20:4n-6 in phospholipids was enhanced with the diets containing fish oil and arachidonic acid, respectively. With the fish oil diet, CPT I was found to be less sensitive to malonyl-CoA inhibition. When animals were fed the diet containing arachidonic acid, the enzyme activity was lowered, but its sensitivity to malonyl-CoA was unaffected. In this group, the liver mitochondrial content appeared to be reduced both when expressed per unit of weight and on a whole organ basis. The results show that a low level of dietary PUFAs supplied to normal Wistar rats is sufficient to influence the content of n-3 and n-6 PUFAs in mitochondrial outer membranes and to alter some of the characteristics of CPT I. However, the data suggest that factors other than the PUFA content of membrane phospholipids must be involved in mediating the changes in CPT I kinetic characteristics observed in this study. (J. Nutr. Biochem. 6: 626–634, 1995.)

Keywords: polyunsaturated fatty acids; mitochondrial outer membranes; carnitine acyltransferase I; Wistar rats

Introduction

Marine oils that are rich in fatty acids (FA) of the n-3 series, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been shown to reduce the rate of very low density lipoprotein (VLDL) production by the liver.[1–4] Previous studies have already shown that α-linolenic acid (18:3n-3) is preferentially used for β-oxidation in vivo[5] and in isolated liver mitochondria.[6] Moreover, dietary fish oils were found to decrease the activity of enzymes involved in FA and triacyglycerol synthesis[7–10] and to stimulate peroxisomal and mitochondrial FA oxidative pathways in the liver.[11,12] Wong et al.[11] reported that the specific activity of carnitine palmitoyltransferase I (CPT I) was not altered in rats maintained on a diet containing 15% fish oil (wt/wt) but was found to be significantly less sensitive to malonyl-CoA inhibition. However, other studies have shown that diets enriched in fish oil enhance CPT I activity in rats[13] and mice.[14] In obese Zucker rats, low levels of dietary fish oil increase markedly the proportion of DHA in phospholipid (PL) fatty acids of mitochondrial outer membranes[15] in

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which CPT I resides. The specific activity of CPT I was found to be slightly decreased and the enzyme was less sensitive to malonyl-CoA inhibition. With a diet containing a very low level of arachidonic acid (20:4n-6), the specific activity of CPT I was strongly depressed and the mitochondrial content of the liver was lower compared with the control diet both when expressed per unit of weight and on a whole-liver basis.[15]

These observations, which may appear inconsistent with each other, may all be involved in the etiology of hyperlipidemia. The lipemia of animals studied was always elevated due either to the relatively high percentage of oil added to the diet (20, 15, and 10%, respectively)[10,11,14] or to inborn deficiency that caused low FA oxidation[16,17] and increased VLDL secretion.[18] Under these conditions, n-3 or n-6 polyunsaturated fatty acids (PUFA) added to the diet would be relatively less abundant in the circulating lipids than in rats that are either normolipidemic or fed diets poor in fat. It is well known that FA with 16 to 18 carbons are better substrates for β-oxidation than are very long chain PUFA.[19,20] These latter are expected to be preferentially esterified into membrane PL with consequent effects on the biophysical and biochemical properties of membranes.[21] However, what has been observed in obese rats[15] may not apply to Wistar rats whose lipidemia and rate of hepatic FA oxidation are normal. In addition, CPT I activity may be modulated not only by membrane FA composition, but also by cellular signals due to the nature and the amount of cytoplasmic FA.

Therefore the observations made with a low level of dietary PUFA in the liver of obese Zucker rats[15] prompted us to apply similar nutritional experiments to normolipidemic Wistar rats that are likely to react differently. The study was specifically designed to investigate whether very low levels of dietary fish oil (n-3 FA) and arachidonic acid concentrate (n-6 FA), such as occur in human nutrition, are still capable of altering FA composition of mitochondrial outer membranes and CPT I characteristics in the liver of normal Wistar rats.

Methods and materials

Animals

Male Wistar rats were bred in the Centre de Sélection et d'Elevage d'Animaux de Laboratoire, C.N.R.S. (Orléans-la-Source, France). They were 5 weeks old on arrival at the Station de Recherches de Nutrition (INRA-CRJ, Jouy-en-Josas, France). All received a control diet containing (g/kg): casein, 220; DL-methionine, 1.6; sucrose, 218; corn starch, 440; cellulose powder, 20; vitamin mixture, 10; mineral mixture, 40 (for details[15]); and oil mixture, 50 (50% "primor" oil, which is an acid erucic-free rapeseed oil, 20% sunflower oil, and 30% hydrogenated palm oil). The control diet was provided ad libitum. After 5 weeks, the animals were divided into three groups. One group (control) continued to receive the control diet. The other two groups were fed a similar diet in which the oil mixture was replaced either by one containing fish oil (FO: 35% sunflower oil, 50% hydrogenated palm oil, and 15% salmon oil) or arachidonic acid (AA: 45% "primor" rapeseed oil, 14% sunflower oil, 35% hydrogenated palm oil, and 6% AA concentrate). *Table 1* shows that the three oil mixtures had a similar n-6/n-3 FA ratio close to 6. Only the FO and AA oil mixtures contained fatty acids whose carbon numbers were greater than 18, so that the FO mixture was characterized by the presence of n-3 PUFA (20:5 and 22:6) and the AA mixture by the presence of n-6 PUFA (20:4). Food intake was limited to 20 g/day (70% of ad libitum). After 7 weeks, the rats were starved for 16 hr and sacrificed between 7 and 8 hr, and the livers were quickly removed and weighed.

Table 1 Fatty acid composition* of the oil mixtures present in the three diets†

Fatty acid	FO mixture	Control mixture	AA mixture
14:0	1.7	0.8	0.6
16:0	27.2	16.5	16.8
18:0	6.0	4.4	3.9
20:0	0.3	0.4	0.4
22:0	0.4	0.3	0.3
Total saturated FA	35.6	22.4	22.0
16:1n-7	1.5	—	—
18:1n-9	30.9	43.9	44.7
18:1n-7	1.0	3.2	1.8
20:1n-9	1.1	0.6	0.7
22:1n-11	0.5	—	—
Total monounsaturated FA	35.0	47.7	47.2
18:2n-6	25.3	25.6	21.5
20:4n-6	—	—	4.2
Total n-6 unsaturated FA	25.3	25.6	25.7
18:3n-3	0.2	4.3	3.9
18:4n-3	0.3	—	—
20:5n-3	1.6	—	—
22:5n-3	0.4	—	0.4
22:6n-3	1.6	—	—
Total n-3 unsaturated FA	4.1	4.3	4.3
n-6/n-3 ratio	6.2	6.0	6.0

*The composition in fatty acids (FA) is given as a weight percentage of total fatty acids in oil mixtures added to the three diets.
†FO (fish oil) refers to the oil mixture containing salmon oil. Arachidonic acid (AA) refers to the oil mixture enriched in arachidonic acid.

Preparation of subcellular fractions

The liver was transferred to aqueous medium containing 0.25 mol/L of sucrose, 10 mmol/L of tris (hydroxymethyl)-aminomethane (Tris)/ HCl, pH 7.4, and 1 mmol/L of ethyleneglycol-bis (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA) at 4°C, minced, and rinsed several times. The blotted tissue was then homogenized in 10 vol of sucrose medium containing 1% albumin[22] (Paesel-Lorei, Frankfurt, Germany) with three strokes of a loose-fitting Teflon pestle at 300 rpm in an ice-cooled Potter-Elvehjem homogenizer. The homogenate was used to prepare a crude mitochondrial fraction (CMF) as previously detailed.[15] The CMF was purified on a gradient of Percoll (Pharmacia, LKB Biotechnology, Uppsala, Sweden) as the procedure of Zammit et al.,[23] slightly modified,[17] and gave the Percoll-purified mitochondrial fraction (PPMF). Mitochondrial outer membranes (OM) were prepared from the PPMF according to the general procedure of Parsons et al.[24] The microsomal fraction was obtained from the supernatant of the crude mitochondrial pellet and purified on a discontinuous gradient of sucrose.[15]

Laboratory assays

Enzyme assays. The presence of mitochondrial and microsomal organelles was estimated by the activities of monoamine oxidase[25] (outer membranes), glutamate dehydrogenase[26] (matrix) for mito-

chondria, and aryl-ester hydrolase[27] for microsomes. Measurement of CPT I activity was performed as described by Bremer[28] with slight modifications,[15] using 200 μg of PPMF proteins in a medium containing 80 mmol/L mannitol, 75 mmol/L of KCl, 25 mmol/L of HEPES, 0.2 mmol/L of EGTA, 0.5 mmol/L of dithiothreitol, 2 mmol/L of KCN, and 1% fatty acid-free albumin. Mitochondria were preincubated for 2 min (a) for the determination of CPT I activity, in the presence of the indicated concentrations of palmitoyl-CoA (Sigma Chemical Co., St. Louis, MO USA) and (b) for the determination of the sensitivity of CPT I to malonyl-CoA, in the presence of 40 μmol/L of palmitoyl-CoA and the indicated concentrations of malonyl-CoA. The reaction was then initiated by adding 400 μmol/L of (1 Ci/mol) L-[^{3}H]carnitine (Amersham Radiochemical Centre, Buckinghamshire, UK). After 4 min, the reaction was stopped by addition of acid and the acyl-[^{3}H]carnitine was extracted with butan-1-ol and quantitated.[28]

Lipid analysis. Total lipids of liver and subcellular fractions were extracted by the procedure of Folch et al.[29] The separation of PL classes was achieved by high-performance liquid chromatography (HPLC)[30] on a Varian apparatus (Walnut Creek, LA USA), using a Hibar 250-4 column (Merck, Darmstadt, Germany) packed with Lichrosorb Si-60 (particle diameter 5 μm), and each fraction was added with a known amount of heptadecanoic acid (NuChek Prep., Elysian, MN USA). PL FA were directly transesterified with methanol/H_2SO_4 after drying the samples and analyzed by capillary gas–liquid chromatography (CGLC) on a Becker-Packard apparatus (model 419) fitted with a 30 m × 0.3 mm internal diameter (i.d.) glass capillary column coated with carbowax 20 M. CGLC peaks were identified based on their retention time relative to methyl heptadecanoate. Peak areas were measured using a Delsi model Enica 21 computing integrator (Delsi instruments, Suresnes, France). The cholesterol content of the lipid extracts was measured by CGLC[31] on a model 419 Becker-Packard chromatograph fitted with a 10 m × 0.3 mm i.d. glass capillary column coated with SE-30. Epicoprostanol and α-cholestane (NuChek Prep., Elysian, MN USA) were added as internal standards.

Protein. Rapid protein estimations were performed by spectrophotometry[6] (A280) just before starting the incubations and were later more accurately carried out in the presence of sodium dodecyl sulfate using the BCA procedure.[32]

Statistics

Results are expressed as means ± SEM. Statistical comparisons of differences between groups were analyzed by one-way ANOVA and Fisher's test.

Results and Discussion

Body and liver weights

One of the main observations made in this study is that n-3 PUFA of fish oil, even when present at a level of only 0.2% of the diet (n-3 unsaturated FA constitute 4.1% of the FO mixture; see *Table 1*) are able to reduce the body weight of normal Wistar rats (*Table 2*). There was no effect on liver weight. The data also suggest that FO at a low level in the diet may stimulate catabolic pathways in organs other than the liver, such that body weight is diminished without a decrease in the proportion represented by the liver. Such a catabolic effect was suggested previously from experiments performed under more drastic conditions with rats fed 21% fat (by weight) as lard essentially.[33] Thus when 6.5% of the lard was replaced by ethyl esters of EPA and DHA, the mean respiratory quotient of the animals was found to be significantly increased. The complete degradation of fatty acids to CO_2 and H_2O gives rise to calculated respiratory quotients that depended on the degree of unsaturation: 0.7 for palmitic acid (16:0), 0.67 for stearic acid (18:0), and 0.76 for either EPA (20:5) or DHA (22:6). Consequently, the difference between the calculated respiratory quotients for saturated FA and PUFA may account in part for the actual difference observed in vivo (between 0.02 and 0.03) and thus for the preferential oxidation of n-3 PUFA.

In contrast, liver weight was increased in rats fed the diet enriched in arachidonic acid. Altogether, the results are similar to those obtained in obese rats, although the changes observed were less marked. It is to be emphasized that food intake was very similar for animals on all three diets (see Methods and materials section). Therefore, the lower body weight observed in FO rats indicates that the fatty acids of fish oil result in a less efficient utilization of the energy contained in the diet. With the AA diet, the increased liver weight might correspond, at least in part, to fat accumulation as already observed in obese rats.[15] Since the effects of FO and AA diets on liver and body weights were less marked than those obtained with obese rats, it was interesting to examine whether these moderate effects correspond to discreet changes in enzyme activities and membrane fatty acid composition.

Table 2 Effects of diets* enriched in n-3 and n-6 fatty acids on body and liver weights†

	FO	Control	AA
Body weight (g)			
initial (g)	370 ± 12	375 ± 10	385 ± 12
final (g)	455 ± 16^a	485 ± 12^b	492 ± 16^b
Liver weight			
(g)	9.55 ± 0.40^a	10.3 ± 0.5^a	11.7 ± 0.8^b
(% of body weight)	2.08 ± 0.03^a	2.11 ± 0.05^a	2.38 ± 0.11^b

*FO and AA refer to the diets enriched in n-3 fatty acids with fish oil and in n-6 fatty acids with the arachidonic acid concentrate, respectively.
†Results are means ± SEM (n = 5). The values in a row with different superscript letters ^{a,b} are significantly different at $P < 0.05$, as determined by ANOVA and Fisher's test comparisons. The absence of these superscript letters indicates that the values in a row are not significantly different.

Enzyme activities in liver tissue and mitochondrial fractions

Monoamine oxidase and glutamate dehydrogenase activities measured in liver homogenates and mitochondrial fractions allowed the calculation of the mitochondrial protein content per gram of liver. There was no difference between the FO and control rats, but the values in AA rats were found to be between 40% and 30% lower, respectively, than those of the two other series (*Table 3*). The present data indicated that a very low level of dietary arachidonic acid was capable of changing the mitochondrial density in the liver, even in

Table 3 Effects of diets* on marker enzyme activities† in hepatic tissue and subcellular fractions‡ isolated from liver of Wistar rats

	FO	Control	AA
Monoamine oxidase activity			
per gram of dry weight liver (nkat)	66.1 ± 4.0[a]	58.8 ± 5.0[a]	40.1 ± 3.4[b]
per mg of PPMF‡ (pkat)	273 ± 20[a]	210 ± 16[b]	237 ± 15[a,b]
per mg of OM fraction‡ (nkat)	4.30 ± 0.20[a]	3.58 ± 0.30[b]	4.03 ± 0.22[a,b]
mg PPMF per gram of dry wt liver§ (monoamine oxidase basis)	243 ± 17[a]	281 ± 23[a]	170 ± 17[b]
Glutamate dehydrogenase activity			
per g of dry weight liver (μkat)	14.1 ± 0.6[a]	13.5 ± 1.0[a]	10.2 ± 0.7[b]
per mg of PPMF (nkat)	51.6 ± 2.0	46.6 ± 3.0	52.5 ± 2.0
mg PPMF per g of dry wt liver§ (glutamate dehydrogenase basis)	273 ± 21[a]	289 ± 24[a]	194 ± 18[b]
Aryl-ester hydrolase activity			
per mg of MCSF‡ (nkat)	108 ± 5[a]	77 ± 3[b]	79 ± 3[b]
per mg of PPMF (nkat)	2.43 ± 0.33	1.97 ± 0.37	2.08 ± 0.42
per mg of OM fraction	4.67 ± 0.66	4.63 ± 0.58	5.07 ± 0.48
Microsomal contamination‖			
in PPMF	2.3	2.6	2.7
in OM fraction	4.3	6.1	6.5

*FO and AA as in Table 2.
†Results are means ± SEM ($n = 5$). The values in a row with different superscript letters [a,b] are significantly different at $P < 0.05$, as determined by ANOVA and Fisher's test comparisons. The absence of these superscript letters indicates that the values in a row are not significantly different.
‡PPMF, Percoll-purified mitochondrial fraction; OM fraction, outer membrane fraction (isolated from PPMF); MCSF, microsomal fraction.
§Values obtained by dividing the monoamine oxidase or glutamate dehydrogenase activity per gram of liver by that of 1 mg of PPMF protein.
‖Microsomal contamination is expressed as milligrams of microsomal protein per 100 mg of protein of the studied mitochondrial fraction and was calculated as follows: the specific activity of aryl-ester hydrolase in the studied mitochondrial fraction was divided by that in the microsomal fraction of the same liver and multiplied by 100.

animals oxidizing normally fatty acids. A lower number of mitochondria could lower oxidative capacity and make fatty acids more available for triacylglycerol formation,[34] fat storage, and weight gain compared especially with rats on the FO diet (*Table 2*).

Since mitochondrial fractions (whole mitochondria and OM) usually contain other organelles likely to mask the actual lipid composition of the fractions, the level of contamination by peroxisomes and microsomes has to be assessed. Peroxisomes were almost completely removed after Percoll gradient centrifugation, so that peroxisomal activities such as uricase and catalase were detected very slightly in Percoll-purified mitochondrial fractions and were totally absent in outer membrane fractions.[35] For microsomes present in mitochondrial fractions, the specific activity of aryl-ester hydrolase found in mitochondrial and microsomal fractions enabled us to calculate that the Percoll-purified fractions contained very low amounts of microsomes (between 2 and 3%; *Table 3*). This unavoidable microsomal contamination was half of that found in obese Zucker rats and appeared to be independent of the diet (*Table 3*).[15] Therefore, in Wistar rats the comparable level of contamination of each fraction (for the three diets) by nonmitochondrial organelles cannot change the accuracy of fatty acid composition and cholesterol content of the mitochondrial fractions to any great extent. However, the composition of fatty acids (*Table 6*) and the content in cholesterol (see below) of microsomal fractions are given to emphasize the similarities of lipid composition between microsomes and mitochondrial OM. The residual microsomal contamination was presumably due to particles tightly bound to OM, as already suggested.[36–39] The existence of a differential contamination of mitochondrial fractions by microsomes according to the strain of rats (obese Zucker and normal Wistar) is not yet understood, but could be of physiological significance since this contamination has been reported to affect CPT I activity[35] and thus the β-oxidative pathway.

Fatty acid composition of total mitochondrial lipids

It is well documented that dietary fatty acids directly influence the composition of biological membranes and the corresponding enzymatic activities.[21] Measurements performed on whole mitochondria (*Table 4*) reflect primarily the composition of the inner membranes because of their extensive surface area. In these organelles, the percentage of 20:4n-6 was similar for all diets, while that of 22:6n-3 was higher in FO rats. It is clear that the FO diet favored the accumulation of docosahexaenoic acid into mitochondrial membranes but only to a small extent (+1.5%). This may appear insignificant compared with the increase observed with a diet containing 20% sardine oil (corn oil as a control), which results in the enhancement of the proportion of DHA by up to 2 to 3 times in the relevant phospholipids of liver mitochondrial membranes.[40] In the present study, the proportions of 20:4n-6 and 22:6n-3 were always lower than those found in obese rats.[15] These differences may arise from the fact that very long chain fatty acids are poor substrates for mitochondrial fatty acid oxidation[19,20] and accumulate preferentially in PL, but this accumulation was not substantial because the oxidative pathway in normal Wistar rats was more active than in obese Zucker rats.[16,17] With the FO diet, 20:5n-3 and 22:5n-3 were found in proportions

Table 4 Fatty acid composition* of total lipids of mitochondrial fractions† isolated from liver of Wistar rats fed diets‡ enriched in PUFA

Fatty acid	FO	Control	AA
16:0	14.6 ± 1.4	16.4 ± 1.4	15.0 ± 1.8
16:1	—	—	0.5 ± 0.2
18:0	13.2 ± 0.9	14.0 ± 0.6	13.9 ± 0.5
18:1	13.5 ± 0.5	14.6 ± 0.5	14.4 ± 0.4
18:2n-6	17.4 ± 0.4^{a}	13.4 ± 0.3^{b}	13.5 ± 0.4^{b}
20:4n-6	19.1 ± 2.1	22.2 ± 1.9	22.4 ± 2.0
20:5n-3	1.4 ± 0.3	—	—
22:5n-3	1.0 ± 0.2^{a}	0.4 ± 0.2^{b}	0.7 ± 0.2^{a,b}
22:6n-3	12.3 ± 0.8^{a}	10.8 ± 0.6^{b}	10.1 ± 0.5^{b}

*Values are means ± SEM (n = 5) and are given as weight percentage of total fatty acids. The values in a row with different superscript letters ^{a,b} are significantly different at $P < 0.05$, as determined by ANOVA and Fisher's test comparisons. The absence of these superscript letters indicates that the values in a row are not significantly different.
†Percoll-purified mitochondrial fractions.
‡FO and AA as in Table 2.

very close to those of the original FO oil mixture (see *Tables 1 and 4*), but since they did not accumulate (*Table 4*) they may have contributed through desaturation-elongation reactions to the significant enhancement of the level of DHA. Besides, the AA diet did not enrich mitochondrial lipids in arachidonic acid, which suggests that the requirement for arachidonic acid was easily met in animals fed the other two diets through synthesis starting from 18:2n-6 and that excess arachidonic acid supplied in the diet is utilized through β-oxidation[19] and hydroxylation reactions.[41]

Fatty acid composition of phospholipids in OM fractions

Owing to the fact that CPT I is located in the mitochondrial outer membrane and that its activity seems to be influenced by surrounding phospholipids,[42] fatty acid composition of the main two PL classes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), was determined. Irrespective of the diet, PC and PE were shown to represent about 57 and 33% of total PL, respectively (*Table 5*). In contrast to the attenuated effects of the three diets on fatty acid composition of whole mitochondria total lipids (*Table 4*), the fatty acid composition of PC and PE in outer membranes appeared to be much more directly related to that of the original diet. For instance, the percentage of 20:4n-6 was always significantly greater in PC and PE from rats fed the AA diet and conversely the percentage of 22:6n-3 was found to be higher in either PL from rats fed the FO diet. The same observation can be made from the fatty acid composition of microsomal PL (*Table 6*), which was close to that of OM fractions, with the PL differing in proportion only (about 63 and 26% in PC and PE, respectively). The results in *Tables 5 and 6* also clearly show that the FO diet repressed the biosynthetic pathway leading to n-6 PUFA since it lowered the percentage of 20:4n-6 and kept more elevated the percentage of its precursor, linoleic acid (18:2n-6), as previously observed.[43] Therefore, the fatty acid composition of pure OM should be very similar to that of the actual OM fractions minimally contaminated by microsomes (*Table 3*). It was interesting to note that the differences in percentages between the three diets for either 20:4n-6 or DHA were of the same range as those obtained from obese rats. However, particularly with the FO diet, the

Table 5 Fatty acid compositions* of PC and PE of mitochondrial outer membranes† isolated from liver of Wistar rats fed diets‡ enriched in PUFA

Fatty acid	PC			PE		
	FO	Control	AA	FO	Control	AA
16:0	21.9 ± 1.7	20.7 ± 1.6	22.0 ± 1.9	19.2 ± 1.7	17.5 ± 1.5	17.8 ± 1.4
16:1	1.5 ± 0.2^{a}	0.4 ± 0.2^{b}	1.8 ± 0.3^{a}	0.6 ± 0.2^{a}	1.2 ± 0.4^{a,b}	1.5 ± 0.4^{b}
18:0	16.9 ± 1.6^{a,b}	17.5 ± 1.7^{a}	13.8 ± 1.3^{b}	18.3 ± 1.6	15.6 ± 1.9	15.9 ± 1.5
18:1	10.8 ± 1.2	11.2 ± 1.2	13.1 ± 1.5	8.0 ± 0.9^{a}	9.8 ± 1.4^{a,b}	12.6 ± 1.3^{b}
18:2n-6	12.9 ± 0.5^{a}	9.1 ± 0.5^{b}	7.2 ± 0.5^{c}	7.2 ± 0.5^{a}	5.3 ± 0.5^{b}	5.6 ± 0.4^{b}
20:4n-6	23.6 ± 2.2^{a}	30.0 ± 2.0^{b}	34.5 ± 1.8^{c}	21.7 ± 1.3^{a}	26.4 ± 1.7^{b}	30.9 ± 1.6^{c}
20:5n-3	1.8 ± 0.3^{a}	0.9 ± 0.3^{b}	0.2 ± 0.1^{c}	2.6 ± 0.4^{a}	1.2 ± 0.4^{b}	0.4 ± 0.1^{c}
22:5n-3	0.6 ± 0.1	0.4 ± 0.2	—	1.3 ± 0.2	1.3 ± 0.4	0.7 ± 0.3
22:6n-3	8.8 ± 1.2^{a}	7.6 ± 1.3^{a}	4.8 ± 1.1^{b}	20.2 ± 2.0^{a}	17.7 ± 1.8^{a}	11.0 ± 1.2^{b}
Σ saturated	38.8 ± 1.8	38.2 ± 1.9	35.8 ± 1.9	37.5 ± 1.9^{a}	33.1 ± 1.8^{b}	33.7 ± 1.7^{b}
Σ n-6	36.5 ± 2.0^{a}	39.1 ± 1.8^{a,b}	41.7 ± 1.9^{b}	28.9 ± 1.0^{a}	31.7 ± 1.5^{b}	36.5 ± 1.4^{c}
Σ n-3	11.2 ± 1.3^{a}	8.9 ± 1.3^{a}	5.0 ± 1.0^{b}	24.1 ± 1.9^{a}	20.2 ± 1.8^{b}	12.1 ± 1.3^{c}
Σ ≥ C_{20} unsaturated	34.8 ± 2.0^{a}	38.9 ± 1.9^{a,b}	39.5 ± 2.0^{b}	45.8 ± 2.1	46.6 ± 1.9	43.0 ± 1.5

*PC and PE were separated by HPLC. As weight percentage of total phospholipids, PC constituted 56.8 ± 0.5, 58.0 ± 0.4, and 56.5 ± 0.4, and PE 33.9 ± 0.4, 32.7 ± 0.3, and 31.2 ± 0.3 for FO, control, and AA diets, respectively. Their fatty acid compositions were determined by CGLC. Values are means ± SEM (n = 5) and are given as weight percentage of total fatty acids. The values in a row for one PL with different superscript letters ^{a,b,c} are significantly different at $P < 0.05$, as determined by ANOVA and Fisher's test comparisons. The absence of these superscript letters indicates that the values in a row are not significantly different. Σ saturated, Σ n-6, Σ n-3, and Σ unsaturated ≥ C_{20} refer to the sum of saturated, of n-6, n-3, and ≥ C_{20} unsaturated fatty acids, respectively.
†Membranes isolated from Percoll-purified mitochondrial fractions.
‡FO and AA as in Table 2.

Table 6 Fatty acid compositions* of PC and PE of microsomal fractions isolated from liver of Wistar rats fed diets† enriched in PUFA

Fatty acid	PC			PE		
	FO	Control	AA	FO	Control	AA
16:0	22.4 ± 1.2	21.1 ± 1.1	21.5 ± 1.2	18.4 ± 1.0	18.4 ± 1.1	17.7 ± 1.2
16:1	1.2 ± 0.2	1.3 ± 0.3	1.6 ± 0.3	0.6 ± 0.3^{a,b}	0.2 ± 0.1^{a}	1.0 ± 0.3^{b}
18:0	17.3 ± 1.3^{a}	17.7 ± 1.3^{a}	14.1 ± 1.2^{b}	18.7 ± 1.6	17.4 ± 1.2	15.9 ± 1.3
18:1	10.2 ± 0.5^{a}	10.9 ± 0.6^{a}	13.2 ± 0.8^{b}	7.4 ± 1.0^{a}	10.6 ± 0.8^{b}	12.5 ± 1.2^{b}
18:2n-6	11.3 ± 1.1^{a}	8.5 ± 0.9^{b}	6.5 ± 0.9^{b}	6.2 ± 0.8	5.9 ± 0.5	5.7 ± 0.5
20:4n-6	24.2 ± 1.2^{a}	28.7 ± 1.3^{b}	32.5 ± 1.6^{c}	22.5 ± 1.7^{a}	24.8 ± 1.8^{a}	30.1 ± 2.1^{b}
20:5n-3	1.1 ± 0.4	0.6 ± 0.2	0.7 ± 0.2	2.0 ± 0.3	1.7 ± 0.3	2.4 ± 0.4
22:5n-3	0.7 ± 0.2	0.7 ± 0.2	1.3 ± 0.5	1.6 ± 0.5	1.5 ± 0.4	1.5 ± 0.4
22:6n-3	9.0 ± 0.9^{a}	7.2 ± 1.0^{a}	4.8 ± 0.5^{b}	20.8 ± 1.9^{a}	15.2 ± 1.3^{b}	10.1 ± 1.1^{c}
Σ saturated	39.7 ± 1.4^{a}	38.8 ± 1.3^{a}	35.6 ± 1.3^{b}	37.1 ± 1.8	35.8 ± 1.6	33.6 ± 1.6
Σ n-6	35.5 ± 1.1^{a}	37.2 ± 1.3^{a,b}	39.0 ± 1.5^{b}	28.7 ± 1.5^{a}	30.7 ± 1.6^{a}	35.8 ± 1.9^{b}
Σ n-3	10.8 ± 1.0^{a}	8.5 ± 1.0^{a,b}	6.8 ± 0.6^{b}	24.4 ± 2.0^{a}	18.4 ± 1.3^{b}	14.0 ± 1.3^{c}
Σ ≥ C_{20} unsaturated	35.0 ± 1.3^{a}	37.2 ± 1.3^{a,b}	39.3 ± 1.7^{b}	46.9 ± 2.1	43.2 ± 1.9	44.1 ± 1.8

*PC and PE were separated by HPLC. As weight percentage of total phospholipids, PC constituted 62.5 ± 0.6, 63.8 ± 0.8, and 63.5 ± 0.7, and PE 26.6 ± 0.5, 25.3 ± 0.4, and 24.9 ± 0.4 for FO, control, and AA diets, respectively. Their fatty acid compositions were determined by CGLC. Values are means ± SEM (n = 5) and are given as weight percentage of total fatty acids. The values in a row for one PL with different superscript letters ^{a,b,c} are significantly different at $P < 0.05$, as determined by ANOVA and Fisher's test comparisons. The absence of these superscript letters indicates that the values in a row are not significantly different. Σ saturated, Σ n-6, Σ n-3, and Σ unsaturated ≥ C_{20} refer to the sum of saturated, of n-6, n-3, and ≥ C_{20} unsaturated fatty acids, respectively.
†FO and AA as in Table 2.

percentages of DHA in PL from Wistar rats were always found to be lower than those from obese Zucker rats (about −5 and −9% in PC and PE, respectively).[15] These data raise the question of whether a sufficient amount of DHA or a sufficient difference in the percentages of DHA due to the diets has to be attained in order to influence membrane enzyme activities. Interestingly, it may be calculated from *Table 5* that the 18:1/18:2n-6 ratio increased from 0.84 to 1.23 and 1.82 in FO, control, and AA groups, respectively, in PC, and similarly from 1.11 to 1.84 and 2.25, in PE. At this stage, these data cannot be related to changes in the enzymatic activities described below.

Cholesterol content in mitochondrial and microsomal fractions

The cholesterol content was found to be highest in microsomal fractions (50.6, 40.9, and 40.8 μg/mg of protein with FO, control, and AA diets, respectively) and lowest in Percoll-purified mitochondrial fractions (around 1.3 μg/mg of protein). This content ranged about 11 μg/mg of protein of OM fractions. Since the amount of contaminating microsomes in these fractions was known (*Table 3*), the cholesterol content of these fractions devoid of microsomes could be calculated. The corrected values were found to be extremely low and rather comparable in the Percoll-purified fractions (0.38, 0.25, and 0.14 μg/mg of protein with FO, control, and AA diets, respectively). The OM fractions exhibited comparable contents, 9.1, 8.7, and 8.3 μg/mg of protein with FO, control, and AA diets, respectively.

Carnitine palmitoyltransferase I activity and its sensitivity to malonyl-CoA

The specific activity of CPT I in the absence of malonyl-CoA was found to be comparable in mitochondria from rats on the FO and control diets and lower on the AA diet (*Figure 1*). Malonyl-CoA is known to inhibit specifically CPT I, and for a given concentration the inhibition may be higher (high sensitivity as in the fed state) or lower (low sensitivity as in the fasted state). Consequently, a higher sensitivity of CPT I to malonyl-CoA results in a decrease of the β-oxidative pathway, which renders fatty acids more available for the triacylglycerol formation. The FO diet was shown to reduce the sensitivity of CPT I to malonyl-CoA, while the AA diet did not alter this sensitivity (*Figure 2*). When outer membranes were submitted to membrane fluidizers such as benzyl alcohol, CPT I activity was found to be slightly enhanced and its sensitivity of malonyl-CoA was lowered.[44] Therefore, some alterations in lipid composition may have an effect on CPT I activity.[45] The similar cholesterol content found in outer membranes with the three diets cannot account for any differential effect of this molecule considered to increase membrane organization.[21] Despite the fact that the treatment of outer membranes by fluidizers altered two CPT I parameters, i.e., enzymatic activity and sensitivity to malonyl-CoA,[44] the FO diet only succeeded in lowering the sensitivity to malonyl-CoA. If the difference in the sensitivity of CPT I to malonyl-CoA between the FO and control rats was due to differences in the fatty acid composition of PUFA in outer membranes (DHA particularly), it would appear surprising that the sensitivity did not differ in control and AA rats despite relatively large differences in DHA and/or total n-3 fatty acid composition, independent of the PL class. Hence, any apparent relationship cannot be demonstrated between CPT I characteristics and fatty acid composition of PL of membranes in which the enzyme is located.

The results did not exclude the possibility that n-3 PUFA were in close proximity to part of the CPT I protein and only affected one feature of the enzyme, but the data suggested

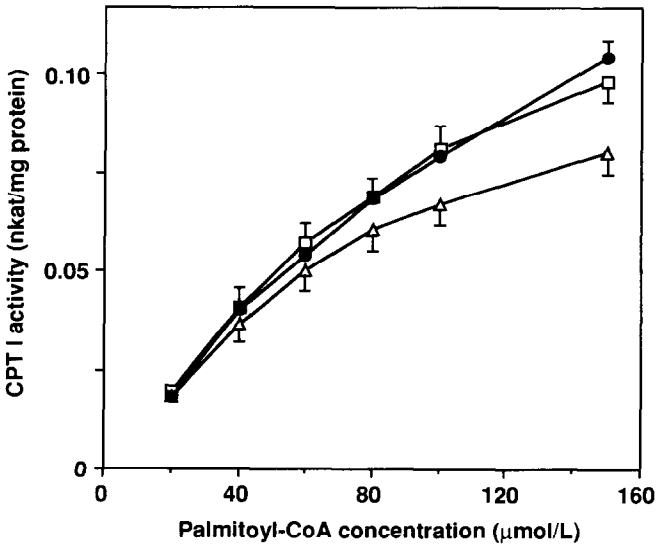

Figure 1 Effect of increasing palmitoyl-CoA concentrations on the activity of carnitine palmitoyltransferase I (CPT I) in Percoll-purified mitochondria fractions isolated from liver of Wistar rats fed the control diet (□), the fish oil (FO) diet (●), or the arachidonic acid (AA) diet (△). Mitochondria (200 μg) were preincubated 2 min in the presence of the indicated concentrations of palmitoyl-CoA. The reaction was initiated by adding L-[^{3}H]carnitine and stopped after 4 min. The radioactivity of the butanolic extract corresponded to carnitine-bound compounds. Results are expressed as nanomoles of palmitoylcarnitine formed per second (nkat) per milligram of protein. T-bars show SEM (n = 5). Values obtained for 80 to 150 μmol/L of palmitoyl-CoA between the control and AA groups are significantly different at $P < 0.05$, as determined by analysis of variance and Fisher's test comparisons, but not between the control and FO groups.

that other factors contained in or triggered by FO, still to be determined, may alter this enzyme activity. In contrast, the AA diet succeeded in decreasing CPT I activity only. It could be thought that CPT I activity was lowered because 20:4n-6 was in larger proportion in PL FA of outer membranes, but in this case the specific activity of CPT I in FO rats should be equally different from that in controls. Since this was not the case, another role for dietary arachidonic acid may be suggested.

The data showed that rats fed the control and FO diets were capable of producing 20:4n-6 from shorter terms. This means that arachidonic acid was supplied in excess to the cell by the AA diet. AA and its acyl-CoA form have been demonstrated to inhibit fatty acid synthetase and acetyl-CoA carboxylase.[46,47] Thus a lower rate of synthesis of malonyl-CoA would have allowed the CPT I enzyme to be less inhibited and therefore more effective in the cell despite its lower specific activity (*Figure 1*). In addition, the continuous intake of arachidonic acid (which is the main membrane PUFA) should save a large part of the energy usually required for its synthesis. Dietary γ-linolenic acid, which is a precursor of arachidonic acid, has been shown to enhance fatty acid β-oxidation in rats,[48] perhaps owing to a higher requirement of energy for AA synthesis. The lower mitochondrial mass found per total liver in AA rats might correspond, at least in part, to a lower need for energy. In this respect, excessive dietary arachidonic acid might constitute, by itself and/or through its derivatives, effective signals to induce metabolic change altering, in particular, mitochondrial turnover.

The main finding of this study was that the activity of CPT I and, therefore, the capacity for fatty acid oxidation were altered in the liver of normal Wistar rats when diets containing very low levels of n-3 and n-6 PUFA were supplied. The altered composition of mitochondrial outer membranes in n-3 and n-6 PUFA, but not in cholesterol, could influence, via the kinetic characteristics of CPT I, the pathway of fatty acid oxidation. However, the data also suggest that factors other than membrane PUFA content will need to be taken into account in order to understand the changes observed in the characteristics of CPT I.

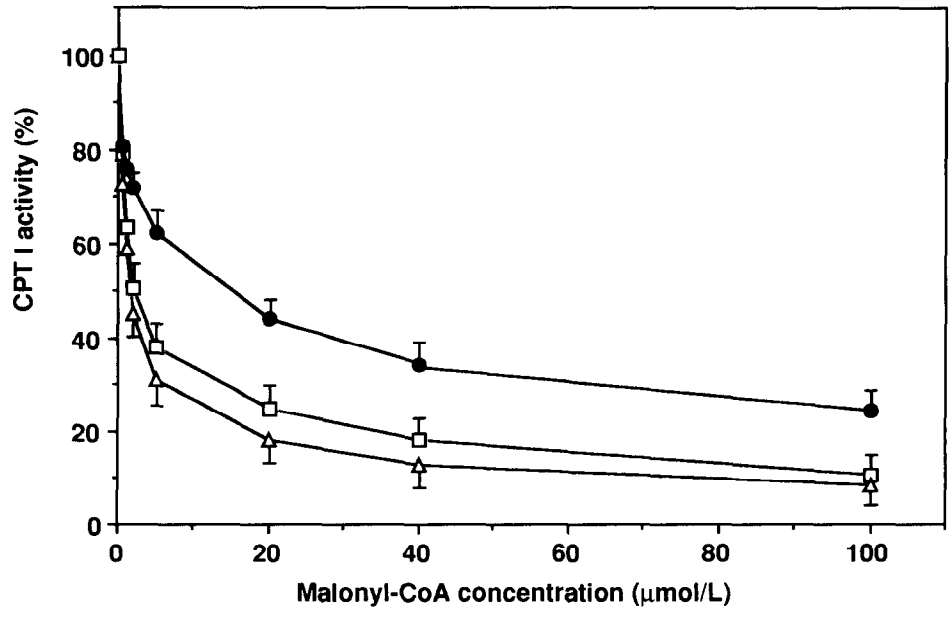

Figure 2 Inhibitory effect of malonyl-CoA on the activity of carnitine palmitoyltransferase I in Percoll-purified mitochondrial fractions isolated from liver of Wistar rats fed the control diet (□), the fish oil diet (●), or the arachidonic acid diet (△). Mitochondria (200 μg) were preincubated for 2 min in the presence of 40 μmol/L of palmitoyl-CoA and the indicated concentrations of malonyl-CoA. The reaction was initiated by adding labeled L -carnitine and stopped after 4 min. Results are given as percentage of the CPT I activity obtained in the absence of malonyl-CoA. A 50% inhibition by malonyl-CoA was seen at the concentration of 2 μmol/L with the control diet, at 15 μmol/L with the FO diet, and 1.5 μmol/L with the AA diet. T -bars show SEM (n = 5). Values obtained for 5 to 100 μmol/L of malonyl-CoA between the FO and control groups are significantly different at $P < 0.05$, as determined by analysis of variance and Fisher's test comparisons, but not the values between the control and AA groups. See Figure 1 for abbreviations.

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