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Comparison of vegetable and fish oil in the provision of N-3 polyunsaturated fatty acids for nervous tissue and selected organs

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Rats were fed (as were their mothers from 2 weeks before mating) with a semi-synthetic diet deficient in alpha-linolenic acid (peanut oil-based) but containing linoleic acid. At age 5 weeks, the animals were divided into several groups that were fed for 3 weeks varying amounts of (n-3) series polyunsaturated fatty acids, either as alpha-linolenic acid (by addition of rapeseed oil to the peanut oil) or as very long chain acids (by addition of fish oil). Compared with vegetable oil, equal quantities of fish oil gave a higher concentration of docosahexaenoic acid (DHA) in all the tissues. About two-fold more vegetable oil than fish oil was needed to obtain the same quantity of DHA in all the tissues. Three weeks of the new diet were not enough to stabilize DHA concentrations in rat nervous tissue, but recovery was more rapid with fish oil than with vegetable oil. For non-nervous tissue, increases in DHA levels were lower with vegetable oil than with the same amount of fish oil, and fish oil provided more very long chain (n-3). (J. Nutr. Biochem. 8:472–478, 1997) © *Elsevier Science Inc. 1997*

Keywords: fish oil; vegetable oil; nervous system; nutrition; brain

Introduction

Fatty acids found in membranes are very long chain fatty acids derived from linoleic and alpha-linolenic acids. For the (n-3) fatty acids, the question is whether the truly essential fatty acid, at least for certain tissues, is alpha-linolenic acid or a longer chain fatty acid. This hypothesis is important for the brain because cerebral cells in culture require the presence of DHA to differentiate, divide, multiply, and to take up and release neuromediators.[1,2] Brain and retina DHA, especially during development,[3] is either supplied by the liver[4] or the diet. Some vegetable oils contain alpha-linolenic acid, and mammals elongate and desaturate this acid. However, in the brain, delta-6-desaturase activity begins to decrease after the development period and reaches an extremely low level during aging.[5,6] Moreover, the enzymatic activity is reduced under a number of pathological conditions. Thus it is possible that hepatic synthesis of polyunsaturated very long chains is not sufficient to ensure the physiological turnover of membrane fatty acids, including those of the brain. Consequently, very long polyunsaturated chains, especially (n-3) fatty acids found in fish oil, could be of interest for biological membrane structure and function, besides having pharmacological interest.[7,8]

The essential nature of alpha-linolenic acid is beyond doubt.[9,10] A deficiency leads to anomalies in the composition of nervous membranes[11–15] and in their architecture and function[16–18] and leads to perturbation of electrophysiological parameters, alteration in learning tests, and greater sensitivity to neurotoxins.[19–24]

Nutritional deficiency in (n-3) fatty acids also alters brain structure and function in humans, and the biochemical correlation between dietary fatty acids, milk composition and possibly brain composition has been demonstrated in both pigs[17–25] and humans.[26,27] As very long polyunsaturated chains are present in human milk, and in view of the vast amount of these fatty acids deposited in nervous tissue during the perinatal period,[28] artificial milk formulas have

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This work was supported by Inserm, Inra, Onidol, and Cetiom (France). The authors are grateful to A. Strickland for reviewing the manuscript.
Received October 25, 1996; accepted April 22, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00059-4

very recently been supplemented with fish oil, with benefits for membranes in infants.[29–30] As a result both non-nervous and nervous tissues are enriched in (n-3) fatty acids, and retinal, visual cortex, and intellectual functions are improved.[31–34]

Although the infant is able to synthesize DHA, the amount produced is inadequate to support the DHA level observed in breast-fed infants;[35] moreover, in animals, during development, different regions within the brain may vary in their capacity to synthesize DHA, in correlation with regional growth rate.[36] Some neurological differences have been found between 9-year-old children fed breast milk as babies and formula-milk babies, it has been suggested that very long polyunsaturated fatty acids may explain this discrepancy.[37]

We have previously shown for some tissues that when the dietary content of alpha-linolenic acid is increased, the level of DHA increases.[19] We have also shown that phospholipids derived from brain and given in the diet are a good source of (n-3) polyunsaturated fatty acids for rat nervous tissue.[38] The question thus raised is what level of dietary fish oil would give the same membrane DHA concentration in tissue as vegetable oil does (and, perhaps also, what is the rate of conversion of alpha-linolenic acid into DHA in the organism). In this study, we sought to answer these questions by varying the amount of dietary (n-3) either in the form of rapeseed oil (providing the alpha-linolenic precursor) or fish oil (providing very long polyunsaturated chains, EPA and DHA) and comparing the results from two series of experiments.

Methods and materials

To stabilize the fatty acid composition of the tissues, two weeks before mating, female rats were fed a diet deficient in alpha-linolenic acid (dietary lipids consisted of peanut oil). At 5 weeks, animals were divided into 2 × 7 groups (10 animals per group).

The complete composition of the diet (casein, DL-methionine, cellulose, starch, saccharose, oil, vitamin and mineral mixture) has been previously published.[11,19,38] The oil and (n-3) polyunsaturated fatty acid content of the diets is given in *Table 1*.

In the first group, the different amounts of alpha-linolenic acid in the diets were obtained by adding to peanut oil (which contains only trace amounts of alpha-linolenic acid: 0.01% of total fatty acids, i.e., 4.5 mg/100 g oil) increasing amounts of rapeseed oil (whose alpha-linolenic acid content represents 9% of total fatty acids). In the second group, different amounts of (n-3) series very long chain fatty acids were obtained by adding to peanut oil increasing amounts of cod liver oil. This oil contains 27% (n-3) fatty acids: (g/100 g fatty acids) 0.9% 18:3 (n-3), 3.1% 18:4 (n-3), 0.6% 20:4 (n-3), 11.5% 20:5 (n-3), 1.6% 22:5 (n-3), 9.2% 22:6 (n-3). The total amount of lipids in all diets was maintained at 5%.

Animals were switched to the different diets at the age of 5 weeks because the fatty acid composition of nerve endings and mitochondria does not stabilize in control animals before this time.[38] Animals were killed by decapitation 3 weeks after the diet change [we have previously shown that the rate of recovery after switching from a deficient to a normal diet (from a sunflower to a soybean oil-based diet) varies depending on the organ]. This recovery was measured by the increase in DHA (and the decrease in 22:5 (n-6) and its replacement by 22:6 (n-3).[39,40] Techniques for removal of organs, lipid extraction, transmethylation, and analysis of fatty acids by capillary column gas chromatography have been described previously.[19] Statistical significance of mean differences between dietary groups was tested by analysis of variance (multiple factor ANOVA, alpha = 0.05).

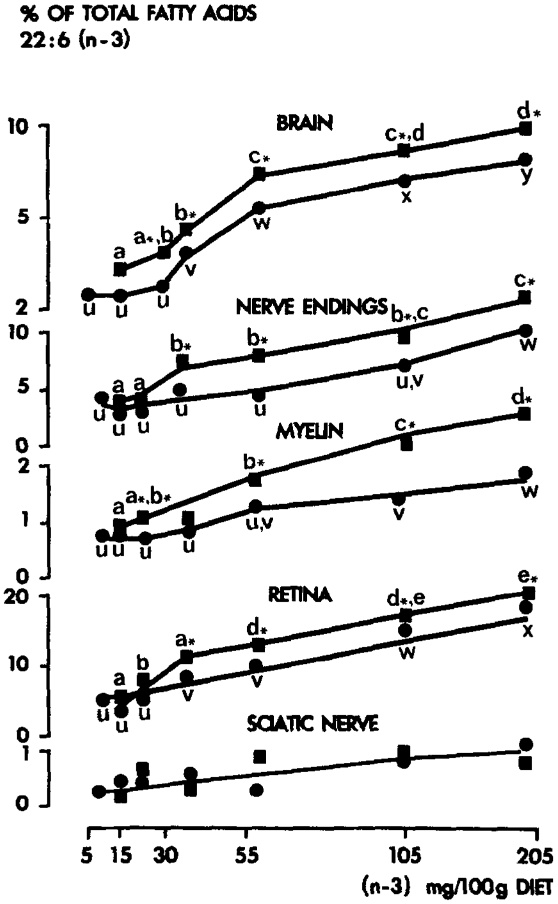

Figure 1 DHA in nervous tissue in response to increasing amounts of either cod liver oil or rapeseed oil. For each individual organ or organelle, values not bearing the same superscript are significantly different. a, b, c, d are related to animals receiving cod liver oil; u, v, w, x, y, to animals receiving rapeseed oil. *: significant difference between cod-liver-oil-fed animals and rapeseed-oil-fed animals. DHA: docosahexaenoic acid (22:6 (n-3)). When the graph has one line, it means that the two lines are identical (no statistical difference between the values) (*squares*, cod liver oil; *circles*, rapeseed oil).

Values in the figures for the various organs are the means of at least five different animals from at least three different litters. For myelin and nerve endings, each value is the mean of at least four different preparations; each density gradient required at least four animals. Thus, each value represents at least 16 animals (from at least three different litters).

Experimental protocols were approved and meet government guidelines (Ministry of Agriculture, authorization N 03007, June 4, 1991).

Results and discussion

Figure 1 shows that in all nervous structures examined the DHA level increased linearly with dietary alpha-linolenic acid concentration, in agreement with our previous results.[19] However, the present results were obtained with rapeseed oil, whereas soybean oil was generally used in previous experiments. It should be noted that the linoleic/

Table 1 Oils used for the different diets (g/kg diet) and fatty acid composition of the different diets (% by weight)

	Peanut oil	Mixture of peanut and rapeseed oil						Mixture of peanut and cod liver oil					
Peanut oil	50.0	49.4	48.8	47.0	44.0	38.0	26.1	49.8	49.6	49.0	48.0	46.0	42.1
Rapeseed oil	—	0.6	1.2	3.0	6.0	12.0	23.9	—	—	—	—	—	—
Cod liver oil	—	—	—	—	—	—	—	0.2	0.4	1.0	2.0	4.0	7.9
Fatty acid	A	B	C	D	E	F	G	H	I	J	K	L	M
14:0	—	—	—	—	—	—	—	—	—	0.1	0.2	0.3	0.6
16:0	9.4	9.5	9.4	9.4	9.2	8.5	7.6	9.8	9.8	9.7	9.6	9.9	10.2
18:0	3.3	3.5	3.5	3.5	3.5	3.2	2.9	4.0	3.8	3.7	3.7	3.8	3.3
20:0	1.3	1.5	1.5	1.4	1.4	1.3	1.0	1.5	1.5	1.4	1.4	1.4	1.4
22:0	2.8	2.8	2.8	2.6	2.4	2.4	2.4	3.0	2.9	3.0	3.5	2.9	1.8
24:0	1.4	1.4	1.4	1.4	1.1	1.1	0.7	1.4	1.4	1.4	1.4	1.4	1.4
16:1n-7	—	—	—	—	—	—	—	—	—	0.2	0.3	0.6	1.3
18:1n-9	60.0	60.0	59.9	60.0	60.3	60.1	60.6	59.4	58.4	58.5	56.8	56.1	54.8
20:1n-9	0.9	1.2	1.2	1.1	1.1	1.2	1.1	1.1	1.1	1.2	1.3	1.8	2.3
22:1n-11	—	—	—	—	—	—	—	0.2	0.2	0.2	0.6	0.5	0.8
22:1n-9	—	—	—	—	—	—	—	—	—	—	0.6	0.1	0.2
18:2n-6	20.1	20.0	20.1	20.1	20.2	20.3	20.7	20.1	20.1	20.0	19.7	19.3	19.1
20:4n-6	—	—	—	—	—	—	—	—	—	—	—	0.1	0.2
18:3n-3	0.1	0.2	0.3	0.6	1.2	2.2	4.3	0.1	0.1	0.1	0.1	0.1	0.2
18:4n-3	—	—	—	—	—	—	—	—	—	—	0.1	0.2	0.4
20:5n-3	—	—	—	—	—	—	—	0.1	0.1	0.3	0.5	1.0	2.0
22:5n-3	—	—	—	—	—	—	—	—	—	—	—	0.1	0.2
22:6n-3	—	—	—	—	—	—	—	—	0.1	0.2	0.4	0.7	1.4
PUFA n-3	—	**0.2**	**0.3**	**0.6**	**1.2**	**2.2**	**4.3**	**0.2**	**0.3**	**0.6**	**1.1**	**2.1**	**4.2**
18:2n-6/18:3n-3	—	100.0	67.0	33.5	16.8	9.2	4.8	201.0	201.0	200.0	197.0	193.0	95.5
n-6/n-3	201.0	100.0	67.0	33.5	16.8	9.2	4.8	100.5	67.0	33.3	17.9	9.2	4.5
mg/100g diet													
n-6	945.0	940.0	945.0	945.0	949.0	954.0	973.0	945.0	945.0	940.0	926.0	912.0	907.0
n-3	4.7	9.7	14.7	29.7	54.7	104.7	204.1	9.7	10.7	29.5	54.0	104.7	204.7

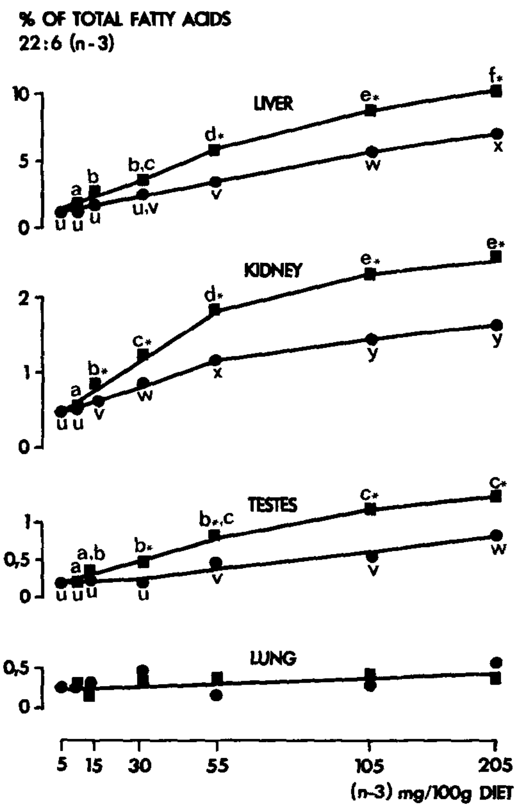

Figure 2 DHA in liver, kidney, testes and lung in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

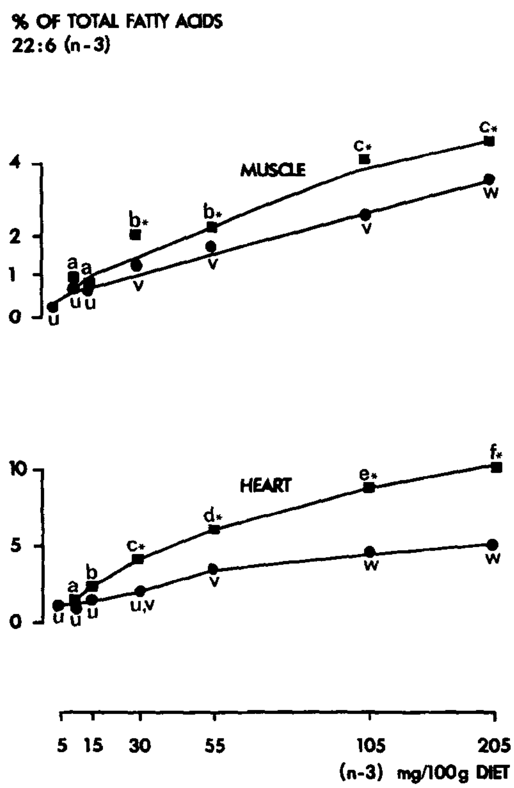

Figure 3 DHA in muscle and heart in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

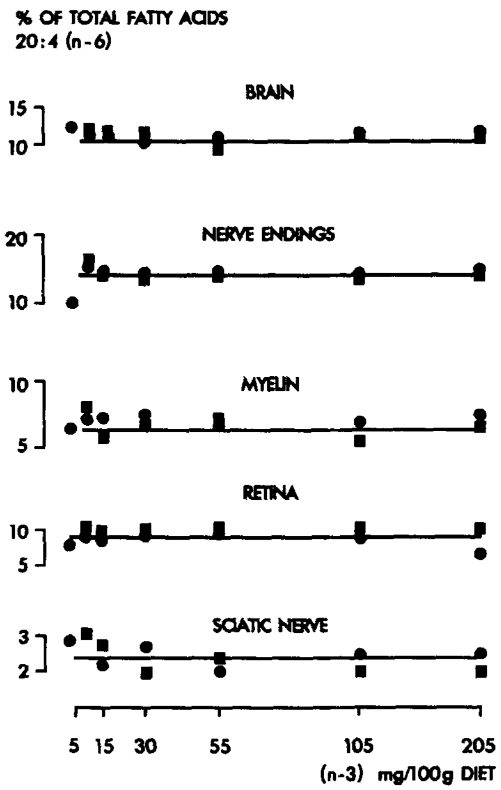

Figure 4 20:4(n-6) in nervous tissue in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

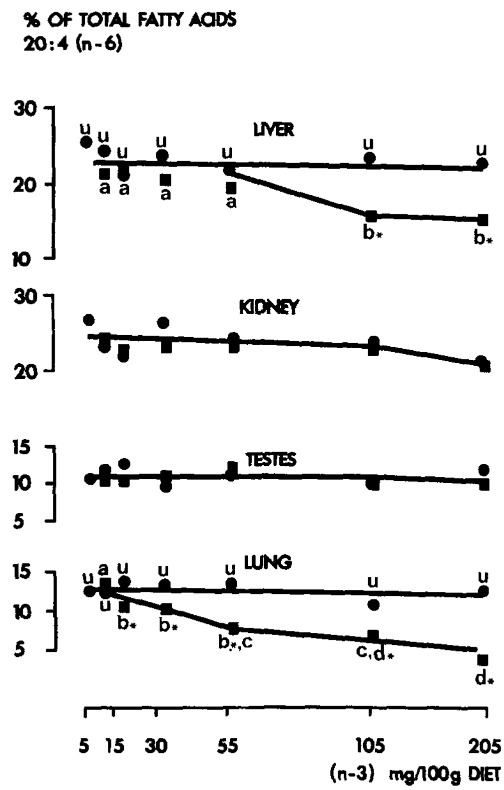

Figure 5 20:4(n-6) in liver, kidney, testes and lung in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

alpha-linolenic acid ratio is 9.8 and 2.8 in soybean oil and rapeseed oil, respectively. As it is known that the rate of conversion of alpha-linolenic acid to EPA and DHA depends on the ratio of linoleic acid to alpha-linolenic acid these ratios are given in *Table 1*. For brain, myelin, nerve endings, and retina, the DHA concentration increased with the amount of fish oil in the diet. In all cases, values obtained with 105 and 205 mg (n-3) fish oil/100 g diet were not significantly different. The plateau was thus reached with 105 mg (n-3) fish oil 100 g diet compared with 200 mg/100 g diet for (n-3) vegetable oil. The efficiency ratio was thus about 2.

In addition, values obtained with rats fed fish oils were always higher than in those fed vegetable oils. The maximal level of DHA found in cerebral membranes with fish oil was higher than with vegetable oil. Nonetheless, we have previously shown that about 3 months are necessary for animal cerebral membranes deficient in alpha-linolenic acid to recover a normal level of DHA. Because this study only lasted 3 weeks, the cerebral structures did not have time to recuperate. However, results show that the rate of recovery was more rapid with very long chain fatty acids derived from fish oils than with alpha-linolenic acid. For sciatic nerve, values were too low for any differences to be demonstrated.

Figure 2 and *3* show similar phenomena for various organs: liver, kidney, testes, heart, and muscle (for lung, values were too low for differences to be determined). During the experiment (3 weeks after dietary change) these levels had time to partially recover.[40] For a given amount of (n-3) in the diet, results show that the recovery was obtained with lower quantities of fish oil (n-3) fatty acids than with alpha-linolenic acid from vegetable oil.

Figure 4 shows that whatever the dietary content of (n-3) series fatty acids, whether of fish or vegetable origin, the concentration of arachidonic acid in the nervous structures was unchanged. In contrast, in liver, lung, and muscle (*Figure 5* and *6*) the content of arachidonic acid was significantly decreased as from 105 mg (n-3) fish oil/100 g diet. For kidney and heart, the decrease in arachidonic acid with 205 mg fish or vegetable oil/100 g diet was not statistically significant.

Figure 7 shows that the decrease in 22:5 (n-6) was more rapid (as a function of (n-3) fatty acids in the diet) with fish oil than with vegetable oil in brain, nerve endings, myelin, and retina. The concentration of 22:5 (n-6) stabilized at 105 mg n-6 fish oil/100 g diet (no significant difference between the 105 and 205 data points).

Figure 8 and *9* show that 22:5 (n-6) decreased significantly more rapidly (as a function of (n-3) fatty acids in the diet) with the fish-peanut oil mixture than with the rapeseed-peanut oil mixture for liver, kidney, lung, heart, and muscle. For testes, the level of 22:5 (n-6) did not depend on dietary (n-3), whatever the length of the chain. It is interesting to note that for 205 mg n-3/100 g diet the concentration of 22:5 (n-6) was the same with both fish or vegetable oil, whereas the concentration of 22:6 (n-3) was different (Fig. 1–3).

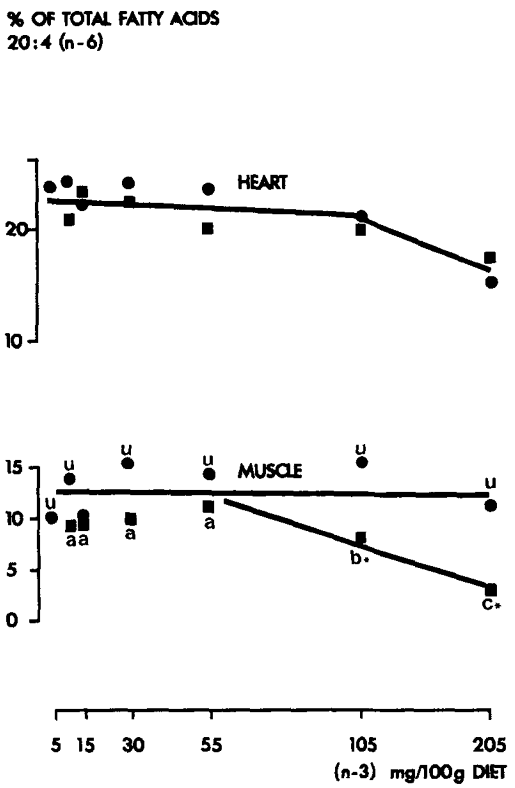

Figure 6 20:4(n-6) in heart and muscle in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

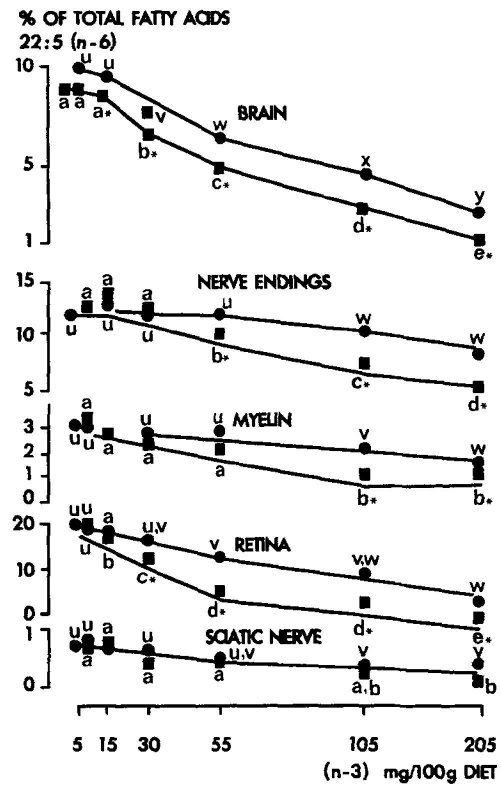

Figure 7 22:5(n-6) in nervous tissue in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

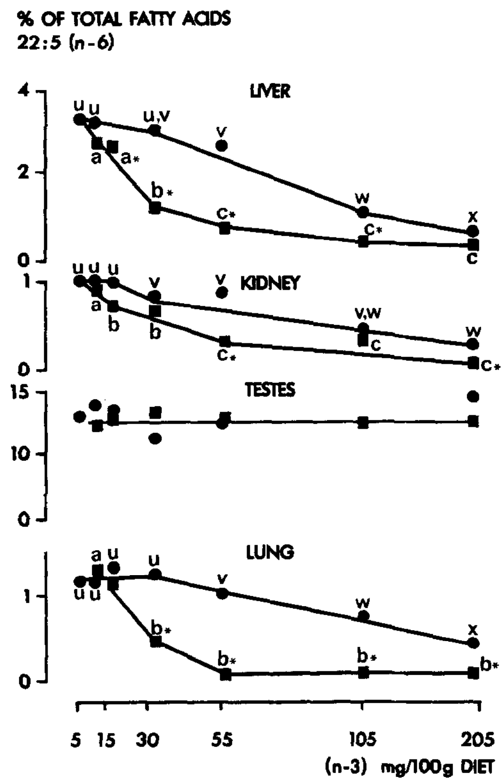

Figure 8 22:5(n-6) in liver, kidney, testes and lung in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

The concentration of 22:6 (n-3) increased more rapidly (as a function of (n-3) fatty acids in the diet) in practically all structures with increasing amounts of (n-3) fatty acids of fish rather than vegetable origin. For non-nervous tissue, the 22:6 (n-3) plateau was reached at 205 mg alpha-linolenic acid/100 g diet (confirming previous data). This plateau was not reached at lower dietary concentrations of fish oil. Moreover, at 205 mg (n-3) fish oil/100 g diet the concentration of 22:6 (n-3) in the membranes was higher than that obtained with 205 mg alpha-linolenic acid/100 g diet.

For nervous structures, 3 weeks of the new diet (containing alpha-linolenic acid) was not enough to stabilize their DHA content. The only conclusion that can be drawn is that the rate of recovery was more rapid with n-3 oil of fish origin rather than vegetable origin.

In previous studies,[41] we found that in alpha-linolenic-deficient rats refed either vegetable oil containing alpha-linolenic acid or fish oil, the recovery of the fatty acid composition in liver was more rapid and more complete with long chain refeeding (fish oil). Concurrently, the delta-6-desaturase activity in liver was lowered by the addition of fish oil in the diet. Moreover, in 2-month-old rats after 4 weeks of alpha-linolenic refeding, the delta-6-desaturase activity decrease stabilized, whereas it continued to decrease after 12 weeks with fish oil refeeding. Thus, we speculate that fish oil is more potent than vegetable oil at down-regulating the delta-6-desaturase activity by increasing DHA incorporation in phospholipids.

Nonetheless, it cannot be excluded that intake of alpha-

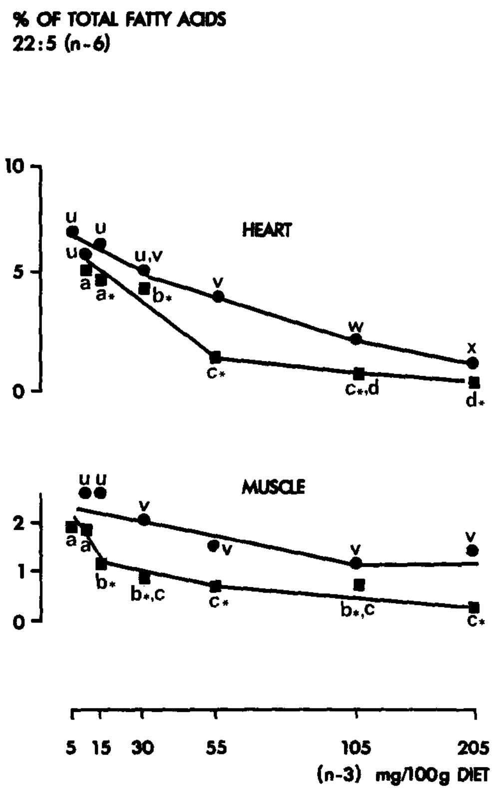

Figure 9 22:5(n-6) in heart and muscle in response to increasing amounts of either cod liver oil or rapeseed oil. Same remarks as in *Figure 1* (*squares*, cod liver oil; *circles*, rapeseed oil).

linolenic acid by itself is preferable for two reasons: the organism can probably regulate excess alpha-linolenic acid by degradation. One study[42] showed that alpha-linolenic acid is as well oxidized as oleic acid and much better than linoleic or gamma-linolenic acids (but the nature and lipid content of the diets were not specified by the authors). In fact, too much fish oil in the diet alters the fatty acid composition of organs in rat, including brain;[43,44] in mice, increasing the dietary (n-3)/(n-6) ratio results in an increase in (n-3) fatty acids with a corresponding decrease in (n-6) fatty acids.[15] Thus it is probable that the organism has more difficulty regulating excess very long chain polyunsaturated fatty acids. However, moderate (n-3) intake does not seem to alter cerebral structure nor induce toxic peroxidation.[45] The hypothesis that organisms might regulate alpha-linolenic better is a question that has not been answered, and has been discussed by Budowski and Crawford.[46]

It can probably be excluded that the higher level of DHA in the tissues of rats fed fish oil can be explained by fish oil providing a more rapid incorporation of DHA into the tissue since animals of both groups were fed the oils for 3 weeks. It should be noted that this time period was derived from previous experiments with rats.[40] Indeed, this 3 weeks period is not adequate for other species, specially for human.[47]

A note of caution must be sounded regarding fish oils. It has previously been shown by us and others that an excess of fish oil in the diet can alter brain fatty acid composition,[43–45,48] and the amount of fish oils in the human diet thus needs to be carefully controlled.

In conclusion, vegetable oils do not ensure the maximal level of DHA in membranes, since fish oils provide higher levels. It remains to be demonstrated whether these higher levels correspond to better function. Moreover, as differences were found in tissues between rats fed fish oil and those fed vegetable oils, the fatty acid profile of purified phospholipids must be examined. Finally, the question arises whether some effects could be attributed to the monoenes and saturated fatty acids found in fish oil. The answer will be obtained using purified triglycerides from fish oil, excluding monoenes and saturated fatty acids.

However, the methods employed for these investigations (using fish oil), do not allow an assesment of the relative efficiency of DHA per se in the fish oil versus the other precursors of (n-3) polyunsaturated fatty acids also present in fish oil with respect to their relative abilities to enhance the DHA levels in the organs measured.

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