

.....

Omega-6/Omega-3 Ratio and Brain-Related Functions

Shlomo Yehuda

Psychopharmacology Laboratory, Department of Psychology,
Bar Ilan University, Ramat Gan, Israel

While the general public typically perceives lipids and fats as ‘harmful’ components of the diet (as in the popular slogan: ‘fat kills’), the scientific story is in fact very different.

Among the major scientific research efforts of the recent period, in the area of neuroscience, we find the recognition of the ‘essential fatty acids’ (EFA). The profound effects of various fatty acids, and equally profound effects of their deficits, are appreciated by a variety of disciplines, including (but not necessarily limited to) lipid biochemistry, physiology, nutrition, psychology, psychiatry, and neurosciences at large. Recently, the issue of lipids, and fatty acids ratio in diets, became an important issue. Simopoulos [1] showed the historical shift from a ‘balanced’ omega-6 and omega-3 ratio diet, towards a marked and significant reduction in the omega-3 intake, and explained that the general ‘Western diet’ of today can in actual fact be considered an omega-3-deficient diet. Her concern is that this ‘deficiency’ may lead to coronary heart disease and high cancer mortality.

Linoleic acid (LA; omega-6; $\omega 6$; 18:2 $\omega 6$) is the parent fatty acid of the omega-6 group. Linoleic acid must be supplied to the body by the diet, because the body is unable to synthesize it. All other members of the omega-6 group are derivatives of LA. Similarly, the parent compound of the omega-3 group is alpha-linolenic acid (ALA, omega-3, $\omega 3$, 18:3 $\omega 3$). All other members of the omega-3 group are merely derivatives of ALA, and together they form the polyunsaturated fatty acids (PUFA). In each group, the derivatives can convert to longer chain fatty acids by using two mechanisms: desaturation and elongation.

The enzymes that are involved in these mechanisms have the same functions in the two fatty acid groups, and therefore the omega-6 and omega-3 fatty acids 'compete' for the same enzymes.

While from a chemical point of view the differences between omega-6 and omega-3 acids are very small, and may appear insignificant, they exert different and sometimes even opposite effects. These opposing effects are not easily explained. It was recently suggested [2] that the distinction between omega-6 and omega-3 PUFA is based on the differential capacity of protein in large, and membrane-bound protein in particular, to 'recognize' various PUFA. The dietary deficiency of omega-3 fatty acids, as well as the particular roles of omega-6 and omega-3, will become obvious as we take a deeper look into the ratio issue.

The Importance of the Ratio

There are several aspects to the issue of the optimal recommended ratio between omega-6 and omega-3 fatty acids. One aspect is the recommendation for total daily dietary intake in various phases of life (e.g. infancy, pregnancy, adulthood and old age). Another aspect is the optimal ratio of PUFA as a food supplement or treatment. PUFA are used in the body in a variety of conditions, such as in dermatological diseases and in cardiovascular disorders. One particular area is the role of PUFA in the brain and the utility of PUFA to protect and stabilize the neuronal membrane in health and in disease. Our comments in this chapter will be limited to PUFA in the central nervous system (CNS) and CNS conditions.

The effects of PUFA on brain function can be divided into at least five categories: (1) modification of neuronal membrane fluidity; (2) modification of membrane activity-bound enzymes; (3) modification of the number and affinity of receptors; (4) modification of the function of neuronal membrane ionic channels, and (5) modification of the production of neurotransmitters and brain peptides [3].

Many studies have demonstrated that various PUFA mediate, or are associated with, several aspects of brain activity, ranging from the role of EFA in neuronal structure and functions, long-term potentiation (LTP), specific brain activation, prostaglandin activity, to neurological and mental disorders, to mood control. Unfortunately, the vast majority of these studies merely test one or two specific fatty acids, and there are very few solid studies that experimentally examine a wide range of ratios between omega-6 and omega-3. The following review will summarize the areas in which studies on ratios were performed, and

will subsequently have to leave out some of the most fascinating areas such as depression, psychosis and pain.

Essential Fatty Acids, the Blood-Brain Barrier, and the Brain

Since EFA must be supplied via the diet, two major issues arise:

Firstly, do EFA and PUFA have the possibility to cross the blood-brain barrier (BBB)? Recently, Rapoport [4] and Edmond [5] provided a detailed discussion on the complex mechanism of delivery of essential PUFA, as it progresses from the blood into the brain. In two developmental periods the involvement of the BBB is crucial, i.e. in infancy and in aging. The human infant is born with immature BBB and during these periods the structure and functions of the BBB are not at their optimal levels. There have been reports of structural changes in the BBB complex, in aging and in Alzheimer's patients [6, 7], but despite the knowledge about structural changes, the knowledge of functional changes is quite limited. Most of the studies did not find changes in the rate of transport of PUFA into the brain during aging [e.g. 8, 9]. The important, but so far unanswered question is whether omega-6 and omega-3 fatty acids have different rates of transport into the brain.

The second issue regarding the BBB is the brain's ability to convert linoleic and alpha-linolenic acids into longer chain fatty acids, arachidonic acid (AA) and docosahexaenoic acid (DHA). Some researchers suspect that the immature brain of the infant is unable to convert these fatty acids to longer chain fatty acids. However, the majority of studies agree that even the infant brain indeed does have such capacity [4, 10].

Brain Neurotransmitters and PUFA

The relationships between PUFA ratios and the various neurotransmitters, as described before [11], are of special interest. It is important to note that omega-3 deficiency induced reduction in dopamine vesicle density in the cortex [12], and malfunction of the dopaminergic mesocorticolimbic pathway [13]. The ability to recover from the dopaminergic effects of omega-3 deficiency is age-dependent [14–16]. Also, the effects of alpha-linolenic acid for recovering from omega-3 deficiency is dependent on spatial configuration. The trans-isomer of alpha-linolenic acid is inactive and if not enough *cis*-isomer is supplied, a state of omega-3 deficiency will occur [16]. Some studies indicate similar effects on the serotonergic system [17].

Membrane Fluidity and Myelin

Before considering the effects of PUFA on brain-mediated functions, the effects of PUFA on brain structural components must be elucidated. Fatty acids and lipids are major components in brain structure. Very high levels of fatty acids and lipids can be found in two structural components; in the neuronal membrane and in the myelin sheaths. The neuronal membrane is composed of two lipid layers. The ratio between the proteins and the lipids is about 50–50%, while lipids (about 70%) constitute the majority in the myelin sheaths (about 30%). The protein component is especially stable, while the lipid component has a relatively high turnover rate.

In order to understand the diverse functions, which seem to be mediated by the various PUFA and by the ratio of omega-6 to omega-3, we [18, 19] proposed that the membrane fluidity index is the common denominator for the various effects of PUFA. Some molecules are able to change the physical state (e.g. the fluidity index) of the membrane. For example, alcohol fluidizes the membrane, while cholesterol hardens it. There are two basic questions regarding the hypothesis that PUFA are able to modify the neuronal membrane fluidity index; firstly, whether changes in the lipid component of the neuronal membrane (e.g. different ratio of various fatty acids) would lead to changes in the neuronal functions, and secondly whether supplementation of various fatty acids would affect the composition and the function of the neuronal membrane. A number of studies have shown that EFA supplementation, under certain conditions of composition and time, indeed modifies both the structure and membrane function (summary can be found in Yehuda et al. [20]). (More detailed studies will be described below.)

The integrity of the myelin is of utmost importance for the proper functions of axons in the nervous system. Breakage or lesions in the myelin can lead to disintegration of many of the nervous system functions. Recent studies emphasize the major role of dietary EFA to the normal functions of myelin. Moreover, the EFA are very important in the active phase of the myelin synthesis. If EFA are not available in this phase, or metabolically blocked, amyelination, dysmyelination or demyelination may occur [21, 22]. If EFA deficiency occurs during the postnatal period, a major delay in the myelination process will occur, accompanied by impaired learning, motor, vision, and auditory abnormalities [23]. It is of great interest to note that similar impairments in the myelination process and in the cognitive function can also be found in postnatally iron-deficient rodents and humans [24]. Disorders, which are associated with myelin malfunction or with dysmyelination, can also occur during the adult period. One such disorder is multiple sclerosis. The rate of myelin lipids turnover is age-dependent. The turnover rate is very slow during

aging, and therefore the rate of repairing damaged sections of myelin is slower in aging [25].

Prostaglandins

One major issue among the PUFA researchers is what particular fatty acid among the omega-6 or omega-3 PUFA group is preferable to study. On the one hand, some prefer to study linoleic and alpha-linolenic acids, as these are the precursors to all other PUFA molecules and they have a powerful effect on the neuronal membrane. Others prefer to study longer chain fatty acids, as they are the precursors to the special group of prostaglandins.

Essential fatty acids are considered to be a special class of unsaturated fatty acids that also act as precursors of yet other types of fatty acids. Most of the prostaglandins are derivatives of AA (arachidonic acid; itself derived from omega-6) or from DHA (docosahexanoic acid, omega-3) and all of them have a high physiological, hormone-like, activity level. A review on prostaglandins was recently published [19]. The various prostaglandins are involved in numerous brain functions, sometimes with conflicting or opposing effects. Prostaglandins are involved in functions such as regional blood flow and permeability of various biological membranes. It has been suggested that prostaglandins are also involved in the functional level of the activity of cAMP (a second messenger) in the cells. The behavioral and physiological effects of a specific ratio of an omega-6/omega-3 compound (in a ratio of 4:1) correlates with changes in the fatty acid profile and with changes in the cholesterol level [20]. It may well be that such a compound has an effect on the prostaglandin system as well and mediates the behavioral and biochemical changes that have been observed in the rats. There is evidence that prostaglandin D₂ has a profound beneficial effect on sleep. On the other hand, other prostaglandins enhance CRF (corticotropin-releasing factor) activity, which enhances wakefulness. CRF, in turn, induces the release of prostaglandins. Prostaglandins also enhance TRH (thyrotropin-releasing hormone) release, and stimulate the dopaminergic and noradrenergic receptor activity, while beta-endorphin inhibits the prostaglandin synthesis [11]. Very few studies examine the effect of different ratios on production and/or activity of various prostaglandins.

Cholesterol and Fatty Acids

The membrane fluidity index is dependent on two major factors: (1) the level and the composition and percentage of PUFA in the membrane, and

(2) the level of membrane cholesterol. An increase in the PUFA level will result in fluidizing of the neuronal membrane, while an increase in cholesterol will harden the membrane. The membrane should be at an optimal physiological gel state. Therefore, cholesterol, which is a complex lipid, is involved in many functions in the membrane. It is well established that cholesterol decreases the membrane fluidity index, which affects the activity of ion channels and receptor functions, as well as the dopamine release. Moreover, cholesterol is a key molecule in the end product of the CRF-ACTH (adreno-cortico-tropic-hormone) axis. Considering that steroids are derivatives of cholesterol, it is of great interest to find that various fatty acids have differential effects on cholesterol metabolism. Many reliable studies confirm that the administration of omega-6 fatty acids reduces the level of cholesterol in the blood. However, omega-6 fatty acids and omega-3 fatty acids differ in their mode of action in cholesterol reduction, such that omega-6 fatty acids redistribute cholesterol while the omega-3 fatty acids actually reduce the level of cholesterol in the neuronal membrane. This may explain why an increase in the cholesterol level in the blood is found in humans who consume omega-3 fatty acid supplements. It has been demonstrated that omega-3 essential fatty acids are more effective in reducing cholesterol levels in macrophages than omega-6 essential fatty acids. This is most probably due to the differential effect on the enzyme acyl-coenzyme A activity. However, some studies have indicated that cholesterol-esterifying enzymes that incorporate free fatty acids into cholesterol esters, without the participation of CoA, are also present in the rat brain [26].

The mechanism by which omega-6 or omega-3 fatty acids are able to reduce the cholesterol level in the blood or in the neuronal membrane is still unclear, although several hypotheses have been proposed. For example, Bourre et al. [27] claimed that alpha-linolenic acid controls the composition of nerve membranes, which implies an inverse relationship between alpha-linolenic acid and cholesterol level. Salem et al. [28] proposed that docosahexanoic acid (DHA) controls the level of cholesterol as well as the composition and function of the neuronal membrane. Another approach [unpubl. data] suggests that the differential effects of omega-6 or omega-3 on the cholesterol level, depends on the activity of PUFA on reduced LDL-receptor activity. A negative correlation was found between membrane cholesterol level and improvement in learning capacity. A number of studies provide support for reducing neuronal membrane cholesterol by dietary supplementation of an omega-6/omega-3 compound in a ratio of 4:1 [29]. Such correlation was not found with other ratios. It is possible that this specific ratio (4:1) optimizes uptake of PUFA into the brain and promotes fatty acid incorporation into the neuronal membranes, while displacing cholesterol out of the membrane. The issue of the cholesterol neuronal membrane level is very important, as the level of cholesterol (and

cholesterol metabolites) in the aging and in the Alzheimer's patient's brain is very high.

Omega-3 Deficiency

The method of inducing omega-3 deficiency via diet is a powerful tool to investigate the role of omega-3 in various brain functions. While most studies in this area involved the omega-3 deficiency issue, by definition, the ratio of omega-6 and omega-3 in experimental diets was different from the ratio in normal or balanced diets. Studies have shown that omega-3-deficient rats (mostly the 3rd generation with deficiency) exhibited poor learning and memory performances in a variety of tests, such as Morris Water Maze [30, 31], and olfactory-based learning and memory tasks, mainly in complex (vs. simple) learning [32, 33]. In addition, sensory deficits were evident in those rats, such as visual problems [34, 35]. One of Salem's studies [28] is of specific interest in this aspect; his omega-3-deficient rats showed very poor performance in spatial tasks and in olfactory-cued reversal learning tasks. However, he did not find any difference in the hippocampus gross morphology. Several hypotheses can be offered to explain the finding of poor learning. Omega-3 deficiency induces a significant decrease in the neuron size in the hippocampus, hypothalamus and cortex [36] – brain areas that mediate spatial and serial learning. In addition, omega-3 deficiency induces a significant reduction in cerebral catecholamines [37], in glucose transport capacity and glucose utilization in the brain [38], in the cyclic AMP level in the hippocampus [39], and in brain phospholipid synthesis capacity in the brain and in hypothalamus [40, 41]. Each one of those changes (in the levels of catecholamines, glucose, cAMP and phospholipids) can induce learning deficit.

The above-mentioned studies demonstrate two points; firstly, the essentiality of omega-3 fatty acids to the structure and normal function of the brain. Secondly, they demonstrate the importance of the ratio. It is impossible to induce omega-3 deficiency without offsetting the ratio between omega-6 and omega-3 in the diet. Though the authors did not specifically discuss the ratio of their control diet, our own calculations of the dietary information in the above-mentioned studies showed that they in fact used the ratio of omega-6/omega-3 of 4:1–5:1.

Early Development

Most studies on omega-3 deficiency singled the early development periods as an important, and almost 'critical' period for brain development.

Studies stress the importance of the influence of the fatty acids profile in mothers' milk on brain maturation. Jumpsen et al. [42, 43] showed that even small changes in the ratio of omega-6 to omega-3 in the diet, during neuronal and glial cell development, have significant effects on the development. He showed that a ratio of 4:1 was the optimal ratio for the development of the frontal cortex, hippocampus, cerebellum and glial cell number in developing rats. Small changes in the ratio, such as 6:1, impaired the rate of development.

Though the discussion whether the addition of long chain PUFA (LCPUFA) to baby formula is recommended is outside of the scope of this chapter, it seems that the importance of the level of PUFA and the omega-6/omega-3 ratio in the diet of the infant, in this sensitive development period, is emphasized by a great number of studies [44, 45, 23].

Learning and Memory

The above discussion demonstrated that various fatty acids serve different roles in the nervous system and in the body and it has been suggested that the nervous system has an absolute molecular species requirement for proper function. Studies in our laboratory confirm this finding, and even suggest an added qualifying requirement, viz. the need for a proper ratio between the essential fatty acids. Many studies examine the effects of various fatty acids on learning and memory, but very few examine the ratio between various PUFA. We experimentally tested our hypothesis that the ratio of omega-6 and omega-3 may be a key factor in modulating behavioral and neuropharmacological effects of polyunsaturated fatty acids. Therefore, we attempted to identify the optimal ratio. To avoid the variations that occur in the composition of fatty acids in commercially prepared oils, and to exclude the possible confounding effects of other fatty acids or lipid mixtures, we used highly purified linoleic and alpha-linolenic acids. We tested a wide range of ratios of linoleic/alpha-linolenic acid (3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1 (vol/vol)), which were administered as dietary supplements. All animal studies were conducted on rats, fed normal diets, as recommended by the American Institute of Nutrition (AIN). We found that a mixture of linoleic (omega-6) and alpha-linolenic acids (omega-3) with a ratio of 4:1 was the most effective in improving learning performance (as assessed by the Morris Water Maze, and passive avoidance), elevating pain threshold, improving sleep, and improving thermoregulation [46]. This ratio was also able to correct learning deficits induced by the neurotoxins AF64A and 5,7-dihydroxytryptamine [47], treatments that decrease the acetylcholine and serotonin brain levels. Similarly, this ratio overcame learning deficit induced by 6-OH-DA (e.g. reduction in brain dopamine level) [48]. Treatment with a single fatty acid was less successful [49, 50].

EFA, Aging and Alzheimer's Disease

Aging is a special period during development. The aging brain is different in many aspects from the adult brain. Among the many brain changes (e.g. decrease in the level of most neurotransmitters and hormones and an increase in cholesterol in the neuronal membrane) is that the level and the ratio of EFA is modified. PUFAs are major molecules responsible for regulating cellular differentiation and apoptosis [51]. Most of the studies on aging report a significant decrease in the level and turnover of PUFA [52–55]. This major change is a significant decrease in omega-3 fatty acids, such as α -linolenic and DHA [56]. The magnitude of the decrease in the ratio is not uniform in all brain areas. While the decrease is significant in the cortex, striatum and hypothalamus, the most profound decrease was found in the hippocampus [57]. During aging, there is a significant change in the transition temperature of the lipids, a change which is more profound in Alzheimer's patients [58]. This change causes the membrane to be more rigid. The most studied fatty acids, in this respect, are DHA (docosahexanoic, omega-3) and AA (arachidonic, omega-6). While the level of both fatty acids is very low in the neuronal membrane of the aged hippocampus in rats, treatment with omega-3 fatty acids improves the membrane status [59, 60]. Basically, there are two ways to explain the low level of PUFA in the aging brain, viz. the low rate of transport of PUFA from the blood into the brain, and the impaired biochemical machinery that normally is expected to incorporate and elongate the fatty acids. These two alternatives are directly related to their respective parent issues: the problem of the blood brain barrier and the dynamics of FA brain metabolism.

The brain of an Alzheimer's patient undergoes more severe changes than the brain of a healthy elderly person. Among the major changes, that are relevant to our issue, is (1) the decrease in the neurotransmitter acetylcholine in the brain (and in particular in the hippocampus), and (2) the major double change in the brain lipids, whereof one is the significant increase in cholesterol level in the neuronal membrane, accompanied by a decrease in total PUFA level. And the other is the change in the omega-6 and omega-3 ratio. The level of omega-3 fatty acids (mainly DHA) is severely reduced [61]. Those changes have direct effect on the membrane fluidity index, causing the membrane to become more rigid and eventually to malfunction.

Alzheimer's disease is a progressive and degenerative age-related dementia. Among the major symptoms are: short- and long-term memory loss, impairment of speech and language, decline of abstract reasoning, and mood change. The hallmark sign of Alzheimer's disorder is the loss of spatial orientation. Most researchers agree that the hippocampus is responsible for this function, and indeed postmortem studies showed shrinkage of the hippocampus in

Alzheimer's patients. The etiology of this disorder is not known, but many of the theories have been summarized in [62]. Our preliminary studies show that administration of a mixture of linoleic and alpha-linolenic acids, in a ratio of 4:1, improves the Mini Mental State Examination (MMSE) and quality of life test scores of Alzheimer's patients [62]. Treatments with other ratios did not improve the Alzheimer's patients' condition. Administrations of a single fatty acid, i.e. DHA, did not improve the Alzheimer's condition by much [63, 64]. These results reflect similar results that were obtained in animal experimental models of Alzheimer's.

Seizure

The relationship between lipids and fatty acid metabolism on the one hand, and seizures on the other, has been previously described [65, 66]. The general finding shows a gross disturbance in the fatty acid metabolisms [67–72]. One of the effects of a seizure is the transient disruption of the blood-brain barrier structure and functions [73, 74]. A well-known application of fatty acids in the diet for the treatment of epilepsy is represented by the ketogenic diet for children with refractory seizures [75, 76]. The promise of therapeutic effects that may be realized from ketogenic diets continues to be reaffirmed by many clinical investigators [77, 78].

The possible mode of action to account for the involvement of brain lipids in seizures was recently summarized [65]: Lipids are important constituents of the neuronal membrane and changes in the fatty acid profile may alter membrane functions.

1. Essential fatty acids and phospholipids may offset the deleterious effects of substances that induce seizures, such as iron, that have been shown to increase lipid peroxidation.
2. Essential fatty acids and phospholipids may offer stability in membrane fluidity that may otherwise be associated with seizures.
3. Essential fatty acids and phospholipids may control the alteration in neuronal membrane phospholipid metabolism that may result from the high prevalence of excitatory amino acid receptors in the epileptic focus.
4. A genetic link may exist between epilepsy and PUFA deficit [79], and a mixture of DHA and EPA may provide some measure of correction.
5. Essential fatty acids may serve as neuroprotectors in the brain, similar to effects observed in the heart, as shown in recent studies where alpha-linolenic acid (but not palmitic acid) was found to protect against ischemic-induced neuronal death, and to prevent kainic-induced seizures and its associated neuronal death [80, 81].

Apart from the interest in PUFA as a stabilizing agent for the neuronal membrane in seizure, is a growing interest in the use of the ketogenic diet in epileptic children. This diet is high-fat and low-carbohydrate, and is used to control intractable seizures in children. The abandoned ketogenic diet was very popular in the past and is currently being re-examined [82–85].

Multiple Sclerosis

Multiple sclerosis (MS) is characterized by active degradation of the central nervous system myelin, with clinical symptoms depending on the brain areas that are undergoing the demyelination. The etiology of MS is unknown; however, one of the major symptoms associated with MS is the deterioration of cognitive functions [86]. While an ideal animal model of MS unfortunately is unavailable, experimental allergic encephalomyelitis (EAE) is considered to be the best available substitute [87].

Relationships between MS disease and lipids and fatty acids have been proposed in the past [88, 89], with changes in lipid metabolism reported for MS patients [90, 91]. In addition, changes in the level of cholesterol in the brain of MS patients have been described [92].

Studies show that administration of a mixture of 4:1 omega-6 to omega-3 fatty acids, exerts beneficial effects in rats given a diluted dose of the EAE toxin. The EAE rats showed learning and motor deficits as well as major changes in the fatty acids profile and the cholesterol level in frontal cortex synaptosomes. This treatment was, to a significant degree, able to rehabilitate the changes induced by EAE, though not to completely reverse the deficits to the level of normal control. None of the other PUFA ratios were as effective as the ratio of 4:1 [93].

EFA and Sleep

Sleep quality is a major problem in the modern society. Vast numbers of ‘healthy people’ complain about the quality of their sleep. One of the major complaints is that sleep does not refresh them enough. In addition, many disease states are associated with objective or subjective sleep disturbances. The presumed neurochemical basis for the relationships between EFA and the different sleep stages was recently reviewed [11, 94].

We already showed that the particular EFA ratio of 4:1, when given to Alzheimer’s patients, significantly reduced their complaints about sleep problems, and the quality of their sleep indeed improved [62].

Table 1. Effects of 4:1 ratio on rat sleep profile

	Before	After
Sleep quality	0.7 ± 0.3	0.5 ± 0.2*
Sleep latency	1.3 ± 0.4	0.7 ± 0.5**
Sleep duration	1.2 ± 0.3	0.6 ± 0.4**
Sleep efficiency	1.3 ± 0.4	0.7 ± 0.3**
Sleep disturbance	0.9 ± 0.4	0.4 ± 0.3**
Sleep medications	0.4 ± 0.2	0.3 ± 0.2 NS
Daytime dysfunction	1.4 ± 0.6	0.6 ± 0.3**
Sleep index total	7.2 ± 1.6	3.8 ± 1.8**

The numbers in each cell represent the mean and standard deviations of the score in each category. *p < 0.05; **p < 0.01.

In addition to the above, we have more data that has not yet been published. The new data includes three separate studies. In all the studies, a wide range of EFA ratios were tested, and in all the studies, the ratio of 4:1 was shown to be the optimal ratio. Two human studies were also performed:

(A) In an open trial, the 4:1 ratio was administered for 1 month (48 subjects, aged 24–46, 55% males and 45% females). The results showed improvement in the subjective feeling about sleep (as measured by interview). Only 3 subjects (out of the 48 subjects) said that their sleep was not improved. No other ratio had this high rate of success.

(B) 48 students (age: 21–27 years, 24 males and 24 females) were included in the study. All subjects were healthy, with no history of depression or sleep disturbances. In addition to the medical examination, each subject answered the Hebrew version of the sleep index (Pittsburgh Sleep Quality Index) before entering the study as well as on the 28th day of the study. The sleep index is intended to measure sleep quality and to identify good and bad sleepers. This index does not provide clinical diagnosis.

The Hebrew sleep index (Bar Ilan University addition, 2001) has 21 questions, examining seven subscales of sleep (three questions for each subscale). The subscales are: sleep quality; sleep latency; sleep duration; sleep efficiency; sleep disturbance; sleep medication; daytime dysfunction. The total score of the sleep index is between 0 and 21. The higher scores indicate poorer sleep. The researchers claim that a global score of above 5 may indicate that the subject has severe difficulties in two areas or moderate difficulties in three areas.

Table 1 shows the results of the study both as sleep index total scores and in each subscale. The total sleep index score decreases from 7.2 at the

Table 2. Effects of 4:1 ratio on rat sleep profile

	Before (n = 36)	Control no treatment (n = 12)	Control saline (n = 12)	1:4 ratio (n = 12)
Wake	740.5 ± 28	740.9 ± 35	745.2 ± 29	770.4 ± 39*
Non-REM sleep	565.5 ± 22	584.9 ± 30	595 ± 42	540.9 ± 40*
REM sleep	100.7 ± 9	90.4 ± 12	102.7 ± 12	128.7 ± 14*
Motor activity 24 h	1,450 ± 100	1,528 ± 110	1,349 ± 136	1,772 ± 98*

The results of sleep profile are presented here as means in seconds. The data for motor activity are presented here as the means and standard deviation of total motor movements for a 24-hour period. * $p < 0.05$.

beginning of the study to 3.8 after 28 days ($p < 0.001$). In all the subscales, except one, statistical significant differences ($p < 0.001$) were found. Only the subscale of 'sleep medication' was not affected in this study. The use of sleep medications in this age group is rare, and the baseline was very low.

In conclusion, the 4:1 ratio showed beneficial effects in this group of students. This is a group which is generally highly stressed and the general comment was that the 4:1 ratio helped them to study more effectively.

In order to investigate the effects of the 4:1 ratio on sleep parameters in rats, a group of Sprague-Dawley male (about 200 g body weight) rats ($n = 36$) were used. EEG electrodes were fixed to the rats' heads. All materials and methods are described in the paper by Conrad et al. [95]. The cage of each rat was on an activity meter. The original group of 36 rats was divided randomly into three groups: (1) no treatment for 4 weeks; (2) a daily i.p. injection of saline for 4 weeks, and (3) a daily injection of 40 mg/kg 4:1 ratio for 4 weeks. Measurements of EEG profile and motor activity were done on days 0 and 28. The results are presented in table 2.

The results show that the 4:1 ratio treated rats improved their sleep profile. They were awake for a longer period of time, the REM sleep periods were longer, and they had shorter non-REM periods. The total motor activity level was higher in the 4:1 ratio treated group. The difference between the 4:1 ratio group at day 28 and the other groups is statistically significant ($p < 0.05$).

The combined results of human and rat studies clearly indicate that the 4:1 ratio only possesses beneficial effects on sleep quality and therefore on daytime performance.

Stress, Cortisol and Learning

The importance of the differentiation among the various types of fatty acids may be appreciated by noting their effects on immunological and endocrinological factors. For example, omega-3 fatty acids suppress the synthesis of interleukin-1 and 6 and enhance the synthesis of interleukin-2, while omega-6 fatty acids have the opposite effect. It should be recalled that both interleukin-6 and interleukin-1 (and to a lesser degree interleukin-2) promote the corticotropin-releasing factor (CRF) release via arachidonic acid. However, CRF inhibits the stimulating effect of interleukin-1 on the prostaglandin synthesis.

Cortisol exerts powerful effects on all body tissues. It increases the level of glucose in the blood, stimulates the breakdown of proteins into amino acids, inhibits the uptake of glucose by muscle tissues (except in the brain) and regulates the response of the cardiovascular system to persistent high blood pressure. All of these actions constitute the 'fight or flight' response, which helps the organism to cope with stress situations. Recent studies, however, indicate that cortisol may have some damaging effects, including deterioration of learning and memory. Human studies on normal, aged, depressed, Cushing's syndrome patients, as well as mentally ill patients, have demonstrated a strong negative correlation between cortisol levels and learning and memory in a wide range of cognitive tasks. In rats, cortisol induces deterioration in spatial task performance as measured by the Morris Water Maze (MWM) test. These negative effects may be explained by the fact that cortisol interferes with physiological mechanisms crucial to the structure, and function of the hippocampus. For example, stress is known to cause atrophy of the hippocampal dendrites, damage to the pyramidal neurons, and to interfere with synaptic activity. It is most likely that this morphological and functional hippocampal damage results from high levels of cortisol.

Recently we demonstrated that a specific mixture of free essential fatty acids [linoleic (18:2 ω 6) and alpha-linolenic (18:3 ω 3)] is able to reduce the levels of cholesterol in brain neuronal membranes [96, 20].

Hippocampal functions, which include spatial learning abilities, can be assessed through the MWM. This popular test is used to evaluate potential drugs for Alzheimer's disorder, since Alzheimer's patients exhibit great difficulties in spatial orientation. Independent of hippocampal effects, hypothermia was also shown to impair learning, as evaluated by using various tests, including the MWM performance test, which is specially meant for spatial learning.

Further interest in the relationships between cortisol and learning arose when recent findings showed an increase in the level of cortisol in Alzheimer's patients, who were also shown to have elevated levels of interleukin-6 (IL-6). It should be noted that IL-6 receptors were found in the adrenal cortex, and that the level of cortisol is raised by IL-6, which in turn is increased by general stress and cold.

Our study [97] showed that a mixture of linoleic and alpha-linolenic acids, administered for 3 weeks prior to injection of cortisol (10 mg/kg), or prior to immersion of rats in a 10°C saline bath, blocked the elevation of cortisol and cholesterol blood levels. In addition, this treatment protected the rats from the spatial learning deficits that usually accompany the stressful conditions in the Morris Water Maze. Moreover, a recent study showed that certain anti epileptic drugs induce an increase in the cortisol level. The PUFA mixture at a 4:1 ratio can reduce the elevated cortisol level and has anti convulsant effects [98]. Only this ratio has the effect of decreasing elevated cortisol levels.

Conclusions

This review related mainly to linoleic and alpha-linolenic acids. The ratio between omega-6 and omega-3 can also be maintained by using longer chain fatty acids, such as AA and DHA. However, very few studies have been carried out on the balance between the LCPUFA balance or ratio and brain function. The few studies, that in fact did examine this area, were recently summarized [99, 100], and demonstrated the importance of the ratio between omega-6 and omega-3.

It might very well be that the required ratio of omega-6 and omega-3 may differ when used for different tissues or functions. One can understand that a ratio of 1:1 is the optimal ratio for preventing cardiovascular diseases, and another ratio would be optimal for cancer prevention. We are merely suggesting that a ratio of 4:1 is the optimal ratio for brain-mediated functions. The question is; how can it be that PUFA is of help to organisms that are only capable of obtaining it from the diet? Our hypothesis is that omega-6 and omega-3, in a ratio of 4:1, act within the neuronal membrane and improves the membrane fluidity index, which is the key to all neuronal activity. Other researchers have also recommended the ratio of 4:1. The results of those studies were earlier summarized by Yehuda and Carasso [46], and again more recently by Horrocks and Yeo [101]. We have difficulties explaining why this particular ratio is best. One possibility is that the preferred ratio for omega-6 and omega-3 PUFA depends on the competition of the same enzymes for desaturation and elongation. Another possibility is that PUFA, in this ratio, are able to create micella, which protects them. Whatever the biochemical basis could be, the results showed that the 4:1 ratio has protective and stabilizing effects on the neuronal membrane.

Acknowledgements

I would like to thank Dr. Sharon Rabinovitz-Shenkar and Ms. Ingrid Muller for their very helpful comments on this manuscript. Furthermore, I would like to thank the Rose K. Ginsburg

References

- 1 Simopoulos AP: Evolutionary aspects of omega-3 fatty acids in the food supply. *Prostaglandins Leukot Essent Fatty Acids* 1999;60:421-429.
- 2 Feller SE, Gawrisch K, MacKerell AD Jr: Polyunsaturated fatty acids in lipid bilayers: Intrinsic and environmental contributions to their unique physical properties. *J Am Chem Soc* 2002;124:318-326.
- 3 Yehuda S, Rabinovitz S, Mostofsky DI: PUFA: Mediators for the Nervous, endocrine, and Immune System; in Mostofsky DI, Yehuda S, Salem N (eds): *Fatty Acids: Physiological and Behavioral Functions*. New York, Humana Press, 2001, pp 403-420.
- 4 Rapoport SI: In vivo fatty acid incorporation into brain phospholipids in relation to plasma availability, signal transduction and membrane remodeling. *J Mol Neurosci* 2001;16:243-261.
- 5 Edmond J: Essential polyunsaturated fatty acids and the barrier to the brain. *J Mol Neurosci* 2001;16:181-193.
- 6 de la Torre JC, Mussivand T: Can disturbed brain microcirculation cause Alzheimer's disease? *Neurol Res* 1993;15:146-153.
- 7 Ginsberg L, Xeureb JH, Gershfeld NL: Membrane instability plasmalogen content and Alzheimer's disease. *J Neurochem* 1998;70:2533-2538.
- 8 Strosznajder J, Samochocki M, Duran M: Aging diminishes serotonin-stimulated arachidonic acid uptake and cholinergic receptor-activated acid release in rat brain cortex membrane. *J Neurochem* 1994;62:1048-1054.
- 9 Terracina L, Brunetti M, Avellini L, de Medio GE, Trovarelli G, Gaiti A: Linoleic acid metabolism in brain cortex of aged rats. *Ital J Biochem* 1992;41:225-235.
- 10 Su HM, Huang MC, Saad NM, Nathanielsz PW, Brenna JT: Fetal baboons convert 18:3n-3 to 22:6n-3 in vivo: A stable isotope tracer study. *J Lipid Res* 2001;42:581-586.
- 11 Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI: Fatty acids and brain peptides. *Peptides* 1998; 19:407-419.
- 12 Zimmer L, Delpal S, Guilloteau D, Aioun J, Durand G: Chronic n-3 polyunsaturated fatty acid deficiency alters dopamine vesicle density in the rat frontal cortex. *Neurosci Lett* 2000;284:25-28.
- 13 Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, Durand G, Chalon S: The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. *Am J Clin Nutr* 2002;75:662-667.
- 14 Kodas E, Vancassel S, Lejeune B, Guilloteau D, 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.
- 15 Chalon S, Delion-Vancassel S, Belzung C, Guilloteau D, Lequisquet AM, Besnard JC, Durand G: Dietary fish oil effects monoaminergic neurotransmission and behavior in rats. *J Nutr* 1998;128: 2512-2519.
- 16 Acar N, Chardigny JM, Darbois M, Pasquis B, Sebedio JL: Modification of the dopaminergic neurotransmitters in striatum, frontal cortex and hippocampus of rats fed for 21 months with trans isomers of alpha-linolenic acid. *Neurosci Res* 2003;45:375-382.
- 17 Farkas E, de Wilde MC, Kiliaan A, Meijer J, Keijsers JN, Luiten PGM: Dietary long chain PUFAs differentially effect hippocampal muscarinic 1 and serotonergic 1A receptors in experimental cerebral hypoperfusion. *Brain Res* 2002;954:32-41.
- 18 Yehuda S, Rabinovitz S, Mostofsky DI: Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J Neurosci Res* 1999;15:565-570.
- 19 Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DT: The Role of PUFA in restoring the aging neuronal membrane. *Neurobiol Aging* 2002;23:843-853.
- 20 Yehuda S, Rabinovitz S, Mostofsky DI: Modulation of learning and neuronal membrane composition in the rat by essential fatty acids preparation: Time course analysis. *Neurochem Res* 1998; 23:631-638.

- 21 Auestad N: Infant nutrition – brain development – disease in later life. *Dev Neurosci* 2000;22:472–473.
- 22 Salvati S, Attorri L, Avellino C, Di Biase A, Sanchez M: Diet, lipids and brain development. *Dev Neurosci* 2000;22:481–487.
- 23 Stockard JE, Saste MD, Benford VJ, Barness L, Auestad N, Carver JD: Effect of docosahexaenoic acid content of maternal diet on auditory brainstem conduction times in rat pups. *Dev Neurosci* 2000;22:494–499.
- 24 Youdim MBH, Yehuda S: The neurochemical basis of cognitive deficits induced by brain iron deficiency: Involvement of dopamine-opiate system. *Cell Molec Biol* 2000;46:491–500.
- 25 Ando S, Tanaka Y, Toyoda Y, Kon K: Turnover of myelin lipids in aging brain. *Neurochem Res* 2003;28:5–13.
- 26 Horrocks LA, Harder HW: Fatty acids and cholesterol; in Lajtha A (ed): *Handbook of Neurochemistry*. Plenum, New York, 1983, pp 1–16.
- 27 Bourre JM, Dumont O, Piciotti M, Clement M, Chaudiere J, Bonneil M, et al: Essentiality of w3 fatty acids for brain structure and function. *World Rev Nutr*. Basel, Karger, 1991, vol 66, pp 103–117.
- 28 Salem N, Moriguchi T, Greiner RS, McBride K, Ahmad A, Catalan JN, Slotnick B: Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. *J Mol Neurosci* 2001;16:299–307.
- 29 Yehuda S, Rabinovitz S, Mostofsky DI: Effects of essential fatty acids preparation (SR-3) on brain biochemistry and on behavioral and cognitive functions; in Yehuda S, Mostofsky DI (eds): *Handbook of Essential Fatty Acids Biology: Biochemistry Physiology and Behavioral Neurobiology*. New York, Humana Press, 1997, pp 427–452.
- 30 Moriguchi T, Greiner RS, Salem N: Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. *J Neurochem* 2000;75:2563–2573.
- 31 Wainwright PE: Dietary essential fatty acids and brain function: A developmental perspective on mechanisms. *Proc Nutr Soc* 2002;61:61–69.
- 32 Greiner RS, Moriguchi T, Slotnick BM, Hutton A, Salem N: Olfactory discrimination deficits in n-3 acid-deficient rats. *Physiol Behav* 2001;72:379–385.
- 33 Catalan J, Moriguchi T, Slotnick B, Murthy M, Greiner RS, Salem N: Cognitive deficits in docosahexaenoic acid-deficient rats. *Behav Neurosci* 2002;116:1022–1031.
- 34 Jeffrey BG, Mitchell DC, Gibson RA, Neuringer M: n-3 fatty acid deficiency alters recovery of the rod photoresponse in rhesus monkeys. *Invest Ophthalmol Vis Sci* 2002;43:2806–2814.
- 35 Jeffrey BG, Mitchell DC, Hibbeln JR, Gibson RA, Chedester AL, Salem N: Visual acuity and retinal function in infant monkeys fed long-chain PUFA. *Lipids* 2002;37:839–848.
- 36 Ahmad A, Murthy M, Greiner RS, Moriguchi T, Salem N: A decrease in cell size accompanies a loss of docosahexaenoate in the rat hippocampus. *Nutr Neurosci* 2002;5:103–113.
- 37 Takeuchi T, Fukumoto Y, 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.
- 38 Ximenes da Silva A, Lavielle F, Gendrot G, Guesnet P, Alessandri JM, 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.
- 39 Nanjo A, Kanazawa A, Sato K, Banno F, Fujimoto K: Depletion of dietary n-3 fatty acid affects the level of cyclic AMP in rat hippocampus. *J Nutr Sci Vitaminol* 1999;45:633–641.
- 40 Gazzah N, Gharib A, Croset M, Bobillier P, Lagarde M, Sarda N: Decrease of brain phospholipid synthesis in free-moving n-3 fatty acid deficient rats. *J Neurochem* 1995;64:908–918.
- 41 Murthy M, Hamilton J, Greiner RS, Moriguchi T, Salem N, Kim HY: Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus. *J Lipid Res* 2002;43:611–617.
- 42 Jumpson J, Lien EL, Goh YK, Clandinin MT: Small changes of dietary (n-6) and (n-3)/ fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. *J Nutr* 1997;127:724–731.
- 43 Jumpson JA, Lien EL, Goh YK, Clandinin MT: During neuronal and glial cell development diet n-6 to n-3 fatty acid ratio alters the fatty acid composition of phosphatidylinositol and phosphatidylserine. *Biochim Biophys Acta* 1997;1347:40–50.

- 44 Wainwright PE, Xing HC, Mutsaers L, McCutcheon D, Kyle D: Arachidonic acid offsets the effects on mouse brain and behavior of a diet with a low (n-6):(n-3) ratio and very high levels of docosahexaenoic acid. *J Nutr* 1997;127:184–193.
- 45 Xiang M, Alfven G, Blennow M, Trygg M, Zetterstrom R: Long-chain polyunsaturated fatty acids in human milk and brain growth during early infancy. *Acta Paediatr* 2000;89:142–147.
- 46 Yehuda S, Carasso RL: Modulation of learning, pain thresholds, and thermoregulation in the rat by preparations of free purified α -linolenic and linoleic acids: Determination of optimal n-3 to omega-6 ratio. *Proc Natl Acad Sci USA* 1993;90:10345–10349.
- 47 Yehuda S, Carasso RL, Mostofsky DI: Essential fatty acid preparation (1:4 ratio) rehabilitates learning deficits induced by AF64A and 5,7-DHT. *NeuroReport* 1995;6:511–515.
- 48 Yehuda S, Rabinovitz S, Mostofsky DI: Polyunsaturated fatty acids mixture treatment prevents deleterious effects of Ro4–1284. *Eur J Pharmacol*, 1999;365:27–34.
- 49 Ikemoto A, Ohishi M, Sato Y, Hata N, Misawa Y, Fujii Y, Okuyama H: 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.
- 50 Umezawa M, Kogishi K, Tojo H, Yoshimura S, Seriu N, Ohta A, Takeda T, Hosokawa M: High-linoleate and high- α -linolenate diets affect learning ability and natural behavior in SAMR1 mice. *J Nutr* 1999;129:431–437.
- 51 Sawazaki S, Hamazaki T, Yazawa K, Kobayashi M: The effect of docosahexaenoic acid on plasma catecholamine concentrations and glucose tolerance during long-lasting psychological stress: A double blind placebo-controlled study. *J Nutr Neurosci Vitaminol* 1999;45:655–665.
- 52 Favreliere S, Stadelman-Ingrand S, Huguette F, De Javel D, Piriou A, Tallineau C, et al: Age-related changes in ethanolamine glycerophospholipid fatty acid levels in rat frontal cortex and hippocampus. *Neurobiol Aging* 2000;21:653–660.
- 53 Regev R, Assaraf YG, Eytan GD: Membrane fluidization by ether other anesthetics, and certain agents abolishes P-glycoprotein ATPase activity and modulates efflux from multidrug-resistant cells. *Eur J Biochem* 1999;258:18–24.
- 54 Vreugdenhil M, Bruehl C, Voskuyl RA, Kang JX, Leaf A, Wadman WJ: Polyunsaturated fatty acids modulate sodium and calcium currents in CA1 neurons. *Proc Natl Acad Sci USA* 1996;93:12559–12563.
- 55 Zaidi A, Michaelis ML: Effects of reactive oxygen species on brain synaptic plasma membrane Ca^{2+} -ATPase. *Free Rad Biol Med* 1999;27:810–821.
- 56 Favreliere S, Perault MC, Huguette F, De Javel D, Bertrand N, Piriou A, Durand G: DHA-enriched phospholipid diets modulate age-related alterations in rat hippocampus. *Neurobiol Aging* 2003;24:233–243.
- 57 Ulmann L, Mimouni V, Roux S, Porsolt R, Poisson JP: Brain and hippocampus fatty acid composition in phospholipid classes of age-related cognitive deficit rats. *Prostaglandins Leukot Essent Fatty Acids* 2001;64:189–195.
- 58 Giorgi PL, Biraghi M, Kantar A: Effect of desmopressin on rat brain synaptosomal membrane: A pilot study. *Curr Therap Res* 1998;59:172–178.
- 59 McGahon BM, Murray CA, Horrobin DF, Lynch MA: Age-related changes in oxidative mechanisms and LTP are reversed by dietary manipulation. *Neurobiol Aging* 1999;20:643–653.
- 60 Meehan E, Beauge F, Choquart D, Leonard BE: Influence of an n-6 polyunsaturated fatty acid-enriched diet on the development of tolerance during chronic ethanol administration in rats. *Alcohol Clin Exp Res* 1995;1:1441–1446.
- 61 Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH: Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. *Lipids* 2000;35:1305–1312.
- 62 Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI: Essential fatty acids preparation (1:4 ratio) improved Alzheimer's patients quality of life. *Inter J Neurosci* 1996;87:141–149.
- 63 Hashimoto M, Hossain S, Shimada T, Sugioka K, Yamasaki H, Fujii Y, Ishibashi Y, Oka JI, Shido O: Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *J Neurochem* 2002;81:1084–1091.
- 64 Das UN: Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: But, why and how? *Prostaglandins Leukot Essent Fatty Acids* 2000;63:351–362.

- 65 Yehuda S, Carasso RL, Mostofsky DI: Essential fatty acid preparation (1:4 ratio) raises the seizure threshold in rats. *Eur J Pharmacol* 1994;254:193–198.
- 66 Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF: The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res* 2001;40:1–94.
- 67 Bazan NG: The neuromessenger platelet-activating factor in plasticity neurodegeneration. *Prog Brain Res* 1986;118:281–291.
- 68 Pediconi MF, Rodriguez de Turco EB, Bazan NG: Reduced labeling of brain phosphatidylinositol, triacylglycerols, and diacylglycerols by [¹⁴C]arachidonic acid after electroconvulsive shock: Potentiation of the effect by adrenergic drugs and comparison with palmitic acid labeling. *Neurochem Res* 1986;11:1–14.
- 69 Van Rooijen LA, Vadnal R, Dobard P, Bazan NG: Enhanced inositol turnover in brain during bicuculline-induced status epilepticus. *Biochem Biophys Res Commun* 1986;136:827–834.
- 70 Flynn CJ, Wecker L: Concomitant increases in the levels of choline and free fatty acids in rat brain: evidence supporting the seizure-induced hydrolysis of phosphatidylcholine. *J Neurochem* 1987;48:1178–1184.
- 71 Visioli F, Rodriguez de Turco EB, Kreisman NR, Bazan NG: Membrane lipid degradation is related to interictal cortical activity in a series of seizures. *Metab Brain Dis* 1994;9:161–170.
- 72 Birkle DL: Regional and temporal variations in the accumulation of unesterified fatty acids and diacylglycerols in the rat brain during kainic acid induced limbic seizures. *Brain Res* 1993;613:115–122.
- 73 Cornford EM, Oldendorf WH: Epilepsy and the blood-brain barrier. *Adv Neurol* 1986;44:787–812.
- 74 Cervos-Navarro J, Kannuki S, Nakagawa Y: Blood-brain barrier (BBB). Review from morphological aspect. *Histol Histopathol* 1988;3:203–213.
- 75 Stafstrom CE, Bough KJ: The ketogenic diet for the treatment of epilepsy: A challenge for nutritional neuroscientists. *Nutr Neurosci* 2003;6:67–79.
- 76 Freeman JM, Vining EP, Pillas DJ, Pyzik PL, Casey JC, Kelly LM: The efficacy of the ketogenic diet-1998: A prospective evaluation of intervention in 150 children. *Pediatrics* 1998;102:1358–1363.
- 77 Ben-Menachem E: New antiepileptic drugs and non-pharmaceutical treatments. *Curr Opin Neurol* 2000;13:165–170.
- 78 Helmstaedter C, Kurthen: Memory and epilepsy: Characteristics, course and influence of drugs and surgery. *Curr Opin Neurol* 2001;14:211–216.
- 79 Søvik O, Mansson JE, Bjørke Monsen AL, Jellum E, Berge RK: Generalized peroxisomal disorder in male twins: Fatty acid composition of serum lipids and response to n-3 fatty acids. *J Inher Metab Dis* 1998;21:662–670.
- 80 Xiao Y, Li X: Polyunsaturated fatty acids modify mouse hippocampal neuronal excitability during excitotoxic or convulsant stimulation. *Brain Res* 1999;846:112–121.
- 81 Lauritzen I, Blondeau N, Heurteaux C, Widmann GR, Lazdunski M: Polyunsaturated fatty acids are potent neuroprotectors. *EMBO J* 2000;19:1784–1793.
- 82 Thavendiranathan P, Chow C, Cunnane S, Burnham WM: The effect of the ‘classic’ ketogenic diet on animal seizure models. *Brain Res* 2003;959:206–213.
- 83 Cunnane SC, Musa K, Ryan MA, Whiting S, Fraser DD: Potential role of polyunsaturates in seizure protection achieved with the ketogenic diet. *Prostaglandins Leukot Essent Fatty Acids* 2002;67:131–135.
- 84 Musa-Veloso K, Rarama E, Comeau F, Curtis R, Cunnane S: Epilepsy and the ketogenic diet: Assessment of ketosis in children using breath acetone. *Pediatr Res* 2002;52:443–448.
- 85 Klepper J, Leiendecker B, Bredahl R, Athanassopoulos S, Heinen F, Gertsen E, Flörcken A, Metz A, Voit T: Introduction of a ketogenic diet in young infants. *J Inher Metab Dis* 2002; 25:449–460.
- 86 Ron AM, Feinstein A: Multiple sclerosis and the mind. *J Neurol Neurosurg Psychiatry* 1992;55:1–3.
- 87 Werkele H: Lymphocyte traffic to the brain; in Partridge WM (ed); *The Blood Brain Barrier*. Raven Press, New York, 1993, pp 67–83.
- 88 Swank RL, Grimsgaard A: Multiple sclerosis: The lipid relationship. *Am J Clin* 1988;48:1387–1393.
- 89 Williams KA, Deber, CM: The structure and function of central nervous system myelin. *Crit Rev Clin Lab Sci* 1993;30:29–64.
- 90 Holman RT, Johnson SB, Kokmen E: Deficiencies of polyunsaturated fatty acids and replacement by nonessential fatty acids in plasma lipids in multiple sclerosis. *Proc Natl Acad Sci USA* 1989; 86:4720–4724.

- 91 Nightingale S, Woo, E, Smith AD, French JM, Gale MM, Sinclair HM, Bates D, Shaw DA: Red blood cell and adipose tissue fatty acids in mild inactive multiple sclerosis. *Acta Neurol Scand* 1990;82:43–50.
- 92 Nicholas, HJ, Taylor J: Central nervous system demyelinating diseases and increased release of cholesterol into the urinary system of rats. *Lipids* 1994;29:611–617.
- 93 Yehuda S, Rabinovitz S, Mostofsky DI, Huberman M, Carasso RL, Sredni B: Essential fatty acid preparation improves biochemical and cognitive functions in EAE rats. *Eur J Pharmacol* 1997; 328:23–29.
- 94 Yehuda S, Rabinovitz S, Mostofsky DI: Essential fatty acids and sleep: Mini review and hypothesis. *Med Hypothesis* 1998;50:139–145.
- 95 Conrad A, Bull DF, King MG, Husband AJ: The effects of lipopolysaccharide (LPS) on the fever response in rats at different ambient temperatures. *Physiol Behav* 1997;62:1197–1201.
- 96 Yehuda S, Brandys Y, Mostofsky DI, Blumenfeld A: Essential fatty acid preparation reduces cholesterol and fatty acids in rat cortex. *Inter J Neurosci* 1996;86:249–256.
- 97 Yehuda S, Rabinovitz S, Carasso RL, Mostofsky DI: Fatty acid mixture counters changes in cortisol, cholesterol and impair learning. *Int J Neurosci* 2000;101:73–87.
- 98 Rabinovitz S, Mostofsky DI, Yehuda S: Anticonvulsant efficiency, behavioral performance and cortisol level: A comparison of carbamazepine (CBZ) and a fatty acid compound (SR-3). *Psychoneuroendocrinology* 2003;in press.
- 99 Simopoulos AP: Human requirement for N-3 polyunsaturated fatty acids. *Poult Sci* 2000;79:961–970.
- 100 Youdim KA, Martin A, Joseph JA: Essential fatty acids and the brain: Possible health implications. *Int J Dev Neurosci* 2000;18:383–399.
- 101 Horrocks LA, Yeo YK: Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res* 1999; 40:211–225.

Prof. Shlomo Yehuda, Psychopharmacology Laboratory
Department of Psychology, Bar Ilan University
Ramat Gan 52900 (Israel)
Tel. +972 3 5318583, Fax +972 3 5353327, E-Mail yehudas@mail.biu.ac.il