Biochimica et Biophysica Acta, 960 (1988) 458–461
Elsevier

BBA 50231

BBA Report

High dietary fish oil alters the brain polyunsaturated fatty acid composition

Jean-Marie Bourre [a], Michelle Bonneil [b], Odile Dumont [a], Michèle Piciotti [a], Gilles Nalbone [b] and Huguette Lafont [b]

[a] *INSERM Unité 26, Hôpital Fernand Widal, Paris and* [b] *INSERM Unité 130, Marseille (France)*

(Received 31 December 1987)

Key words: Polyunsaturated fatty acid; Arachidonic acid; Icosapentaneoic acid; Docosahexaenoic acid; Fish oil; (Rat brain)

Feeding adult rats a 17% corn-oil diet for 8 weeks did not change brain polyunsaturated fatty acids (PUFA) compared to rats fed 2.2% corn oil (with 2.2% lard added). When the corn-oil diet was supplemented with 14.5% cod liver oil or 12.5% salmon oil, the fatty acid composition of brain PUFA was significantly altered, even if α-tocopherol was added to the salmon-oil diet. Comparing salmon-oil- and cod-liver-oil-fed animals with corn-oil-fed animals, arachidonic acid 22:4($n-6$) and 22:5($n-6$) were reduced, and 20:5($n-3$), 22:5($n-3$) and 22:6($n-3$) were increased. Liver fatty acids were also significantly altered. Thus, the brain is not protected against a large excess of very-long-chain $n-3$ PUFA, which increase $n-3/n-6$ ratio and could lead to abnormal function, and which might be difficult to reverse.

Consumption of fish oils containing $n-3$ polyunsaturated fatty acids (PUFA) may have beneficial effects on ischemic heart disease and thrombosis [1–3].

As the ingestion of large amounts of $n-3$ PUFA in experimental animals gives rise to adverse effects (i.e., vitamin E deficiency symptoms), it is possible that a diet abundant in fish oil may be harmful in man. Not much is known about human susceptibility to $n-3$ PUFA with respect to disturbances in vitamin E metabolism. In contrast, it is known that various animals species differ greatly in susceptibility to $n-3$ PUFA; moreover, young animals are more sensitive than old. The PUFA composition of the diet regulates the fatty acid composition of the liver endoplasmic reticulum [4], and this, in turn, is an important factor controlling the rate and extent of lipid peroxidation in vitro and possibly in vivo [5,6]. 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. Maintenance of membrane fluidity within narrow limits is presumably a prerequisite for proper functioning of a cell. Lipids play a key role in determining membrane fluidity, and changes in lipid composition have been reported to alter important cellular functions. It is known that alterations in the fatty acyl composition of membranes can affect numerous cellular functions [7,8]. Therefore, dietary modification of membrane phospholipids by fish oil feeding may have significant effects, unrelated to either plasma lipids or arachidonic acid metabolism.

However, the most unsaturated fatty acids present in brain, namely, ($n-3$)20:5, -22:5 and -22:6, have received relatively little attention. These fatty acids are nevertheless found at high levels in many membranes, such as those from brain and retina [9,10]. They are found in phos-

Abbreviations: PUFA, polyunsaturated fatty acids.

Correspondence: J.M. Bourre, INSERM Unité 26, Hôpital Fernand Widal, 200 rue du Faubourg St-Antoine, 75475 Paris Cedex 10, France.

pholipids (as brain contains nearly no acylglycerols, cholesterol esters or free fatty acids).

It would be useful to know what effects an elevation of $n-3$ PUFA has on membrane lipid dynamics and organization of various organs, and especially the brain. The brain is the organ with the highest lipid concentration after adipose tissue. All brain lipids are localized in membranes, and the effect of PUFA deficiency on brain composition and function is well documented [11–15]. Dietary linolenic acid controls the composition of nerve membrane, the level of enzymatic activities, the amplitude of electrophysiological parameters, the resistance to poisons and the performance of learning tasks [10,16]. Brain contains high amounts of $n-3$ PUFA, and it is well known that fish oil alters the PUFA composition of various organs, especially liver and heart [17,18]. However, in the brain, no such study has been performed, except our previous work showing that moderate uptake of Menhaden oil has little effect on brain PUFA levels [19].

Four groups of male Wistar rats (IFFA-Credo, l'Arbresle, France) weighing 190–210 g were housed, two per cage. Group 1 (10 rats) was fed a diet containing 4.4% (w/w) fat consisting of a lard (2.2%) and corn-oil (2.2%) mixture. Experimental animals were fed a 17% lipid diet; group 2, which served as control (10 rats), received a corn-oil diet (17% w/w); group 3 (10 rats) was fed 2% corn oil with 15% cod-liver oil; group 4 (12 rats) received a salmon-oil-enriched diet (12.5% w/w) supplemented with 4.5% of corn oil. Diet compositions have been previously described [20]. Table I presents the fatty acid composition of the various diets. Corn oil, as supplied, contained 45 mg of α-tocopherol per 100 g oil. Salmon oil was supplemented with 100 mg α-tocopherol/100 g oil. Therefore, the amount of vitamin E supplied by the salmon-oil-enriched diet was 295 mg/kg of diet. The low-fat and corn-oil diets were prepared to last 1 month, and stored at $-20\,^{\circ}C$ in plastic bags, and the salmon-oil-enriched diet was prepared every 2 weeks and stored at $-20\,^{\circ}C$ in sealed containers flushed with nitrogen. Rats were fed ad libitum and uneaten food was discarded in the morning; they had free access to water and the feeding period was 8 weeks. At the beginning of the feeding period, animals ate daily 1.66 mg eicosapentaenoic acid/g animal and 1.22 mg docosahexaenoic acid/g animal, and at the end, they ate daily 0.75 mg eicosahexaenoic acid/g animal and 0.55 mg docosahexaenoic acid/g animal.

TABLE I

FATTY ACID COMPOSITION OF OILS (%/mg per 100 g)

Fatty acid	Corn oil + lard	Corn oil	Cod liver oil	Salmon oil
14:0	–	–	5.2	4.4
15:0	–	–	0.4	0.3
16:0	20.2	11.6	10.8	12.4
16:1($n-7$)	0.3	–	9.8	5.3
17:0	–	–	0.3	0.5
18:0	9.0	0.7	2.0	1.8
18:1($n-9$)	35.1	26.8	17.0	14.3
18:1($n-7$)	–	–	4.4	2.5
18:2($n-6$)	33.6	59.5	1.8	16.8
18:3($n-3$)	0.7	0.8	0.9	0.6
18:4($n-3$)	–	–	2.6	2.0
20:0	0.3	–	0.2	0.3
20:1($n-9$)+($n-11$)	0.5	–	11.9	9.7
20:4($n-6$)	0.3	0.1	0.4	0.4
20:4($n-3$)	–	–	0.7	0.7
20:5($n-3$)	–	–	8.6	9.8
22:1($n-11$)	–	–	6.1	6.6
22:1($n-9$)+($n-11$)	–	–	7.4	7.1
22:5($n-3$)	–	–	2.5	1.2
22:6($n-3$)	–	–	9.9	7.1
24:1($n-9$)	–	–	0.2	0.4
P/S [a]	1.2	5.0	1.5	2.0
$n-6/n-3$	46.0	71.0	0.09	0.8

[a] Polyunsaturated/saturated ratio.

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 [11].

Table II shows that cod liver oil as well as salmon oil supplemented with α-tocopherol (100 mg/100 g) induce similar alterations in forebrain PUFA levels (but saturated and monounsaturated fatty acids are little, if at all, affected).

Although the brain is considered as being heavily protected, a 8-week fish-oil diet in 60-day-old animals increased the $n-3$ series and decreased the $n-6$ series. When cod-liver-oil- and salmon-oil-fed animals are compared with those fed corn oil, brain 20:4($n-6$), 22:4($n-6$) and 21:5($n-6$) were decreased by 16–19, 37–40,

TABLE II

FATTY ACID COMPOSITION OF THE FOREBRAIN FROM ANIMALS FED THE FOUR OIL DIETS: COMPARISON WITH LIVER

Each value is the mean of two determinations on ten different forebrains (20 measurements). For liver, two cod-liver-fed animals were used, four salmon-fed animals (8 measurements), four corn-fed animals (8 measurements), and four controls (8 measurements). Symbols of significance (* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$) are expressed in comparison with the control (ANOVA).

Fatty acid	Brain				Liver		
	control	corn	cod liver	salmon	control	corn	cod liver
14:0	0.1	0.1	0.1	0.1	0.44 *	0.24 *	1.3 *
15:0	–	–	–	–	0.1	0.1	0.5 *
16:0	18.4	18.1	17.7	19.4	22.32	20.1 *	45.1 ***
16:1($n-9$)	0.2	0.2	0.2	0.2	0.26	0.34	0.6 *
16:1($n-7$)	0.75	0.6	0.77	0.87	2.8	0.92 *	3.5 *
17:0	0.21	0.27	0.2	0.45 *	0.2	0.2	0.8 **
18:0	19.0	18.9	18.8	19.8	13.84	10.8	16.8 **
18:1($n-9$)	19.4	19.4	20.5	20.8	13.84	11.8	9.7 *
18:1($n-7$)	5.4	4.2	4.2	4.1	3.86	1.94	3.7 **
18:2($n-6$)	0.62	1.3 *	0.82	0.85	14.0	30.6 **	7.8 ***
18:3($n-6$)	–	–	0.2	–	0.3	0.57	–
18:3($n-3$)	–	0.1	–	0.1	0.2	0.24	–
18:4($n-3$)	–	–	–	–	–	–	0.1
20:0	1.1	0.88	1.38	0.62 *	0.6	0.2	0.9 **
20:1($n-9$)	2.8	2.6	2.8	2.05	0.1	0.16	1.1 ***
20:1($n-7$)	1.5	0.94	1.0	0.67	0.26	0.18	0.4 **
20:2($n-6$)	0.28	0.38	0.26	0.2	0.32	0.53	–
20:3($n-6$)	0.37	0.41	0.65 *	0.55 *	0.3	0.36	0.3
20:4($n-6$)	8.8	9.1	7.2 **	7.7	20.36	18.34	3.2 ***
20:5($n-3$)	0.2	0.2	0.35	0.27	0.13	0.1	1.0 ***
22:0	1.0	1.0	1.02	1.02	0.1	0.12	0.2
22:1($n-9$)	0.34	0.35	0.34	0.35	–	–	0.2
22:1($n-7$)	0.4	0.8	0.28	0.27	–	–	0.1
23:0	–	0.1	0.1	–	–	–	–
22:4($n-6$)	3.15	3.5	2.1 **	2.2 **	0.52	1.16 *	–
22:5($n-6$)	0.75	0.78	0.17 ***	0.2 ***	1.0	1.06	–
22:5($n-3$)	0.22	0.27	0.98 ***	0.65 ***	0.5	0.32	0.3
24:0	1.4	1.6	1.6	1.17	0.34	0.32	0.1 *
22:6($n-3$)	10.7	10.8	13.0 ***	13.9 ***	3.72	2.9 *	1.6 *
24:1($n-9$)	2.7	2.9	2.7	2.3	0.24	0.12 *	0.7 **
24:1($n-7$)	0.5	0.5	0.48	0.47	0.3	–	–

64–79%, respectively, whereas brain 20:5($n-3$), 22:5($n-3$) and 22:6($n-3$) were increased by 35–75, 141–22, 20–29%, respectively. In the cod-liver-oil-fed animals, all liver fatty acids were significantly affected.

Interestingly, dietary fish oil at levels up to 10% has been reported to increase 22:6($n-3$) in the liver [6]. However, in the present experiment, levels were found to decrease. Thus, the critical point between pharmacology and toxicology appears to be between 10 and 14 g/100 g.

Yellow fat disease [21,22] is considered to be an expression of excess intake of PUFA, especially of those derived from plant and fish oil that have three or more double bonds, and results in vitamin E deficiency. The disorder has been reported in horses, pigs [23,24], minks [25], cats [26] and rabbits [27]. The horse, pig and mink are considered to be especially susceptible [21]. Experimentally induced yellow fat disease has been described in the rat [21]. The disease is primarily a generalized disorder of fat deposition. It is usually characterized by extensive adipose-cell degeneration (steatosis), inflammation and fibrosis of adipose

tissue (steatitis) and accumulation of lipofuscin pigment. However, the brain was not examined.

We have previously shown that the nervous system recovers slowly, if at all, after $n-3$ PUFA deficiency in both young and adult animals, and that the duration of impaired function is the same, whatever the cell and the organelle [28,29], in contrast to other organs [30]. Thus, it is possible that the effects of increased $n-3$ PUFA and decreased $n-6$ PUFA will be difficult for the brain to reverse after the fish-oil diet has been changed to a normal one. Moreover, at least for brain cell cultures, the essential PUFA are 20:4 and 22:6 (and not 18:2 and 18:3), which can either be produced by the liver or are supplied directly by the diet [31]. If excess fish oil alters hepatic function, brain recovery will be even more difficult.

In experiments in human volunteers [32], as well as in animals [33], it has been found that a diet containing fatty fish decreased blood serum cholesterol and, in particular, triacylglycerol levels. These results may indicate that ingestion of $n-3$ acids has a beneficial effect on blood lipid composition, and hence on the condition of blood vessels [32]. Thus, $n-3$ fatty acids are being promoted in pharmacological doses for the prevention of coronary artery disease. However, the use of fish-oil supplements in patients should be considered equivalent to drug therapy, and further studies of their long-term efficacy, toxicity and the possibility of overdosage [33] must be conducted before recommendations can be made about their general use [34].

This work was supported by INSERM. The authors are extremely grateful to G. Durand and G. Pascal for running some gas-liquid chromatography analysis, and to P. Garzino for breeding the animals.

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