

STRUCTURAL AND FUNCTIONAL ROLES OF UNSATURATED FATTY ACIDS IN THE BRAIN. EFFECTS OF AGEING

Rôle des acides gras poly-insaturés sur la structure et la fonction du cerveau. Effet du vieillissement

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Résumé : Parmi tous les organes, et pour ce qui touche spécifiquement le cerveau, ce sont les acides gras $\omega 3$ qui ont été particulièrement. Le déficit en acide alpha-linolénique (18 : 3 $\omega 3$) altère la structure et la fonction des membranes, et entraîne de légers dysfonctionnements cérébraux, comme cela a été montré sur les modèles animaux puis chez les nourrissons humains. Les résultats récents ont montré que son déficit alimentaire induit des anomalies plus marquées dans certaines structures cérébrales que dans d'autres, le cortex frontal et l'hypophyse étant les plus touchés. Ces singularités sont accompagnées de perturbations comportementales qui touchent plus particulièrement certains tests (habituation, adaptation à une situation nouvelle). Les anomalies biochimiques et comportementales sont partiellement réversées par une supplémentation avec des phospholipides, notamment extraits d'œuf enrichis en $\omega 3$ ou de cervelle de porc. Une étude effet-dose montre que les phospholipides d'origine animale sont plus efficaces que ceux d'origine végétale pour réverser les conséquences du déficit, dans la mesure, entre autres, où ils apportent les très longues chaînes pré-formées. La carence en acide alpha-linolénique diminue la perception du plaisir, en altérant légèrement l'efficacité des organes sensoriels et en affectant certaines structures cérébrales. Au cours du vieillissement, la baisse de l'audition, de la vue et de l'odorat résultent tout autant de la diminution de l'efficacité des parties concernées du cerveau que des perturbations des récepteurs sensoriels, en particulier de l'oreille interne ou de la rétine. Un niveau de perception donné du goût du sucré exige, par exemple, une quantité de sucre plus grande chez ceux qui sont déficients en cet acide gras. Compte tenu des habitudes alimentaires des populations occidentales, le déficit en $\omega 6$ n'étant pas encore observé, son impact sur le cerveau n'a pas été étudié. En revanche, une carence en acides gras $\omega 9$, spécifiquement en acide oléique, induit une réduction de cet acide dans nombre de tissus, excepté le cerveau (mais le nerf sciatique est touché). Ainsi, cet acide ne serait pas synthétisé en















quantité suffisante, au moins pendant la période de gestation-lactation, impliquant une obligation d'apport alimentaire. Il convient de rappeler que la mise en place des neurones est pratiquement terminée quelques semaines avant la naissance, et que ceux-ci resteront toute la vie durant. De ce fait, une perturbation dans celle-ci, une altération de leurs connectivités, et un mauvais renouvellement de leurs composants à n'importe quel stade de la vie, ne pourront qu'accélérer le vieillissement. Les activités enzymatiques de synthèse des acides gras poly-insaturés à longues chaînes carbonées à partir des acides linoléique et alpha-linolénique sont très faibles dans le cerveau : cet organe dépend donc d'un approvisionnement exogène. De ce fait, les acides gras essentiels pour le cerveau sont les acides arachidonique et cervonique, qui proviennent de l'alimentation, à moins qu'ils ne soient synthétisés par le foie à partir des acides linoléique et alpha-linolénique. De ce fait la diminution des activités de désaturases hépatiques au cours du vieillissement (qui participent à la synthèse des longues chaînes, avec les élongases) peuvent induire des altérations du renouvellement des membranes du cerveau. Or, dans de nombreuses structures, dont surtout le cortex frontal, une diminution des acides cervonique et arachidonique est observée au cours du vieillissement, associée préférentiellement à une réduction des phosphatidyl-éthanolamines (principalement sous leur forme de plasmalogènes). Les activités d'oxydations peroxy-somales des acides gras poly-insaturés diminuent dans le cerveau au cours du vieillissement, participant au moindre renouvellement des acides gras membranaires, qui sont par ailleurs moins bien protégés contre les per-oxydations radicalaires.

Abstract : Among various organs, in the brain, the fatty acids most extensively studied are $\omega 3$ fatty acids. Alpha-linolenic acid (18 : 3 $\omega 3$) deficiency alters the structure and function of membranes and induces minor cerebral dysfunctions, as demonstrated in animal models and subsequently in human infants. Recent results have shown that dietary alpha-linolenic acid deficiency induces more marked abnormalities in certain cerebral structures than in others, as the frontal cortex and pituitary gland are more severely affected. These selective lesions are accompanied by behavioural disorders more particularly affecting

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certain tests (habituation, adaptation to new situations). Biochemical and behavioural abnormalities are partially reversed by a dietary phospholipid supplement, especially ω 3-rich egg yolk extracts or pig brain. A dose-effect study showed that animal phospholipids are more effective than plant phospholipids to reverse the consequences of alpha-linolenic acid deficiency, partly because they provide very long preformed chains. Alpha-linolenic acid deficiency decreases the perception of pleasure, by slightly altering the efficacy of sensory organs and by affecting certain cerebral structures. Age-related impairment of hearing, vision and smell is due to both decreased efficacy of the parts of the brain concerned and disorders of sensory receptors, particularly of the inner ear or retina. For example, a given level of perception of a sweet taste requires a larger quantity of sugar in subjects with alpha-linolenic acid deficiency. In view of occidental eating habits, as ω 6 fatty acid deficiency has never been observed, its impact on the brain has not been studied. In contrast, ω 9 fatty acid deficiency, specifically oleic acid deficiency, induces a reduction of this fatty acid in many tissues, except the brain (but the sciatic nerve is affected). This fatty acid is therefore not synthesized in sufficient quantities, at least during pregnancy-lactation, implying a need for dietary intake. It must be remembered that organization of the neurons is almost complete several weeks before birth, and that these neurons remain for the subject's life time. Consequently, any disturbance of these neurons, an alteration of their connections, and impaired turnover of their constituents at any stage of life, will tend to accelerate ageing. The enzymatic activities of synthesis of long-chain polyunsaturated fatty acids from linoleic and alpha-linolenic acids are very limited in the brain: this organ therefore depends on an exogenous supply. Consequently, fatty acids that are essential for the brain are arachidonic acid and cervonic acid, derived from the diet, unless they are synthesized by the liver from linoleic acid and alpha-linolenic acid. The age-related reduction of hepatic desaturase activities (which participate in the synthesis of long chains, together with elongases) can impair turnover of cerebral membranes. In many structures, especially in the frontal cortex, a reduction of cervonic and arachidonic acids is observed during ageing, predominantly associated with a reduction of phosphatidylethanolamines (mainly in the form of plasmalogens). Peroxisomal oxidation of polyunsaturated fatty acids decreases in the brain during ageing, participating in decreased turnover of membrane fatty acids, which are also less effectively protected against peroxidation by free radicals.

Figure 1. Nomenclature of the main fatty acids.

	COOH	Lauric acid	12:0
	COOH	Myristic acid	14:0
	COOH	Palmitic acid	16:0
	COOH	Stearic acid	18:0
	COOH	Oleic acid	18:1 (n-7)
	COOH	Linoleic acid	18:2 (n-6)
	COOH	Alpha-linolenic acid	18:3 (n-3)
	COOH	Arachidonic acid	20:4 (n-6)
	COOH	EPA	20:5 (n-3)
	COOH	DHA, cervonic acid	22:6 (n-3)
	COOH	Lignoceric acid	24:0
	COOH	Nervonic acid	24:1 (n-9)
	COOH	Cerebronic acid	24:2 (n-8)
	COOH	Phytanic acid	25:0

Introduction

The brain is composed of three main types of cells: neurons, astrocytes and oligodendrocytes. Other cells also have important roles, such as endothelial cells of cerebral capillaries. Neurons account for only about 1/4 of the weight of the brain. Nervous tissue has the highest lipid concentration, immediately after adipose tissue. These fatty acids, almost all structural and not energetic, participate directly in the architecture and therefore in the functioning of cerebral cell membranes, including those which ensure compartmentalization of the cell and individualization of its organelles. Life is strictly impossible in the absence of the two essential fatty acids because cell membranes can no longer be formed or maintained. At one time, these essential fatty acids were called "vitamin F". The cell membrane - composed of large quantities of phospholipids - ensures the individuality of cells and acts as a support for a large number of specific physiological activities. The fatty acid composition of a phospholipid is specific to the membrane (and cell) to which it belongs.

Fatty acids can be classified into two categories: non-essential fatty acids that are synthesized by all organs, including the brain (saturated or mono-unsaturated, possibly alpha-hydroxylated fatty acids), and essential fatty acids that must be provided in the diet. In fact, cerebral membranes are not composed of the dietary precursors, linoleic acid and alpha-linolenic acid, but their longer chain and more unsaturated derivatives.

An average of one in three fatty acids in the nervous system is polyunsaturated. Position 2 of phospholipids is generally occupied by a polyunsaturated fatty acid, which is usually 20:4 ω 6 (arachidonic acid), 22:4 ω 6 (adrenic acid), 22:5 ω 3 and especially 22:6 ω 3 (DHA, cervonic acid). Polyunsaturated fatty acids of the ω 3 series have very special roles in cell membranes, especially in the nervous system: all brain cells and organelles contain high levels of these fatty acids, but they are not present in sufficient quantities in modern French diets.

The importance of dietary ω 3 polyunsaturated fatty acids is well known, as these compounds decrease the incidence of cardiovascular disease and constitute precursors of biologically active derivatives. However, their structural role in membranes, including cerebral membranes, is also qualitatively and quantitatively very important. Among other roles, fatty acids control the composition of membranes and therefore their fluidity and consequently their enzymatic activities, binding between molecules and receptors, cellular interactions, and nutrient transport.

In fact, the first studies demonstrating the relationship between the effect of a nutrient and the structure and function of the brain were based on alpha-linolenic acid. A first discovery in 1984 demonstrated that alpha-linolenic acid deficiency induces abnormalities of the composition of the various cell types and organelles of the nervous system: neurons, astrocytes, oligodendrocytes, myelin, nerve endings, endoplasmic reticulum (1). These subjects present a very marked cervonic acid deficiency, which is generally compensated by an excess 22:5 ω 6 (fatty acids described by Galli (2)). The total quantity of polyunsaturated fatty acids is therefore relatively

normal; saturated and monounsaturated fatty acids are virtually not affected. Dietary alpha-linolenic acid is preserved in nervous tissues (and its very-long-chain derivatives are reutilized), as a 21-fold decrease in dietary intake results, at worst, in only a 5-fold decrease in alpha-linolenic acid levels in the various organs examined, and only a twofold decrease in neurons.

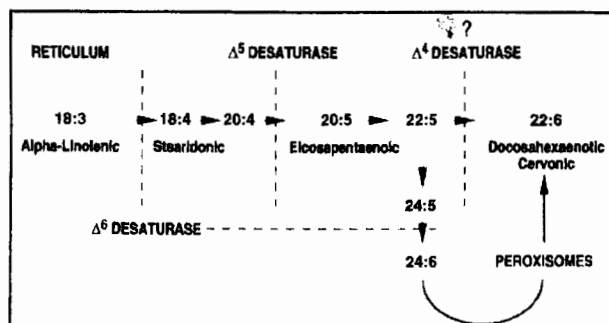
It was subsequently demonstrated, in 1989, in a study of the biochemistry, physicochemistry, toxicology, electrophysiology and behaviour in the same series of animals, that these fatty acids can control certain electrophysiological parameters, and certain higher functions (learning). A reduction of these fatty acids induces an alteration of membrane functioning (enzyme, receptor, transporter activities), and an increased susceptibility of these membranes to aggression. A dose-effect relationship has been demonstrated between the quantity of dietary alpha-linolenic acid and the DHA content of cerebral structures (3), in young as well as adult animals (4). The rate of recovery after correction of the deficiency is very slow (5, 6), including in the cerebral microcirculation (7). These results have been confirmed many times on many different models (8, 9). Brainfood have been documented in some books (10, 11).

Simultaneous linoleic acid and alpha-linolenic acid deficiency is obviously incompatible with life, as it alters the fatty acid composition of all organs, including the brain (2). In the course of evolution, the dietary acquisition of DHA largely contributed to the development of the human brain (12).

Studies can only be conducted on animal models, as it is obviously impossible to study the effects of nutrients on the human brain. Animal studies provide certain leads that can sometimes be partially validated in man, but they usually only provide confirmation.

This review will analyse most of the studies concerning unsaturated fatty acids and ageing. However, after a brief reminder of the general relationships between fatty acids of the three families and the brain, this review will only present recent data, i.e. studies conducted since 2000 until the end of January 2003. Only results concerning mammals will be presented, while data derived from birds (particularly hens, eggs, and possibly the chicks derived from these eggs) were not taken into account. Schematically, the very great majority of the published results concern $\omega 3$ fatty acids, and only a very small minority of studies are strictly devoted to $\omega 6$ fatty acids; when

Figure 2. Anabolism of long-chain polyunsaturated fatty acids from precursors, essential fatty acids (linoleic acid and alpha-linolenic acid).



$\omega 6$ fatty acids are mentioned, it is only in reference to $\omega 3$ fatty acids. Several interesting studies have been published on $\omega 6$ fatty acids, particularly oleic acid.

Nomenclature

Fats are mainly composed of molecules called triglycerides, in turn composed of fatty acids. The type of fatty acid determines the nutritional characteristics of each type of fat. "Saturated" fats are predominantly composed of saturated fatty acids; monounsaturated fats are predominantly composed of monounsaturated fatty acids (mainly oleic acid) and polyunsaturated fats contain useful quantities of polyunsaturated fatty acids. Two polyunsaturated fatty acids are essential, i.e. mammals, and therefore man, are unable to synthesize them or even transform them from one to the other. These fatty acids must therefore be supplied in the diet. Before identification of their chemical structure, they were grouped under the term "vitamin F". These essential fatty acids are called linoleic acid and alpha-linolenic acid, and each one constitutes the head of two families of fatty acids called $\omega 6$ (omega-6) and $\omega 3$ (omega-3) or (n-6) and (n-3) according to chemical nomenclature. Chemically, it would be more accurate to speak of polyethylene chains rather than polyunsaturated chains. The head of the $\omega 3$ family is therefore alpha-linolenic acid. Its derivatives are timnodonic acid (alias eicosapentaenoic acid (EPA), 22 : 5 $\omega 3$), which participates in the pharmacological effect of fish oil in the context of prevention and treatment of cardiovascular disease, and cervonic acid (alias docosahexaenoic acid (DHA), 22 : 6 $\omega 3$), as the brain is the living structure, which contains the greatest quantities of this fatty acid, which explains why it was discovered in this organ. The frontal brain, specific to man, is the region of the brain with the highest levels of DHA. All fatty acids in the $\omega 3$ family share a common characteristic : the first unsaturated bond, corresponding to a double chemical bond, is always situated on the 3rd carbon from the metabolically non-reactive methyl end of the chain. This facilitates description, as it is unnecessary to decline all of the names of all fatty acids of the family, presenting double bonds at different points along the carbon chain when counting from the biochemically reactive carboxyl end (according to chemical nomenclature) that undergoes various modifications.

Omega-3 fatty acids

Analysis of the fatty acid composition of total phospholipids of 11 regions of the murine brain shows that the level of 22 : 6 $\omega 3$, under normal conditions, is significantly higher in the frontal cortex. Alpha-linolenic acid deficiency does not affect all cerebral structures to the same degree : the pituitary gland, frontal cortex and corpus striatum are the structures most severely affected, with an approximate 40 % reduction of 22 : 6 $\omega 3$. An egg yolk or pig brain phospholipid supplement can restore a normal fatty acid composition in all regions of the brain, except for the frontal cortex. There is therefore a regional distribution of fatty acids in the brain and the impact of a fatty acid deficiency is consequently "region-specific" (13).

Under conditions of deficiency, $\omega 3$ fatty acids are preserved and reutilized by recycling mediated by deacylation and reacylation reactions, which is

decreased by only 30 % to 70 %, while transfer between the blood and the brain is reduced 40-fold (14), which explains the 50 % DHA deficiency initially observed in neurons (1).

Alpha-linolenic acid deficiency induces a reduction of DHA in the hippocampus, associated with a reduction of the size, but not the number of neurons (15), and a specific reduction of $\omega 3$ fatty acids associated with a reduction of a particular phospholipid, phosphatidylserine (16). In elderly rats, administration of fish oil increases the transcription of transthyretin in this structure (17) and decreases NGF ("nerve growth factor") (Ikemoto *et al.*, 2000). Inhibition of production of the second messenger controlled by protein-kinase constitutes a target for $\omega 3$ fatty acids, at least *in vitro* (18).

Possibilities of biochemical and behavioural recovery

The dietary use $\omega 3$ alone is unsatisfactory, as a diet enriched in fish oil enhances alertness and acquisition of learning in young mice, but decreases motor activity and learning in elderly mice (19).

Exploratory activity is significantly reduced in $\omega 3$ deficient mice. On the elevated plus-maze (a test which measures anxiety), the time spent in the open arms of the apparatus was significantly lower in $\omega 3$ deficient mice than in controls. The use of the learning protocol on the maze showed that $\omega 3$ deficiency impaired learning. A phospholipid supplement (in this case eggs enriched with $\omega 3$, but also containing $\omega 6$) reversed the biochemical and behavioural alterations induced by $\omega 3$ polyunsaturated fatty acid deficiency in mice, as exploratory activity and learning were totally restored after egg or pig brain phospholipid supplementation, while the level of anxiety was not restored to the same level as that of the controls. Recovery was therefore incomplete (20).

There is a time lag between incorporation of fatty acids in the brain and improvement of learning performances (21). Note that egg extracts enriched in $\omega 3$ and $\omega 6$ have been used for several years to prepare special milk formulas and that pig brain phospholipids have been used in small-for-dates infants (22).

A DHA-rich phospholipid supplement improves behaviour, learning and visual function in control elderly mice and mice with $\omega 3$ polyunsaturated fatty acid deficiency (23). Pure DHA (in the form of ethyl ester) provides similar results (24).

An intra-amniotic injection in $\omega 3$ fatty acid deficient animals corrects $\omega 3$ fatty acid deficiency of the foetal brain in 24 hours (25).

Overall, in man, $\omega 3$ fatty acids could reduce cognitive decline and the risk of dementia (26). In various types of dementia, including the Alzheimer type, low plasma levels of $\omega 3$ fatty acids (including in DHA) constitute a risk factor for these diseases (27). Abnormal phospholipid turnover has been described in schizophrenia (28); specific treatment with EPA would be useful in schizophrenia (29), and also in depression (30). In another field, neurologically disabled children absorb insufficient quantities of $\omega 3$ fatty acids, as reflected by the presence of serum markers of $\omega 3$ deficiency, 20 : 3 $\omega 9$ and 22 : 5 $\omega 6$, which interferes with effective renewal of their already damaged cerebral structures (31).

Dose-effects : triglycerides or phospholipids?

Alpha-linolenic acid is probably elongated and desaturated by the liver into longer chains that actually constitute the essential fatty acids for the brain, as initially discovered in nerve cell cultures, which only differentiate, multiply, bind and release neurotransmitters when the culture medium contains 20 : 4 $\omega 6$ and 22 : 6 $\omega 3$; but not in the presence of 18 : 2 $\omega 6$ and 18 : 3 $\omega 3$ (32). A recent dose-effect study showed that egg or pig brain phospholipids are better sources of polyunsaturated fatty acids than soybean phospholipids, as they directly provide long-chain polyunsaturated fatty acids (33). Similar results have been observed with $\omega 6$ fatty acids (34).

Neurotransmission, signalling, transport

Alpha-linolenic acid deficiency results in disorders of monoaminergic **neurotransmission** in the rat frontal cortex, that could be linked to the observed behavioural and cognitive deficits. There is obviously a relationship between polyunsaturated fatty acids, neurotransmission and behaviour (35, 36), but neither the density nor the function of dopamine transporters are affected (37). In alpha-linolenic acid deficient animals, the disorder of dopaminergic neurotransmission (in the prefrontal cortex, among others) is only partially reversed after correction of the dietary deficiency (38).

A PET study in conscious monkeys showed that modulation of cholinergic neurotransmission by DHA not only involves cerebral structures, but also cerebral blood flow (39).

In terms of **signalling**, intracellular and inter-cellular relationships may be affected by $\omega 3$ fatty acid deficiency, which decreases accumulation of phosphatidylserine that can induce modifications of signal contents (40).

A very important finding is that $\omega 3$ fatty acid deficiency can modify the energy metabolism of the brain by altering glucose **transport** (41).

Electrophysiological studies show that alpha-linolenic acid deficiency induces electroencephalographic changes (36).

Sense organs : vision, hearing, smell and taste

Vision is dependent on the nature of dietary fatty acids. The retina, part of the central nervous system, is one of the tissues with the highest levels of $\omega 3$ polyunsaturated fatty acids. $\omega 3$ fatty acid deficiency induces modifications of the distribution of membrane fatty acids of the retina associated with alterations of the amplitude of "a" and "b" waves on the electroretinogram (3).

In $\omega 3$ fatty acid deficient mice, phospholipid supplementation induces a significant increase in the amplitude of the "b" wave in both controls and deficient mice. It also restores a normal fatty acid composition of the retina (23). In fact, DHA plays an important role in vision, involving the retina and brain : photoreceptors, neurotransmission, activation of rhodopsin, development of cones and rods, neuronal synapses and maturation of cerebral structures (42).

As many behavioural tests directly or indirectly depend on vision, it is fundamental to observe that

learning deficits in alpha-linolenic acid deficient mice are not due to visual impairment (43).

The concentrations of DHA-rich phospholipids in the retina decrease during ageing (44).

Similar results have been reported for smell: alteration of tests assessing the sense of smell is not due to a reduction of olfactory function *per se*, but to an alteration of cerebral structures (45).

For the first time, it has been shown that $\omega 3$ fatty acid deficiency alters **hearing**, particularly the cerebral response to auditory stimuli. It also induces premature or more accelerated ageing of the auditory nervous system. Fatty acids affect the efficacy of sensory receptors, but also cerebral receptor structures (46). Phosphatidylcholine (lecithin) could preserve mitochondrial function of the cochlea, and consequently protect against age-related hearing loss (47).

$\omega 3$ fatty acid deficiency alters **taste**. For example, a given level of perception of a sweet taste requires a larger quantity of sugar in animals with alpha-linolenic acid deficiency (48).

Omega-6 fatty acids

Specific linoleic acid deficiency does not appear to occur in man, as, fortunately, apart from exceptional cases of artificial diet lasting several months, it would be difficult not to absorb linoleic acid, as this fatty acid is present in variable quantities in the majority of foods. Selective, serious linoleic acid deficiency has therefore never been observed in man, but perhaps because sufficiently detailed investigations have not been conducted.

Almost all of the studies listed in the references did not deal directly with $\omega 6$ fatty acids, but with their relationships with $\omega 3$ fatty acids. Only the minimum dietary requirements have been determined in animals (49). The effect of an increase of linoleic acid on tissue concentration of ceronic acid and consequently on alpha-linolenic requirement have been documented (50). In baboons, 22:4 $\omega 6$ is mainly formed from arachidonic acid (20:4 $\omega 6$). A reduction of the arachidonic acid content is observed during ageing, related to glutamate receptors (51).

Recovery after $\omega 3$ fatty acid deficiency is only possible if $\omega 6$ fatty acids do not induce excessive competition (52).

$\omega 6$ and $\omega 3$ fatty acids modulate neurotransmitter metabolism, at least in piglets (53) and, in rats, they participate in the hippocampus receptor density, which could account for the effects of these fatty acids on memory (54). Competition between $\omega 3$ and $\omega 6$ fatty acids does not concern recycling, but elongation and desaturation reactions (55).

In mice, maternal exposure to a diet very rich in $\omega 6$ fatty acids during pregnancy induces increased alcohol consumption in females of the litter (56), in line with observations showing that alcohol interferes with the fatty acids of nerve endings (57).

Consumption of foods with a well defined $\omega 3$: $\omega 6$ ratio is effective against various components of stress (58).

Omega-9 fatty acids: oleic acid

The nutritional value of oleic acid in the context of a balanced diet has been the subject of a number of studies, with particular emphasis on the cardiovascular system. However, very recent research has demonstrated that this fatty acid is also important for the brain (59). In the brain, $\omega 9$ fatty acids consist of oleic acid, but also very large quantities of longer-chain derivatives, mainly 24:1, especially in the myelin sheath.

Commercial vegetable oils cannot be used to precisely determine the effect of the presence and the concentration of oleic acid in the diet on the fatty acid composition of various organs, as they always contain oleic acid. Sufficiently large quantities of triglycerides to be compatible with nutritional investigations were therefore synthesized chemically and enzymatically; they were composed of either oleic acid, alpha-linolenic acid, or linoleic acid. Globally, in rats, dietary oleic acid deficiency leads to a reduction of the oleic acid concentration in many organs, including the sciatic nerve, but not in the brain. In many organs, endogenous synthesis therefore does not compensate for the absence of oleic acid in food (60). This fatty acid is therefore partially essential, especially during pregnancy and lactation, at least in rats.

The absence of modifications of the 18:1 $\omega 9$ concentration of cerebral structures according to the oleic acid content of the diet raises several hypotheses. Either the nervous system selectively binds oleic acid, suggesting the possibility of specific, active transport mechanisms across the blood-brain barrier, or it is able to synthesize all of the oleic acid that it needs, independently of its presence in the diet (59), as a steryl desaturase has been demonstrated to be active in the brain (61). In mice presenting signs of accelerated ageing, a reduction of this delta-9-desaturase is detected in the hippocampus, which could account for the observed behavioural disturbances (62).

Free fatty acids (including oleic acid) were assayed in the cerebrospinal fluid of a large series of patients (63). An oleic acid derivative, oleamide, modulates sleep (64) and reduces apoptosis of cerebellar neurons (65).

Ageing and fatty acid composition

A reduction of arachidonic acid incorporation in the brain and a reduction of its turnover are observed during ageing (66).

A recent study examined the composition of the human cortex between the ages of 2 years and 88 years (67). Globally, DHA and monounsaturated fatty acid concentrations increase up until the age of 18 years. During ageing, the levels of polyunsaturated fatty acids, especially arachidonic acid, decrease, while the level of alpha-linolenic acid increases. The polyunsaturated fatty acid content of phosphatidylethanolamine is markedly decreased in Alzheimer's disease (68).

The rat cortex and hippocampus present major changes in their polyunsaturated fatty acid composition, especially affecting the DHA content of phos-

phatidylethanolamine plasmalogens (69), associated with an alteration of their participation in membrane structures resulting in a reduction of cognitive performances, their participation in neurotransmission and also their antioxidant role during ageing (70).

Globally, during ageing, a reduction of the 20 : 4 ω 6, 22 : 4 ω 6 and 22 : 6 ω 3 concentration is observed in the cortex and cerebellum, associated with an increase in the 18 : 1 ω 9 and 20 : 1 ω 9 concentrations, mainly in phosphatidylethanolamines and phosphatidylserines (71, 72). These defects can be corrected by an appropriate diet (73, 74). A modification of the exchanges of choline and serine bases is also observed during ageing (75). The microsomal synthesis of phosphatidylethanolamines and phosphatidylserines is decreased during ageing (76). Another important finding is that phosphatidylserine decarboxylase decreases during ageing, reducing the production of phosphatidylethanolamine (77).

In the hippocampus of ageing rats, dietary alpha-linolenic acid deficiency induces a selective reduction of phosphatidylserine associated with increased MAO-B activity, but has no effect on the serotonin and noradrenaline contents (78).

An increase in the concentration of pro-inflammatory cytokine (interleukin-1-beta) is observed in the brain during ageing (79), which may be responsible for a certain degree of deterioration of certain cellular functions, especially as binding of interleukin-1 to its receptor inhibits the release of glutamate in hippocampal nerve endings of young rats, but not in ageing rats (80).

The reduction of cerebral glucose levels induces modifications of cerebral metabolism, resulting in peroxidation in ageing rats (81).

Turnover of phospholipids (especially phosphatidylcholine and phosphatidylethanolamine) and cholesterol in synaptic membranes is decreased during ageing (82). The lipid composition of mitochondria present in synapses is also affected, especially their linoleic acid content (83).

The increased cholesterol content of cerebral membranes induces an increased physical rigidity of these membranes, inducing functional disturbances; membrane fluidity can be restored by a carefully defined polyunsaturated fatty acid supplement (58); the optimal ratio between ω 6 fatty acids and ω 3 fatty acids is 5 : 1.

Ageing and desaturase activities

The essential fatty acids for the brain are arachidonic acid and cervonic acid, which are either provided in the diet or are synthesized by the liver from linoleic acid and alpha-linolenic acid. Consequently, the effects of ageing on desaturases (which, together with elongases, participate in the synthesis of long-chain fatty acids) can alter the turnover of cerebral membranes. Changes in hepatic desaturase activities during ageing vary according to the species : they are slightly modified (84, 85) or remain unchanged (86) in rats, and are decreased by one half in mice (87). This reduction is not observed in alpha-linolenic acid

deficient ageing animals (88), which probably allows better preservation of the few remaining ω 3 fatty acids. The specific activity of enzyme, but also the total desaturase capacity of the whole liver must be taken into account (89).

The reduction of delta-6-desaturase activity can be compensated by the addition of gamma-linolenic acid to the diet (90), which results in an increase in delta-6-desaturase kinetic parameters (V_m) for linoleic acid and alpha-linolenic acid in ageing animals (91). Blackcurrant seed oil contains stearidonic acid (18 : 4 ω 3) (92) and beneficial effects on health have been observed in man (93).

Hepatic desaturase activities depend on a number of factors, especially hormonal factors and vitamin B6 (94); hypertension can also accelerate the ageing process (95). The combination of vitamin B12 and phosphatidylcholine appears to attenuate the age-related learning deficit, at least in a murine model of accelerated ageing (96). In Alzheimer's disease, modifications of the cerebral fatty acid composition have been correlated with alterations of desaturase activities (97).

All mechanisms involved in the production and activity of desaturases can be influenced by the nature of membrane fatty acids. For example, the activity of an enzyme of the nuclear membrane of hepatocytes is dependent on the presence of ω 3 fatty acids (98).

The fatty acid composition of human adipose tissue is generally altered during ageing, sometimes independently of the composition of the diet (99).

Ageing and peroxisomal activities

Peroxisomes participate in oxidation of long-chain fatty acids, but they are also involved in the synthesis of very-long-chain polyunsaturated fatty acids, as, in the absence of delta-4 desaturase (which would have transformed 22 : 5 ω 3 into 22 : 6 ω 3), 22 : 5 ω 3 are elongated into 24 : 5 ω 3 in the endoplasmic reticulum and then desaturated by a delta-6-desaturase into 24 : 6 ω 3, which is then transformed into 22 : 6 ω 3 in peroxisomes (100) (Figure 2).

Peroxisomal oxidation of polyunsaturated fatty acids in the brain decreases during ageing (101).

trans fatty acids

As these fatty acids are non-physiological, it is reasonable to suppose that their incorporation into membranes would interfere with the functioning of these membranes, possibly accelerating the ageing process. Their harmful effect on foetal and child development has been suspected (102), especially as the levels of these fatty acids are equivalent in maternal and foetal plasma, indicating permeability of the placenta to these compounds (103). *trans* fatty acids are known to alter cholinesterases in various organs, including the brain (104).

The *trans* isomer of oleic acid is incorporated into the brain (105). In mice, the presence of this

trans fatty acid in the diet, but associated with a borderline essential fatty acid deficiency, only has a limited effect on cerebral development, but it alters behavioural tests (106).

trans polyunsaturated fatty acids are incorporated into the brain (107), in mitochondria and microsomes (108), in various cerebral structures, including microvessels, nerve endings and the retina, which contain the highest concentrations, twice the levels observed in myelin and the sciatic nerve. Alpha-linolenic acid deficient animals incorporate twice as much *trans* polyunsaturated fatty acids (109); however, the brain appears to be relatively protected (110), at least in animal models.

trans monounsaturated fatty acids must be distinguished from *trans* polyunsaturated fatty acids. One *trans* polyunsaturated fatty acid, CLA (conjugated linoleic acid), presenting important favourable physiological effects, can be integrated into phospholipids, but only to a limited extent in the brain (111), less than in adipose tissue, muscle, liver and kidneys (112). However, the effects of CLA on cerebral ageing have not yet been studied.

The incorporation of *trans* fatty acids modifies the level of monoamines in the cerebral cortex; this effect is reversible for the cortex, but not for the hippocampus (113).

Although the production of conjugated polyunsaturated fatty acids (especially the formation of oxidation products) indicates destruction of membrane fatty acids, this is not the only process which alters the fatty acid composition of membranes during ageing; a balanced dietary intake of $\omega 6$ and $\omega 3$ fatty acids must be taken into account (114).

Blood-brain fatty acid transport

By definition, essential polyunsaturated fatty acids must cross the blood-brain barrier, but the mechanisms of this transport are unknown. Several hypotheses have been proposed (115), including possible transport via the choroid plexus (116).

Age-related changes in the microcirculation include changes in the concentrations of trace elements, antioxidant enzymes (catalase is decreased, but not glutathione peroxidase) and fatty acids: the level of monounsaturated fatty acid increases, while the level of polyunsaturated fatty acids decreases and the level of saturated fatty acids remains stable (117).

The specific role of astrocytes in the synthesis of DHA from alpha-linolenic acid has not been fully elucidated (118, 119). A possible co-operation between astrocytes and endothelial cells of micro-vessels has been proposed (120). Depending on the conditions, serum free fatty acids or lipoprotein phospholipids could supply the brain with polyunsaturated fatty acids (121). A particular phospholipid, phosphatidylcholine, may act as a fatty acid transporter to the brain (122).

The major problem in terms of nutritional physiology concerns determination of the bioavailability of alpha-linolenic acid and DHA (123).

Oleic acid does not cross the blood-brain barrier (124).

Antioxidants and unsaturated fatty acids in the brain

A Medline database search reveals a small number of studies in this field: the combination of "polyunsaturated", "brain" and "ageing" provided only about one hundred references!

Although antioxidants are known to be important, particularly in the brain, the roles of the various components of vitamin E have been poorly elucidated. However, vitamin E deficiency is known to alter the fatty acid profile of the brain (125), and alpha-tocopherol is the only effective membrane antioxidant (126). It is difficult to increase the quantity of vitamin E in the brain, which is unfortunate, as its 20:4 $\omega 6$ and 22:6 $\omega 3$ fatty acids are easily peroxidized (127). The efficacy of protection against reactive oxygen species clearly decreases during ageing, accelerating destruction of membrane polyunsaturated fatty acids, which are less and less effectively renewed, constituting a vicious circle. An optimal dietary balance between $\omega 6$ fatty acids and $\omega 3$ fatty acids is therefore essential (128).

What about saturated fatty acids?

The mechanisms of synthesis of saturated fatty acids have been documented for many years in the brain (129, 130) and in peripheral nerves (131). Their intimate mechanisms are currently under investigation (132). These mechanisms are now universally accepted, except that lignoceric acid (C24:0) can be partially derived from the diet (133, 134), as illustrated by the reduction of lignoceric acid accumulation in the brain by the presence of monounsaturated fatty acids in the diet in patients with adrenoleukodystrophy (135). These studies have mainly concerned myelination, rather than the ageing process. Polyunsaturated fatty acids could possibly be re-utilized to synthesize all types of fatty acids (136).

Practical applications

The polyunsaturated fatty acid requirement of the human brain is considerable during the neonatal period and remains high throughout life, in order to ensure turnover of cell membranes and to preserve the integrity of cell functions, otherwise the ageing process would be accelerated. However, these very long chains are not synthesized in the brain, or only in small amounts.

During the antenatal period, they cross the placenta in only small quantities and are not synthesized by the placenta. The brain synthesizes only very small quantities in adults and almost none in the elderly (87). It is therefore probably essential to provide them in the diet. However, baboon foetal tissue has been shown to synthesize DHA from alpha-linolenic acid (137). Indeed, infant cerebellar grey and white matter acids is in relation to age and diet (138, 139).

For all ages in Human, proposed ratio $\omega 6/\omega 3$ by authorities is 5 to 6 (140), this number is also valuable for preterm infant formulas (141).

Cover of nutritional requirements in France : pregnant women and nursing mothers

During pregnancy, 600 grams of essential fatty acid are transferred from the mother to the foetus, which represents an enormous quantity of 2.2 grams per day, on average.

A study recently conducted in the Aquitaine region shows that women of childbearing age consume 90 % of their linoleic acid requirement in their diet, but only 40 % of their alpha-linolenic acid requirement (142). Similar results have been found in other countries, such as Canada (143) and Sweden (144). This deficiency could be prevented by the use of rapeseed, soybean or walnut oil. According to the recommended daily allowance during pregnancy (140), the dietary fat consumption by pregnant women must be globally increased by 15 %, but with doubling of the consumption of DHA and cervonic acid. Fish with a real nutritional quality could be useful in this situation, but this may not be the case for fish derived from fish farms.

The increased requirements for various nutrients during pregnancy are not considerable, except for several minerals such as iron, zinc or certain vitamins, such as vitamin D, but they are important (145). Following the example of supplementation of milk, it could be useful to add vitamin D to a seasoning oil, in view of its liposolubility.

However, the recommended daily allowances suppose that the future mother's diet is satisfactory, which is unfortunately not always the case, as illustrated by the SUVIMAX study. A significant proportion of women consume less than 2/3 of the recommended allowances for many vitamins, minerals and trace elements, including omega-3 fatty acids, as demonstrated by the Aquitaine study. The quality of the woman's diet obviously determines the quantities of ω 3 and ω 6 fatty acids received by the neonate (146). However, the use of linseed oil in nursing mothers does not increase the DHA level of her milk (147). In contrast, ω 3 long-chain fatty acid supplementation during pregnancy and breastfeeding increases the child's IQ (148, 149). In an animal model, addition of long-chain fatty acids combined with a reduction of precursors ensures a neuronal and glial profile similar to that obtained with maternal breastfeeding (150).

Blood lipid concentrations of docosahexaenoic and arachidonic acids at birth determine their relative postnatal changes in term infants fed breast milk or formula (151). Moreover, alpha-linolenic acid in cholesterol esters is a marker of alpha-linolenic acid intake in newborns (152).

Better dietary polyunsaturated fatty acids for the brain

What is the better diet for the brain, a diet containing precursors (alpha-linolenic acid) or long-chain fatty acids (DHA, cervonic acid)? In other words, is it better to eat vegetables or fish, bearing in mind that eggs (so-called omega-3) are situated between the two in terms of omega-3, as they can contain both. In fact, alpha-linolenic acid and DHA are both necessary, but it would be preferable to consume the precursor that can be utilized by normal metabolism or oxidized when it is present in excess (153), rather than long-chain fatty acids, which are

less effectively metabolized (toxic effects in the form of haemorrhages at very high doses, such as those consumed by Eskimos), as the brain is sensitive to excess levels (154, 19), although peroxidation is not increased provided vitamin E is present (155).

In view of current dietary habits, the most effective way to increase monounsaturated omega-9 and omega-3 fatty acids in the form of **alpha-linolenic acid** would be to eat rapeseed oil alone or mixed with other oils, provided they contain certain substantial quantities of rapeseed oil, which is not the case at the present time. The other oils rich in alpha-linolenic acid are soybean oil and walnut oil, but they have a much lower oleic acid content.

Fish and "omega-3" eggs provide substantial quantities of long-chain ω 3 fatty acids (**EPA and DHA**), but farmed fish must have been fed appropriate fats : the ω 3 content of their flesh can vary by a factor of 1 to 40 depending on whether they are fed vegetable oils (copra, etc.) or fish oils.

The nutritional quality of animal products can be improved by modifying the feed given to these animals. However, this measure is not very effective in ruminants, as the microbial activity of the rumen hydrolyses a considerable fraction of dietary polyunsaturated fatty acids, transforming them into non-essential fatty acids. However, this approach could be useful in monogastric species (such as pigs), substantial in poultry, spectacular for eggs, and essential in fish, especially carnivorous fish (75 % of all fish).

Tables 1 and 2 show the multiplication factors obtained by providing alpha-linolenic acid (in the form of rapeseed or linseed) or DHA (in the form of fish oil). The use of such products induces positive changes in consumers, especially on blood lipid parameters (156).

Table 1. Several examples of the multiplication factor obtained by feeding with alpha-linolenic acid (rapeseed or linseed).

	Alpha-linolenic acid	Omega-3 long-chain fatty acids
Pork rib-eye steak	3 to 6	0.9
Beef rib-eye steak	1 to 2	1
Chicken thigh	9	3
Egg	1 to 40	2 to 5

Table 2. Factor of multiplication by feeding with oils of fish.

	Alpha-linolenic acid	Omega-3 long-chain fatty acids
Beef loin	1.3	1 to 2
Chicken thigh	1	2 to 7
Salmon	-	5 to 20
Egg	4 to 8	2 to 6

According to 11 : (157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 156).

In fact, the most spectacular results presented in the above two tables are obtained by a very careful selection of the feed given to animals and would be relatively expensive. Fortunately, in practice, a team working in Brittany (156) has shown that replacement of part of the animal's feed by cooked linseed increases the cost of feed by about only 5 % for beef cattle or poultry; which corresponds to an increase in the total cost of production of about 1 to 2 %, for an extremely favourable result. It would be possible to exactly double the alpha-linolenic acid content of the meal, thereby

reaching recommended daily allowances : for example, eggs would contain 12 times more of than this precious fatty acid, rabbit would contain 10 times more, chicken would contain 4 times more, and pork and milk would contain 3 times more!

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REFERENCES

- Bourre J.M., Pascal G., Durand G., Masson M., Dumont O. and 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.
- Galli C., White H.B. and Paoletti R. Lipid alterations and their reversion in the central nervous system of growing rats deficient in essential fatty acids. *Lipids* 1971; 6 : 378-387.
- Bourre J.M., Francois M. and 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.* 1989a; 119 : 1880-1892.
- Bourre J.M., Dumont O., Pascal G. and Durand G. Dietary alpha-linolenic acid at 1.3 g/kg maintains maximal docosahexaenoic acid concentration in brain, heart and liver of adult rats. *J. Nutr.* 1993; 123 : 1313-1319.
- Youyou A., Durand G., Pascal G., Piciotti M., Dumont O. and Bourre J.M. 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.
- Bourre J.M., Durand G., Pascal G. and Youyou A. Brain cell and tissue recovery in rats made deficient in n-3 fatty acids by alteration of dietary fat. *J. Nutr.* 1989b; 119 : 15-22.
- Homayoun P., Durand G., Pascal G. and Bourre J.M. 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.
- Salem N., Moriguchi T. and Greiner R.S. *et al.* Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. *J. Mol. Neurosci.* 2001; 16 : 299-307.
- Wainwright P.E. Dietary essential fatty acids and brain function : a developmental perspective on mechanisms. *Proc. Nutr. Soc.* 2002; 61 : 61-69.
- Bourre J.M. La diététique du cerveau. Editions Odile Jacob. 1990a France. Comida inteligente, a dietética do cérebro, Gradiva. De la inteligencia y el placer, la dietética del cerebro, Biblioteca Mondadori. Brainfood, Little Brown, USA. La dietética del cervello, Sperling Kupfer, Italy. Intelligenz und Ernährung, Econ, Germany (1991).
- Bourre J.M. Diététique du cerveau : la nouvelle donne. Editions Odile Jacob. 2003.
- Crawford M., Bloom M. and Cunnane S. *et al.* Docosahexaenoic acid and cerebral evolution. *Word Rev. Nur. Diet.* 2001; 88 : 6-17.
- Carrie I., Clement M., de Javel D., Frances H. and Bourre J.M. Specific phospholipid fatty acid composition of brain regions in mice. Effects of n-3 polyunsaturated fatty acid deficiency and phospholipid supplementation. *J. Lipid Res.* 2000; 41 : 465-472.
- Contreras M.A., Greiner R.S., Chang M.C., Myers C.S., Salem N. and Rapoport S.I. Nutritional deprivation of alpha-linolenic acid decreases but does not abolish turnover and availability of unacylated docosahexaenoic acid and docosahexaenoyl-CoA in rat brain. *J. Neurochem.* 2000; 75 : 2392-2400.
- Ahmad A., Murthy M., Greiner R.S., Moriguchi T. and Salem N. A decrease in cell size accompanies a loss of docosahexaenoate in the rat hippocampus. *Nutr. Neurosci.* 2002; 5 : 103-113.
- Murthy M., Hamilton J., Greiner R.S., Moriguchi T., Salem N. and Kim H.Y. Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus. *J. Lipid Res.* 2002; 43 : 611-617.
- Puskas L., Kitajka K., Nyakas C., Barcelo-Coblijn G. and Farkas T. Short-term administration of omega 3 fatty acids from fish oil results in increased tranthyretin transcription in old rat hippocampus. *Proc. Natl. Acad. Sci. USA* 2003; 100 : 1580-1585.
- Mirnikjoo B., Brown S.E., Kim H.F., Marangell L.B., Sweatt J.D. and Weeber E.J. Protein kinase inhibition by omega-3 fatty acids. *J. Biol. Chem.* 2001; 276 : 10888-10896.
- Carrie I., Guesnet P., Bourre J.M. and Frances H. Diets containing long-chain n-3 polyunsaturated fatty acids affect behaviour differently during development than ageing in mice. *Br. J. Nutr.* 2000; 83 : 439-447.
- Carrie I., Clement M., de Javel D., Frances H. and Bourre J.M. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. *J. Lipid Res.* 2000; 41 : 473-480.
- Lim S. and Suzuki H. Changes in maze behavior of mice occur after sufficient accumulation of docosahexaenoic acid in brain. *J. Nutr.* 2001; 131 : 319-324.
- Ramirez M., Gallardo E.M., Souto A.S., Weissheimer C. and Gil A. Plasma fatty-acid composition and antioxidant capacity in low birth-weight infants fed formula enriched with n-6 and n-3 long-chain polyunsaturated fatty acids from purified phospholipids. *Clin. Nutr.* 2001; 20 : 69-76.
- Carrie I., Smirnova M., Clement M., de Javel D., Frances H. and Bourre J.M. Docosahexaenoic acid-rich phospholipid supplementation : effect on behavior, learning ability, and retinal function in control and n-3 polyunsaturated fatty acid deficient old mice. *Nutr. Neurosci.* 2002; 5 : 43-52.
- Lim S.Y. and Suzuki H. Dose-response effect of docosahexaenoic acid ethyl ester on maze behavior and brain fatty acid composition in adult mice. *Int. J. Vitam. Nutr. Res.* 2002; 72 : 77-84.
- Schiefermeier M. and Yavin E. n-3 Deficient and docosahexaenoic acid-enriched diets during critical periods of the developing prenatal rat brain. *J. Lipid Res.* 2002; 43 : 124-131.
- Kalmijn S. Fatty acid intake and the risk of dementia and cognitive decline : a review of clinical and epidemiological studies. *J. Nutr. Health Aging* 2000; 4 : 202-207.
- Conquer J.A., Tierney M.C., Zecevic J., Bettger W.J. and Fisher R.H. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids* 2000; 35 : 1305-1312.
- Fenton W.S., Hibbeln J. and Knable M. Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. *Biol. Psychiatry* 2000; 47 : 8-21.
- Puri B.K., Richardson A.J. and Horrobin D.F. *et al.* Eicosapentaenoic acid treatment in schizophrenia associated

- with symptom remission, normalisation of blood fatty acids, reduced neuronal membrane phospholipid turnover and structural brain changes. *Int. J. Clin. Pract.* 2000; 54 : 57-63.
30. Puri B.K., Counsell S.J., Hamilton G., Richardson A.J. and Horrobin D.F. Eicosapentaenoic acid in treatment-resistant depression associated with symptom remission, structural brain changes and reduced neuronal phospholipid turnover. *Int. J. Clin. Pract.* 2001; 55 : 560-563.
 31. Hals J., Bjerve K.S., Nilsen H., Svalastog A.G. and Ek J. Essential fatty acids in the nutrition of severely neurologically disabled children. *Br. J. Nutr.* 2000; 83 : 219-225.
 32. Bourre J.M., Faivre A. and Dumont O. *et al.* Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium. *J. Neurochem.* 1993; 41 : 1234-1242.
 33. Bourre J.M. and Dumont O. The administration of pig brain phospholipids versus soybean phospholipids in the diet during the period of brain development in the rat results in greater increments of brain docosahexaenoic acid. *Neurosci. Lett.* 2002; 335 : 129-133.
 34. Wijendran V., Huang M.C., Diao G.Y., Boehm G., Nathanielsz P.W. and Brenna J.T. Efficacy of dietary arachidonic acid provided as triglyceride or phospholipid as substrates for brain arachidonic acid accretion in baboon neonates. *Pediatr. Res.* 2002; 51 : 265-272.
 35. Chalon S., Vancassel S., Zimmer L., Guilloteau D. and Durand G. Polyunsaturated fatty acids and cerebral function: focus on monoaminergic neurotransmission. *Lipids* 2001; 36 : 937-944.
 36. Takeuchi T., Fukumoto Y. and Harada E. Influence of a dietary n-3 fatty acid deficiency on the cerebral catecholamine contents, EEG and learning ability in rat. *Behav. Brain Res.* 2002; 131 : 193-203.
 37. Kodas E., Page G. and Zimmer L. *et al.* Neither the density nor function of striatal dopamine transporters were influenced by chronic n-3 polyunsaturated fatty acid deficiency in rodents. *Neurosci. Lett.* 2002; 321 : 95-99.
 38. Kodas E., Vancassel S., Lejeune B., Guilloteau D. and Chalon S. Reversibility of n-3 fatty acid deficiency-induced changes in dopaminergic neurotransmission in rats: critical role of developmental stage. *J. Lipid Res.* 2002; 43 : 1209-1219.
 39. Tsukada H., Kakiuchi T., Fukumoto D., Nishiyama S. and Koga K. Docosahexaenoic acid (DHA) improves the age-related impairment of the coupling mechanism between neuronal activation and functional cerebral blood flow response: a PET study in conscious monkeys. *Brain Res.* 2000; 862 : 180-186.
 40. Hamilton L., Greiner R., Salem N. and Kim H.Y. n-3 fatty acid deficiency decreases phosphatidylserine accumulation selectively in neuronal tissues. *Lipids* 2000; 35 : 863-869.
 41. Ximenes D.S., Lavielle F., Gendrot G., Guesnet P., Alessandri J.M. and Lavielle M. Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. *J. Neurochem.* 2002; 81 : 1328-1337.
 42. Uauy R., Hoffman D.R., Peirano P., Birch D.G. and Birch E.E. Essential fatty acids in visual and brain development. *Lipids* 2001; 36 : 885-895.
 43. Carrie I., Clement M., de Javel D., Frances H. and Bourre J.M. Learning deficits in first generation OF1 mice deficient in (n-3) polyunsaturated fatty acids do not result from visual alteration. *Neurosci. Lett.* 1999; 266 : 69-72.
 44. Rotstein N.P., Illicheta de Boschero M.G., Giusto N.M. and Avelano M.I. Effects of aging on the composition and metabolism of docosahexaenoate-containing lipids of retina. *Lipids* 1987; 22 : 253-260.
 45. Catalan J., Moriguchi T., Slotnick B., Murthy M., Greiner R.S. and Salem N. Cognitive deficits in docosahexaenoic acid-deficient rats. *Behav. Neurosci.* 2002; 116 : 1022-1031.
 46. Bourre J.M., Durand G., Erre J.P. and Aran J.M. Changes in auditory brainstem responses in alpha-linolenic acid deficiency as a function of age in rats. *Audiology* 1999; 38 : 13-18.
 47. Seidman M.D., Khan M.J., Tang W.X. and Quirk W.S. Influence of lecithin on mitochondrial DNA and age-related hearing loss. *Otolaryngol. Head Neck Surg.* 2002; 127 : 138-144.
 48. Frances H., Drai P., Smirnova M., Carrie I., Debray M. and Bourre J.M. Nutritional (n-3) polyunsaturated fatty acids influence the behavioral responses to positive events in mice. *Neurosci. Lett.* 2000; 285 : 223-227.
 49. Bourre J.M., Piciotti M., Dumont O., Pascal G. and Durand G. Dietary linoleic acid and polyunsaturated fatty acids in rat brain and other organs. Minimal requirements of linoleic acid. *Lipids* 1990; 25 : 465-472.
 50. Bourre J.M., Dumont O. and Durand G. Does an increase in dietary linoleic acid modify tissue concentrations of ceronic acid and consequently alter alpha-linolenic requirements? Minimal requirement of linoleic acid in adult rats. *Biochem. Mol. Biol. Int.* 1996; 39 : 607-619.
 51. Ulmann L., Mimouni V., Roux S., Porsolt R. and Poisson J.P. Brain and hippocampus fatty acid composition in phospholipid classes of aged-relative cognitive deficit rats. *Prostaglandins Leukot. Essent. Fatty Acids* 2001; 64 : 189-195.
 52. Ikemoto A., Ohishi M. and Sato Y. *et al.* Reversibility of n-3 fatty acid deficiency-induced alterations of learning behavior in the rat: level of n-6 fatty acids as another critical factor. *J. Lipid Res.* 2001; 42 : 1655-1663.
 53. de La Presa O.S. and Innis S.M. Diverse, region-specific effects of addition of arachidonic and docosahexaenoic acids to formula with low or adequate linoleic and alpha-linolenic acids on piglet brain monoaminergic neurotransmitters. *Pediatr. Res.* 2000; 48 : 125-130.
 54. Farkas E., de Wilde M.C., Kiliaan A.J., Meijer J., Keijser J.N. and Luiten P.G. Dietary long chain PUFAs differentially affect hippocampal muscarinic 1 and serotonergic 1A receptors in experimental cerebral hypoperfusion. *Brain Res.* 2002; 954 : 32-41.
 55. Contreras M.A., Chang M.C. and Rosenberger T.A. *et al.* Chronic nutritional deprivation of n-3 alpha-linolenic acid does not affect n-6 arachidonic acid recycling within brain phospholipids of awake rats. *J. Neurochem.* 2001; 79 : 1090-1099.
 56. Cabanes A., de Assis S., Gustafsson J.A. and Hilakivi-Clarke L. Maternal high n-6 polyunsaturated fatty acid intake during pregnancy increases voluntary alcohol intake and hypothalamic estrogen receptor alpha and beta levels among female offspring. *Dev. Neurosci.* 2000; 22 : 488-493.
 57. Zerouga M., Beauge F., Niel E., Durand G. and Bourre J.M. Interactive effects of dietary (n-3) polyunsaturated fatty acids and chronic ethanol intoxication on synaptic membrane lipid composition and fluidity in rats. *Biochim. Biophys. Acta* 1991; 1086 : 295-304.
 58. Yehuda S., Rabinovitz S., Carasso R.L. and Mostofsky D.I. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. *Neurobiol. Aging* 2002; 23 : 843-853.
 59. Bourre J.M. and Dumont O. Dietary oleic acid not used during brain development and in adult in rat, in contrast with sciatic nerve. *Neurosci. Lett.* 2003; 336 : 180-184.
 60. Bourre J.M., Dumont O.L., Clement M.E. and Durand G. A. Endogenous synthesis cannot compensate for absence of dietary oleic acid in rats. *J. Nutr.* 1997; 127 : 488-493.
 61. Carreau J.P., Daudu O., Mazliak P. and Bourre J.M. Palmitoyl-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.
 62. Kumar V.B., Vyas K., Buddhiraju M., Alshaher M., Flood J.F. and Morley J.E. Changes in membrane fatty acids and delta-9 desaturase in senescence accelerated (SAMP8) mouse hippocampus with aging. *Life Sci.* 1999; 65 : 1657-1662.
 63. Pilitis J.G., Diaz F.G. and Wellwood J.M. *et al.* Quantification of free fatty acids in human cerebrospinal fluid. *Neurochem. Res.* 2001; 26 : 1265-1270.
 64. Huitron-Resendiz S., Gombart L., Cravatt B.F. and Henriksen S.J. Effect of oleamide on sleep and its relationship to blood pressure, body temperature, and locomotor activity in rats. *Exp. Neurol.* 2001; 172 : 235-243.
 65. Yang J.Y., Abe K., Xu N.J., Matsuki N. and Wu C.F. Oleamide attenuates apoptotic death in cultured

- rat cerebellar granule neurons. *Neurosci. Lett.* 2002; 328 : 165-169.
66. Gatti C., Noremborg K., Brunetti M., Teolato S., Calderini G. and Gaiti A. Turnover of palmitic and arachidonic acids in the phospholipids from different brain areas of adult and aged rats. *Neurochem. Res.* 1986; 11 : 241-252.
 67. Carver J.D., Benford V.J., Han B. and Cantor A.B. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Res. Bull.* 2001; 56 : 79-85.
 68. Edlund C., Soderberg M., Kristensson K. and Dallner G. Ubiquinone, dolichol, and cholesterol metabolism in aging and Alzheimer's disease. *Biochem. Cell Biol.* 1992; 70 : 422-428.
 69. Favrele S., Stadelmann-Ingrand S. and Huguet F. *et al.* Age-related changes in ethanolamine glycerophospholipid fatty acid levels in rat frontal cortex and hippocampus. *Neurobiol. Aging* 2000; 21 : 653-660.
 70. Brosche T. and Platt D. The biological significance of plasmalogens in defense against oxidative damage. *Exp. Gerontol.* 1998; 33 : 363-369.
 71. Lopez G.H., Ilincheta de Boschero M.G., Castagnet P.I. and Giusto N.M. Age-associated changes in the content and fatty acid composition of brain glycerophospholipids. *Comp Biochem. Physiol B Biochem. Mol. Biol.* 1995; 112 : 331-343.
 72. Giusto N.M., Salvador G.A., Castagnet P.I., Pasquare S.J. and Ilincheta de Boschero M.G. Age-associated changes in central nervous system glycerolipid composition and metabolism. *Neurochem. Res.* 2002; 27 : 1513-1523.
 73. McGahon B.M., Martin D.S., Horrobin D.F. and Lynch M.A. Age-related changes in synaptic function: analysis of the effect of dietary supplementation with omega-3 fatty acids. *Neuroscience* 1999; 94 : 305-314.
 74. McGahon B.M., Murray C.A., Horrobin D.F. and Lynch M.A. Age-related changes in oxidative mechanisms and LTP are reversed by dietary manipulation. *Neurobiol. Aging* 1999; 20 : 643-653.
 75. Ilincheta de Boschero M.G., Lopez G.H., Castagnet P.I. and Giusto N.M. Differential incorporation of precursor moieties into cerebral cortex and cerebellum glycerophospholipids during aging. *Neurochem. Res.* 2000; 25 : 875-884.
 76. Montanini I., Gatti C., Woelk H. and Porcellati S. The influence of polyunsaturated phosphatidylcholine on brain lipid synthesis during aging. *Farmacol. Sci.* 1983; 38 : 376-382.
 77. Salvador G.A., Lopez F.M. and Giusto N.M. Age-related changes in central nervous system phosphatidylserine decarboxylase activity. *J. Neurosci. Res.* 2002; 70 : 283-289.
 78. Delion S., Chalon S., Guilloteau D., Lejeune B., Besnard J.C. and Durand G. Age-related changes in phospholipid fatty acid composition and monoaminergic neurotransmission in the hippocampus of rats fed a balanced or an n-3 polyunsaturated fatty acid-deficient diet. *J. Lipid Res.* 1997; 38 : 680-689.
 79. Martin D.S., Lonergan P.E. and Boland B. *et al.* Apoptotic changes in the aged brain are triggered by interleukin-1beta-induced activation of p38 and reversed by treatment with eicosapentaenoic acid. *J. Biol. Chem.* 2002; 277 : 34239-34246.
 80. McGahon B., Murray C.A., Clements M.P. and Lynch M.A. Analysis of the effect of membrane arachidonic acid concentration on modulation of glutamate release by interleukin-1: an age-related study. *Exp. Gerontol.* 1998; 33 : 343-354.
 81. Benzi G., Pastoris O., Tentoni S. and Villa R.F. Modifications in cerebral lipid metabolism by severe glucose deprivation during aging. *Neurobiol. Aging* 1987; 8 : 457-463.
 82. Ando S., Tanaka Y., Toyoda nee O.Y., Kon K. and Kawashima S. Turnover of synaptic membranes: age-related changes and modulation by dietary restriction. *J. Neurosci. Res.* 2002; 70 : 290-297.
 83. Ruggiero F.M., Cafagna F., Petruzzella V., Gadaleta M.N. and Quagliariello E. Lipid composition in synaptic and nonsynaptic mitochondria from rat brains and effect of aging. *J. Neurochem.* 1992; 59 : 487-491.
 84. Bourre J.M., Piciotti M. and Dumont O. Delta 6 desaturase in brain and liver during development and aging. *Lipids* 1990; 25 : 354-356.
 85. Dinh L., Bourre J.M., Dumont O. and Durand G. Comparison of recovery of previously depressed hepatic delta 6 desaturase activity in adult and old rats. *Ann. Nutr. Metab.* 1995; 39 : 117-123.
 86. Bezdard J., Blond J.P., Bernard A. and Clouet P. The metabolism and availability of essential fatty acids in animal and human tissues. *Reprod. Nutr. Dev.* 1994; 34 : 539-568.
 87. Bourre J.M. and Piciotti M. Delta-6 desaturation of alpha-linolenic acid in brain and liver during development and aging in the mouse. *Neurosci. Lett.* 1992; 141 : 65-68.
 88. Dinh T.K., Bourre J.M. and Durand G. Effect of age and alpha-linolenic acid deficiency on delta 6 desaturase activity and liver lipids in rats. *Lipids* 1993; 28 : 517-523.
 89. Maniongui C., Blond J.P., Ulmann L., Durand G., Poisson J.P. and Bezdard J. Age-related changes in delta 6 and delta 5 desaturase activities in rat liver microsomes. *Lipids* 1993; 28 : 291-297.
 90. Biagi P.L., Bordoni A., Hrelia S., Celadon M. and Horrobin D.F. Gamma-linolenic acid dietary supplementation can reverse the aging influence on rat liver microsome delta 6-desaturase activity. *Biochim. Biophys. Acta* 1991; 1083 : 187-192.
 91. Hrelia S., Bordoni A., Motta P., Celadon M. and Biagi P.L. Kinetic analysis of delta-6-desaturation in liver microsomes: influence of gamma-linolenic acid dietary supplementation to young and old rats. *Prostaglandins Leukot. Essent. Fatty Acids* 1991; 44 : 191-194.
 92. Ulmann L., Poisson J.P., Blond J.P. and Bezdard J. Incorporation into liver microsomal lipids of linoleic and stearic acids and of their respective products of delta 6 and delta 9 desaturation, gamma-linolenic and oleic acids: effect of age and of blackcurrant seed oil. *Biochim. Biophys. Acta* 1991; 1086 : 230-236.
 93. Hornych A., Oravec S., Girault F., Forette B. and Horrobin D.F. The effect of gamma-linolenic acid on plasma and membrane lipids and renal prostaglandin synthesis in older subjects. *Bratisl. Lek. Listy* 2002; 103 : 101-107.
 94. Bordoni A., Hrelia S., Lorenzini A. *et al.* Dual influence of aging and vitamin B6 deficiency on delta-6-desaturation of essential fatty acids in rat liver microsomes. *Prostaglandins Leukot. Essent. Fatty Acids* 1998; 58 : 417-420.
 95. Narce M., Asdrubal P., Delachambre M.C., Gresti J. and Poisson J.P. Influence of spontaneous hypertension on n-3 delta-6-desaturase activity and fatty acid composition of rat hepatocytes. *Mol. Cell Biochem.* 1995; 152 : 7-12.
 96. Hung M.C., Shibasaki K., Yoshida R., Sato M. and Imaizumi K. Learning behaviour and cerebral protein kinase C, antioxidant status, lipid composition in senescence-accelerated mouse: influence of a phosphatidylcholine-vitamin B12 diet. *Br. J. Nutr.* 2001; 86 : 163-171.
 97. Nakada T., Kwee I.L. and Ellis W.G. Membrane fatty acid composition shows delta-6-desaturase abnormalities in Alzheimer's disease. *Neuroreport* 1990; 1 : 153-155.
 98. Ammouche A., Youyou Y., Durand G. and Bourre J.M. Effects of dietary fats on nucleoside triphosphatase activity and nuclear membrane fatty acid composition of rats during development. *Ann. Nutr. Metab.* 1994; 38 : 132-140.
 99. Bolton-Smith C., Woodward M. and Tavendale R. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. *Eur. J. Clin. Nutr.* 1997; 51 : 619-624.
 100. Sprecher H. The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. *Prostaglandins Leukot. Essent. Fatty Acids* 2002; 67 : 79-83.
 101. Bourre J.M. and Piciotti M. Alterations in eighteen-carbon saturated, monounsaturated and polyunsaturated fatty acid peroxisomal oxidation in mouse brain during development and aging. *Biochem. Mol. Biol. Int.* 1997; 41 : 461-468.
 102. Carlson S.E., Clandinin M.T., Cook H.W., Emken E.A. and Filer L.J. trans Fatty acids: infant and fetal development. *Am. J. Clin. Nutr.* 1997; 66 : 715S-736S.
 103. Koletzko B. and Muller J. Cis- and trans-isomeric fatty acids in plasma lipids of newborn infants and their mothers. *Biol. Neonate* 1990; 57 : 172-178.

104. Mahfouz M.M., Osman M.Y. and El Habet A.E. Effect of dietary trans fatty acids on cholinesterase and monoamine oxidase activities in different organs of rats. *Acta Biol. Med. Ger.* 1982; 41 : 355-363.
105. Cook H.W. The influence of trans-acids on desaturation and elongation of fatty acids in developing brain. *Lipids* 1981; 16 : 920-926.
106. Wauben I.P., Xing H.C., McCutcheon D. and Wainwright P.E. Dietary trans fatty acids combined with a marginal essential fatty acid status during the pre- and postnatal periods do not affect growth or brain fatty acids but may alter behavioral development in B6D2F(2) mice. *J. Nutr.* 2001; 131 : 1568-1573.
107. Pettersen J. and Opstvedt J. trans fatty acids. 5. Fatty acid composition of lipids of the brain and other organs in suckling piglets. *Lipids* 1992; 27 : 761-769.
108. Tahin Q.S., Blum M. and 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.
109. Grandgirard A., Bourre J.M. and Julliard F. *et al.* Incorporation of trans long-chain n-3 polyunsaturated fatty acids in rat brain structures and retina. *Lipids* 1994; 29 : 251-258.
110. Larque E., Zamora S. and Gil A. Dietary trans fatty acids in early life : a review. *Early Hum. Dev.* 2001; 65 : S31-S41.
111. Beyers E.C. and Emken E.A. Metabolites of cis,trans, and trans,cis isomers of linoleic acid in mice and incorporation into tissue lipids. *Biochim. Biophys. Acta* 1991; 1082 : 275-284.
112. Alasnier C., Berdeaux O., Chardigny J.M. and Sebedio J.L. Fatty acid composition and conjugated linoleic acid content of different tissues in rats fed individual conjugated linoleic acid isomers given as triacylglycerols small star, filled. *J. Nutr. Biochem.* 2002; 13 : 337-345.
113. Acar N., Chardigny J.M., Berdeaux O., Almanza S. and Sebedio J.L. Modification of the monoaminergic neurotransmitters in frontal cortex and hippocampus by dietary trans alpha-linolenic acid in piglets. *Neurosci. Lett.* 2002; 331 : 198-202.
114. Yamamoto N., Okaniwa Y., Mori S., Nomura M. and Okuyama H. Effects of a high-linoleate and a high-alpha-linolenate diet on the learning ability of aged rats. Evidence against an autoxidation-related lipid peroxide theory of aging. *J. Gerontol.* 1991; 46 : B17-B22.
115. Magret V., Elkhaili L. and Nazih-Sanderson F. *et al.* Entry of polyunsaturated fatty acids into the brain : evidence that high-density lipoprotein-induced methylation of phosphatidylethanolamine and phospholipase A2 are involved. *Biochem. J.* 1996; 316 : 805-811.
116. Bourre J.M., Dinh L., Boithias C., Dumont O., Piciotti M. and Cunnane S. Possible role of the choroid plexus in the supply of brain tissue with polyunsaturated fatty acids. *Neurosci. Lett.* 1997; 224 : 1-4.
117. Tayarani I., Cloez I., Clement M. and Bourre J.M. Antioxidant enzymes and related trace elements in aging brain capillaries and choroid plexus. *J. Neurochem.* 1989; 53 : 817-824.
118. Innis S.M. and Dyer R.A. Brain astrocyte synthesis of docosahexaenoic acid from n-3 fatty acids is limited at the elongation of docosapentaenoic acid. *J. Lipid Res.* 2002; 43 : 1529-1536.
119. Williard D.E., Harmon S.D., Kaduce T.L. and Spector A.A. Comparison of 20-, 22-, and 24-carbon n-3 and n-6 polyunsaturated fatty acid utilization in differentiated rat brain astrocytes. *Prostaglandins Leukot. Essent. Fatty Acids* 2002; 67 : 99-104.
120. Moore S.A. Polyunsaturated fatty acid synthesis and release by brain-derived cells in vitro. *J. Mol. Neurosci.* 2001; 16 : 195-200.
121. Spector A.A. Plasma free fatty acid and lipoproteins as sources of polyunsaturated fatty acid for the brain. *J. Mol. Neurosci.* 2001; 16 : 159-165.
122. Lagarde M., Bernoud N. and Brossard N. *et al.* Lyso-phosphatidylcholine as a preferred carrier form of docosahexaenoic acid to the brain. *J. Mol. Neurosci.* 2001; 16 : 201-204.
123. Pounes-Ballihaut C., Langelier B. and Houlier *et al.* Comparative bioavailability of dietary alpha-linolenic and docosahexaenoic acids in the growing rat. *Lipids* 2001; 36 : 793-800.
124. Edmond J. Essential polyunsaturated fatty acids and the barrier to the brain : the components of a model for transport. *J. Mol. Neurosci.* 2001; 16 : 181-193.
125. Clement M. and Bourre J.M. Alteration of brain and liver microsomal polyunsaturated fatty acids following dietary vitamin E deficiency. *Neurosci. Lett.* 1993; 164 : 163-166.
126. Clement M. and Bourre J.M. Graded dietary levels of RRR-gamma-tocopherol induce a marked increase in the concentrations of alpha- and gamma-tocopherol in nervous tissues, heart, liver and muscle of vitamin-E-deficient rats. *Biochim. Biophys. Acta* 1997; 1334 : 173-181.
127. Zanetti R. and Gatala A. Changes in n-6 and n-3 polyunsaturated fatty acids during lipid-peroxidation of mitochondria obtained from rat liver and several brain regions : effect of alpha-tocopherol. *Prostaglandins Leukot. Essent. Fatty Acids* 2000; 62 : 379-385.
128. Youdim K.A., Martin A. and Joseph J.A. Essential fatty acids and the brain : possible health implications. *Int. J. Dev. Neurosci.* 2000; 18 : 383-399.
129. Bourre J.M., Daudu O. and Baumann N. [Ontogenesis of three fatty acid synthesizing systems in cerebral microsomes : relation to myelination]. *Biochimie* 1976; 58 : 1277-1279.
130. Murad S. and Kishimoto Y. Chain elongation of fatty acid in brain : a comparison of mitochondrial and microsomal enzyme activities. *Arch. Biochem. Biophys.* 1978; 185 : 300-306.
131. Salles J., Sargueil F. and Knoll-Gellida A. *et al.* Fatty acid synthase expression during peripheral nervous system myelination. *Brain Res. Mol. Brain Res.* 2002; 101 : 52-58.
132. Knoll A., Sargueil F., Salles J., Garbay B., Lucet-Levannier K. and Cassagne C. Hydroxyacyl-CoA dehydrase and trans-2,3-enoyl-CoA reductase activities are consistent with long-chain fatty acid accumulation during rat brain development. *Neurosci. Lett.* 1999; 263 : 5-8.
133. Bourre J.M., Paturneau-Jouas M.Y., Daudu O.L. and Baumann N.A. Lignoceric acid biosynthesis in the developing brain. Activities of mitochondrial acetyl-CoA-dependent synthesis and microsomal malonyl-CoA chain-elongating system in relation to myelination. Comparison between normal mouse and dysmyelinating mutants (quaking and jimpy). *Eur. J. Biochem.* 1977; 72 : 41-47.
134. Singh I., Moser A.E., Goldfischer S. and Moser H.W. Lignoceric acid is oxidized in the peroxisome : implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. *Proc. Natl. Acad. Sci. USA* 1984; 81 : 4203-4207.
135. Moser H.W. and Borel J. Dietary management of X-linked adrenoleukodystrophy. *Annu. Rev. Nutr.* 1995; 15 : 379-397.
136. Menard C.R., Goodman K.J., Corso T.N., Brenna J.T. and Cunnane S.C. Recycling of carbon into lipids synthesized de novo is a quantitatively important pathway of alpha-[U-13C]linolenate utilization in the developing rat brain. *J. Neurochem.* 1998; 71 : 2151-2158.
137. Su H.M., Huang M.C., Saad N.M., Nathanielsz P.W. and Brenna J.T. Fetal baboons convert 18 : 3n-3 to 22 : 6n-3 in vivo. A stable isotope tracer study. *J. Lipid Res.* 2001; 42 : 581-586.
138. Jamieson E.C., Farquharson J. and Logan R.W. *et al.* Infant cerebellar gray and white matter fatty acids in relation to age and diet. *Lipids* 1999; 34 : 1065-1071.
139. Martinez M. and Mougan I. Fatty acid composition of human brain phospholipids during normal development. *J. Neurochem.* 1998; 71 : 2528-2533.
140. Legrand P., Bourre J.M., Descamps B., Durand G. and Renaud S. Apports nutritionnels conseillés pour la population française. *Lipides. Tec et doc Lavoisier.* 2000; 63-82.
141. Billeaud C., Bougle D. and Sarda P. *et al.* Effects of preterm infant formula supplementation with alpha-linolenic acid with a linoleate/alpha-linolenate ratio of 6 : a multicentric study. *Eur. J. Clin. Nutr.* 1997; 51 : 520-526.
142. Combe N. and Boué C. Apports alimentaires en acides linoléique et alpha-linoléique d'une population d'Aquitaine. *OCL.* 2001; 8 : 118-121.
143. Innis S.M. and Elias S.L. Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. *Am. J. Clin. Nutr.* 2003; 77 : 473-478.

144. Xiang M., Alfvén G., Blennow M., Trygg M. and Zetterstrom R. Long-chain polyunsaturated fatty acids in human milk and brain growth during early infancy. *Acta Paediatr.* 2000; 89: 142-147.
145. Martin A. Apports nutritionnels conseillés pour la population française. 2000; Tec et doc Lavoisier.
146. Heird W.C. The role of polyunsaturated fatty acids in term and preterm infants and breastfeeding mothers. *Pediatr. Clin. North Am.* 2001; 48: 173-188.
147. Francois C.A., Connor S.L., Bolewicz L.C. and Connor W.E. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am. J. Clin. Nutr.* 2003; 77: 226-233.
148. Agostoni C., Marangoni F., Giovannini M., Galli C. and Riva E. Prolonged breast-feeding (six months or more) and milk fat content at six months are associated with higher developmental scores at one year of age within a breast-fed population. *Adv. Exp. Med. Biol.* 2001; 501: 137-141.
149. Helland I.B., Smith L., Saarem K., Saugstad O.D. and Drevon C.A. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* 2003; 111: e39-e44.
150. Jumpsen J., Lien E.L., Goh Y.K. and Clandinin M.T. Small changes of dietary (n-6) and (n-3)/fatty acid content ration alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. *J. Nutr.* 1997; 127: 724-731.
151. Guesnet P., Pugo-Gunsam P. and Maurage C. *et al.* Blood lipid concentrations of docosahexaenoic and arachidonic acids at birth determine their relative postnatal changes in term infants fed breast milk or formula. *Am. J. Clin. Nutr.* 1999; 70: 292-298.
152. Babin F., Rodriguez A., Sarda P., Vandeputte B., Mendy F. and Descomps B. Alpha linolenic acid in cholesterol esters: a marker of alpha linolenic acid intake in newborns. *Eur. J. Clin. Nutr.* 2000; 54: 840-843.
153. Cunnane S. New developments in alpha-linolenate metabolism with emphasis on the importance of beta-oxidation and carbon recycling. *World Rev. Nutr. Diet.* 2001; 88: 178-183.
154. Bourre J.M., Bonneil M., Dumont O., Piciotti M., Nalbone G. and Lafont H. High dietary fish oil alters the brain polyunsaturated fatty acid composition. *Biochim. Biophys. Acta* 1988; 960: 458-461.
155. Ando K., Nagata K., Yoshida R., Kikugawa K. and Suzuki M. Effect of n-3 polyunsaturated fatty acid supplementation on lipid peroxidation of rat organs. *Lipids* 2000; 35: 401-407.
156. Weill P., Schmitt B., Chesneau G., Daniel N., Safradou F. and Legrand P. Effects of introducing linseed in livestock diet on blood fatty acid composition of consumers of animal products. *Ann. Nutr. Metab.* 2002; 46: 182-191.
157. Chanmugam P., Boudreau M. and Boutte T. *et al.* Incorporation of different types of n-3 fatty acids into tissue lipids of poultry. *Poult. Sci.* 1992; 71: 516-521.
158. Specht-Overholt S., Romans J.R. and Marchello M.J. *et al.* Fatty acid composition of commercially manufactured omega-3 enriched pork products, haddock, and mackerel. *J. Anim. Sci.* 1997; 75: 2335-2343.
159. Simopoulos A.P. New products from the agri-food industry: the return of n-3 fatty acids into the food supply. *Lipids* 1999; 34: S297-S301.
160. Mandell I.B., Buchanan-Smith J.G. and Holub B.J. Enrichment of beef with omega 3 fatty acids. *World Rev. Nutr. Diet.* 1998; 83: 144-59.
161. Mandell I.B., Buchanan-Smith J.G. and Campbell C.P. Effects of forage vs grain feeding on carcass characteristics, fatty acid composition, and beef quality in Limousin-cross steers when time on feed is controlled. *J. Anim. Sci.* 1998; 76: 2619-2630.
162. Baucells M.D., Crespo N., Barroeta A.C., Lopez-Ferrer S. and Grashorn M.A. Incorporation of different polyunsaturated fatty acids into eggs. *Poult. Sci.* 2000; 79: 51-59.
163. Surai P.F., MacPherson A., Speake B.K. and Sparks N.H. Designer egg evaluation in a controlled trial. *Eur. J. Clin. Nutr.* 2000; 54: 298-305.
164. Scollan N.D., Choi N.J., Kurt E., Fisher A.V., Enser M. and Wood J.D. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *Br. J. Nutr.* 2001; 85: 115-124.
165. Galobart J., Barroeta A.C., Cortinas L., Baucells M.D. and Codony R. Accumulation of alpha-tocopherol in eggs enriched with omega3 and omega6 polyunsaturated fatty acids. *Poult. Sci.* 2002; 81: 1873-1876.
166. Howe P.R., Downing J.A., Grenyer B.F., Grigonis-Deane E.M. and Bryden W.L. Tuna fishmeal as a source of DHA for n-3 PUFA enrichment of pork, chicken, and eggs. *Lipids* 2002; 37: 1067-1076.