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Incorporation of n-3 fatty acids into phospholipids of rat liver and white and brown adipose tissues: A time-course study during fish-oil feeding

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The aim of this study was to determine the time-course incorporation of dietary n-3 polyunsaturated fatty acids into phospholipids of tissues highly involved in lipid and energy metabolism: the liver and the white (WAT) and brown (BAT) adipose tissues. Rats were fed a diet supplemented with 19% fish oil for up to 4 weeks. Minor changes in the relative proportions of tissue phospholipids were observed in the three tissues. Fish-oil feeding induced rapid and large replacements of n-6 fatty acids by n-3 fatty acids. In liver, the 22:6n-3 level increased progressively and reached a plateau after 3 (phosphatidylethanolamine and phosphatidylserine) or 7 days (phosphatidylcholine and phosphatidylinositol). In contrast, the 20:5n-3 level transiently peaked in all liver phospholipids at days 1–3 before reaching a plateau after day 7. In WAT as in BAT the level of n-3 fatty acids increased progressively and reached in all phospholipids a plateau after day 7. As a general trend, in each phospholipid class the 22:6n-3/20:5n-3 ratio was higher in liver than in the two adipose tissues. This study shows that each dietary n-3 fatty acid is incorporated very rapidly into liver, WAT, and BAT phospholipids but according to time courses and at levels that depend simultaneously on the tissue and phospholipid class considered. (J. Nutr. Biochem. 6:673–680, 1995.)

Keywords: n-3 fatty acids; phospholipids; liver; white adipose tissue; brown adipose tissue

Introduction

The important biological effects of n-3 fatty acids on several pathways predisposing to vascular diseases are well documented since the early discovery of a very low incidence of atherosclerotic diseases among Greenland Eskimos.[1] Later epidemiological studies of fish-eating populations have indicated that n-3 fatty acid consumption is related to a very low incidence of diabetes mellitus.[2] Animal studies have shown that n-3 fatty acids influence insulin sensitivity of muscle and adipose tissue[3,4] and hepatic fatty acid synthesis.[5] Previous studies have also shown that eating unsaturated fatty acid–rich diets alters adipose tissue sensitivity to catecholamines.[6,7] Similarly, n-3 fatty acids were reported to promote a stimulation of lipolysis by isoproterenol in rat adipose cells.[8] Although the mechanisms by which n-3 fatty acids modulate hormone actions at the cellular level remain to be defined, these polyunsaturated fatty acids can increase nonspecifically the cellular membrane fluidity which in turn is able to influence the activity of receptors.[9] Modifications of the fatty acid composition of phospholipids under the influence of dietary n-3 fatty acids have been reported in liver[10–12] as in several organs of the rat.[11,13–17] However, despite some studies on white adipose tissue (WAT),[18,19] no detailed information of the time-related incorporation of n-3 fatty acids into phospholipids are available for white or brown adipose tissue. Liver, WAT, and brown adipose tis-

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sues (BAT) are, in mammals, the major organs involved in lipid metabolism, lipid storage, and energy utilization, respectively. Thus these important functions may be greatly affected by the incorporation of n-3 fatty acids in membrane phospholipids as suggested by their lowering effect on hepatic triacylglycerol synthesis[12] and adipocyte fat storage.[8,20]

It was therefore of interest to examine how dietary n-3 fatty acids modulate the membrane phospholipid composition of liver, WAT, and BAT. This was done by studying simultaneously in these three tissues of rats the detailed time-course changes in the phospholipid fatty acid composition induced by fish-oil feeding. Particular attention was paid to the incorporation of the two major n-3 fatty acids, 20:5n-3 and 22:6n-3, into various phospholipid classes.

Methods and materials

Animals and diet

Thirty-five 8-week-old male Wistar rats were individually housed in a room with a 12 hr light/dark cycle at 25°C. These rats were fed a basal diet (AO4, Usine d'Alimentation Rationnelle, Villemoisson, France) from weaning up to 8 weeks of age. When the animals reached 250 ± 2.8 g, 30 rats were shifted to a semisynthetic diet containing 19% (wt/wt) purified fish oil (MaxEPA, R.P. Scherer, Beinheim, France) and 1% (wt/wt) sunflower oil. The composition of the diets has been previously described.[21] The lipid moieties of the experimental diet contained 32.1% n-3 fatty acids, including 15.9% 20:5n-3 and 9.4% 22:6n-3, 33.4% saturated, 11.8% n-9, 13.6% n-7, and 5.4% n-6 fatty acids. Five rats fed the basal diet were killed at the onset of the experiment (0 day) and those fed the fish-oil diet after 1 day (249 ± 4 g), 3 days (279 ± 2.9 g), 1 week (280 ± 1.7 g), 2 weeks (331 ± 4.7 g), 3 weeks (375 ± 5.1 g), and 4 weeks (388 ± 5.1 g) of feeding (5 rats at a time). Samples of liver, of retroperitoneal WAT, and interscapular BAT were immediately excised and extracted. Blood was collected by heart puncture, placed into a heparinized test tube, and plasma was separated by centrifugation.

Lipid analysis

Lipids were extracted from the different tissues according to the Folch et al. procedure.[22] Phospholipids were purified from about 300 mg of total lipids on a short silica gel column and separated into individual classes by thin-layer chromatography.[23] Fatty acids were analyzed by gas liquid chromatography (CP9000 gas chromatograph, Chrompack, Les Ulis, France; AT-WAX capillary column, 60m × 0.25 mm i.d., Alltech, Templeuve, France) after BF_3-catalyzed transmethylation. Quantitative analysis of phospholipids was made by phosphorus estimation according to the malachite green micromethod.[24] Results were expressed as the mean ± SE. Statistical analyses were performed by the Student's *t*-test. Values were considered to be significantly different when $P < 0.05$.

Results

To simplify the description and discussion of the complex analytical data on fatty acid composition, results are presented as levels of the five fatty acid series and of the three main indices (unsat/sat ratio, double bond index, and n-3/n-6 ratio). For liver, WAT, and BAT, the composition of only the two main phospholipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), is given (*Tables 3–5*). The levels of the two main n-3 fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), and of the two main n-6 fatty acids, linoleic acid (18:2n-6) and arachidonic acid (20:4n-6), expressed as molar percentage of total fatty acids in plasma lipids (*Figure 1*), and in tissue PC, PE, phosphatidylinositol (PI), and phosphatidylserine (PS) are also presented (*Figures 2–4*).

Changes in plasma lipids

Since the overall changes in the fatty acid composition were similar for plasma triacylglycerols and phospholipids, only the effects of fish-oil supplementation on the fatty acid composition of total plasma lipids are shown (*Table 1, Figure 1*). Alterations in the fatty acid pattern were evident within only 1 day, minor changes being seen later. The plateau levels of n-3 fatty acids, EPA and DHA were 7.5, 23, and 3 times higher than in the basal state ($P < 0.001$), respectively, the DHA/EPA ratio being similar to that found in the diet. The rapid incorporation of n-3 fatty acids in plasma lipids was mainly at the expense of 18:2n-6 and n-9 fatty acids.

Changes in liver phospholipids

Feeding a fish-oil diet resulted in minor changes in the relative proportion of liver phospholipids, only a transient decrease of PS (week 1), and an increase of cardiolipins (CL) (week 4) being observed (*Table 2*). *Table 3* shows that the global fatty acid composition of liver glycerophospholipids changed markedly. The DB index and the n-3/n-6 ratio in both PC and PE increased during the first 3 days and remained steady but the DB index of PE decreased to initial levels thereafter.

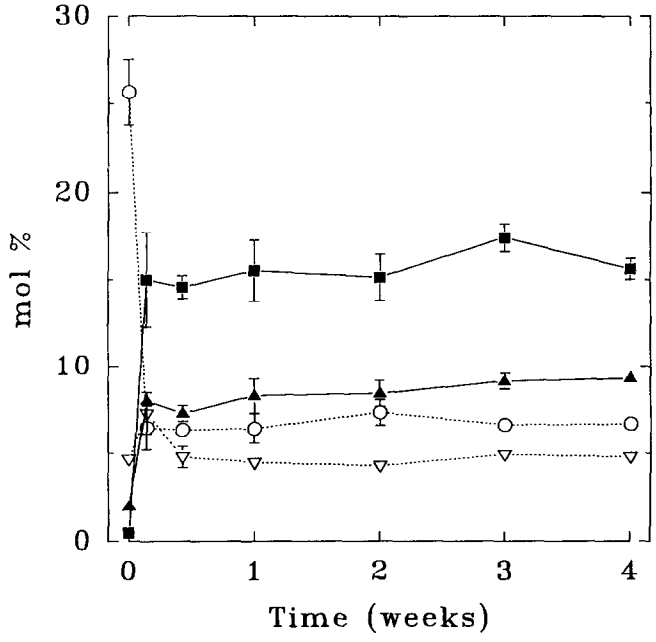

Figure 1 Effect of fish-oil feeding on the fatty acid composition of the rat plasma total lipids. Means ± SE ($n = 5$). 18:2(n-6) ○, 20:4(n-6) ▽, 20:5(n-3) ■, 22:6(n-3) ▲.

Table 1 Fatty acid composition of plasma total lipids*

Fatty acids	Days on diet				
	0	1	3	7	28
S.SAT	38.6 ± 2.4	33.4 ± 2.5	41.1 ± 1.4	39.7 ± 2.4	37.2 ± 1.1
S.n-9	17.2 ± 0.4^{a}	12.4 ± 1.8^{b}	9.2 ± 0.3^{b}	11.3 ± 1.0^{b}	10.3 ± 0.2^{b}
S.n-7	9.0 ± 0.8^{a}	10.5 ± 1.2^{ac}	11.6 ± 0.3^{c}	9.4 ± 0.9^{a}	10.2 ± 0.3^{a}
S.n-6	31.3 ± 2.2^{a}	15.0 ± 1.6^{b}	11.9 ± 0.7^{b}	12.0 ± 0.9^{b}	13.0 ± 0.5^{b}
S.n-3	3.9 ± 0.3^{a}	28.7 ± 3.1^{b}	26.2 ± 1.1^{b}	27.6 ± 3.0^{b}	29.3 ± 1.0^{b}
UNSAT/SAT	1.6 ± 0.2	2.0 ± 0.3	1.4 ± 0.1	1.5 ± 0.2	1.7 ± 0.1
DB index	118.2 ± 6.0^{a}	216.0 ± 12.4^{b}	190.0 ± 7.0^{b}	198.2 ± 15.0^{b}	209.8 ± 5.2^{b}
n-3/n-6	0.1 ± 0.0^{a}	1.9 ± 0.2^{b}	2.2 ± 0.1^{b}	2.3 ± 0.4^{b}	2.2 ± 0.2^{b}

*Values (mole percent) are means ± standard error of 5 determinations. Within a line, values without a common superscript are significantly different at $P < 0.05$ or less.
S.SAT: sum of saturated fatty acids; S.n-9: sum of n-9 fatty acids; S.n-7: sum of n-7 fatty acids; S.n-6: sum of n-6 fatty acids; S.n-3: sum of n-3 fatty acids; UNSAT/SAT: ratio of unsaturated to saturated fatty acids; DB index: number of double bonds per 100 mole fatty acids; n-3/n-6: ratio of n-3 to n-6 fatty acids.

Alterations in the fatty acid patterns were rapid, being completed after 1 week or less, and showed thereafter low amplitude changes. The level of n-3 fatty acids increased 5 times in PC ($P < 0.001$) and in PE ($P < 0.05$), being steady after 1–3 days (*Table 3*). EPA showed a rapid increase with transient peaks at day 1 (PS) and days 1–3 (PE, PC, PI) followed after 1 week by a steady level in all phospholipids (*Figure 2*). The DHA increase was slower than that of EPA and reached a plateau level after 3–7 days. At plateau, and according to the phospholipid class, the DHA/EPA ratio was 1.5–10 times higher than in the diet. The incorporation of n-3 fatty acids in liver phospholipids was mainly at the expense of 20:4n-6 and 18:2n-6 in PE and PC but of only 20:4n-6 in PS and PI (*Figure 2*).

Table 2 Phospholipid composition of liver, white and brown adipose tissues*

	Days on diet		
	0	7	28
Liver			
CL	2.8 ± 0.5^{a}	2.8 ± 0.6^{a}	5.0 ± 0.7^{b}
PE	19.4 ± 2.2	13.0 ± 2.3	15.3 ± 1.4
PS	5.8 ± 0.7^{a}	3.2 ± 0.8^{b}	5.8 ± 0.4^{a}
PI	10.7 ± 2.0	8.4 ± 1.7	12.2 ± 0.7
PC	53.6 ± 3.4	65.8 ± 6.0	52.7 ± 1.7
SM	7.7 ± 1.2	6.8 ± 1.2	9.0 ± 0.7
White adipose tissue			
CL	1.6 ± 0.7	1.5 ± 0.3	2.1 ± 0.4
PE	18.7 ± 0.3^{a}	16.6 ± 0.8^{b}	19.2 ± 2.2^{ab}
PS	9.3 ± 0.4^{a}	5.7 ± 0.2^{b}	5.2 ± 1.7^{b}
PI	8.9 ± 1.1^{a}	8.4 ± 0.3^{a}	4.7 ± 1.2^{b}
PC	44.3 ± 4.2	49.1 ± 0.9	51.0 ± 1.7
SM	17.2 ± 2.0	18.6 ± 1.6	17.8 ± 0.9
Brown adipose tissue			
CL	7.8 ± 0.5^{a}	5.6 ± 1.0^{ab}	5.7 ± 0.5^{b}
PE	24.2 ± 1.9	23.8 ± 1.5	19.6 ± 2.4
PS	7.0 ± 1.3^{a}	3.0 ± 0.5^{b}	6.0 ± 0.5^{a}
PI	10.6 ± 0.9	8.4 ± 0.6	10.1 ± 0.5
PC	40.6 ± 3.0^{a}	50.7 ± 2.1^{b}	50.3 ± 1.4^{b}
SM	9.7 ± 0.7	8.6 ± 1.5	8.4 ± 0.4

*Mole percent values given are means ± standard error of 5 determinations. Within a line, values without a common superscript are significantly different at $P < 0.05$ or less.
CL, cardiolipin; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine; SM, sphingomyelin.

Changes in WAT phospholipids

Feeding the fish-oil diet resulted in only minor changes in the distribution of WAT phospholipids, with a decrease of PS and PI, respectively, after 1 and 4 weeks being observed (*Table 2*). *Table 4* shows that the global fatty acid composition of PC and PE changed rapidly and markedly. The DB index increased rapidly for PC (1 day) and progressively for PE (4 weeks) while the n-3/n-6 ratio increased largely for these phospholipids during the first week. The changes in the level of the various fatty acids were rapid and completed within 1 week. The level of n-3 fatty acids increased by up to 11 and 4 times in PC and PE ($P < 0.001$; *Table 4*), respectively. In contrast with liver, EPA and DHA increased progressively in all phospholipids during the first week. Thereafter they remained at a nearly steady level, the corresponding DHA/EPA ratio being lower (PC, PI), similar (PE), or higher (PS) than in the diet. The incorporation of n-3 fatty acids was mainly at the expense of 18:2n-6 and 20:4n-6 in all WAT phospholipids and also of n-9 fatty acids in PE (*Table 4, Figure 3*).

Changes in BAT phospholipids

Feeding the fish-oil diet resulted in minor changes in the distribution of BAT phospholipids, with only a decrease of CL and an increase of PC being observed after 1 week (*Table 2*). *Table 5* shows that the global fatty acid composition of BAT changed rapidly and very markedly. The DB index increased progressively during the first week for PC and PE and then plateaued. The n-3/n-6 ratio increased during the first 3 days for PC and the first week for PE. The changes in the levels of the various fatty acids were rapid and completed within 1 week. The level of n-3 fatty acids increased by up to 11 (PC) and 5 (PE) times ($P < 0.001$; *Table 5*). During the first week, the increase in the levels of EPA and DHA was progressive until a plateau was reached

Table 3 Fatty acid composition of liver phosphatidylcholine and phosphatidylethanolamine*

Fatty acids	Days on diet				
	0	1	3	7	28
Phosphatidylcholine					
S.SAT	45.6 ± 2.3^{a}	36.0 ± 3.2^{b}	41.7 ± 0.6^{ab}	53.5 ± 1.8^{c}	41.3 ± 2.8^{ab}
S.n-9	5.0 ± 0.3^{a}	2.8 ± 0.1^{b}	2.7 ± 0.3^{b}	3.6 ± 0.2^{c}	4.7 ± 0.2^{a}
S.n-7	5.8 ± 0.5^{ad}	8.3 ± 0.7^{b}	6.0 ± 0.2^{ac}	5.8 ± 0.1^{ac}	4.8 ± 0.4^{d}
S.n-6	38.1 ± 1.1^{a}	27.3 ± 1.0^{b}	22.2 ± 0.5^{c}	15.0 ± 0.5^{d}	22.0 ± 1.1^{c}
S.n-3	5.5 ± 0.4^{a}	25.5 ± 3.1^{bc}	27.4 ± 0.9^{b}	22.0 ± 1.6^{c}	27.2 ± 2.1^{bc}
UNSAT/SAT	1.1 ± 0.1^{ac}	1.9 ± 0.3^{b}	1.4 ± 0.1^{ab}	0.9 ± 0.1^{c}	1.5 ± 0.2^{ab}
DB index	153 ± 6^{a}	239 ± 16^{b}	229 ± 4^{b}	180 ± 10^{a}	229 ± 14^{b}
n-3/n-6	0.1 ± 0.0^{a}	0.9 ± 0.1^{b}	1.2 ± 0.1^{bc}	1.5 ± 0.1^{c}	1.2 ± 0.1^{bc}
Phosphatidylethanolamine					
S.SAT	31.4 ± 3.6^{a}	31.3 ± 2.8^{a}	27.1 ± 3.3^{a}	52.0 ± 2.1^{b}	44.2 ± 7.4^{ab}
S.n-9	3.9 ± 0.3^{a}	3.2 ± 0.2^{a}	1.9 ± 0.1^{b}	1.3 ± 0.1^{c}	1.8 ± 0.2^{bc}
S.n-7	3.5 ± 0.5^{a}	5.1 ± 0.2^{b}	2.5 ± 0.2^{a}	2.4 ± 0.1^{a}	3.0 ± 0.6^{a}
S.n-6	44.4 ± 3.5^{a}	27.8 ± 2.0^{b}	18.7 ± 0.8^{c}	10.0 ± 0.7^{d}	12.7 ± 2.1^{d}
S.n-3	16.8 ± 2.9^{a}	32.6 ± 2.6^{b}	49.8 ± 3.4^{c}	34.3 ± 1.6^{b}	38.3 ± 6.4^{b}
UNSAT/SAT	2.3 ± 0.4^{ac}	2.3 ± 0.3^{a}	2.9 ± 0.4^{a}	0.9 ± 0.1^{bc}	1.3 ± 0.2^{c}
DB index	255 ± 21^{ab}	292 ± 14^{a}	352 ± 19^{b}	237 ± 11^{a}	266 ± 44^{ab}
n-3/n-6	0.4 ± 0.1^{a}	1.2 ± 0.1^{b}	2.7 ± 0.2^{c}	3.4 ± 0.1^{d}	3.0 ± 0.5^{cd}

*See legend to *Table 1*.

in all phospholipids as observed in WAT (*Figure 4*). At plateau, the DHA/EPA ratio was lower (PC, PI) or higher (PE, PS) than in the diet. The incorporation of n-3 fatty acids in BAT phospholipids was mainly at the expense of n-6 and n-9 fatty acids. (*Table 5, Figure 4*).

Discussion

While changes in plasma and tissue fatty acid composition in response to a diet enriched in n-3 fatty acids are well recognized in rats,[11,25–27] the rates at which these changes occur are less well known.[10,17,28] As previously observed for serum lipids,[10] our experiment shows a rapid and sustained incorporation of dietary n-3 fatty acids into plasma lipids, thus providing a continuous fatty acid supply to liver and adipose tissues. These results are in accordance with the recent observation of a rapid absorption of n-3 polyunsaturated fatty acids from marine oils over a 24 hr period.[29] Similarly our finding of limited changes in the proportions of the phospholipid classes in liver, WAT, and BAT is in agreement with the observation that the phospholipid class distribution in tissues and membranes is generally not sensitive to dietary modulation including n-3 fatty acid feeding.[11,18,28]

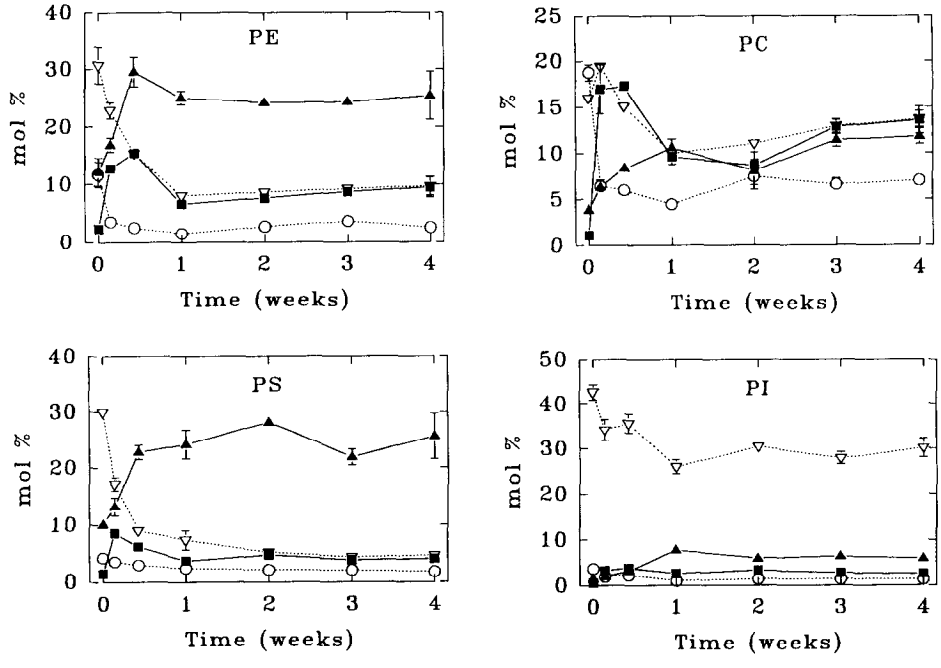

Figure 2 Effect of fish-oil feeding on the fatty acid composition of rat liver phospholipids. Means ± SE (n = 5). 18:2(n-6) ○, 20:4(n-6) ▽, 20:5(n-3) ■, 22:6(n-3) ▲. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol.

Table 4 Fatty acid composition of WAT phosphatidylcholine and phosphatidylethanolamine*

Fatty acids	Days on diet				
	0	1	3	7	28
Phosphatidylcholine					
S.SAT	45.7 ± 1.1^{a}	40.7 ± 1.7^{b}	49.0 ± 1.7^{ac}	50.1 ± 0.9^{c}	52.1 ± 1.9^{c}
S.n-9	11.8 ± 0.7^{a}	10.9 ± 0.8^{ab}	10.0 ± 0.4^{ab}	9.9 ± 0.1^{b}	9.3 ± 0.2^{b}
S.n-7	3.5 ± 0.3^{a}	4.8 ± 0.5^{ab}	4.6 ± 0.1^{b}	6.1 ± 0.5^{c}	7.3 ± 0.3^{c}
S.n-6	37.3 ± 1.1^{a}	31.8 ± 3.3^{a}	20.0 ± 0.9^{b}	15.5 ± 0.7^{c}	22.1 ± 0.9^{b}
S.n-3	1.7 ± 0.5^{a}	11.8 ± 2.2^{bd}	16.4 ± 2.0^{bc}	18.4 ± 1.2^{c}	9.2 ± 1.8^{d}
UNSAT/SAT	1.2 ± 0.1^{ab}	1.5 ± 0.1^{a}	1.1 ± 0.1^{ab}	1.0 ± 0.0^{ab}	0.9 ± 0.1^{b}
DB index	125 ± 4^{a}	173 ± 8^{b}	165 ± 12^{b}	156 ± 6^{b}	125 ± 10^{a}
n-3/n-6	0.1 ± 0.0^{a}	0.4 ± 0.1^{b}	0.8 ± 0.1^{c}	1.2 ± 0.1^{d}	0.4 ± 0.1^{b}
Phosphatidylethanolamine					
S.SAT	35.3 ± 1.3^{ab}	36.1 ± 2.2^{ab}	40.0 ± 2.5^{a}	33.1 ± 1.1^{bc}	27.6 ± 2.9^{c}
S.n-9	18.3 ± 0.8^{a}	13.9 ± 1.4^{b}	12.3 ± 1.5^{bc}	13.7 ± 0.7^{b}	8.6 ± 1.6^{c}
S.n-7	5.4 ± 0.4^{a}	4.3 ± 0.5^{ab}	4.4 ± 0.3^{ab}	3.7 ± 0.3^{b}	7.3 ± 2.1^{ab}
S.n-6	31.9 ± 0.6^{a}	31.4 ± 2.4^{a}	21.1 ± 0.6^{b}	17.9 ± 0.7^{c}	22.1 ± 1.3^{b}
S.n-3	9.1 ± 0.4^{a}	14.3 ± 1.2^{b}	22.2 ± 1.9^{c}	31.6 ± 1.7^{d}	34.4 ± 2.8^{d}
UNSAT/SAT	1.8 ± 0.1^{a}	1.8 ± 0.2^{ab}	1.5 ± 0.2^{a}	2.0 ± 0.1^{ab}	2.8 ± 0.4^{b}
DB index	181 ± 4^{a}	202 ± 9^{a}	210 ± 12^{a}	252 ± 9^{b}	275 ± 19^{b}
n-3/n-6	0.3 ± 0.0^{a}	0.5 ± 0.1^{b}	1.1 ± 0.1^{c}	1.8 ± 0.1^{d}	1.6 ± 0.1^{d}

See legend to Table 1.

The present study demonstrates for the first time how rapidly dietary n-3 fatty acids are able to alter the fatty acid profiles of liver and adipose tissue phospholipids in the rat. The observed changes in n-3 fatty acid contents are consistent with the dietary treatment, although their time course and extent depend on the phospholipid and the organ considered. The initial transient rise observed for the EPA content in liver PE and PC is more likely related to a specific metabolism than to intestinal absorption or hepatocyte bioavailability of this fatty acid since its plasma level was steady after 1 day of fish-oil feeding. The observed decrease in the relative amount of EPA in liver phospholipids following the initial rise may reflect the lag time needed to develop an efficient fatty acid oxidative activity in connection with the administration of a hyperlipidic diet enriched in n-3 fatty acids.[8,30,31] The recent observation of an early proliferation of peroxisomes[32] as well as a rapid increase in acyl-CoA oxidase[30] after administration of n-3 fatty acids suggest that these mechanisms play an important role in regulating the fatty acid supply for phospholipid synthesis. The very rapid acylation of EPA in liver PC is in agreement with the demonstration of a more efficient handling of EPA than DHA by the 1-acyl-glycerol 3-phosphocholine acyltransferase system of rat liver microsomes.[33] Fatty acid

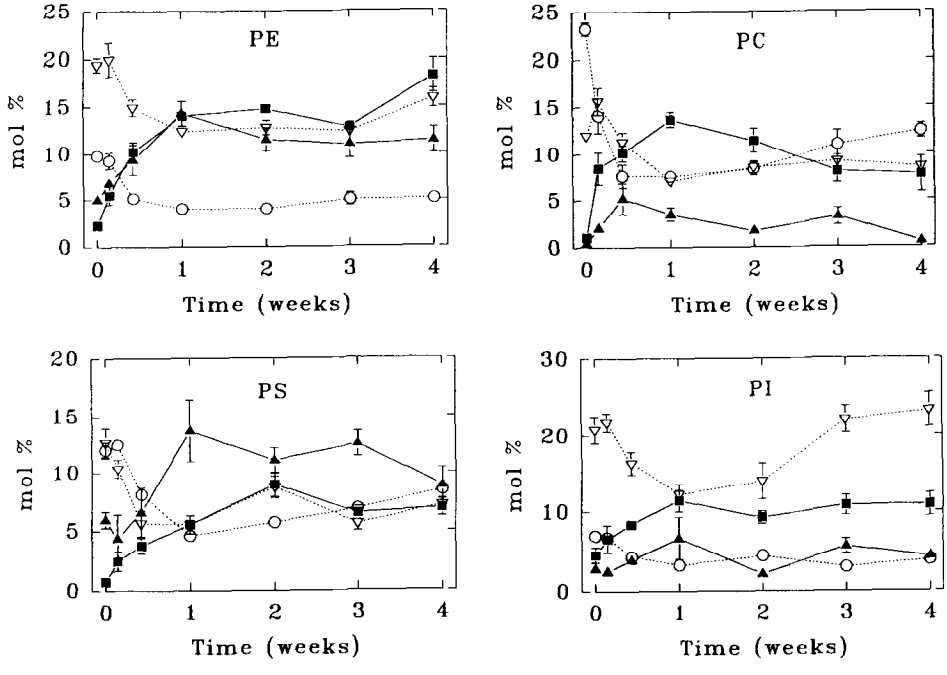

Figure 3 Effect of fish-oil feeding on the fatty acid composition of rat white adipose tissue phospholipids. Means ± SE (n = 5). 18:2(n-6) ○, 20:4(n-6) ▽, 20:5(n-3) ■, 22:6(n-3) ▲. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol.

Table 5 Fatty acid composition of BAT phosphatidylcholine and phosphatidylethanolamine*

Fatty acids	Days on diet				
	0	1	3	7	28
Phosphatidylcholine					
S.SAT	49.1 ± 1.2^{a}	49.5 ± 1.1^{a}	57.4 ± 2.1^{b}	52.5 ± 2.3^{ab}	53.6 ± 1.3^{b}
S.n-9	17.6 ± 0.9^{a}	13.6 ± 1.4^{b}	9.1 ± 0.8^{c}	6.3 ± 0.6^{d}	6.0 ± 0.3^{d}
S.n-7	3.1 ± 0.5^{ac}	5.1 ± 0.3^{b}	4.2 ± 0.1^{a}	3.4 ± 0.3^{c}	5.2 ± 0.3^{b}
S.n-6	28.3 ± 1.5^{a}	20.5 ± 1.4^{b}	12.6 ± 0.8^{c}	16.5 ± 1.7^{bc}	14.3 ± 0.9^{bc}
S.n-3	1.9 ± 0.3^{a}	11.3 ± 1.2^{b}	16.7 ± 0.7^{c}	21.3 ± 0.8^{d}	20.9 ± 0.8^{d}
UNSAT/SAT	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	0.7 ± 0.1^{b}	0.9 ± 0.1^{ab}	0.9 ± 0.0^{ab}
DB index	104.0 ± 5.0^{a}	136.0 ± 3.0^{b}	138.0 ± 6.0^{b}	174.0 ± 8.0^{c}	166.0 ± 6.0^{c}
n-3/n-6	0.1 ± 0.0^{a}	0.6 ± 0.1^{b}	1.3 ± 0.1^{c}	1.4 ± 0.1^{c}	1.5 ± 0.1^{c}
Phosphatidylethanolamine					
S.SAT	42.3 ± 1.4	39.6 ± 1.3	39.8 ± 1.1	42.7 ± 1.6	38.5 ± 1.4
S.n-9	15.2 ± 0.9^{a}	14.4 ± 0.9^{a}	10.8 ± 0.8^{b}	5.0 ± 0.5^{c}	6.2 ± 0.3^{c}
S.n-7	3.6 ± 0.3^{ac}	4.4 ± 0.2^{a}	4.6 ± 0.4^{a}	2.9 ± 0.3^{bc}	4.6 ± 0.4^{ab}
S.n-6	31.9 ± 0.5^{a}	27.2 ± 1.4^{a}	18.2 ± 1.2^{b}	17.1 ± 1.3^{b}	16.4 ± 0.6^{b}
S.n-3	7.0 ± 0.6^{a}	14.4 ± 0.9^{b}	26.6 ± 0.8^{c}	32.3 ± 1.0^{d}	34.3 ± 1.0^{d}
UNSAT/SAT	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.6 ± 0.1
DB index	168.0 ± 3.0^{a}	198.0 ± 3.0^{b}	230.0 ± 6.0^{c}	248.0 ± 6.0^{cd}	257.0 ± 5.0^{d}
n-3/n-6	0.2 ± 0.0^{a}	0.6 ± 0.1^{b}	1.5 ± 0.1^{c}	1.9 ± 0.1^{d}	2.1 ± 0.1^{d}

*See legend to *Table 1*.

transport, CoA activation, and phospholipids interconversion may also be involved in this complex regulation. Since lipoprotein metabolism was shown to be closely related to liver PC composition in fish-oil fed rats,[35] our results suggest that the present approach is valuable for examining more precisely these relationships.

Despite the different size and physiological specializations of WAT and BAT, the modifications in their phospholipid fatty acid profile were similar, reaching near steady-state levels 1 week after the beginning of fish-oil ingestion. The fatty acid alterations in adipose tissue phospholipids may have relevance to modifications in specialized membrane-mediated processes. Thus, the modulation of insulin binding and responsiveness by the fatty acid composition of the adipocyte plasma membrane has been well documented[34,36] but the specific role of n-3 fatty acids in this field remains poorly investigated.[37,38] It was recently reported that these fatty acids can stimulate catecholamine-mediated lipolysis[8] as well as modulate body fat mobilization.[39] Furthermore, lipid accretion in adipose tissue might be greatly affected by cellular membranes enriched in n-3 fatty acids. Increases in long-chain n-3 fatty acids in plasma membrane and mitochondria have been associated with increased permeability to ions and hence may have important

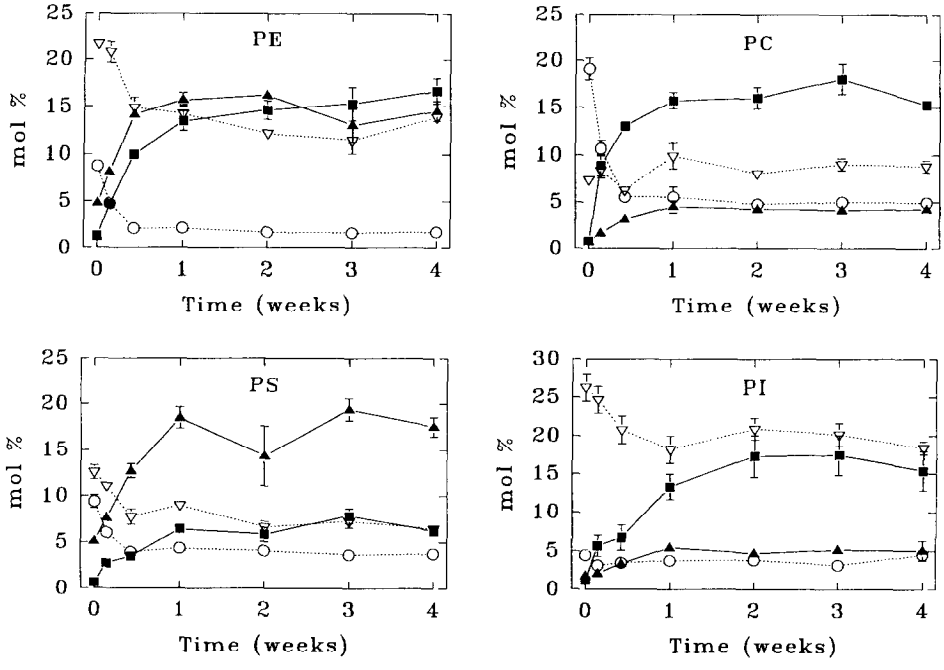

Figure 4 Effect of fish-oil feeding on the fatty acid composition of rat brown adipose tissue phospholipids. Means ± SE (n = 5). 18:2(n-6) ○, 20:4(n-6) ▽, 20:5(n-3) ■, 22:6(n-3) ▲. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol.

influence on the overall metabolic activity.[40] Since energy demand is mainly met by the utilization of lipid fuels, a greater mobilization of endogenous fat stores may be expected thus contributing to the reported limitation of fat cell hypertrophy in fish-oil fed rats.[20] Moreover, considering the possible regulation of food intake or energy balance by prostaglandins,[41] variations in eicosanoid production after ingestion of n-3 fatty acids may modulate the rate of lipogenesis-lipolysis, glucose metabolism, and even insulin secretion.[42] Thus, changes in membrane fatty acids associated with a fish-oil–enriched diet are strong and potential candidates in the causal link between the dietary treatment and the observed protection against insulin resistance and obesity.[38]

In conclusion, our study shows that the ingestion of fish oil induces slight changes in the phospholipid distribution of liver, WAT and BAT but alters rapidly and profoundly their fatty acid profiles. All phospholipids are affected but to different extents, according to the tissue examined, and reach a near steady composition after feeding the animals 1 week or less with the fish-oil–enriched diet. These changes are expected to affect simultaneously membrane functions through key control enzymes, signal transduction, and transport efficiency.

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