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Effect of increasing amounts of dietary fish oil on brain and liver fatty composition

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Increasing dietary fish oil in rat had the following effect on brain lipids: Arachidonic acid regularly decreased; eicosapentanenoic acid, normally nearly undetectable, was present; 22:5($n-3$), dramatically increased but remained below 1% of total fatty acids; cervonic acid was increased by 30% at high fish oil concentration. Saturated and monounsaturated fatty acids were not affected regardless of chain-length. In contrast, in the liver, nearly all fatty acids (saturated, monounsaturated and polyunsaturated) were affected by high dietary content of fish oil, but liver function was normal: serum vitamin A and E, glutathione peroxidase, alkaline phosphatase, transaminases were not affected. Serum total cholesterol, unesterified cholesterol and phosphatidylcholine were slightly affected. In contrast, triacylglycerols were dramatically reduced in proportion to the fish oil content of the diet.

Introduction

Dietary $n-3$ fatty acids (such as EPA and DHA) can greatly affect the fatty acid composition of the various membrane phospholipids of the nervous system within a relatively short time, both in weanlings [1] and in adult animals [2]. These biochemical effects may result in functional changes including alterations in membrane architecture and fluidity, enzymatic activities, cellular response, ion transport and membrane electrophysiology, resistance to poisons and even for the nervous tissue, altered biosynthesis of arachidonic and EPA-derived prostaglandins and leukotrienes.

The replacement of cell membrane $n-6$ fatty acids by dietary $n-3$ fatty acids and the subsequent alterations of membrane composition remain to be elucidated. Indeed, maintenance of membrane fatty acid composition and fluidity within a narrow limit is probably a prerequisite for the proper function of the cell, including nerve cells, as it is known that alterations of the fatty acyl composition of membrane can alter numerous cellular function [3,4].

Our previous results have shown that fish oil (even if added with sufficient amounts of vitamin E) in the form of cod liver oil or salmon oil alters dramatically the polyunsaturated fatty acid composition of the brain and the liver [2] and heart [5] and leads to lipofuscin accumulation in this last organ. In contrast, pharmacological doses of fish oil (below 1% of the calories) have little effect on brain fatty acid composition and do not provoke peroxidation [6].

The brain changes could alter brain function; moreover, the speed of recovery could be very slow, as shown by nutritional studies using a diet deficient in α-linolenic acid [7–9].

Consumption of large amounts of $n-3$ fatty acid could be important, as fish oils have a beneficial effect on ischaemic heart disease and thrombosis [10–12]. In human volunteers [13] as well as in animals [14], fish oil decreased blood lipids and, in particular, triacylglycerol levels, showing that ingestion of $n-3$ fatty acids may have a beneficial effect on the condition of the blood vessels [13]. However, the use of fish oil in patients is equivalent to drug therapy and further studies on their long-term efficacy, toxicity and the possibility of overdosage must be conducted before precise recommendations can be made on their general use [15].

This work was undertaken to determine whether increasing the amount of dietary fish oil results in a

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TABLE I

Composition of diets (weight %)

	Diet %				
	A	B	C	D	E
Casein	27.4	27.4	27.4	27.4	27.4
Starch	30.5	30.5	30.5	30.5	30.5
Glucose	21.6	21.6	21.6	21.6	21.6
Minerals	5	5	5	5	5
Vitamins	1	1	1	1	1
Cellulose	4.5	4.5	4.5	4.5	4.5
Corn oil	10	8.5	6	3	–
Salmon oil	–	1.5	4	7	10
Vit. E, mg/kg	215	223	237	253	270
$(n-6)/(n-3)$	75	10	3	1	0.1

parallel increase in the amount of $n-3$ and decrease in the amount of $n-6$ fatty acids in brain as well as in liver.

TABLE II

Total lipid fatty acid composition (weight %) of brain of rats fed various diets

Values (mean ± S.D., $n=6$) not bearing the same superscript letter are significantly different at $P<0.05$. If no superscript appears, values are not different. S.D. did not exceed 10% of the mean values.

Fatty acids	A	B	C	D	E
14:0	0.1	0.1	0.1	0.1	0.1
15:0	0.1	0.2	0.1	0.1	–
16:0	20.1	19.9	20.3	19.6	20.2
16:1($n-9$)	0.2	0.2	0.2	0.2	0.2
16:1($n-7$)	0.5	0.5	0.6	0.6	0.7
17:0	0.4	0.4	0.4	0.4	0.2
18:0	21.6	21.2	20.9	20.6	20.8
18:1($n-9$)	17.5 [a]	17.8 [a]	17.8 [a]	19.2 [b]	20.4 [b]
18:1($n-7$)	3.7	3.7	3.6	3.7	3.8
18:2($n-6$)	0.9 [a]	1 [a]	0.9 [a]	0.7 [a]	0.3 [b]
18:3($n-6$)	0.1	0.2	0.1	0.1	–
18:3($n-3$)	–	–	–	0.1	–
20:0	0.7	0.6	0.6	0.5	0.5
20:1($n-9$)	1.7	1.6	1.4	1.6	1.7
20:1($n-7$)	0.5	0.5	0.6	0.5	0.5
20:2($n-6$)	0.2	0.2	0.2	0.1	0.1
20:3($n-6$)	0.9	1	0.9	0.9	0.1
20:4($n-6$)	10.2 [a]	9.1 [a]	8.5 [a]	7.7 [b]	7.8 [b]
20:5($n-3$)	–	–	0.1 [a]	0.2 [a]	0.3 [b]
22:0	0.6	0.6	0.6	0.6	0.6
22:1($n-9$)	0.2	0.2	0.2	0.3	0.3
22:1($n-7$)	0.2	0.2	0.2	0.2	0.2
22:4($n-6$)	3.5 [a]	2.7 [b]	2.7 [b]	3.0 [b]	2.7 [b]
22:5($n-6$)	0.8 [a]	0.4 [b]	0.3 [b]	0.3 [b]	0.3 [b]
22:5($n-3$)	0.1 [a]	0.2 [a]	0.4 [b]	0.6 [c]	0.7 [c]
24:0	0.5	0.7	0.8	0.8	0.4
22:6($n-3$)	12.1 [a]	12.3 [a]	15.4 [b]	14.6 [b]	15.6 [b]
24:1($n-9$)	2.1	2.1	1.5	2	2
24:1($n-7$)	0.2 [a]	0.4 [a]	0.5 [a]	0.1 [b]	0.1 [b]
24:4($n-6$)	0.4	0.4	0.5	0.3	0.3
24:5($n-6$)	0.2	0.1	–	–	–

Materials and Methods

Five groups of 12 male wistar rats (IFFA-Credo, l'Arbresle, France) weighing 190–200 g were housed two per cage. All groups received for 8 weeks the same semi-synthetic diet (Table I) having the same total amount of lipids, but varying in fish oil (increasing salmon oil was compensated for by decreasing corn oil). Group A received 10% corn oil; group B, 8.5% corn oil supplemented with 1.5% fish oil; group C, 6% corn oil and 4% fish oil; group D, 3% corn oil and 7% fish oil; group E, 10% fish oil. Diet composition has been previously published [5]. The diet were largely supplemented with vitamin E that ranged between 170 to 220 mg total tocopherol per kg of diet.

Animals were killed by decapitation and the exsanguinated forebrain and liver were dissected out. Lipid extraction and fatty acid analysis were performed as previously described [16].

Animal weights were not statistically different between the various dietary groups. As previously pub-

TABLE III

Total lipid fatty acid composition (weight %) of liver of rats fed various diets

Values (mean ± S.D., $n=6$) not bearing the same superscript letter are significantly different at $P<0.05$. If no superscript appears, values are not different. S.D. did not exceed 10% of the mean values.

Fatty acids	A	B	C	D	E
14:0	0.35 [a]	0.55 [a]	0.7 [b]	0.6 [a]	0.8 [bc]
15:0	0.15	0.15	0.2	0.3	0.3
16:0	22.4 [a]	22.8 [a]	27.7 [b]	28 [b]	28.4 [b]
16:1($n-9$)	0.35 [a]	0.4 [a]	0.5 [a]	0.4 [a]	3.3 [b]
16:1($n-7$)	1.7 [a]	2.9 [b]	4.5 [c]	3.3 [bc]	6.1 [d]
17:0	0.35 [a]	0.25 [a]	0.6 [a]	0.5 [a]	4.3 [b]
18:0	15	13.6	13.7	16.3	15.2
18:1($n-9$)	13.7 [a]	11.2 [b]	11.2 [b]	9.7 [b]	11.4 [b]
18:1($n-7$)	2.7 [a]	3.5 [b]	3.6 [b]	4.2 [b]	4 [b]
18:2($n-6$)	18 [a]	19.4 [a]	15.4 [b]	10.4 [c]	2.2 [d]
18:3($n-6$)	0.35 [a]	0.25 [a]	1.3 [b]	0.3 [a]	0.1 [a]
18:3($n-3$)	0.2	0.2	0.6	0.3	0.1
20:0	0.2	–	–	0.1	–
20:1($n-9$)	0.5	0.40	0.6	0.6	0.8
20:1($n-7$)	0.25	0.2	0.2	–	0.16
20:2($n-6$)	0.4	0.3	0.2	0.3	0.1
20:3($n-6$)	0.65 [a]	1.05 [b]	1.1 [b]	0.7 [a]	0.46 [a]
20:4($n-6$)	14.1 [a]	13.2 [a]	7 [b]	6.4 [b]	5.9 [c]
20:5($n-3$)	0.2 [a]	1.9 [b]	2.9 [c]	5.8 [d]	9.5 [e]
22:0	0.4 [a]	0.5 [a]	0.1 [b]	0.2 [b]	–
22:1($n-9$)	0.2	0.3	–	–	–
22:1($n-7$)	0.3	0.2	–	–	–
22:4($n-6$)	0.8 [a]	0.1 [b]	0.1 [b]	0.1 [b]	0.2 [b]
22:5($n-6$)	0.5	–	–	0.2	0.2
22:5($n-3$)	–	0.85 [a]	1.3 [b]	2.2 [c]	2.7 [c]
24:0	0.5	0.2	0.13	0.2	0.3
22:6($n-3$)	5.5 [a]	5.7 [a]	5.4 [a]	8.3 [b]	9.7 [c]
24:1($n-9$)	0.5	0.3	0.3	0.6	0.5
24:1($n-7$)	–	–	–	0.1	0.3
24:4($n-6$)	0.1	–	–	–	0.1

lished [17], plasma alanine aminotransferase (ALAT) (EC 2.6.1.2) and aspartate aminotransferase (ASAT) (EC 2.6.1.1) were assayed according to the method of Kessler et al. [18], using the Technicon SMAC analyzer system. Alkaline phosphatase (EC 3.1.3.1) was assayed according to the method of Morgenstern et al. [19]. Liver (20 μg protein) GSH-PX activity (EC 1.11.1.9) was assayed, as described by Levander et al. [20] by using *t*-butylhydroperoxide to initiate the reaction. Serum vitamin E and A concentrations were determined after HPLC separation, according to the method of De Leenheer et al. [21].

The triacylglycerol (TG) concentration of serum and heart lipid extracts was determined by using the Technicon-SMAC system (Technicon, Tarrytown, NY) according to the method of Bucolo and David [22]. Serum cholesterol was determined with the Technicon-SMAC system according to the method of Lie et al. [23]. Serum phosphatidylcholine were assayed with the Biolyon kit (Ref. 44711, Wako Chemicals, Neuss, F.R.G.) with the Technicon RA 1000 (Technicon).

Results

Brain

Table II shows that saturated and monounsaturated fatty acids regardless of chain-length were not affected by the different diets, except oleic acid, which was slightly but significantly increased. The 20, 22 and 24 carbon atom saturated and monounsaturated fatty acids were not altered, showing that myelination was not affected, at least that sphingolipids were normal (these myelin markers are formed only with these very-long-chain fatty acids).

Arachidonic acid ($20:4(n-6)$) decreased proportionately to the increasing dietary fish oil content (and decreasing corn oil): approx. 25% reduction between diets A and D. We have previously shown that arachidonic acid content is independent of dietary linoleic acid content after the minimum indispensable dose (0.3% of the calories) [24]; this is largely the case in diets A, B, C and D. Thus, the decrease of $20:4(n-6)$ in brain membranes is due only to the increase of fish oil in the diet. $22:4(n-6)$ was less affected than arachidonic acid, $22:5(n-6)$ which was reduced by about 60%. The high value of this latter fatty acid with diet A could be due to the high amount of dietary corn oil, since $22:5(n-6)$ levels in membranes parallels dietary excess of linoleic acid [24] as well as the deficiency in α-linolenic acid [16,25,26].

EPA was nearly undetectable in diet A and B, and increased in diet C, D and E, but the content was still extremely low, even in the diet containing a very high amount of fish oil.

$22:5(n-3)$ was increased 7-fold between diet A and D, but the brain content was always below 1% of the total fatty acids. Cervonic acid, $22:6(n-3)$, was increased by about 30% between diet A and D.

As subtle changes in brain membrane polyunsaturated fatty acids determined by dietary alterations in α-linolenic acid provoke alterations in brain membrane polyunsaturated fatty acids, membrane fluidity, en-

TABLE IV

Liver plasma parameters of rats fed various diets

GSH-PX: selenium-dependent glutathione peroxidase activity (EC 1.11.1.9) is in μmol/min per mg. Tocopherol and retinol are expressed in mg/l. Other plasma lipids are expressed in g/l. Alkaline phosphatase (EC 3.1.3.1), aspartate aminotransferase (ASAT) (EC 2.6.1.1) and alanine amino-transferase (ALAT) (EC 2.6.1.2) are expressed in IU/l. Values (mean ± S.D. $n = 6$) not bearing the same superscript letter are significantly different at $P < 0.05$. If no superscript appears, values are not different.

Liver and plasma parameters	Diet				
	A	B	C	D	E
Liver					
GSH-PX	1.22 ± 0.22	1.12 ± 0.14	1.04 ± 0.23	1.00 ± 0.25	1.15 ± 0.18
Plasma					
GSH-PX	168.5 ± 14.9	163.3 ± 16.6	149.4 ± 14.1	164.3 ± 12.8	166.5 ± 10.5
α-Tocopherol	12.60 ± 0.57	14.15 ± 1.58	15.83 ± 1.24	14.78 ± 1.70	13.60 ± 0.63
Vitamin A	0.44 ± 0.04	0.50 ± 0.06	0.53 ± 0.07	0.51 ± 0.05	0.44 ± 0.06
Alkaline phosphatase	280.6 ± 20.5 ^{a}	256.2 ± 34.6 ^{a}	275.5 ± 28.8 ^{ac}	368.3 ± 40.7 ^{bc}	272.5 ± 39.8 ^{a}
ASAT	158.7 ± 48	113.3 ± 7.6	134.7 ± 30.0	142.3 ± 15.4	119.8 ± 16.3
ALAT	40.7 ± 8.3	47.2 ± 6.2	40.5 ± 7.0	37.8 ± 3.3	43 ± 6.7
Unesterified cholesterol	0.98 ± 0.22 ^{a}	0.72 ± 0.13 ^{ab}	0.76 ± 0.13 ^{ab}	0.56 ± 0.08 ^{b}	0.56 ± 0.09 ^{b}
Total cholesterol	2.07 ± 0.14 ^{ab}	2.04 ± 0.26 ^{ab}	2.34 ± 0.26 ^{a}	2.15 ± 0.27 ^{ab}	1.79 ± 0.26 ^{b}
Triacylglycerols	3.11 ± 0.34 ^{a}	2.29 ± 0.58 ^{ab}	1.94 ± 0.54 ^{b}	1.51 ± 0.20 ^{b}	1.58 ± 0.46 ^{b}
Phosphatidyl-cholines	2.57 ± 0.14 ^{a}	2.49 ± 0.15 ^{a}	2.39 ± 0.14 ^{a}	2.24 ± 0.24 ^{ab}	1.96 ± 0.17 ^{b}

zymatic activities, electrophysiological parameters, learning tests and resistance to poisons [26], the question can be raised of whether increased fish oil intake leads to functional alterations in the nervous system.

Liver

Table III shows that nearly all fatty acids (saturated, monounsaturated and polyunsaturated fatty acids) were affected in diet D and E; alterations were less important with diet C, and minimal with diet B (except for EPA). Alterations in the fatty acid profile were not paralleled by change in liver function: serum vitamin E and A, selenium-dependent glutathione peroxidase, transaminases (ALAT and ASAT) alkaline phosphatase were normal in all diets (Table IV).

Thus, any change in any parameter with a diet containing between 3–8% fish oil must be considered with attention, as the beneficial effect may be accompanied by, or be due to a pathological effect. Thus, liver function is certainly altered when diet contains more than 10% fish oil. Total serum cholesterol and phosphatidylcholine were reduced only with E diet, unesterified cholesterol with D and E diets. In contrast, triacylglycerols were dramatically reduced with C, D and E diets.

The positive effect of fish oil in the field of cardiovascular diseases is clear [10–13], but the toxic effect of high doses of fish oil must be taken into account. In fact, if the consumption of long-chain polyunsaturated $n-3$ fatty acids has beneficial consequence, the appropriate therapeutic dose is not clearly defined.

Although blood parameters were normal, except triacylglycerol, in all animals, dramatic alteration in the membrane polyunsaturated fatty acids could alter some liver or brain function, not yet determined.

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