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Function of Dietary Polyunsaturated Fatty Acids in the Nervous System

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ABSTRACT. **The brain is the organ with the second greatest concentration of lipids; they are directly involved in the functioning of membranes. Brain development is genetically programmed; it is therefore necessary to ensure that nerve cells receive an adequate supply of lipids during their differentiation and multiplication. Indeed the effects of polyunsaturated fatty acid (PUFA) deficiency have been extensively studied; prolonged deficiency leads to death in animals. Linoleic acid (LA) is now universally recognized to be an essential nutrient. On the other hand, alpha-linolenic acid (ALNA) was considered non-essential until recently, and its role needs further studies.**

In our experiments, feeding animals with oils that have a low alpha-linolenic content results in all brain cells and organelles and various organs in reduced amounts of 22:6(n-3), compensated by an increase in 22:5(n-6). The speed of recuperation from these anomalies is extremely slow for brain cells, organelles and microvessels, in contrast with other organs. A decrease in alpha-linolenic series acids in the membranes results in a 40% reduction in the Na-K-ATPase of nerve terminals and a 20% reduction in 5'-nucleotidase. Some other enzymatic activities are not affected, although membrane fluidity is altered. A diet low in ALNA induces alterations in the electroretinogram which disappear with age; motor function and activity are little affected but learning behaviour is markedly altered. The presence of ALNA in the diet confers a greater resistance to certain neurotoxic agents, i.e. triethyl-lead.

We have shown that during the period of cerebral development, there is a linear relationship between brain content of (n-3) acids and the (n-3) content of the diet up to the point where alpha-linolenic levels reach 200 mg for 100 g food intake. Beyond that level there is a plateau. For the other organs, such as the liver, the relationship is also linear up to 200 mg/100 g, but then there is merely an abrupt change in slope and not a plateau. By varying the dietary 18:2(n-6) content, it was noted that 20:4(n-6) optimum values were obtained at 150 mg/100 g for all nerve structures, at 300 mg for testicle and muscle, 800 mg for the kidney, and 1200 mg for the liver, lung and heart. A deficiency in ALNA or an excess of LA has the same main effect: an increase in 22:5(n-6) levels.

Taking into account that the relative metabolisms of man and the rat, their rates of development, the difference between their brain/body weight ratios, and the similar fatty acid (FA) composition of their nerve membranes, it is possible to affirm that results obtained in the rat are necessarily, and at the very least, valid for man. For the brain and the other organs, the requirement in ALNA acid is 200 mg/100 g food intake (0.4% of calories), provided that LA requirements of 1200 mg/100 g food intake (2.4% of calories) are met.

During pre and postnatal development, delta-6 desaturase in brain decreases dramatically (12-fold) up to postnatal day 21 and remains nearly constant thereafter. In liver, the activity increases approximately 9-fold between day 3 before birth and day 7 after birth. Then, it decreases slightly up to weaning and is approximately constant up to 4 months. From then on delta-6-desaturase decreases with age (-40% between 4 and 17 months). The question remains whether the residual delta-6 desaturase activity after day 21 is sufficient to support the turnover of brain membranes. If it is not, the very-long-chain FA would have to be synthesized by the liver. As liver synthesis decreases during aging this source may be insufficient. It should be noted that cultured nerve cells differentiate, multiply, take up and release neurotransmitters only if the medium contains 20:4(n-6) and 22:6(n-3), but not if it contains 18:2(n-6) and 18:3(n-3). Thus, the FA that are essential for the brain could be those with very long chains. They are probably synthesized in the liver from ALNA and LA. They can also be furnished directly by the diet.

However a dietary excess of fish oil can prove to be toxic due to perturbation of the composition of cerebral membranes. Pharmacological doses of fish oil do not alter FA composition of liver and brain, and do not change protection against peroxidation. In contrast, increasing dietary fish oil in rat had the following effects on brain lipids: 20:4(n-6) regularly decreased; cervonic acid was increased by 30% at high fish oil concentration.

PUFA in membranes are protected against peroxidation, mainly by vitamin E. In peripheral nervous system during development and aging a highly significant correlation between vitamin E and (n-6) PUFA was observed but not between (n-3) PUFA and vitamin E.

INTRODUCTION

Nervous tissue contains high amounts of lipids which play a role in modulating the structure, fluidity and function of brain membranes (1–3). Brain lipids contain polyunsaturated fatty acids (PUFA) derived from dietary essential linoleic (LA) and alpha-linolenic acids (ALNA). More than one-third of the brain fatty acids (FA) are polyunsaturated with a prevalence of acids containing very long chains (mainly arachidonic acid (AA) 20:4 (n-6), and cervonic acid, 22:6 (n-3)) (4–14). Essential FA deficiency is known to have dramatic effects on various organs. Long and highly unsaturated chains, in particular AA, are the precursors of important hormonal substances (prostaglandins and leukotrienes), but their structural role is also important (3) since they play a major role in the structure and function of the membrane (1, 2–19). Thus, either the dietary precursors FA are rapidly and completely transformed into the longer chain FA after crossing the blood-brain barrier, or the FA essential for the brain are in fact the very-long-chain FA which are either synthesized in the liver or are provided with the diet.

It must be taken into account that cerebral development is genetically programmed. Moreover the renewal of neurons and oligodendrocytes is nil, and that of nervous membranes is often very slow. Therefore, during differentiation and multiplication, cells require adequate supplies of nutrients, especially lipids and particularly PUFA. Saturated and monounsaturated FA are mainly synthesized by nerve tissue itself, via complex mechanisms that differ according to cell type and organelle (16). A lipid abnormality would lead to an alteration in the function of membranes.

In fact, the PUFA present in the membranes are not the dietary precursors (LA and ALNA) but in fact longer and more desaturated chains (mainly 20:4 (n-6) and 22:6 (n-3)). These control the composition of membranes and hence their fluidity and, as a result, their enzymatic activity, the binding between molecules and their receptors, cellular interactions, and the transport of nutrients. As far as the nervous system is concerned, these FA can also influence certain electrophysiological parameters as well as learning functions. Dietary PUFA to a great extent determine membrane levels of these FA (3, 17) and are particularly important for ensuring harmonious cerebral development (18). There are many reports on the influence of PUFA on the structure and function of the nervous system (4–14, 19–35). However, PUFA of the (n-3) series play a very special role in membranes, especially in the nervous system: all cerebral cells and organelles are extremely rich in these FA. It is therefore extremely important to know precisely what quantity should be supplied by the diet, especially during development.

CONTROL OF THE COMPOSITION AND THE FUNCTION OF NERVOUS MEMBRANES BY ALNA (14)

We have compared animals fed a diet containing a normal amount of (n-6) FA but lacking (n-3) FA (a diet containing sunflower oil) with animals fed a diet containing both types of FA (a diet containing soybean oil).

- Diet deficient in (n-3): two groups of Wistar rats were fed for several generations with a semi-synthetic diet containing either sunflower oil or peanut oil.
- Diet containing (n-3): two other groups of rats were fed diets containing either soybean oil or rapeseed oil.

Rapeseed oil-fed animals were compared with peanut-fed animals, soybean animals with sunflower animals. The brain cells and the intracellular organelles conserve a normal total quantity of PUFA, but the various cell types and organelles show a considerable deficit in cervonic acid (22:6(n-3)) that is compensated for by an excess of docosapentaenoic acid (DPA 22:5(n-6)) as measured for whole brain and other organs (36, 37) (Table 1). Comparison of animals that have been fed for 60 days either a sunflower diet or a soybean diet shows (n-3/n-6) ratios of 1/20 in the diet, 1/16 in the oligodendrocytes, 1/12 in the myelin, 1/2 in the neurons, 1/6 in the synaptosomes and 1/3 in the astrocytes (24). The importance of (n-3) FA has also been shown by a study of phosphatidylethanolamine in animals fed a peanut or rapeseed oil diet (38).

Extremely slow recovery (Fig. 1)

In young animals, after switching from the (n-3)-deficient to the (n-3)-containing diet (39, 40), several months were needed before brain cells and organelles recovered normal levels of 22:6(n-3) and lost the excess

Table 1 Quantities of 22:6(n-3) and 22:5(n-6) in the nervous system of (n-3)-deficient-diet animals

	22:6(n-3)	22:5(n-6)
Neurons	28	214
Synaptosomes	27	1088
Oligodendrocytes	10	240
Myelin	14	1200
Astrocytes	47	344
Mitochondria	25	917
Microsomes	28	592
Retina	36	1280
Sciatic nerve	28	1000

Data from refs 14 and 24. Expressed as percentages of the non-(n-3)-deficient animals.

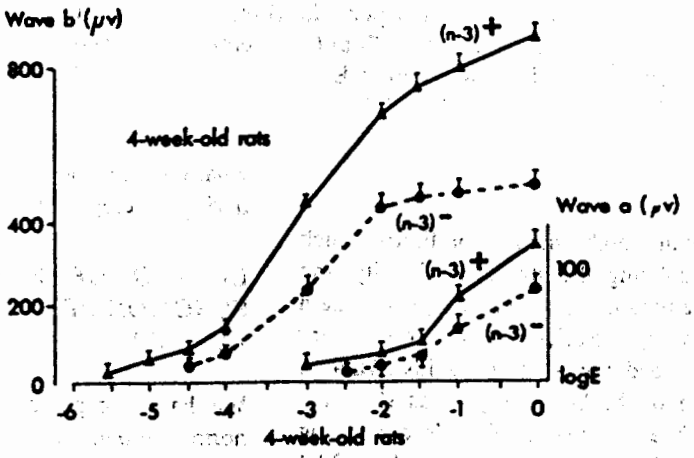

Fig. 1 Electroretinogram.

22:5(n-6). This slow recovery was the same whatever the cell or organelle. It was expected that recuperation would not be rapid in myelin, which has a slow turnover. But it is very surprising that nerve terminals also have a very slow recovery although turnover of their membrane molecules is supposed to be somewhat rapid. Thus, regulation of recuperation occurs either at the level of synthesis of chain ends in the liver, or at the transport across the blood-brain barrier, or at the level of the enzymatic activities of desaturation and elongation. It was very interesting to note that cerebral microvessel and capillaries (41) also have a very slow rate of recuperation, even though they are in contact with plasma lipoproteins of normal composition, since the liver recuperates very rapidly (2 weeks).

ALNA deficiency alters enzymatic activities (24)

Membrane bound enzymatic activities can be altered by PUFA. For instance, the activity of 5'-nucleotidase is decreased by 30% in whole brain, but not in myelin or in nerve terminals, signifying that its activity is probably altered considerably in cell membranes. These results are in agreement with those of Bernsohn et al (42) who have shown that a decrease in the activity of this enzyme produced by simultaneous deficiency in LA and ALNA is only corrected by the addition of ALNA to the diet, showing the very specific effect of this acid.

The very important enzyme that equilibrates ions during nerve impulse, Na-K-ATPase, is reduced nearly by half in the nerve terminals of animals fed an (n-3)-deficient diet compared with those fed the (n-3)-containing diet. On the other hand, simultaneous deficiency in LA and ALNA leads to an increase in Na-K-ATPase activity (33). It consumes half the energy used by the brain.

CNPase (a special phosphodiesterase), which is specific for myelin, decreases as a result of ALNA deficiency, even though the myelin membrane is considered to be very rigid and not very metabolically active. The activity of another enzyme, acetylcholine-esterase, is also modulated by dietary lipids (43).

Membrane fluidity changes

Fluidity in nerve-ending membranes is affected by the diet, depending on the membrane region. Feeding the sunflower oil diet compared to the soybean oil diet results in less fluidity in the surface polar part of the membranes probed by TMA- or PROP-DPH but greater fluidity in the apolar part of the membranes (probed by diphenalhexatriene (DPH)) (Table 2) (44).

Interestingly, the fluidizing effect of ethanol shown with DPH is also decreased significantly in animals fed sunflower oil (Table 3) (44). Concomitantly, rats fed sunflower oil are more sensitive to ethanol-induced hypothermia, illustrating the importance of diet to membrane sensitivity and animal response to ethanol, regardless of the exact mechanisms. Compared to the soya diet, the sunflower diet gives a decrease in fluidity in the polar part of the membrane probed by TMA- or PROP-DPH (–3%) and increase in the apolar part of the membrane probed by DPH (+4%).

Table 2 Effect of diet on membrane organization: fluorescence polarization of DPH, TMA-DPH and PROP-DPH in rat nerve ending membranes

Probe	Diets		Percentage changes
	Soya	Sunflower	
DPH	0.333 ± 0.004	0.320 ± 0.001*	– 4%
TMA-DPH	0.352 ± 0.001	0.359 ± 0.002*	+ 2%
Prop-DPH	0.360 + 0.001	0.370 ± 0.003*	+ 3%

Data from ref. 44.
Data are means ± SD. Statistical significance: *$p < 0.01$.

Table 3 Fluidizing efficacy of ethanol in rat nerve ending membranes. Effect of diet on ethanol membrane tolerance

Probe	Diets		Percentage changes
	Soya	Sunflower	
DPH	0.0185 ± 0.0010	0.0152 ± 0.0012**	–18%
TMA-DPH	0.0088 ± 0.0002	0.0080 ± 0.0012	
Prop-DPH	0.0087 ± 0.0003	0.0070 ± 0.0009	

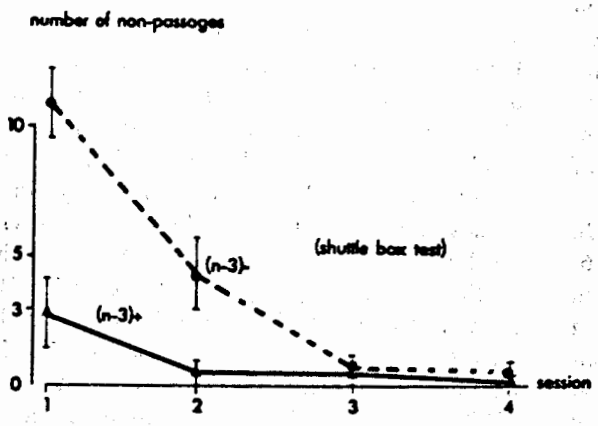

Fig. 2 Learning tests.

Electroretinogram is affected by alpha-linolenic deficiency

In the retina, 22:6 (n-3) level is high (20). Prolonged deficiency in PUFA induces changes in the distribution of membrane FA in the retina which are associated with changes in the electroretinogram (5, 31). In 4-week-old animals, the threshold of detection (10 mV) of wave A required a light stimulation 10 times stronger than that of the non-(n-3)-deficient animals. In 6-week-old animals, electroretinogram changes were less marked; in adult animals, only the A wave remained abnormal (24). Thus, in adult animals, although the biochemical abnormality is still present, electroretinogram is partially returned to normal.

Learning tests are altered by alpha-linolenic deprivation (Fig. 2)

It has been previously shown that a simultaneous deficiency in LA and ALNA affects the learning capacities of animals (29), as does a selective deficiency in ALNA (45). Though motor activity and open field tests were practically normal in animals fed the (n-3)-deficient diet, their learning capacities were severely perturbed, as shown by the shuttle box test. In the first session, animals fed the (n-3)-containing diet made a more rapid association between the light stimulus and the electric shock, since they avoided on average 7 shocks out of 30, whereas (n-3)-deficient diet animals avoided only 2. These differences diminished with further conditioning and disappeared at the fourth session (24). Very interestingly the extinction of learning capacities was significantly longer in animals deficient in dietary ALNA (46, 47).

Mortality in animals tested with the neurotoxic agent triethyltin (24)

The LD50 of animals fed the soybean oil or sunflower oil diets did not differ significantly (6.18 vs 6.02 ml/kg, respectively). But the animals fed the sunflower oil diet died more rapidly than did those fed the soybean oil diet.

MINIMUM REQUIREMENTS

Minimum dietary requirement of ALNA for cerebral membranes (24)

We gave diets with intermediate levels of ALNA 3 weeks before mating to 12 groups of rats. Increasing the amount of 18:3 (n-3) led to an overall increase in 22:6 (n-3) in 21-day-old pups, and inversely a decrease in 22:5 (n-6). In fact, in brain, levels of 22:6 (n-3) increased linearly at an intake of 18:3 (n-3) that varied from 0-200 mg/100 g diet and then reached a plateau (the opposite was observed for 22:5(n-6)). In liver, kidney, lung, heart and muscle the same threshold was found but the plateau was less pronounced (Fig. 3).

Minimum dietary requirement of LA (48)

3 weeks before mating, 12 groups of female rats were

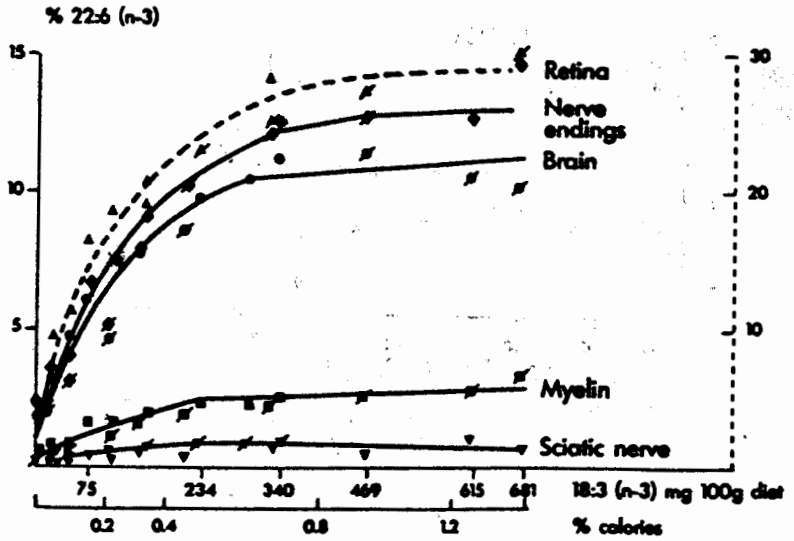

Fig. 3 Relationship between dietary ALNA and cervonic acid level in nervous tissue.

fed different amounts of LA acid between 150 and 6200 mg/100 g food intake. Their male pups were killed when 21 days old. LA levels remained very low in brain, myelin, synaptosomes and retina. In contrast, 18:2(n-6) levels increased in sciatic nerve. In heart, LA levels were high, but were not related to dietary LA intake. Levels of 18:2(n-6) were significantly increased in liver, lung, kidney and testicle and were even higher in muscle. On the other hand, in heart a constant amount of 18:2(n-6) was found at a low level of dietary 18:2(n-6). Constant levels of AA (20:4(n-6)) were reached at 150 mg/100 g diet in all nerve structures, at 300 mg/100 g diet in testicle and muscle, at 800 mg/100 g diet in kidney, and at 1200 mg/100 g diet in liver, lung and heart. Constant adrenic acid (22:4(n-6)) levels were obtained at 150, 900 and 1200 mg/100 g diet in myelin, sciatic nerve and brain, respectively. Minimal levels were difficult to determine. In all fractions examined, accumulation of DPA (22:5(n-6)) was the most direct and specific consequence of excess amounts of dietary 18:2(n-6). Tissue eicosapen- taenoic acid (EPA) (20:5(n-3)) and 22:5(n-3) levels were relatively independent of dietary 18:2(n-6) intake, except in lung, liver and kidney. In several organs (muscle, lung, kidney, liver, heart) as well as in myelin, very low levels of dietary LA led to an increase in 20:5(n-3). Dietary requirements for 18:2(n-6) varied from 150-1200 mg/100 g food intake, depending on the organ and the nature of the tissue FA. Therefore, the minimum dietary requirement is estimated to be about 1200 mg/100 g (i.e. the level that ensures stable and constant amounts of AA in the various tissues and brain fractions examined) (Fig. 4).

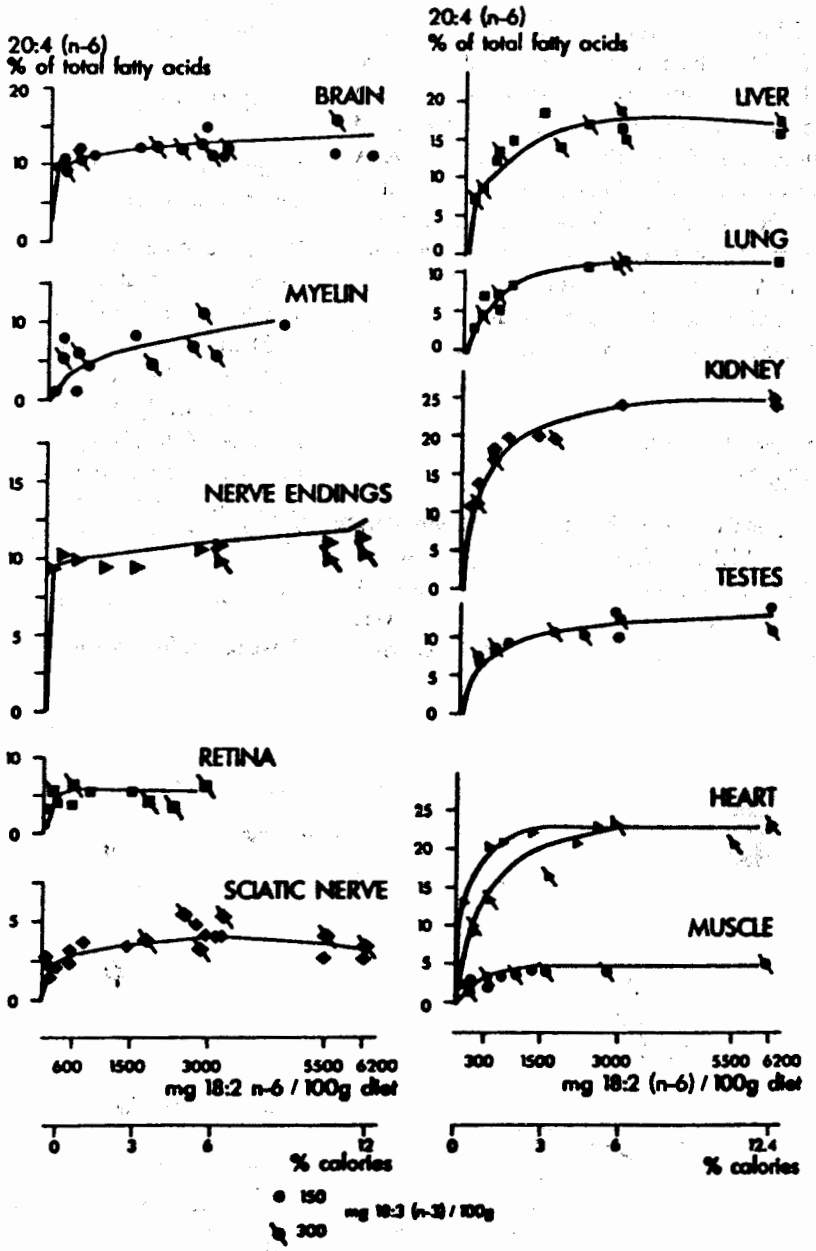

Fig. 4 Relationship between dietary LA and AA in tissues.

ESSENTIAL FA FOR BRAIN CELLS IN CULTURE (49)

Fetal brain cells were dissociated from mouse cerebral hemispheres taken on the 16th day of gestation to determine the biochemical and morphological effects of PUFA on fetal brain cells grown in a chemically defined medium. After cells had grown in chemically defined medium for 8 days, the proportion of PUFA in cultured cells was only one-half of that observed at day 0 and about 1.5 times less than that of cells grown in serum-supplemented medium. FA 20:3(n-9) was present in cultured cells grown in either chemically defined or serum-supplemented medium, demonstrating the deficiency of essential FA. The reduced amount of PUFA in cells grown in the chemically defined medium was balanced by an increase in monounsaturated FA. The saturated FA were not affected. When added at seeding time, LA, ALNA, AA, or docosahexaenoic acid stimulated the proliferation of small dense cells. In addition, we observed that each of the four FA studied was incorporated into phospholipids. Adding FA of the (n-6) series increased the content of (n-6) FA in the cells, but also provoked an increase in the (n-3) FA. Among several combinations of FA, only 20:4 and 22:6, when added to the culture in a ratio of 2/1, restored a FA profile similar to controls (i.e. in vivo tissue taken at postnatal day 5).

Very interestingly, it should be noted that cultured nerve cells differentiate, multiply, take up and release neurotransmitters only if the medium contains 20:4(n-6) and 22:6(n-3), but not if it contains 18:2(n-6) and 18:3(n-3) (49, 50). As a consequence hepatic desaturase must be functional for transformation of dietary precursors into longer chains.

DELTA-6 DESATURASE IN BRAIN AND LIVER DURING DEVELOPMENT AND AGING (Fig. 5)

Thus, delta-4 desaturase was measured in the mouse brain and liver using LA acid as substrate. During pre and postnatal development, delta-6 desaturase in brain decreased dramatically (12-fold) up to postnatal day 21 (51–54) and remained nearly constant thereafter. In liver, the activity increased approximately 9-fold between day 3 before birth and day 7 after birth. Then it decreased slightly up to weaning and was approximately constant up to 4 months. From then on, delta-6 desaturase decreased with age (40% between 4 and 17 months). The mouse was chosen as model because it is known that desaturating activity in mice is lower than that in rats (55, 56) and thus is closer to that in humans, taking into account the ratio of very-long-chain PUFA to their precursors in the blood and liver.

Delta-6 activity in brain is very high during early development up to 7 days after birth. This corresponds to the period of neuronal and glial multiplication, the latter event being at a peak at 3–5 days after birth in the mouse. Early brain development requires large quantities of PUFA for membrane synthesis. Interestingly, delta-6 activity does not peak during myelination, although myelin contains large amounts of PUFA. The same pattern was found for delta-9 desaturase activity (57). Thus, PUFA required for myelination are either accumulated in the oligodendrocytes before myelination or possibly are supplied through the blood stream. This is in contrast to the synthesis of saturated and monounsaturated long-chain and very-long-chain FA which peaks during myelination and is impaired in neurological dysmyelinating mutants (58). Interestingly, chain lengthening of EPA is less affected in these mutants than elongation of erucic and arachidic acids (59).

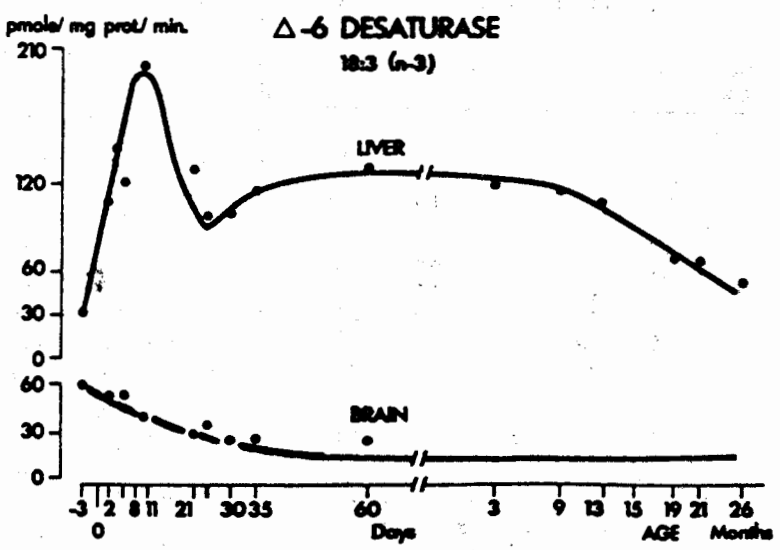

Fig. 5 Delta-6 desaturase in brain and liver during development and aging.

Whether the residual delta-6 desaturase activity after 21 days is sufficient to support the turnover of brain membranes, remains to be determined. If it is not, the very-long-chain FA would have to be synthesized by the liver. As liver synthesis decreases during aging this source may be insufficient. It has been hypothesized that one aspect of aging could be the reduced activity of delta-6 desaturase which would impede membrane renewal (60). Thus, regulation of desaturases must be carefully re-examined.

EFFECTS OF INCREASING AMOUNT OF FISH OIL (62–64)

Fish oils, directly providing very long (n-3) PUFA, could be an important dietary factor of their pharmacological role (however the toxicological role must not be neglected).

We found that increasing dietary fish oil in rat had effects on brain lipids (Table 4): AA decreased progressively; EPA normally nearly undetectable, was present; 22:5(n-3) dramatically increased but remained below 1% of total FA; cervonic acid increased by 30%, at high fish oil concentration. Saturated and monounsaturated FA were not affected regardless of chain length. In contrast, in the liver, nearly all FA (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 and transaminases were not affected. Total serum cholesterol, unesterified cholesterol and phosphatidylcholine were slightly affected. In contrast, triacylglycerols were dramatically reduced in proportion to the fish oil content of the diet as previously shown.

Specifically for brain, Table 4 shows that 20:4(n-6) decreased proportionately with increasing dietary fish oil content (and decreasing corn oil). We have previously shown that 20:4(n-6) content is independent of dietary LA content in excess of minimal level (0.3% of the calories); this is largely true in all diets tested. Thus the decrease of 20:4(n-6) in brain membranes is due only to the increase of fish oil in the diet. While 22:4(n-6) was less affected than AA, 22:5(n-6) was reduced by about 60%. The high amount of dietary corn oil could increase 22:5(n-6), since its level in membranes parallels dietary excess of LA (48) as well as the deficiency in ALNA (30, 24).

Table 4 FA composition of total lipid (weight percent) of rat brain after various diets

Diet composition					
Salmon oil, g/100g	–	1.5	4.0	7.0	10.0
Corn oil, g/100g	10	8.5	6.0	3.0	–
(n-6/n-3)	75	10.0	3.0	1.0	0.1
Brain fatty acids, weight %:					
18:2(n-6)	0.9	1	0.9	0.7	0.3
20:4(n-6)	10.2	9.1	8.5	7.7	7.8
22:4(n-6)	3.5	2.7	2.7	3.0	2.7
22:5(n-6)	0.8	0.4	0.3	0.3	0.3
20:5(n-6)	–	–	0.1	0.2	0.3
22:5(n-3)	0.1	0.2	0.4	0.6	0.7
22:6(n-3)	12.1	12.3	15.4	14.6	15.6

Data from ref. 63. Five groups of 12 male Wistar rats (IFFA-Credo, l'Arbresle, France) weighing 190–200 g were housed two per cage. All groups received the same semi-synthetic diet for 8 weeks having the same total amount of lipids, but varying in fish oil (increasing salmon oil was compensated for by decreasing corn oil).

It must be noted that EPA was nearly undetectable in diet containing low amounts of fish oil, and increased in diet containing 4% or more fish oil, but the content was still extremely low, even in the diet containing a very high amount of fish oil. In 10% fish oil diet, 22:5(n-3) was increased 7-fold but the brain content was always below 1% of the total FA and 22:6(n-3) was increased by about 30%.

Brain is not protected against a large excess of very-long-chain (n-3) PUFA, which increase the (n-3/n-6) ratio and could lead to abnormal function, and which might be difficult to reverse.

PUFA IN MEMBRANES. PERSISTENCE OF HIGH CORRELATIONS OF VITAMIN E WITH TOTAL AND SPECIFIC (n-6) BUT NOT WITH (n-3) FA (61)

PUFA must be protected against oxygen toxicity and free radical agression. Vitamin E, as an integral part of membranes, is seen as a biological antioxidant, which, by sequestering free radicals, functions to terminate the propagation of autooxidation processes such as PUFA peroxidation. In addition, specific effects of alpha-tocopherol that do not involve its antioxidant function and that act on the architecture of membranes by controlling the profiles of their unsaturated phospholipid and cholesterol components have been suggested. It is interesting to determine whether there is a correlation between alteration of vitamin E and PUFA of both series.

We found in developing and aging peripheral nervous system a highly significant correlation between vitamin E and (n-6) PUFA [18:2(n-6), 20:4(n-6), and total (n-6)] was observed but not between (n-3) PUFA and vitamin E. It is suggested that there may be a relationship between vitamin E and (n-6) PUFA in the peripheral nervous system membranes during development and aging.

The sciatic nerve concentration of vitamin E in rats increased rapidly during the postnatal period (approximately 5-fold between days 1 and 8), then decreased dramatically (about 2-fold between days 8 and 30), and further decreased slowly between days 30 and 60 and remained constant up to 2 years. This is in contrast to brain. While the sciatic nerve concentration of vitamin E decreased by 58% between days 8 and 30, the concentration of vitamin E in serum presented a marked decrease (75%). The age-related changes in FA concentration of the endoneurial fraction of the sciatic nerve were characterized by a large increase in content of saturated and

monounsaturated FA up to 6 months (2-fold for saturated and 4-fold for monounsaturated FA). Then, up to 24 months, the amount of these FA decreased very slowly. The content of (n-6) PUFA decreased rapidly up to 1 year and slowly afterwards. In contrast, during development the amount of (n-3) PUFA was relatively stable and decreased during aging.

DISCUSSION

In conclusion, a diet deficient in ALNA caused marked alterations in the FA composition of all cellular and subcellular fractions examined. The total content (number of moles) of PUFA was not altered, the marked decrease in 22:6(n-3) being compensated for by an increase in 22:5(n-6) as previously shown (36). This compensation is quantitative, but total unsaturation remains in deficit. It is evident that PUFA control the fluidity of biological membranes, hence many of their activities. A specific deficiency in (n-3) FA perturbs the activities of membrane enzymes, alters some electrophysiological activities as shown by the electroretinogram and disturbs learning abilities. After switching from a deficient to a normal diet, the rate of recovery is remarkably slow: it is several months before brain cells and organelles recover normal levels of cervonic acid. This rate is the same in all other organelles. It is therefore crucial to supply the FA necessary for cerebral structures at the developmental stage. A deficiency is difficult to correct.

It should be noted that mean FA levels in human brain differ little from those of similar regions in rat brain. Human development involves a greater daily increase in brain mass over a longer period, and the ratio brain weight:total body weight is greater in man, even taking the 2/3 coefficient into account. Consequently, the minimal requirements in rat are a fortiori those in man. In any case, for obvious ethical reasons, it is not possible to determine the effects of increasing dietary FA levels on the composition of human cerebral membranes. Our study is the first to measure simultaneously the variations of all the PUFA levels in several organs as a function of variations in dietary LA content, minimal ALNA requirements being satisfied. In contrast to ALNA requirements, which are the same for all organs (200 mg/100 g food intake) (24), the LA requirements differ according to organ (48). The minimal requirements in man may, therefore, be taken as 1200 mg/100 g food intake (2.4% of calories) for LA and 200 mg/100 g food intake (0.4% of calories) for ALNA.

A pathogenesis of ALNA acid deficiency has been described in the monkey (65) and in humans (30, 66–68). A deficiency in (n-3) FA has been proposed as a syndrome of modern society (69). It is, therefore, very important to verify the precise amount of (n-3) acids in the diet. The results of this study indicate that, in order to avoid deficiency, ALNA should be present at 0.4% of the total dietary energy, in agreement with studies in animals (70) and in humans (30, 71, 72).

Since cerebral structures contain very-long-chain PUFA, it might seem wise to provide these acids directly in the diet, especially since the ability of the organism to transform linoleic and alpha-linolenic precursors diminishes rapidly during development (25, 51–54). However, large quantities (up to 12%) of dietary fish oil, even supplemented with vitamin E, perturb the FA profile of the liver as well as that of the brain. In brain, there is a deficiency of AA and a marked decrease in 22:4(n-6) and 22:5(n-6), associated with excess 22:6(n-3) and 22:5(n-3).

As subtle changes in brain membrane PUFA determined by dietary alterations in ALNA provoke alterations in brain membrane PUFA, membrane fluidity, enzymatic activities, electrophysiological parameters, learning tests and resistance to poisons (24), the question can be raised of whether increased fish oil intake leads to functional alterations in the nervous system.

It is clear that consumption of fish oils containing (n-3) PUFA may have beneficial effects on ischemic heart disease and thrombosis (73–75). However, as the ingestion of large amounts of (n-3) PUFA in experimental animals gives rise to adverse effects, 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. Interestingly, during development and aging, in rat peripheral nerve, (n-3) FA content and vitamin E content are not correlated (61). The PUFA composition of the diet regulates the FA composition of the liver endoplasmic reticulum (77), and this in turn is an important factor controlling the rate and extent of lipid peroxidation in vitro and possibly in vivo (78, 79). The replacement of cell membrane (n-6) FA by dietary (n-3) FA and subsequent alterations of membrane composition remain to be elucidated. Maintenance of membrane fluidity within narrow limits is presumably a prerequisite for proper functioning of the cell. Lipids play a key role in determining membrane fluidity, and changes in lipid and FA composition have been reported to alter important cellular functions (1, 2, 8, 15). Therefore, dietary modification of membrane phospholipids by fish oil supplements may have significant effects. The (n-3) FA 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 must be conducted before reommendations can be made about their general use.

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 (80, 81), thus special attention must be paid to the brain (64). Requirements for (n-3) acids are very high in humans during the neonatal period (21, 22, 82) and must be supplied to the mother during gestation and then to the newborn. Human milk contains ALNA and also cervonic acid,

which are often absent from infant formulas. Human newborns receiving formula milk have red blood cells that are deficient in cervonic acid (83). The FA composition of red blood cells can serve as an index of cerebral membrane composition (84). In addition, there is undoubtedly a relationship between dietary lipids, serum FA (85) and the properties of red blood cells (86) and their structure (87).

References

1. Brenner RR. Effects of unsaturated acids on membrane structure and enzyme kinetics. Prog Lipid Res 1984; 23: 69–96.
2. Farias RN. Membrane cooperative enzymes as a tool for the investigation of membrane structure and related phenomena. Adv Lipid Res 1980; 17: 251–282.
3. Mead JF. The non-eicosanoid functions of the essential fatty acids. J Lipid Res 1984; 25: 1517–1521.
4. Bazan NG. Supply of n-3 polyunsaturated fatty acids and their significance in the central nervous system. In: Wurtman RJ, Wurtman JJ, eds. Nutrition and the Brain. Vol. 8. New York: Raven Press, 1990: 1–24.
5. Connor WE, Neuringer M. Aberrations in membrane structure and function. In: Karnovsky ML, Leaf A, Biological Membranes Bolls LC, eds. New York: Alan R. Liss, 1988: 275–294.
6. Crawford MA, Hassam AG, Williams G, Whitehouse W. Function and biosynthesis of lipids. In: Bazan NG, Brenner RR, Giusto NM, eds. Advances in Experimental Medicine and Biology. New York: Plenum Press, 1977: 135–144.
7. Galli C. In: Gallic, Jacini G, Pecile A, eds. Dietary Lipids and Postnatal Development. New York: Raven Press, 1973: 191–202.
8. Greenwood CE, Craig REA. Current topics in nutrition and disease In: Basic and Clinical Aspects of Nutrition and Brain Development. New York: Alan R. Liss, 1987: 159–216
9. Lamptey MS, Walker LB. Physical and neurological development of the progeny of female rats fed on essential fatty acid–deficient diet during pregnancy and/or lactation. J Nutr 1978; 108: 351–357.
10. McKenna MC, Campagnoni AT. Effect of pre-and postnatal essential fatty acid deficiency on brain development and myelination. J Nutr 1979; 109: 1195–1204.
11. Yamamoto N, Hashimoto A, Takemoto Y, et al. Effect of the dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process and glycolipid compositions. J Lipid Res 1988; 29: 1013–1021.
12. Holman RT. Essential fatty acid deficiency. In: Holman RT, ed. Progress in the Chemistry of Fats and Other Lipids. Vol. 9. Part 2. Oxford: Pergamon Press, 1968: 279.
13. Paoletti R, Galli C. Effects of essential fatty acid deficiency on the central nervous system in the growing rat. In: Lipid Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier/North Holland Press, Amsterdam, 1972: 121–140.
14. Bourre JM, Pascal G, Durand G, Masson M, Dumont O, Piciotti M. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. J Neurochem 1984; 43: 342–348.
15. Stubbs CD, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophys Acta 1984; 779: 89–137.
16. Bourre JM. Origin of aliphatic chains in brain. In: Baumann N, ed. Neurological Mutations Affecting Myelination. INSERM Symposium no. 14. Amsterdam: Elsevier/North Holland Biomedical Press, 1980: 187–206.
17. Holman RT. Control of polyunsaturated acids in tissue lipids. J Am Coll Nutr 1986; 5: 183–211.
18. Menon K, Dhopeshwarkar GA. Essential fatty acid deficiency and brain development. Prog Lipid Res 1982; 21: 309–326.
19. Sanders TA, Rana JK. Comparison of the metabolism of linoleic and linolenic acids in the fetal rat. Ann Nutr Metab 1987; 31: 349–353.
20. Bazan NG, Di Fazio De Escalante S, Careaga M, Bazan HE, Giusto NM. High content of 22: 6 (docosohexaenoate) and active [2-3H] glycerol metabolism of phosphatidic acid from photoreceptor membranes. Biochim Biophys Acta 1982; 712: 702–706.
21. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980; 4: 121–129.
22. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion in infant brain: implications for fatty acid requirement. Early Hum Dev 1980; 4: 131–138.
23. Berkow SE, Campagnoni AT. Essential fatty acid deficiency: effects of cross-fostering mice at birth on myelin levels and composition. J Nutr 1983; 113: 582–591.
24. Bourre JM, Francois M, Youyou A, et al. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr 1989; 119: 1880–1892.
25. Cook HW. In vitro formation of polyunsaturated fatty acids by desaturation in rat brain: some properties of the enzymes in developing brain and comparisons with liver. J Neurochem 1978; 30: 1327–1334.
26. Kaare RN, Drevon CA. Dietary n-3 fatty acids and cardiovascular disease. Arteriosclerosis 1986; 6: 352–352.
27. Crawford MA, Hassam AG, Stevens PA. Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. Prog Lipid Res 1981; 20: 31–40.
28. Philbrick DJ, Mahadevappa VG, Ackman G, Holub J. Ingestion of fish oil or a derived n-3 fatty acid concentrate containing eicosapentaenoic acid (EPA) affects fatty acid compositions of individual phospholipids of rat brain, sciatic nerve and retina. J Nutr 1987; 117: 1663–1670.
29. Lamptey M S, Walker BL. Learning behaviour and brain lipid composition in rat subjected to essential fatty acid deficiency during gestation, lactation and growth. J Nutr 1978; 108: 358–367.
30. Holman RT, Walker BL. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982; 35: 617–623.
31. Neuringer M, Connor WE. N-3 fatty acids in the brain and retina: evidence for their essentiality. Nutr Rev 1986; 44: 285–294.
32. Paoletti R, Galli C. Effects of essential fatty acid deficiency on the central nervous system in the growing rat. In: Lipids Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier/North Holland, Amsterdam, 1972: 121–140
33. Sun GY, Sun AY. Synaptosomal plasma membranes: acyl group composition of phosphoglycerides and (Na+/ K+) ATPase activity during fatty acid deficiency. J Neurochem 1974; 22: 15–18.
34. Sprecher H. Biochemistry of essential fatty acids. Prog Lipid Res 1981; 20: 13–22.
35. Svennerholm L, Alling C, Bruce A, Karlsson I, Sapia O. Effects of offsprings of maternal malnutrition in the rat. In: Lipids, Malnutrition and the Developing Brain. Ciba Foundation Symposium. Elsevier/North Holland, Amsterdam, 1972: 141–157
36. Galli C, Trzeciak HI, Paoletti R. Effects of dietary fatty acids on the fatty acid composition of brain ethanolamine – phosphoglyceride : reciprocal replacement of n-6 and n-

3 polyunsaturated fatty acids. Biochim Biophys Acta 1971; 248: 449–454.
37. Alling C, Bruce A, Karlsson I, Sapia O, Svennerholm I. Effect of maternal essential fatty acid supply on fatty acid composition of brain, liver, muscle and serum in 21-day-old rats. J Nutr 1972; 102: 773–782.
38. Nouvelot A, Delbart C, Bourre JM. Hepatic metabolism of dietary alpha-linolenic acid in suckling rats and its possible importance in polyunsaturated fatty acid uptake by the brain. Ann Nutr Metab 1986; 30: 316–323.
39. Bourre JM, Durand G, Pascal G, Youyou A. Brain cell and tissue recovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. J Nutr 1989; 119: 15–22.
40. Youyou A, Durand G, Pascal G, Piciotti M, Dumont O, Bourre JM. Recovery of altered fatty acid composition induced by a diet devoid of n-3 fatty acids in myelin, synaptosomes, mitochondria, and microsomes of developing rat brain. J Neurochem 1986; 46: 224–228.
41. Homayoun P, Durand G, Pascal G, Bourre JM. Alteration in fatty acid composition of adult rat brain capillaries and choroid plexus induced by a diet deficient in n-3 fatty acids: Slow recovery after substitution with a nondeficient diet. J Neurochem 1988; 51: 45–48.
42. Bernshohn J, Spitz FJ. Biochem Biophys Res Commun 1974; 57: 293–
43. Foot M, Cruz TF, Clandinin MT. Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. Biochem J 1982; 208: 631–640.
44. Beaugé F, Zerouga M, Niel E, et al. Effects of dietary linolenate/linoleate balance on the neuronal membrane sensitivity to ethanol. In: Kuriyama K, Takada A, Ishich Y, eds. Biomedical and Social Aspects of Alcohol and Alcoholism. Amsterdam: Elsevier/North Holland. 1988: 291–294
45. Lamptey MS, Walker BL. A possible essential role for dietary linolenic acid in the development of the young rat. J Nutr 1976; 106: 86–93.
46. Yamamoto N, Sarton M, Moriuchi A, Nomura M, Okuyama H. Effect of dietary a linolenate/linoleate balance on brain lipid compositions and learning ability of rats. J Lipid Res 1987; 28: 144–151.
47. Yamamoto N, Hashimoto A, Takemoto Y, et al. Effect of the dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process and glycolipid compositions. J Lipid Res 1988; 29: 1013–1021.
48. Bourre JM, Piciotti M, Dumont O, Pascal G, Durand G. Delta 6 desaturase in brain and liver during development and aging. Lipids 1990; 25: 354–356.
49. Bourre JM, Faivre A, Dumont O, et al. Effect of polyunsaturated fatty acids in fetal mouse brain cells in culture in a chemically defined medium. J Neurochem 1983; 41: 1234–1242.
50. Loudes C, Faivre-Bauman A, Barret A, Grouselle D, Puymirat J, Tixier-Vidal A. Release of immunoreactive TRH in serum-free cultures of mouse hypothalamic cells. Brain Res 1983; 285: 231–234.
51. Bourre JM, Piciotti M, Dumont O. Delta desaturase in brain and liver during development and aging. Lipids 1990; 25: 354–356.
52. Strouve-Vallet C, Pascaud M. Desaturation de l'acide linoleique par les microsomes du foie et du cerveau du rat en développement. Biochimie 1971; 53, 699–703.
53. Bordoni A, Biagi PL, Turchetto E, Hrelia S. Aging influence on delta-6- desaturase activity and fatty acid composition of rat liver microsomes. Biochem Int 1988; 17: 1001–1009.
54. Ulmann L, Blond JP, Maniongui C, et al. Effect of age and dietary essential fatty acids on desaturase activities and on fatty acid composition of liver microsomal phopholipids of adult rats. Lipids 1991; 26: 127–133.
55. Cunnane SC, Keeling PWN, Thompson RPH, Crawford MA. Linoleic acid and arachidonic acid metabolism in human peripheral blood leucocytes: comparison with the rat. Br J Nutr 1984; 51: 209–217.
56. Horrobin DF, Huang YS, Cunnane SC, Manku MS. Essential fatty acids in plasma, red blood cells and liver phospholipids in common laboratory animals as compared to humans. Lipids 1984; 19: 806–811.
57. Carreau JP, Daudu O, Mazliak P, Bourre JM. Palmityl-CoA and stearyl-CoA desaturase in mouse brain microsomes during development in normal and neurological mutants (quaking and jimpy). J Neurochem 1979; 32: 659–660.
58. Bourre JM, Paturneau-Jouas M, Daudu O, Baumann N. Lignoceric acid biosynthesis in the developing brain. Activities of mitocondrial acetyl-CoA-dependent system in relation to myelination. Comparison between normal mouse dysmyelinating mutants (quaking and jimpy). Eur J Biochem 1977; 72: 41–47.
59. Bourre JM, Daudu O, Baumann N. Nervonic acid biosynthesis by erucyl-CoA elongation in normal and quaking mouse brain microsomes. Elongation of other unsaturated fatty acyl-CoA (mono and polyunsaturated). Biochim Biophys Acta 1976; 424: 1–7.
60. Horrobin D. Loss of delta-6-desaturase activity as a key factor in aging. Med Hypotheses 1981; 1: 1211–1220.
61. Clément M, Bourre JM. Alteration of a-tocopherol content in the developing and aging peripheral nervous system: persistence of high correlations with total and specific (n-6) polyunsaturated fatty acids. J Neurochem 1990; 54: 2110–2117.
62. Chaudière J, Clément M, Driss F, Bourre JM. Unaltered brain membranes after prolonged intake of highly oxidizable long-chain fatty acids of the (n–3) series. Neurosci Lett 1987; 82: 233–239.
63. Bourre JM, Bonneil M, Dumont O, et al. Effect of increasing amounts of dietary fish oil on brain and liver fatty composition. Biochim Biophys Acta Lipids 1990; 1043: 149–152.
64. Bourre JM, Bonneil M, Dumont O, Piciotti M, Nalbone G, Lafont H. High dietary fish oil alters the brain polyunsatureted fatty acid composition. Biochim Biophys Acta 1988; 960: 458–461.
65. Fiennes RNT, Sinclair AJ, Crawford MA. Essential fatty acid studies in primates: linolenic acid requirements of capuchins. J Med Primates 1973; 2: 155–169.
66. Anonymous. Combined EFA deficiency in a patient on long term TPN. Nutr Rev 1986; 44: 301–308.
67. Bjerve KS, Mostad IL, Thoresen L. Alpha-linolenic acid deficiency in patients on long-term gastric-tube feeding estimation of linolenic acid and long-chain unsaturated n-3 fatty acid requirement in man. Am J Clin Nutr 1987; 45: 66–77.
68. Bjerve KS, Fisher S, Alme K. Alpha-linolenic acid deficiency in man. Effect of ethyl linolenate on fatty acid composition and biosynthesis of eicosanoids. Am J Clin Nutr 1987; 46: 570–576.
69. Rudin DO. The dominant diseases of modernized societies as omega-3 essential fatty acid deficiency syndrome: substrate beriberi. Med Hypotheses 1982; 8: 17–47.
70. Pudelkewicz C, Seufert J, Holamn RT. Requirements of the female rat for linolenic and linolenic acids. J Nutr 1968; 94, 138–146.
71. Lasserre M, Mendy F, Spielmann D, Jacotot B. Effects of different dietary intake of essential fatty acids on C20: 3 w6 and C20: 4w6 serum levels in human adults. Lipids 1985; 20: 227–233.
72. Kinsella JE. Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oils. Food Technol 1986; 146: 89–97.
73. Ackman RG. Absorption of omega-3 fatty acids. Nutrition 1989; 5: 251–252.
74. Salem N, Kim HY, Yergey JA. Docosahexaenoic acid: membrane function and metabolism. In: Simopoulos AP, ed. Health Effects of Polyunsaturated Fatty Acids in Seafoods. New York: Academic Press. 1986: 263–317.
75. Simopoulos AP. Summary of the conference on the health effects of polyunsaturated fatty acids in seafoods. J Nutr 1986; 116: 2350–2354.
76. Lands WE. Renewed questions about polyunsaturated fatty acids. Nutr Rev 1986; 44: 189–195.
77. Tahin QS, Blum M, Carafoli E. The fatty acid

composition of subcellular membranes of rat liver, heart and brain: diet-induced modifications. Eur J Biochem 1981; 121: 5–13.
78. Mounié J, Faye B, Magdalou J, Goudonnet H, Truchot R, Siest G. Modulation of UDP glucuronosyltransferase activity in rats by dietary lipids. J Nutr 1986; 116: 2034–2043.
79. Hammer CT, Wills ED. Biochem J 1978; 174: 585–
80. Huang YS, Nassar BA, Horrobin DF. Changes of plasma lipids and long-chain n-3 and n-6 fatty acids in plasma, liver, heart and kidney phospholipids of rat fed variable levels of fish oil with or without cholesterol supplementation. Biochim Biophys Acta 1986; 879: 22–27.
81. Swanson JE, Kinsella JE. Dietary n-3 polyunsatureted fatty acids: modification of rat cardiac lipids and fatty acid composition. J Nutr 1986; 116: 514–523.
82. Martinez M. Health effects of ω3 polyunsaturated fatty acids in seafoods. In: Simopoulos AP, Kiffur RR, Maretin RE, Barlow SM, eds. World Review of Nutrition and Diet. Vol. 66. Basel: Karger, 1991: 87–102.
83. Putnam JC, Carlson SE, De Voe PW, Barnes IA. The effect of variations in dietary fatty acids on the fatty acid composition of erythrocyte phosphatidylcholine and phosphatidylethanolamine in human infants. Am J Clin Nutr 1982; 36: 106–114.
84. Carlson SE, Carver JD, House SG. High fat diets varying in ratios of polyunsaturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. J Nutr 1986; 116: 718–725.
85. Guesnet P, Pascal G, Durand G. Effects of dietary a-linolenic acid deficiency during pregnancy and lactation on lipid fatty acid composition of liver and serum in the rat. Reprod Nutr Dev 1988; 28: 275–292.
86. Popp-Snijders C, Schouten JA, De Jong AP, Van Der Veen EA. Effect of dietary cod-liver oil on the lipid composition of human erythrocyte membranes. Scand J Clin Lab Invest 1984; 44: 39–46.
87. Durand G, Guesnet P, Desnoyer F, Pascal G. Effects of alpha-linolenic acid deficiency on the morphology and fatty acid composition of rat erythrocytes. Prog Lipid Res 1986; 25: 395–400