Composition of Nerve Biomembranes and Nutritional Fatty Acids

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Introduction

The brain, the spinal cord, and the peripheral nerves are the tissues that contain the highest concentration of lipids, other than adipose tissue. Since these lipids are practically all structural and not available for energy, they participate directly in the structure and hence, the function of cerebral membranes.

Cerebral development is genetically programmed, if one stage is missed or perturbed, the chances of recovery are extremely small. In addition, the renewal of neurons and oligodendrocytes is practically nil, and that of membranes is often very slow. Therefore, in the course of differentiation and multiplication, cells require adequate supplies of nutrients, particularly polyunsaturated fatty acids. A lipid abnormality leads to an alteration in the function of membranes.

Saturated and monounsaturated fatty acids are mainly synthesized by nerve tissue itself, via complex mechanisms that differ according to cell type and organelle.[1] In the nervous system, on average, one fatty acid of three is polyunsaturated. In fact, the polyunsaturated fatty acids present in the membranes are not the dietary precursors (linoleic and alpha-linolenic acids) but longer and more desaturated chains (mainly arachidonic, 20:4 n-6, and cervonic acids, 22:6 n-3). These control the composition of membranes, their fluidity and, as a result, their enzymatic activity, the binding between molecules and their receptors, cellular interactions, and the transport of nutrients. These fatty acids can also control certain electrophysiological parameters as well as learning functions. It is well known that dietary polyunsaturated fatty acids control the membrane levels of these fatty acids[2,3] and are particularly important for ensuring harmonious cerebral development.[4]

There are many reports on the influence of polyunsaturated fatty acids on the structure and function of the nervous system.[5-22] Polyunsaturated fatty acids 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 fatty acids (Table I).

If animals are given diets with varying amounts of linoleic and alpha-linolenic acid, serum levels of the n-6 fatty acid series are relatively stable while n-3 serum levels are correlated with dietary content. It is therefore extremely important to know precisely what quantity of alpha-linolenic acid should be supplied by the diet because serum levels, and consequently nerve membrane composition, depend on it.

Table I. Richness in Polyunsaturated Fatty Acids of Cerebral Cells and Organelles

	% Total Polyunsaturated Fatty Acids	% 20:4	% 22:6
Neurons	32	15	8
Synaptosomes	33	18	12
Oligodendrocytes	20	9	5
Myelin	15	9	5
Astrocytes	29	10	11
Capillaries	35	16	10
Mitochondria	30	16	12
Microsomes	29	11	12
Retina	45	5	35
Photoreceptor membrane	65	4	56
Peripheral nerve	10	7	2
Schwann cells	22	11	5

Results are expressed as % of total fatty acids ($mg^{C_{20}}$)

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Materials and Methods

Variations in Linoleic Acid

A strain of Wistar rats was submitted to a diet containing 2% sunflower oil. Three weeks before mating, 12 groups of females were submitted to 12 diets differing in their linoleic and alpha-linolenic acid content (6 diets contained 150mg/100g alpha-linolenic acid, the other 6 diets 300mg/100g). In each of the diets (containing 5% lipids), the linoleic acid content was obtained by mixing rapeseed, soybean, linseed, and palm oils. The n-6/n-3 ratio therefore varied from 1 to 10. The maximum dietary linoleic acid content was 3000mg/100g.

Variations in Alpha-linoleic Acid

Diet (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 (n-3) +. Two other groups of rats were fed diets containing either soybean oil or rapeseed oil. Rapeseed-fed animals were compared with peanut-fed animals, soybean animals with sunflower animals.

Either 15 days or 60 days after birth, animals receiving an (n-3)- diet were switched to an (n-3) + diet to study speed of recuperation at these two ages. Varying quantities of alpha-linolenic acid in the diet (for a given quantity of linoleic acid) were obtained by adding increasing amounts of rapeseed oil to African peanut oil (or by adding soybean oil to sunflower oil).

The separation of neurons, oligodendroyctes, astrocytes, myelin, nerve terminals (synaptosomes), mitochondria, and endoplasmic reticulum (microsomes) has been previously described.[10] Purity of the fractions was established by phase contrast microscopy, assay of enzyme markers, analysis of specific proteins (radioimmunoassay), electrophoresis, and lipid analysis.[10] Methods for extraction of lipids from these fractions, and their transmethylation to obtain methyl esters and analysis of the latter by gas phase capillary column chromatography have also been described.[10]

Resistance to Poisons

This was measured by intraperitoneal injection of triethyl lead. The LD_{50} was determined after establishment of LD_0, LD_{100}, and intermediate doses chosen in geometric progression. The LD_{50} was calculated using a linear regression program.

Electroretinogram and Learning Test

These were performed using methods already published.[23]

Results

Effects of Quantity of Dietary Linoleic Acid on the Nature and Quantity of n-6 and n-3 Polyunsaturated Fatty Acids in the Nervous System and Other Organs. Definition of the Minimum Indispensable Dietary Intake of Linoleic Acid

The quantity of arachidonic acid, 20:4 n-6 was very well regulated in nerve tissue. It was independent of dietary 18:3 n-3, provided that these levels were around the optimal value. In fact, the optimum level of 20:4 n-6 in nerve membranes was obtained as 150mg/100g 18:2 n-6 in the diet. If the amount of 18:2 n-6 in the diet was increased, the amount of 20:4 n-6 in brain, retina, sciatic nerve, and synaptosomes remained stable. A very slight increase was found in myelin. For the other organs, the amount of 20:4 n-6 increased parallel to the dietary content of 18:2 n-6 until this level reached 300mg for testicle and muscle, 800mg for kidney and 1200mg for the liver, lung and heart.

22:5 n-6 accumulated when dietary 18:2 n-6 increased. This accumulation depended on the dietary content of 18:3 n-3 for the brain and retina; on the other hand, it was relatively independent for sciatic nerve and myelin. If dietary 18:2 n-6 was excessive, the various organs accumulated 18:2 n-6 as well as 22:5 n-6.

Alpha-linolenic Acid Controls the Function of Nerve Membranes[23]

In animals given the (n-3)- diet, the cells and organelles showed a very marked deficiency in cervonic acid, which was generally compensated for by an excess of 22:5 n-6. The n-3/n-6 ratio was 16 times lower in oligodendrocytes, 12 times lower in myelin, twice as low in neurons, 6 times lower in synaptosomes, 3 times lower in astrocytes, 7 times lower in mitochondria, and 5 times lower in microsomes. On the other hand, saturated and monounsaturated fatty acids were practically unchanged.

Even though the brain is considered to be the best protected organ in the body, the membranes of brain cells and organelles are nearly as vulnerable to a deficiency in alpha-linolenic acid as those of the other organs. At any rate, there is some preservation of dietary alpha-linolenic acid (and reutilization of its very long-chain derivatives) since a 21-fold decrease in the diet only gave rise to a 5-fold decrease in the organs that we examined (Table II).

The importance of fatty acids of the n-3 series has also been demonstrated by specifically studying certain phos-

Table II. Quantities of 22:6 n-3 and 22:5 n-6 in (n-3)⁻ Animals Expressed as % of (n-3)⁺ Animals

	$\frac{(n\text{-}3)^-}{(n\text{-}3)^+} \times 100$	
Nervous System	**22:6 n-3**	**22:5n-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

Effects of (n-3)⁻ and (n-3)⁺ diets on 22:6 n-3 and 22:5 n-6 levels in cerebral cells and organelles. (n-3)⁺ diet: rapeseed or soybean oil. (n-3)⁻ diet: nut or sunflower oil.

pholipids such as phosphatidylethanolamine in animals fed diets containing peanut or rapeseed oil.[12]

Recuperation Rate of Anomalies[24,25]

After switching from the (n-3)- to the (n-3) + diet, several months were needed before brain cells and organelles recovered normal levels of cervonic acid and lost excess 22:5 n-6. This slow recovery was the same whatever the cell or organelle. It could be expected that recuperation would not be rapid in myelin, which has a slow turnover. However, it is surprising that nerve terminals also have slow recuperation because their membrane molecules are known for their rapid turnover rate. It can be suggested that regulation of recovery occurs either at the level of synthesis of chain ends in the liver (cervonic and arachidonic acids), from transport across the blood brain barrier, or from the enzymatic activities of desaturation and elongation which are known to be very weak in the liver after birth.[21,22] It is interesting to note that the endothelium of cerebral microvessel and capillaries also has a very slow rate of recovery even though it is in contact with plasma lipoproteins of normal composition in the liver which recuperate rapidly (within 2 weeks).[24]

Effects of Alpha-linolenic Acid Deficiency on Enzymatic, Electrophysiological, Behavioral and Toxicological Parameters

Enzymatic activities

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 altered in cell membranes (Table III). These results are in agreement with those of Bernsohn *et al.*[26] who have shown that a decrease in the activity of this enzyme produced by simultaneous deficiencies in linoleic and alpha-linolenic acids is only corrected by the addition of alpha-linolenic acid to the diet.

Na-K-ATPase is reduced nearly by half in the nerve terminals of animals fed an (n-3)- diet compared with those fed the (n-3) + diet (Table III). On the other hand, simultaneous deficiencies in linoleic and alpha-linolenic acids lead to increased Na-K-ATPase activity.[19] This enzyme controls ion transport produced by nerve transmission. It consumes half the energy used by the brain.

It is interesting to note that CNPase, which is specific for myelin, decreases as a result of alpha-linolenic acid deficiency, even though this membrane is considered to be very rigid and metabolically inactive. The activity of another enzyme, acetylcholine esterase, is also modulated by dietary lipids.[27] Some cerebral enzymes are unaffected by a deficiency in alpha-linolenic acid; N-methyl-transferase phosphocholinecytidyl-transferase (Fonlupt, personal communication), specific AMP and CMP cyclic nucleotide phosphodiesterase (Prigent, personal communication) as well as the binding of certain ligands such as dihydroalprenolol and diazepam to the membrane receptor (Fonlupt, personal communications). The effects of the nature of membrane fatty acids on enzymatic activities have been studied in many organs,[8,28] and in general the brain does not escape this rule.

Table III. Decrease in Membrane Enzyme Activities Due to a Deficiency in Alpha-linolenic Acid

	Brain	Myelin	Nerve terminals
5' nucleotidase	0.70	0.74	1.2
Na + K + ATPase	0.95	1.10	0.55
CNPase	0.95	0.78	0.00

Values represent enzyme activities obtained with (n-3)⁻ animals divided by those obtained with (n-3)⁺ animals

Electroretinogram[23]

Cervonic acid levels are high in the retina.[6] In the long term, overall deficiencies in polyunsaturated fatty acids induce changes in the distribution of membrane fatty acids in the retina which are associated with changes in the electroretinogram.[17]

In 4-week-old animals, the threshold of detection (10 uV) of wave A required a light stimulation 10 times stronger in the (n-3)- group than in that of the (n-3) + group. In 6-week-old animals, the electroretinogram changes were less marked and in the mature rats only wave A remained abnormal.[23]

Learning Tests

Simultaneous deficiencies in linoleic and alpha-linolenic acids affect the learning capacities of animals,[15,18] as does a selective deficiency in alpha-linolenic acid.[29] Though motor activity and open field tests were practically normal in (n-3)- animals, their learning capacities were severely perturbed, as shown by the shuttle box test. In the first session, (n-3) + animals made a more rapid association between the light stimulus and the electric shock, since they avoided on average 7 shocks of 30, whereas (n-3)- animals only avoided 2 of 30 shocks. These differences diminished with further conditioning and disappeared at the fourth session.[23]

Minimum Requirement of ALA Needed in Cerebral Membranes

When diets with intermediate levels of alpha-linolenic acid were given, increasing the amount of 18:3 n-3 led to an overall increase in 22:6 n-3, and inversely a decrease in 22:5 n-6. In fact, in the brain, levels of 22:6 n-3 increased linearly for an intake of 18:3 n-3 that varied from 0 to 200mg/100g diet and then reached a plateau (the opposite was observed for 22:5 n-6). In liver, kidney, and muscle the same threshold was found but the plateau was less clear. These precursors, linoleic and alpha-linolenic acids, have to be elongated and desaturated by the liver into longer chains, which are in fact the essential fatty acids for the brain, as cell cultures seem to have demonstrated.[31] Nerve cells in culture differentiate, multiply, and capture and liberate neurotransmitters only if the medium contains 20:4 n-6 and 22:6 n-3, but not in the presence of 18:2 n-6 and 18:3 n-3.[31,32]

Discussion

Linoleic Acid

This work is the first to study simultaneously all the polyunsaturated fatty acids of several organs as a function

of variations in dietary linoleic acid, minimum requirements in alpha-linolenic acid being assured. Serious dietary deficiency of linoleic acid results in a reduction in arachidonic acid as well as 22:4 n-6. There is a linear relationship between dietary linoleic acid and the concentration of 20:4 n-6 and 22:4 n-6 in the membranes of various tissues up to a certain threshold. Thereafter, tissue concentrations of these acids are nearly constant regardless of dietary linoleic acid levels. The requirement of linoleic acid is different according to the organ: it varies from 150 to 1200mg/100g food intake. Brain requirements of linoleic acid are very large during the perinatal period in man,[9-13] though it should be noted that high concentrations of linoleic acid during total parenteral nutrition in newborns alters the fatty acid profile of the liver as well as the brain.[33]

Alpha-linolenic Acid

A diet deficient in alpha-linolenic acid (sunflower or peanut oils) caused marked alterations in the fatty acid composition of all cellular and subcellular fractions examined. The total content of polyunsaturated fatty acids was not altered, the marked decrease in cervonic acid being compensated for by an increase in 22:5 n-6. This compensation is quantitative, but total unsaturation remains in deficit. It is evident that polyunsaturated fatty acids control the fluidity of biological membranes, hence many of their activities. A specific deficiency in n-3 fatty acids 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 recuperation is remarkably slow, it is several months before brain cells and organelles recover normal levels of cervonic acid. This rate is the same for all organelles. It is therefore crucial to supply the fatty acids necessary for cerebral structures at the right moment. A deficiency is difficult to correct.

Pathology resulting from alpha-linolenic acid deficiency has been described in the monkey,[34] and in man.[7,16,35,36] A syndrome of modern society has been suggested to be a deficiency in acids of the n-3 series.[37] It is therefore of great importance to verify the exact quantities of the n-3 series in the diet; a minimum must be provided to ensure that cerebral membranes have a normal composition and function. Dietary excess of linoleic acid leads to a specific accumulation of 22:5 n-6, which depends on dietary levels of alpha-linolenic acid. If the diet is deficient in alpha-linolenic acid there is an accumulation of 22:5 n-6, which then replaces the deficient 22:6 n-3.

Bearing in mind that the mean cerebral fatty acid composition of man is similar to that of the rat, in man a greater amount of brain is formed per day over a longer period, and the brain weight/total body weight ratio is greater, even taking into consideration the 2/3 coefficient. It is also evident that minimum levels in the rat are, a fortiori, those in man. At any rate, for evident ethical reasons, it will always be impossible to determine the effects of increasing doses of dietary fatty acids on the composition of cerebral membranes in man. The minimal levels are therefore:

— linoleic acid, 1200mg/100g food intake (2.4% of calories),

— alpha-linolenic acid, 200mg/100g food intake (0.4% of calories).

n-3 acid requirements in man are very large during the neonatal period and must be supplied to the mother during gestation, then to the newborn.[38] It has been shown that the nature of dietary fatty acids determines those in the blood of the infant. It should be noted that human milk contains quantities of alpha-linolenic acid, as well as cervonic acid, which are often absent from artificial milks. Human infants who receive artificial milks have red blood cells deficient in cervonic acid,[39] and red blood cell fatty acid composition can be considered an index of the composition of cerebral membranes.[40] In addition, there is a relationship between dietary lipids and the properties and structure of red blood cells.[41-43] Red blood cells are membranes whose composition is similar to that of cerebral membranes.

Deficiencies in polyunsaturated fatty acids can appear among the inhabitants of the third world, both infants and adults, among the elderly subjected to artificial diets, the ill (parenteral or controlled enteral nutrition), and in postoperative patients in whom requirements are increased.

It should be noted that the position of polyunsaturated fatty acid among the triacylglycerols can modulate its intestinal absorption and thus its serum fatty acid profile.[44]

Finally, membrane polyunsaturated fatty acids need to be heavily protected against peroxidation. Vitamin E is very probably a protector, but the mechanisms are mainly unknown.[45]

Advantages and Dangers of Very Long-Chain Precursors Derived from Fish Oil

Since cerebral structures are formed of very-long-chain polyunsaturated fatty acids, 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. However, it is known that large quantities of fish oil can lead to serious pathology in animals due partly to marked peroxidation of polyunsaturated fatty acids as a result of the absence of protectors such as vitamin E. If the diet of rats is supplemented with menhaden oil (1% low weight added to the normal diet), the profile of cerebral fatty acids is little altered, peroxidized derivatives do not appear, and there is no change in the activity of enzymes that protect against peroxidation: cytosolic superoxide dismutase containing Cu and Zn and mitochondria containing Mn, glutathione peroxidase, glutathione reductase, and catalase.[46]

On the other hand, large quantities of dietary fish oil, even supplemented with vitamin E, seriously perturb the fatty acid profile of the liver as well as that of the brain. In brain, there is a deficiency of arachidonic acid and a marked decrease in 22:4 n-6 and 22:5 n-6, associated with excess cervonic acid and 22:5 n-3.[47] Fish oils are of incontestable value in the prevention and treatment of cardiovascular disease and are used for their pharmacologic properties. The effects of fish oils must therefore be examined more closely from a dietary, pharmacologic and toxicologic point of view.

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