EFFECTS OF POLYUNSATURATED FATTY ACIDS ON BRAIN DEVELOPMENT AND AGING

Ph.D. Thesis

Endre Hőgyes

Department of Medical Chemistry, University of Szeged and Institute of Biochemistry, Biological Research Center, Szeged

2004

Abbreviations

AchE	Acetylcholinesterase
ARA	Arachidonic acid
BBB	Blood-brain barrier
ChAT	Choline acetyltransferase
DHA	Docosahexaenoic acid
DPA	Docosaspentaenoic acid
EPA	Eicosapentaenoic acid
FAS	Fatty acid synthase
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
LNA	Linolenic acid
NBM	Nucleus basalis magnocellularis
NGF	Nerve growth factor
NMDA	*N*-methyl-D-aspartate
MUFA	Monounsaturated fatty acid
SFA	Saturated fatty acid
SHR	Spontaneously hypertensive rat
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
PS	Phosphatidylserine

Contents

List of publications		4.
Introduction		5.
	Structure and biosynthesis of fatty acids	5.
	DHA in the brain	7.
	DHA and the neuroprotection	8.
	Late effect of the maternal DHA on the learning ability	9.
	DHA in the aged brain	10.
	Fatty acids in SHR rats	11.
	Aims	12.
Materials and Methods		14.
	Experiment with the neuroprotective effects of LC-PUFAs	14.
	Measurement of the late effect of maternal LC-PUFA supplementation on learning ability in adult rats	17.
	The experimental procedure of short term fish oil administration in old rats	17.
	Research on the effect of hypertension and long term LC-PUFA feeding on brain fatty acid content and learning ability in SHR rats.	18.
Results and discussion		21.
	The neuroprotective effect of LC-PUFAs	21.
	The late effect of maternal LC-PUFA supplementation	27.
	The study of short term fish oil administration in old rats	32.
	The long term LC-PUFA feeding in SHR rats	34.
Summary		42.
References		43.
Acknowledgement		51.

List of publications

Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats.

de Wilde MC, Hogyes E, Kiliaan AJ, Farkas T, Luiten PG, Farkas E**.**

Brain Res. 2003 Oct 24;988(1-2):9-19.

Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids.

Barcelo-Coblijn G, Hogyes E, Kitajka K, Puskas LG, Zvara A, Hackler L Jr, Nyakas C, Penke Z, Farkas T.

Proc Natl Acad Sci U S A. 2003 Sep 30;100(20):11321-6. Epub 2003 Sep 17.

Neuroprotective effect of developmental docosahexaenoic acid supplement against excitotoxic brain damage in infant rats.

Hogyes E, Nyakas C, Kiliaan A, Farkas T, Penke B, Luiten PG.

Neuroscience. 2003;119(4):999-1012.

Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio.

Barcelo-Coblijn G, Kitajka K, Puskas LG, Hogyes E, Zvara A, Hackler L Jr, Farkas T.

Biochim Biophys Acta. 2003 Jun 10;1632(1-3):72-9.

Introduction

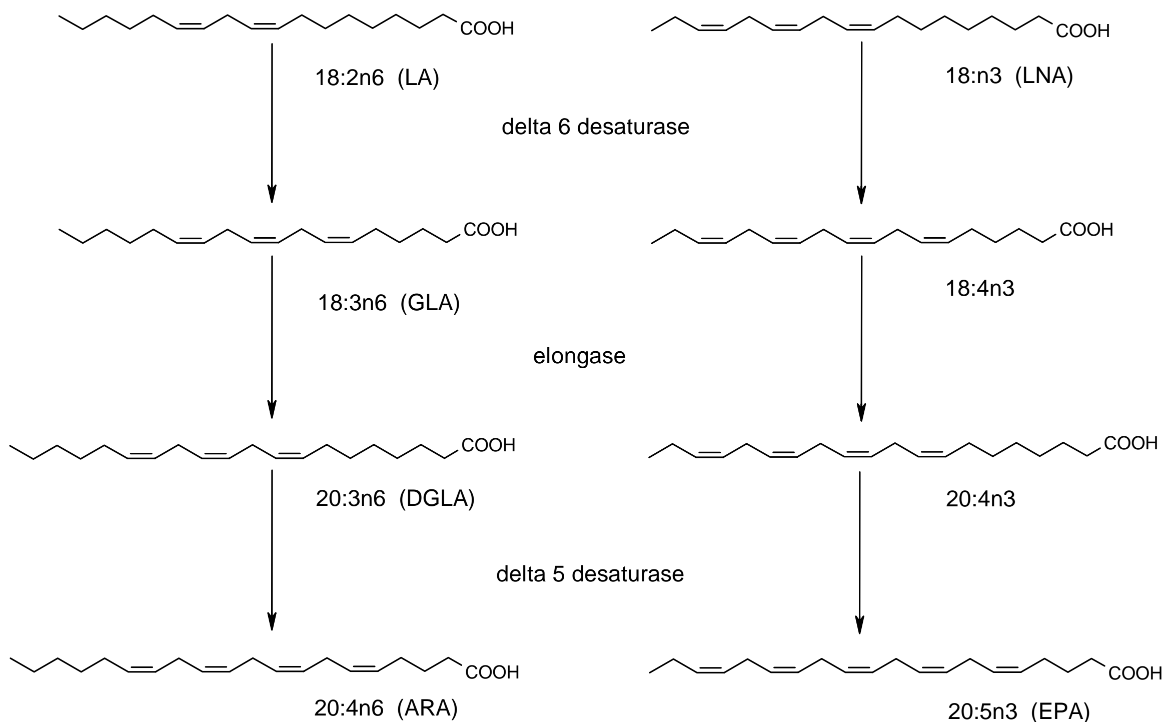
There has been growing interest in fatty acids in both medical and basic science in the last few decades due to various functions in which they are involved either in a single cell or in the whole body. They play important role in metabolic energy production in animals as part of triacylglycerol molecules; build up cell membranes in phospholipids, serve as precursors of eicosanoids, and take part in several pathological diseases like Zellweger and Alzheimer disease. It has been revealed recently, that the brain requires certain types of fatty acids in order to develop and function properly. The lack or low intake of given fatty acid types causes serious problems in learning, memory, visual and auditory functions. These very important fatty acids are the long chain polyunsaturated fatty acids (LC-PUFAs). Their amount, ratio, utilization were have been found important in brain function. Mainly docosahexaenoic acid (DHA) seems crucial for neurons.

Structure and biosynthesis. LC-PUFAs are fatty acids of a chain of 18 or more carbon atoms with two or more double bonds. According to the number of the carbon atom followed by the first double bond from the methyl end, PUFAs are divided into two major groups, the n-6 and n-3 families. Using another nomenclature omega (ω-3, etc.), is also accepted, and means the same. However, the delta (Δ) notation signs the location of double bond from the carboxyl end.

Mammalian cells have an enzyme complex, the cytoplasmic fatty acid synthase (FAS), for synthesizing fatty acids. It is supposed to be a multifunctional dimer, containing a β-ketoacyl-acyl-carrier protein part, and a pantetheine sulfhydryl of acyl-carrier protein part (154). This complex uses malonyl-CoA for synthesis, and the end product of this mechanism is palmitic acid (16:0). It is the precursor of further synthesis, the elongation or desaturation. Elongases are located in both the mitochondria and the endoplasmic reticulum, and synthetize stearic acid (18:0). Using Δ9 desaturase, cells establish a double bond between the ninth and tenth carbon atoms (108), obtaining oleic acid (18:1n9). Due to the lack of Δ12 and Δ15 desaturases in mammalian cells (87), mammalian cells are unable to synthetize either n-6 or n-3 fatty acids from oleic acid. So, this type of fatty acids must be obtained from diet. The precursors of long chain n-6 and n-3 PUFAs are linoleic acid (LA, 18:2n6) and linolenic acid (LNA, 18:3n3), respectively. The investigation of the metabolic pathways of PUFAs began a

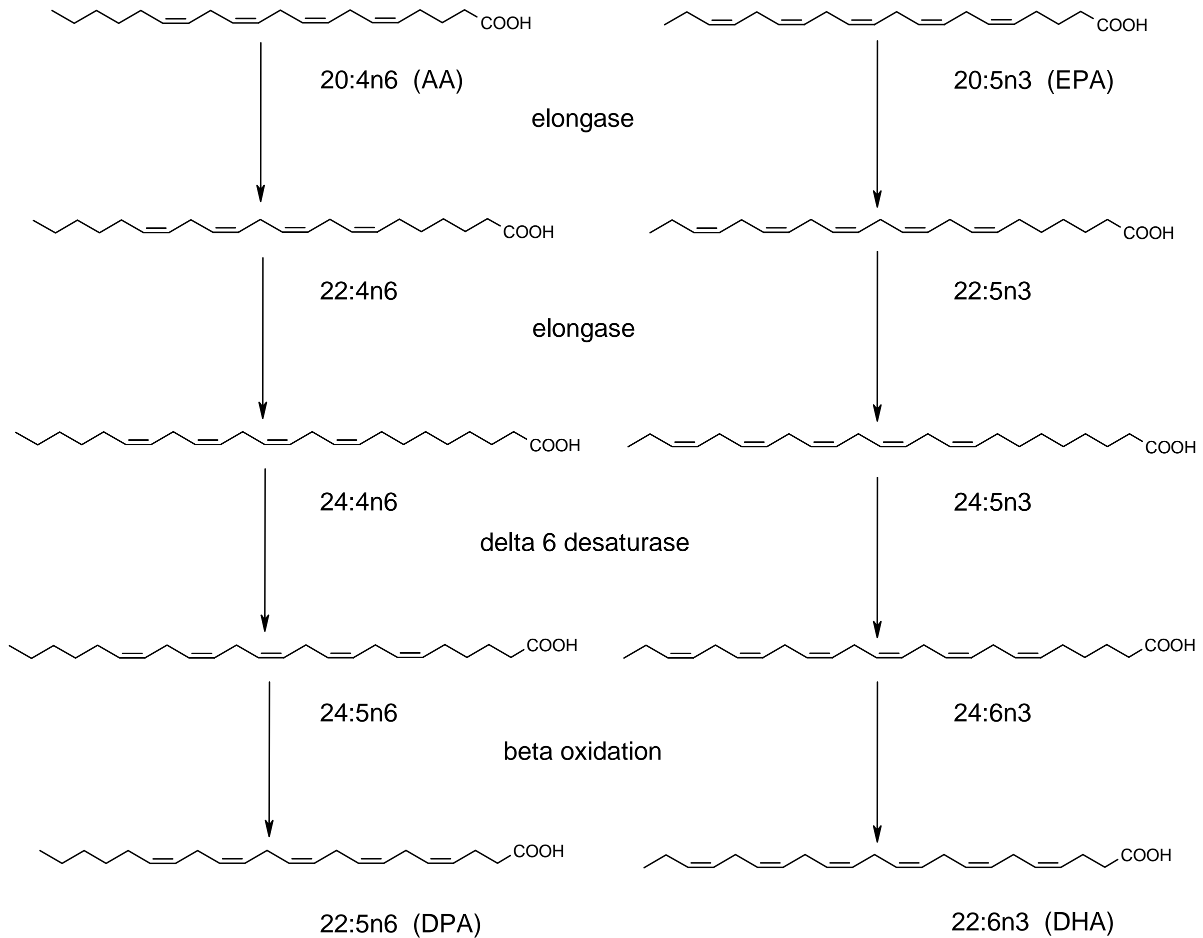
few decades ago (78,103). Fatty acid metabolism is located in both microsomes and peroxisomes (138). Fig. 1. shows the building up of 20-carbon PUFAs in both n-6 and n-3 cases. Using elongase (2,97), and two specific desaturases, Δ6 (4,18,115) and Δ5 (19,82,105,154), mammalian cells are able to convert LA to arachidonic acid (ARA, 20:4n6), and LNA to eicosapentaenoic acid (EPA, 20:5n3), while the two PUFA families are competing for the same enzymes (29,96). Moreover, the n-6 and n-3 fatty acids cannot be converted to each other.

Fig. 1. Synthesis of 20-carbon PUFAs



This is the reason why the diet must contain both PUFA families. 20-carbon PUFAs, like dihomo-γ-linolenic acid (DGLA, 20:3n6), ARA and EPA are the precursors for eicosanoids (146). The mechanisms above are located in microsomes. The lack of Δ4 desaturase in mammalian cells (9), is responsible for the fact that the two end products of PUFA families, the docosapentaenoic acid (DTA, 22:5n6) and docosahexaenoic acid (DHA, 22:6n3) cannot be formed immediately from 22:4n6 and 22:5n3, respectively (Fig. 2.). It requires one cycle of β-oxidation, after a Δ6 desaturation and elongation step (151). The β-oxidation is carried out in the peroxisomes. Fibroblasts from Zellweger diseased patients have a lack of peroxisomes, and are unable to synthetize DHA (20,109). The structure of the two end

Fig. 2. Synthesis of 22-carbon PUFAs



products, DPA and DHA are very similar. They contain the highest number of carbon with the most double bonds. Cells often try to replace DHA with DPA in n-3 deficient conditions.

DHA in the brain. DHA is the most abundant n-3 PUFA in the brain and is found at sn-2 position of phospholipids (129,130). Phosphatidylethanolamine (PE) and phosphatidylserine (PS) are the main pool for DHA, while phosphatidylcholine (PC) and phosphatidylinositol (PI) contain limited amount of DHA. Because there is more PE in brain membrane than PS and PE seems more sensitive for any kind of changes, I focused on PE in some cases in this study. DHA is the active component from n-3 series, responsible for optimal membrane-protein interaction in signal transduction (60,91,107) and gene expression in the brain (77).

DHA is essential for the nervous system. The source of DHA for the brain can be either the diet, or the liver can synthetize DHA (78,119,135-137,142), or astrocytes can produce DHA from its precursors. However, Δ6 desaturation is a limiting step, the enzyme activity decreases after birth in the brain (15). So, it would be preferable for brain to receive DHA from liver or diet. DHA in the diet is more potent than its precursors. For example, the skin prefers utilizing LNA and oxidized it more rapidly than it does 20- and 22-carbon PUFAs (122). To reach the same DHA level in tissues, two times more vegetable oil (18-carbon n-3 source) is needed than fish oil (20- and 22-carbon n-3 source, mainly EPA, DHA) (58).

DHA and the neuroprotection. Brain growth and functional development require adequate supplies of ARA and DHA in infants (30). Lack of both n-6 and n-3 PUFAs disturbs neural integrity and function of fetus and neonates as well (6,65). The main DHA increments in brain parallels brain growth spurt, and synaptogenesis in rats (50). ARA content of brain is also increasing during the maturation of the brain. However, these two main PUFAs have opposite effects on the outgrowth of neurites induced by nerve growth factor (NGF). DHA enhances the effect of NGF, while ARA suppresses it (62). DHA was found to maintain the normal synthesis of NGF in developing hippocampus (63). Fetal alcohol syndrome, cystic fibrosis, aggressive hostility, phenylketonuria, unipolar depression, attention deficit hyperactivity disorder were reported to be related with inadequate DHA intake during brain development (58).

Maternal fatty acid supplementation reflects the fatty acid status of the fetus and newborns. An n-3 fatty acid sufficient diet increases the DHA content of the milk, liver and brain in fetal and neonatal rats (161), on the other hand, an n-3 fatty acid deficient diet decreases their content (14). Reciprocal replacement is also observed between n-6 and n-3 fatty acids depending on the composition of diet (45).

The developing brain is vulnerable for any kind of toxic damage. We investigated whether an excitotoxic brain damage induced by *N*-methyl-aspartate (NMDA) could be reduced by maternal LC-PUFA intake in infant rats. Excitotoxicity, evoked by NMDA is a generally accepted model for mimicking excitotoxic neuronal death (128). It causes glutamate receptor overactivation and elevated Ca^{2+} influx into the neurons, which induces apoptotic cascades like activation of phospholipases, calpains, protein kinases, NO synthase, endonucleases and can also generate free radicals (133). This kind of overactivation is supposed to be involved in acute and chronic neurodegenerative disorders (22,104). NMDA excitotoxicity alters

membrane composition, and finally membrane degradation can be found in neuronal death (38). Interestingly, the first step of the apoptotic mechanism is the inhibition of phospholipid synthesis, not membrane degradation. PE and PC are synthesized in the Kennedy pathway (8,67,72), which, consists of three steps. The last step, where PE and PC are formed from the reaction of cytidine diphosphate-ethanolamine/choline and 1,2-diacylglycerol transferred by the proper transferase, is blocked in the presence of NMDA in cortical neural cell culture derived from embryonic rat brain (48). The distribution of phospholipid molecular species can be modified by diets (60,79). DHA prefers being incorporated into aminophospholipids, mainly into plasmalogens, by increasing its level (156).

Late effect of maternal DHA on the learning ability. Several studies have reported, that two generations are required to deplete DHA content of brain in rats reared on n-3 deficient diet (111,131). It has also been revealed from these studies that decreased DHA concentration in the brain was accompanied with poorer performance in the Morris water maze spatial orientation test and in an olfactory-cued reversal learning task. Furthermore, restricted maternal DHA intake caused decreased levels of dopamine and noradrenaline in the cerebral cortex, hippocampus, and striatum in the pups (144). On the other hand, administration of DHA improved learning performance in DHA depleted offspring (46). Chronic deprivation of DHA was shown to cause a reduced amount of DHA in the brain where the lack of DHA was compensated for with n-6 fatty acids, mainly with DPA. In order to improve learning performance in DHA deprived rats, it is not enough to restore the DHA content to the normal level, n-6 fatty acid levels must also be reduced (64).

Studies above investigated the late effect of DHA deficient diet on offspring. But the opposite case, the late effect of DHA sufficient diet is relatively not well studied. Interestingly, there are more human studies than rat in the literature. Children, whose mother received cod liver oil (n-3 PUFA source) during pregnancy and lactation, reached higher score on cognitive test for children (Kaufman Assessment Battery for Children) at 4 years of age compared to control group (57). Maternal DHA supplementation has been found to have positive correlation with gestational length, cerebral maturation of infants (56) and superior psychomotoric development (84,93,121,127). Conversely, high maternal n-6 PUFA intake can modify behavior and locomotor activity in mice. For example, pups of dams who received high amount of n-6 PUFA during pregnancy and lactation, were found more active in an open field test, more aggressive in the resident-intruder test, and spent less time immobile in the swim

test than their controls (126). Furthermore, it has been demonstrated in rats, that maternal n-3 supplementation makes myelination time longer, and gets the appearance of reflexes delayed (132). Similar negative effect was found in the development of rat auditory system (54).

DHA in the aged brain. There is an age-related LC-PUFA loss in the rat brain. The loss of LC-PUFAs may cause alterations in membrane fluidity leading to a more rigid membrane, which is not ideal for normal lipid-protein interactions. Dietary manipulation may reverse this unfavorable process. It has been shown, that reduced caloric intake increases membrane fluidity in the aged (44,68,98). Possible explanations are associated with lowered lipid peroxidation and decreased amount of free radicals (162). The loss of PUFAs (mainly ARA and DHA) is one of the main reason for membrane rigidity. The hippocampus, cortex, striatum, and hypothalamus were found to be affected largely by the age-related loss of PUFAs (39,124,148,159). Dietary n-3 fatty acid intake improved the membrane condition in the hippocampus in aged rats (101,102). There are two different theories for explaining why PUFAs are disappearing during aging. According to blood-brain barrier (BBB) theory, trafficking of PUFAs through the BBB is diminished in aged brain. However, conflicting evidence has been provided by most of the studies, where no difference was found between the PUFA uptake of young and old rat brain through the BBB (89,90). Still a research group reported less amount of ARA obtained through the BBB in old brain (5). Evidences from Alzheimer patients support definitely the later theory (25,49), where the BBB is compromised, and disturbs fatty acid trafficking. The second theory establishes that the metabolism of PUFAs is altered in the aged brain. For instance, fatty acid incorporation into phospholipids is can be inhibited (61,147). Enzyme activity of desaturases and elongases are found to be very low (83). In addition to loss of PUFAs, the elevation of cholesterol in neural membranes in the aging brain can also cause higher membrane rigidity (31-33,36). Along this line, anti-cholesterol drugs like Lovastatine (32,33) and piracetam (113) are able to alleviate membrane rigidity caused by cholesterol. Normal membrane fluidity is very important for maintaining optimal circumstances for the function of membrane associated-proteins (158). The loss of PUFAs causes more rigid membrane, which may lead to decline in brain function. It is tempting to hypothesize, that due to restoring normal membrane conditions by dietary manipulations, the cognitive functions might improve. To support this theory, the ability of neurons to sustain long-term potentiation is restored after level of ARA is elevated in aged hippocampus (99,100). Furthermore, serotoninergic and dopaminergic neurotransmission can

be impaired in the frontal cortex in n-3 fatty acid deficient old rats (26). Chronic cerebral hypoperfusion carried out by occluding the common carotid arteries of old rats, is a good model for vascular dementia. Diet enriched in n-3 fatty acids, together with antioxidant and vitamin additives, enhanced the density of the serotonergic 1A and the cholinergic muscarinic 1 receptors, and while n-3 fatty acids themselves enhanced only muscarinic 1 receptor density in this rat model (27). Moreover, PUFAs and additives improved the performance of rats in Morris water maze task in these model animals (37). Long term DHA treatment in the case of old mice increased the level of DHA in the expense of ARA and DTA in the brain, while the treated mice performed better in the time maze learning test (88-90,143). On the other hand, short term DHA administration improved only visual function, but not learning performance in the Morris water maze in old mice (16).

Fatty acids in SHR rats. A strong correlation has been extensively characterized between hypertension and fatty acid metabolism. Several studies reported the antihypertensive effect of fish oil in both humans (10,92,112) and spontaneously hypertensive rats (SHR) (59,155). The n-3 PUFAs, DHA and EPA, were found the potent antihypertensive component of fish oil (110). DHA was shown to have antithrombotic (74), and anti-inflammatory effects (1), to reduce serum lipid levels (12), and to have beneficial effect on vascular system (53). These observations may serve as base for the hypotensive effect of DHA. Dietary DHA showed a dose response effect on blood pressure. For example, DHA did not decrease blood pressure given at low doses (21,41,155), and was more active at high doses (74). Interestingly, one study did not show the antihypertensive effect of a dietary mixture of DHA and EPA (116). Finally, DHA made the life span longer, and improved learning performance in stroke-prone spontaneously hypertensive rats (SHR-SP) (74).

On the other hand, it has been revealed that blood pressure has a deep impact on fatty acid metabolism, as well. For instance, the activity of Δ6 and Δ5 desatures was found lowered in the liver of young SHR rats compared to normotensive controls (134). These differences extended between the groups by aging, due to the age related elevation of blood pressure (117). The decreased activity of both desaturases caused alteration of liver fatty acid composition. Furthermore, the amount of precursors increased, while the level of end products decreased. In contrast with the liver, the Δ6 and Δ5 desaturase activities were unaffected by hypertension in the kidney, so blood-pressure-related changes in fatty acid composition of the kidney were not proven (52). Only age-related changes were observed in desaturase activity

in this organ. Results obtained from the aorta were similar to kidney in SHR rats, while there were not found any kind of changes in the enzyme activities and fatty acid composition in the heart (24). Not only PUFAs metabolism was affected in blood-pressure-related changes, but that of SFAs and MUFAs too. DHA intake can reduce the Δ9 activity in hepatocytes in SHR rats causing elevation an in the level 16:0, while a decrease in 16:1n7 content (35).

In summary, the interaction between fatty acid composition and the function of vital organs such as the liver, the kidney and the cardiovascular system were well studied in SHR rats. However, the relationship between blood pressure and brain fatty acid content was poorly investigated. It is well known that cognitive functions were declined in SHR rats compared to normotensives (43,114).

Aims:

1. We proposed that dietary manipulation of first of all DHA supplementation may be able to serve as a neuroprotector in the infant rats. Testing this theory, dams were fed with three types of diet, a PUFA deficient, an n-6 PUFA sufficient with little amount of n-3 PUFA, and a both n-6 and n-3 sufficient. Pups of these dams were injected with NMDA into the nucleus basalis magnocellularis (NBM) at the age of 14 days. Cholinergic neurons of NBM project fibers to the ipsilateral neocortex, and play important role for in maintaining normal function of neurons there (86,94,95,150). Two days after the injection, a decreasing number of neurons in the NBM and, the degeneration of fibers in the neocortex were measured. Fatty acid composition of PE, PC, PS, and PI were also determined.
2. In our experiment, we aimed at showing the beneficial effect of maternal LC-PUFA supplementation on learning performance. Dams were divided into three groups and received three different types of diet during pregnancy and lactation. The three diets contained LC-PUFAs in various amount, Placebo was deficient in both n-6 and n-3 PUFAs, Control showed normal physiological state: high amount of n-6 and little amount of n-3, while Supplement group was enriched in both types of LC-PUFAs. Brain fatty acid content was followed during aging, and the rats performed spatial discrimination learning task at the age of 12 month and 26 month.
3. We aimed at demonstrating whether an increasing level of DHA in neural membranes of old rats could be associated with improved learning performance. 24-moths-old rats were

fed either fish oil supplemented chow or normal chow. After 1 month of feeding, Morris water maze performance was tested, and fatty acid composition of rat brain was also determined.

4. Our goal was to find relationship between hypertension and brain fatty acid content, and their effect on learning performance in aged rats. Rats received either LC-PUFA supplemented diet or control diet through their life. The systolic blood pressure, brain fatty acid content and spatial discrimination learning task were measured, and the data were correlated to reveal beneficial effect of LC-PUFAs on hypertension and learning performance.

Materials and methods

1. **Experiments with the neuroprotective effects of LC-PUFAs.**

Animals and diet. Female Wistar rats were divided into three groups, and received three different types of diets during pregnancy and lactation. The three different diets were: (1) Supplement, (2) Placebo, (3) Control, (Table 1). The Supplement and Placebo were synthetic diets, obtained from Numico Research Company, Wageningen, Netherlands. The Supplement contained very large amount of main LC-PUFAs such as DHA, EPA and ARA, and their precursor, while the Placebo had a lack of these fatty acids, only their precursors LA and LNA were present contained in very little amount. Instead of these, the Placebo contained abundant MUFAs, mainly oleic acid. Control was standard laboratory rat food, purchased from Charles River Co., Budapest Hungary. It contained limited amount of n-3 LC-PUFAs, but there were an excess of n-6 LC-PUFAs in it.

Table 1. Fatty acid composition of different diets used in the experiment

Fatty acids	Supplement %	Placebo %	Control %
14:0	2,0	-	0,6
16:0	12,4	5,4	13,7
18:0	5,1	3,2	3,2
20:0	0,5	0,4	-
22:0	-	0,1	-
24:0	-	0,2	-
Total SFA	20,0	9,3	17,5
16:1	1,2	0,1	0,8
18:1	18,9	68,5	17,1
20:1	0,5	1,7	-
22:1	-	0,9	-
24:1	-	-	-
Total MUFA	20,6	71,2	17,9
18:2n6 (LA)	32,3	15,1	51,2
18:3n6	0,6	0,2	-
20:3n6	0,3	-	-
20:4n6 (ARA)	9,4	-	0,1
Total PUFAn6	42,6	15,3	51,3
18:3n3 (LNA)	3,5	3,3	6,8
20:5n3 (EPA)	2,6	-	0,7
22:6n3 (DHA)	13,6	-	1,2
Total PUFAn3	19,7	3,3	8,7

The aim with these three diets was the following: the supplement was sufficient in DHA and its precursors and contained appropriate amount of n-6 PUFAs. We used this diet to prove the beneficial effect of increased n-3 fatty acid content of the diet. The control served as a normal physiological diet, because of the high n-6/n-3 ratio. The fatty acid composition of this diet differed from the Supplement in the decreased amount of n-3 fatty acids. So, any differences obtained in the results between these two groups, were the consequence of the different n-3 fatty acid content of the diet. Placebo was deficient in both types of LC-PUFAs. We used the Placebo diet to investigate whether the lack of LC-PUFAs had negative effect on neuroprotection.

Excitotoxic lesion of NBM cholinergic neurones. 8, 14 days old pups of dams from each group were exposed to NMDA injection. The injection procedure was described by Luiten et al., and Stuiver et al (94,140). Briefly, 20 nmol of racemic mixture of NMDA (Sigma, ST. Louis, MO, USA) in 0,4 µl phosphate-buffered saline (PBS, pH 7,4) was injected unilaterally in the right hemisphere in a volume of 0,1 µl into the nucleus basalis magnocellularis (NBM) area which includes also the cholinergic neurones projecting to the neocortex ipsilaterally. Surgery was performed under ether anaesthesia. A stereotaxic frame was used for positioning the head of pups. The intact left hemisphere served as control side for each individual case. After 48 h survival period, no pups died.

Tissue processing and anatomical measurements. 48 hours after NMDA injection rats were sacrificed by transcardiac perfusion with 4% paraformaldehyde in 0,1 M phosphate buffer (pH 7,4). The brains were removed and rinsed in 30% sucrose solution for two nights, and sectioned on cryostat microtome at a thickness of 20 µm. Free floating brain sections intended to be stained for the presence of acetylcholinesterase (AchE) were postfixed by immersion in a 2,5% glutaraldehyde solution in PBS overnight at 4 ºC. Thereafter, the cholinergic fibers in the neocortex were visualised by staining for the presence of AchE according to Hedreen et al.(55); using a silver-nitrate intensification procedure.

Cholinergic cell number. Choline acetyltransferase (ChAT) and p75 low-affinity neurotrophin receptor protein ($p75^{NTR}$) were stained with immunocytochemical methods in the NBM to assay cell bodies and dendrites. Polyclonal goat anti-ChAT primary antibody was used for staining ChAT, in a dilution rate of 1:2000, according to Bruce et al (13). Immunostainig of the membrane bound $p75^{NTR}$ was carried out by rabbit anti- $p75^{NTR}$ antibody in a dilution rate of 1:1000 (Chemicon, USA). The total number of stained

cholinergic cell bodies were counted in the NBM region around the level of NMDA lesion in both injected and non-injected sides. The loss of neurones at the injected side was calculated as a percentage of the cell number at the intact side, which was considered as 100%.

Dendrite density. Computerized image analysis system (Quantimet 600HR, Leica, Germany) was used to measure the degree of dendrite arborisation in the penumbra region. We measured both types of cholinergic markers (ChAT and $p75^{NTR}$) for the analysis. A limited amount of neurones (15-20 cells) and their dendritic fields were selected at each brain section and the area of stained dendrites was measured around these neurones. The computer programme was able to select the stained fibers and dendrites, while the stained cell bodies were ignored. The measured dendritic area was divided by the number of neurones included in the selected region to obtain an averaged dendrite density per neurone as the measure of the rescued dendrite arborisation. The involution of dendrite arborisation at the injected side was expressed as a percent decrement to the intact non-injected brain side (100%).

Axon degeneration. The degree of axon degeneration was measured from neocortex, where NBM projects fibers ipsilaterally, after 48 hours survival time. Cholinergic fiber density was assayed on superficial layers (I to IV), and deeper layers (V and VI) separately according to Nyakas et al (118). The fiber density at the injection side was expressed as percentage of the control, non-injected side.

Fatty acid analysis. Forebrain tissue was collected from pups reared in the same cages with those, which were injected with NMDA. The number of groups was the following: Supplement and Placebo n=10, Control n=4. The pups were sacrificed at the age of 12 days, the brains were removed. Lipids were extracted from the forebrains according to Folch et al. (42); using chloroform/methanol (2:1 vol/vol). Lipid classes were separated on TLC plates (20x20, Silica G 60) using methyl-acetate/i-propanol/chloroform/methanol/0,25% KCl (25:25:25:10:9 vol/vol) mobile phase. Lipids were transmethylated in presence of absolute methanol containing 5% HCl for 2,5 h at 80 °C. Fatty acid methyl esters were determined by gas chromatography (Hewlett-Packard Model 6890) with FFAP column (30 mX0,32 mmX0,25 µm film thickness; Supelco, Bellefonte, PA, USA). To identify peaks, Supelco fatty acid standards were used (Catalogue No. 4-7085-U, 4-7015). The same fatty acid analysis protocol was used in all the experiments.

Statistics. Statistical analysis was performed with one-way ANOVA in both case of morphometric and fatty acid data. LSD test was used as post hoc test. The results were expressed as means±S.E.M.s.

2. **Measurement of the late effect of maternal LC-PUFA supplementation on learning ability in adult rats.**

Animals and Diet. Three groups of dams were fed with three different types of diet (Supplement, Placebo, Control) during pregnancy and lactation. Same diets were used as above. After weaning all pups in the three groups were received the same control diet. So, the diet of three groups differed only during pregnancy and lactation. Only females were used in the experiment. The aim of this was to investigate late affect of maternal supplementation during aging. Pups were sacrificed in four different ages, 7 days, 35 days, 13 months, and 26 months of ages.

Spatial learning task. The animals were trained in the holeboard spatial discrimination task at the age of 24 weeks. The experimental procedure was carried out according to Douma et al (28); and van der Zee et al. (149). Briefly, the floor of testing box contained 16 small removable pits in rows of four, and chocolate chips were placed in four of them always in the same pattern. The animals had to learn to find only the baited holes during a training of 7 days (two trials per day.) The trials were ended either after 3 minutes, or when the rats found all four baited holes. Reference memory score was calculated for each trial as: Ref. Memory = Number of visits and revisits to baited holes/ Number of visits and revisits to baited holes + Number of visits to non-baited holes. The equation reflects the learning ability. Female rats performed this task at the age of 12 and 25 moths, after the task the rats were sacrificed.

Statistical analysis. The spatial learning task results were compared by repeated measurement two-way ANOVA, while fatty acid results were analyzed by multivariate general linear model two-way ANOVA. LSD was used as post hoc test in all cases.

3. **The experimental procedure of short term fish oil administration in old rats.**

Animals and Diet. 24-month-old rats were fed either with commercial rat chow or chow enriched with fish oil for a month. Table 2. shows the fatty acid composition of both diets. After feeding and learning task the animals were sacrificed and the brains were removed and stored at -70°C until processing.

Table 2. Main fatty acid composition of diets (wt/wt, of total fatty acids)

Fatty acid	Control	Fish oil
16:0	13,6	13,4
18:0	3,1	7,1
18:1	17,0	23,0
18:2	51,0	21,2
20:4	0,1	0,8
22:6	1,2	11,2

Learning Test. After four weeks of dietary supplementation, rats were trained in the Morris water maze spatial learning task (34). The animals performed four daily trials on five consecutive days. The escape latency reaching the hidden platform was registered at each trial. The place of the hidden platform was unaltered during the experiment. The trials were terminated when the rat climbed on the platform, or 90 s had elapsed. The daily mean escape latencies and first trial at each daily session were evaluated, but only mean escape latencies are shown here.

Statistical analysis. Independent student t-test was used in the learning test.

4. Research on the effect of hypertension and long term LC-PUFA feeding on brain fatty acid content and learning ability in SHR rats.

Diet. Three different types of diets were used; Control, Diet 1, and Diet 2. Control was deficient in LC-PUFAs; Diet 1 and Diet 2 differed in the ratio of n-6 and n-3 PUFAs, and certain extra additives. Table 3. shows the fatty acid content of the different diets, while Table 4. lists the amount the extra additives. Carbohydrate-, protein-, mineral content, as well as caloric value of the three diets were the same.

Table 3. Fatty acid content of the diets.

Fatty Acid	Experimental Diets		
	Control	Diet 1	Diet 2
18:2n-6 (LA)	0.640	1.321	1.661
20:4n-6 (ARA)	0.000	0.118	0.118
Total n-6	0.640	1.439	1.779
18:3n-3 (ALA)	0.155	0.137	0.184
20:5n-3 (EPA)	0.000	0.589	0.589
22:6n-3 (DHA)	0.000	0.382	0.382
Total n-3	0.155	1.108	1.155
(n-6)/(n-3)	**4.13**	**1.30**	**1.54**

Experimental groups. Male SHR and normotensive Wistar-Kyoto (WKY) rats were provided the experimental diets from the age of 4 weeks to age of 80 weeks. The combination of two strains and three diets produced six groups (n=10 per group).

Table 4. Extra additives in the diets.

Nutrient	Component	Control	Diet 1	Diet 2
Antioxidants and vitamins	β carotene		0.02	0.02
	Flavonoids		0.2	0.2
	Folate	0.0004	0.001	0.001
	Selenium	0.000019	0.00004	0.00004
	Vitamin B6	0.00153	0.00172	0.00172
	Vitamin B12	0.00005	0.00012	0.00012
	Vitamin C		0.2	0.2
	Vitamin E	0.0063	0.3	0.3
Other	L-acetylcarnitin			0.6
	Choline	0.15	0.15	0.4
	Phosphatidylcholine			0.2
	Phosphatidylserine			0.2
	Co-Q10			0.2
	Thiamin	0.002	0.002	0.2
	Tyrosine	0.944	0.944	1
	Tryptophan	0.232	0.232	1

Blood-pressure measurement. A tail-cuff method (17,106) was used to measure systolic blood pressure (BP) at the ages of 53, 54, 55 and 79 weeks. The animals were slightly anesthetized by isoflurane gas during the procedure. Blood pressure was measured in each case three consecutive times and values were averaged.

Learning test. Holeboard spatial discrimination task was chosen (see above), but different equation was used for reference memory in this case: [(No. of visits to baited holes)+(No. of revisits to baited holesx0,5)] divided by [(4-No. of visits to baited holes)+(No. of visits and revisits to non-baited holes No. of revisits to baited holes)]. The scores of two daily trials were averaged and corrected for the value of the first training day. The test was repeated at the age of 41 weeks.

Statistics. Repeated measurement two-way analysis of variance (ANOVA) was used in the cases of systolic blood pressure and behavioral tasks. The fatty acid composition in various classes of brain membrane was calculated by multivariate general linear model two-way ANOVA. LSD correction followed the ANOVA in all cases. *F* values were used to express the dietary and blood pressure effects [*F*(diet) and *F*(bp), respectively], where superscripted symbols indicated significance (*P* values: *≤0,05; ** ≤0,01). Correlation analysis was performed by Pearson one-tailed test.

Results and discussion

1. **Results of the neuroprotective effect of LC-PUFAs.**

Fatty acid content of neural membrane. Fatty acid contribution of all four phospholipid classes (PE, PC, PS, PI) were investigated. We grouped fatty acids into SFA, MUFA and LC-PUFA to emphasize the effect of different diets.

The phospholipid classes reacted differently to diets. SFAs, MUFAs and both n-6 and n-3 LC-PUFAs were involved in changing. Due to the high amount of n-3 fatty acids in supplement diet, the ratio of DHA increased in PE (Table 1.) compared to both Control and Placebo. Competing n-3 fatty acids with n-6 for the same place in phospholipids, the increased DHA caused decreased in the level of ARA and 22:5n6 compared to Placebo and Control, while 22:4n6 remained unchanged.

Table 1. Percent fatty acid content of phosphatidylethanolamine derived from forebrain of infant rats

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	7,99±0,44*	9,75±0,32	8,57±0,80	0,019
16:0DMA	6,04±0,34	5,64±0,32	5,29±0,28	0,418
18:0	19,60±0,44	19,97±0,27	19,69±0,71	0,660
18:0DMA	6,14±0,38*§	4,89±0,27	4,52±0,28	0,011
MUFA				
18:1n7	0,79±0,02**	1,13±0,02##	0,88±0,03	0,000
18:1n7DMA	0,54±0,02	0,65±0,04	0,55±0,04	0,070
18:1n9	4,87±0,25*	5,59±0,17	4,73±0,28	0,043
18:1n9DMA	0,87±0,05	1,07±0,07	0,81±0,07	0,051
PUFA				
20:4n6 (ARA)	17,57±0,24**	18,82±0,28	17,83±0,59	0,016
22:4n6	4,70±0,13	4,78±0,11	5,13±0,27	0,233
22:5n6	1,08±0,03**§§	2,89±0,07##	2,26±0,08	0,000
22:6n3 (DHA)	25,19±0,71**§§	19,36±0,39	20,45±1,34	0,000

Means±S.E.M.s are shown; *P<0,05; **P<0,01 versus Placebo; §P<0,05; §§P<0,01 versus Control; ##P<0,01 versus Control.

Placebo which diet contained the least amount of n-3 fatty acids, showed the least ratio of DHA in PE, and the highest ratio of ARA and 22:5n6, both differed markedly to Supplement however the latter altered only significantly compared to Control. LC-PUFA enriched diet modified the SFA and MUFA content of PE. Level of 16:0 decreased in Supplement group compared to Placebo, despite of Supplement diet compared more than Placebo. Main MUFAs

were increased in Placebo, due to the diet. Higher level of 18:0DMA in the Supplement group indicated, that DHA preferred to incorporate to plasmalogens.

Table 2. shows the fatty acid percent in PS, other aminophospholipid containing high amount of DHA.

Table 2. Percent fatty acid content of phoshatidylserine derived from forebrain of infant rats

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	6,82±0,99	9,51±1,00	6,65±1,17	0,119
18:0	38,21±0,83	40,14±1,38	37,53±0,81	0,660
MUFA				
18:1n9	4,95±0,29	6,41±0,27$^{\#}$	4,30±0,47	0,010
18:1n7	0,63±0,05	0,74±0,02	0,67±0,01	0,123
PUFA				
18:2n6 (LA)	0,57±0,07	1,04±0,09	0,65±0,14	0,100
20:4n6 (ARA)	6,93±0,26§§	6,49±0,34$^{\#\#}$	10,17±0,84	0,000
22:4n6	5,08±0,28	5,08±0,24	5,58±0,35	0,534
22:5n6	1,54±0,08**§§	3,59±0,09	3,24±0,20	0,000
22:6n3 (DHA)	31,45±1,53**§	22,38±1,11	25,27±1,16	0,000

Means±S.E.M.s are shown; ***P*<0,01 versus Placebo; $^{\S}P$<0,05; $^{\S\S}P$<0,01 versus Control; $^{\#}P$<0,05, $^{\#\#}P$< 0,01 versus Control.

Alterations were similar to PE concerning to LC-PUFAs like DHA and 22:5n6. Statistical differences were observed in DHA and 22:5n6 in all three groups. The level of DHA was elevated by 140% and 125% in Supplement group compared to Placebo and Control group, respectively. In this case there was no difference between the Supplement and Placebo groups in the level of ARA.

Table 3. Percent fatty acid content of phosphatidylcholine derived from forebrain of infant rats

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
14:0	3,30±0,09§	3,43±0,15$^{\#}$	2,72±0,13	0,016
16:0	51,47±0,56	52,13±0,65	49,13±1,68	0,092
18:0	7,97±0,30	7,27±0,34	8,76±0,40	0,051
MUFA				
16:1	2,84±0,20**	4,00±0,30$^{\#}$	2,52±0,11	0,003
18:1n7	3,10±0,06	3,28±0,06	3,21±0,04	0,132
18:1n9	14,72±0,14**	16,25±0,44$^{\#}$	14,44±0,37	0,003
PUFA				
18:2n6 (LA)	1,01±0,03*§§	1,24±0,04$^{\#\#}$	1,64±0,14	0,000
20:4n6 (ARA)	7,58±0,38*	6,50±0,19	7,83±0,74	0,046
22:4n6	0,55±0,01	0,50±0,01	0,68±0,07	0,001
22:6n3 (DHA)	3,54±0,06**§	2,26±0,09$^{\#\#}$	3,02±0,30	0,000

Means±S.E.M.s are shown; **P*<0,05; ***P*<0,001 versus Placebo, §*P*<0,05, §§*P*<0,01 versus Control; #*P*<0,05; ##*P*<0,01 versus Control.

However, both groups contained lesser amount of ARA (by 32% and 36%, respectively) compared to the Control, which diet contained high amount of n-6, but small amount of n-3 fatty acids. SFA and MUFA membrane concentrations seemed less sensitive to diets. Only the level of 18:1n9 was higher by 33% in Placebo compared to Control.

PC (Table 3.) is relatively poor in n-3 PUFAs but relatively high increment was measured in DHA in the supplement group (152% versus Placebo, and 133% versus Control). On the other hand, Placebo group was found to contain the smallest ratio of LC-PUFAs, mainly DHA and ARA. The latter was decreased by 15% compared to the Supplement group and by 17 compared to Control. While elevated amount of MUFAs (16:1 and 18:1) were observed in Placebo in PC as well as in PE and PS previously.

Table 4. Percent fatty acid content of phosphatidylinositol derived from forebrain of infant rats

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	12,27±1,26§§	15,23±1,27	20,69±1,06	0,018
18:0	35,76±1,14	35,31±1,58	35,81±2,19	0,968
MUFA				
18:1n9	7,46±0,36	8,48±0,65	8,07±0,99	0,435
PUFA				
20:4n6 (ARA)	32,39±1,88§	28,46±2,76	24,81±2,72	0,036
22:6n3 (DHA)	4,90±0,69**§§	0,63±0,28	0,90±0,49	0,000

Means±S.E.M.s are shown; ***P*<0,01 versus Placebo, §*P*<0,05; §§*P*<0,01 versus Control.

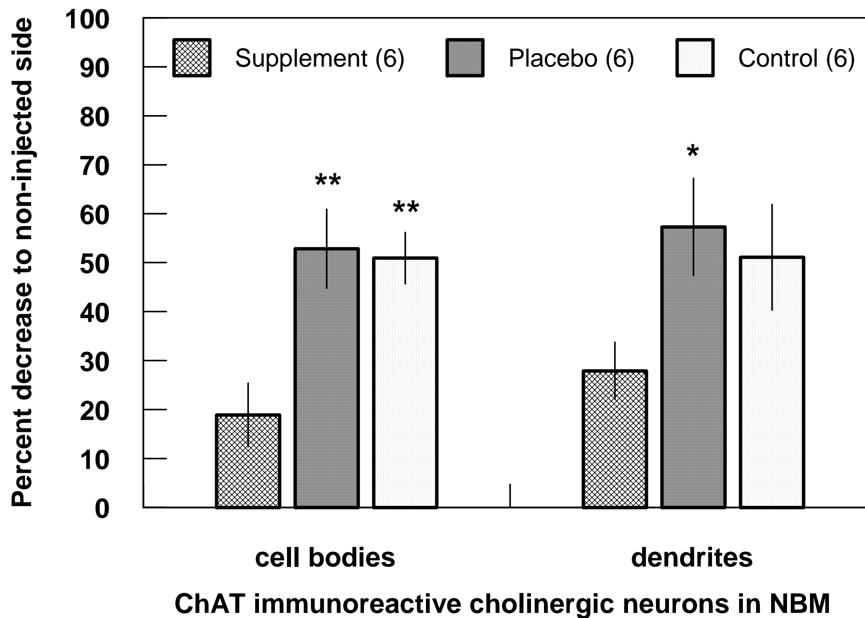
The highest elevation of DHA was found in PI in the Supplement group. (Table 4.) The increment was more than 700% versus Placebo and more than 500% versus Control. This elevation was accompanied to reduction of 16:0 (41% reduction versus Control).

Summarizing the results, DHA increased significantly in all four phospholipid classes in Supplement group compared to Placebo and Control. In aminophospholipids, which are the main pool of DHA, n-6 PUFAs (ARA and mainly 22:5n6) were substituted for DHA in this group. Moreover, level of certain SFAs and MUFAs also decreased in PE. In the placebo group, MUFA increment was measured in PE, PS, and PC, presumably in Placebo compensating the lack of LC-PUFAs.

ChAT immunostaining. Fig. 1. demonstrates that more neurons survived in the Supplement group than in both the Placebo and Control groups (left side, *P*<0,005). Only 18,9±6,5% of neurons died in the Supplement group which was significantly lower than in the case of the other two diets (Placebo: 52,8±8,1%, *P*<0,005 versus Supplement; Control: 50,9±5,3%,

$P<0,005$ versus Supplement). The dendrite arborisation of survived neurons remained more preserved in the Supplement group compared to the Placebo ($P<0,05$ versus Supplement). There was no significant difference between the Placebo and Control groups either survived neurons or dendrite arborisation.

Fig.1.

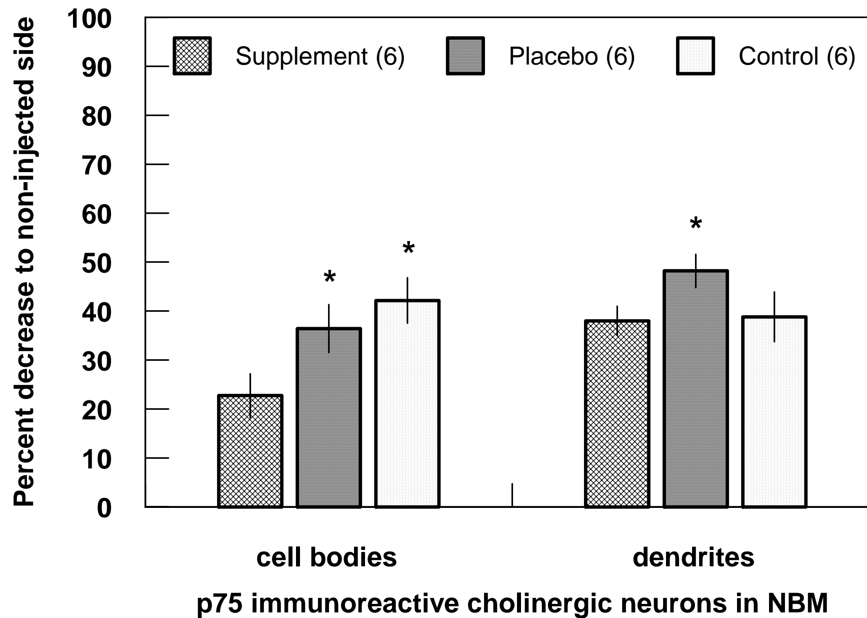


p75^{NTR}* *immunostaining. The results of this cholinergic marker supported the results of the ChAT labeling (Fig. 2.). The degree of lost neurons was markedly lower in the Supplement group than in the Placebo and in the Control ($P=0,025$; post hoc *t*-tests: $P<0,05$ Placebo versus Supplement; $P<0,02$ Control versus Supplement). The dendrite arborisation seemed more vulnerable in the Placebo group, the degree of loss was higher, ($P=0,128$; $P<0,05$ Placebo versus Supplement). No difference was obtained between the Control and Placebo groups in any case with this method either. Comparing the values obtained from the used markers, it showed some variance in the bulk of degeneration (compare Figs 1 and 2). However it was not significant, if the data were compared with ANOVA for repeated measures ($P>0,1$).

AChE fiber staining. The loss of projecting axons to the cortex was measured at superficial layers (I-IV) and deeper layers (V-VI). The effect of diets showed significant effect ($P<0,05$) on the relative loss AChE fiber density. The degree of degeneration was more pronounced in the superficial layers than in the deeper layers ($P<0,001$, Fig. 3.). Lower fiber loss was observed in the superficial layers in the Supplement group compared to the Placebo ($P<0,02$

versus Supplement) and Control ($P<0,05$ versus Supplement). At the deeper layers significant difference was observed only between the Supplement and the Control groups ($P<0,05$).

Fig.2.

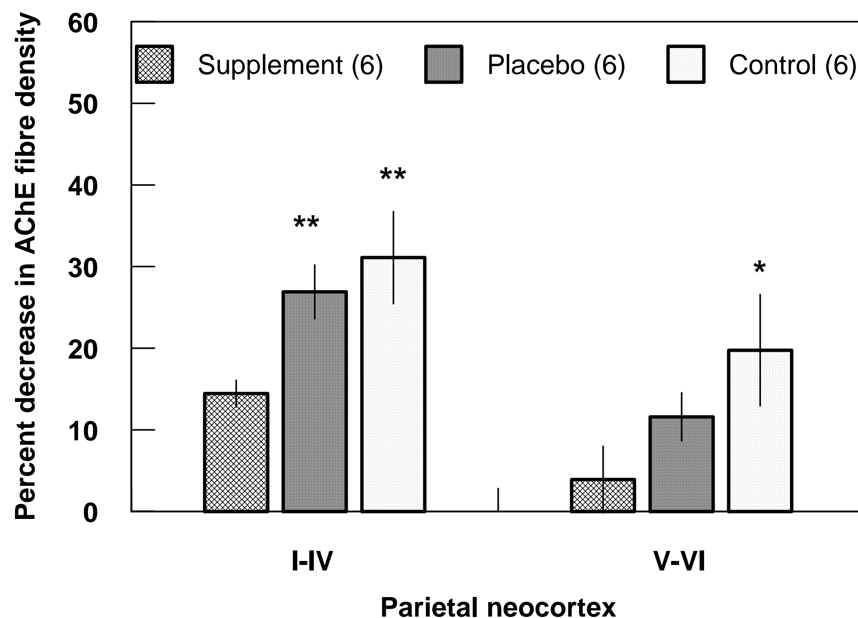


Discussion of the neuroprotective effect of LC-PUFAs.

The goal of the study was to prove the neuroprotective effect of dietary LC-PUFAs. First of all, we found the enhanced incorporation of DHA into neural membranes after dietary supplementation as a basic event. More neurons survived and the dendrite and axon arborisations of survived neurons appeared to be more preserved in the Supplement group. In this group, the level of DHA was higher in all PL classes than in the other two groups. The elevation of DHA was accompanied with reduction of n-6 fatty acids. Mainly the n-6 end product 22:5n-6 was affected; its level decreased markedly in PE and PS which classes contained measurable amount of it. On the other hand, the ARA, which is as important as DHA for the developing brain (23), remained unchanged in the supplement group compared to the Control, and increased to the Placebo group in most phospholipid classes. In the diet, Placebo was deficient in LC-PUFAs, while the Control was abundant in n-6 fatty acid. However, the immunocytochemical data were very similar in these two groups but differed from the Supplement group. That is why we concluded that dietary DHA can cause neuroprotection against NMDA induced neurotoxicity, via its increased level in neural membranes. The MUFAs and SFAs did not play a major role in neuroprotection. The MUFA

and SFA profiles of either in the diets or in neural membranes were similar in the Supplement and the Control groups. MUFAs increased in all classes in the Placebo group, but did not influence neuroprotection. Two cholinergic markers were used for staining cholinergic cell bodies and dendrites: ChAT and $p75^{NTR}$. ChAT is the enzyme for catalyzing the formation of acetylcholine. The $p75^{NTR}$ is a receptor protein, binding NGF and other trophic factors, and can be found in the projecting cholinergic cells (11,76,80). Despite that p75 is a membrane-bound protein, and ChAT is transported constantly to the neurites, they reacted similarly to NMDA toxicity. It is well known, that cholinergic cells cease the synthesis of ChAT enzyme due to any injury. Similarly, it may take place concerning p75. Results obtained from both immunostaining techniques were comparable and supported the neuroprotective effect of the Supplement diet. The exact protective mechanisms of DHA against NMDA neurotoxicity is not clear. It is supposed, that DHA can modify the sensitivity of NMDA receptor or is able to prevent the membrane degradation caused by NMDA toxicity (48). Further possible explanations for the protective effects of DHA are the following: (a) DHA has reported to enhance dendrite outgrowth, while ARA can suppresses it (62,63). (b) DHA has been attributed an antiapoptotic effect. DHA reduced apoptosis induced by serum deprivation, in Neuro-2A cells in vitro (73). (c) DHA can enhance free radical scavenging. For example, ethyl-DHA administration was shown to inhibit the formation of oxidation products in fetal brain (156).

Fig.3.



2. **Results of the late effect of maternal LC-PUFA supplementation.**

Fatty acid composition of rat brain. Table 5-8 summarize the late effect of maternal diet on brain fatty acid content in PE class from four different ages. First, at the youngest age group (right after birth) the level of DHA increased significantly at expense of n-6 PUFAs (specifically DPA and ARA) and MUFAs (18:1n7, 18:1n9) due to the DHA-enriched diet. At the same time, the concentration of n-6 PUFAs and MUFAs reduced significantly in the Supplement group compared to Placebo or Control (Table 5.). However, DTA was not affected by the diet either at this or at older age. The level of 16:0 decreased in the Supplement group compared to Placebo group, too.

Table 5. Fatty acid content of PE from 7 days old rat brain

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	7,99±0,44*	9,75±0,32	8,57±0,80	0,019
16:0DMA	6,04±0,34	5,64±0,32	5,29±0,28	0,418
18:0	19,60±0,44	19,97±0,27	19,69±0,71	0,660
18:0DMA	6,14±0,38*§	4,89±0,27	4,52±0,28	0,011
MUFA				
18:1n7	0,79±0,02**	1,13±0,02$^{\#\#}$	0,88±0,03	0,000
18:1n7DMA	0,54±0,02	0,65±0,04	0,55±0,04	0,070
18:1n9	4,87±0,25*	5,59±0,17	4,73±0,28	0,043
18:1n9DMA	0,87±0,05	1,07±0,07	0,81±0,07	0,051
20:1n9	-	-	-	-
PUFA				
20:4n6 (ARA)	17,57±0,24**	18,82±0,28	17,83±0,59	0,016
22:4n6	4,70±0,13	4,78±0,11	5,13±0,27	0,233
22:5n6	1,08±0,03**§§	2,89±0,07$^{\#\#}$	2,26±0,08	0,000
22:6n3 (DHA)	25,19±0,71**§§	19,36±0,39	20,45±1,34	0,000

Means±S.E.M.s are shown; **P*<0,05; ***P*<0,01 versus Placebo; §*P*<0,05; §§*P*<0,01 versus Control; $^{\#\#}$*P*<0,01 versus Control.

The following age group, where fatty acids were measured, was day 35, the time of weaning. The results demonstrate that, the maternal supplementation determined the brain fatty acid composition, however the effect of the diets was not as prominent as it was in the previous age group (Table 6.) Significant difference was not observed in the case of MUFAs and ARA in the Supplement group compared to the Control and the Placebo groups. DHA remained significantly higher in the Supplement group than in the Control and Placebo groups, while DPA remained significantly lower in the Supplement group than in the Control and Placebo groups. A new fatty acid not detected after birth, 20:1n9 appeared here, the Placebo group contained the higher amount of 20:1n9, and it was significantly elevated compared to Control

group. The Control group the level of 18:0 increased compared to Supplement and Placebo groups.

Table 6. Fatty acid content of PE from 35 days old rat brain

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	5,46±0,21	5,86±0,21	7,24±1,07	0,086
16:0DMA	5,08±0,05	5,02±0,07	4,98±0,25	0,794
18:0	14,73±0,23§§	15,14±0,26$^{\#}$	17,32±1,17	0,018
18:0DMA	9,14±0,21	8,77±0,16	8,94±0,42	0,575
MUFA				
18:1n7	1,59±0,05	1,68±0,41	1,53±0,04	0,140
18:1n7DMA	2,00±0,09	2,00±0,02	1,76±0,08	0,078
18:1n9	10,73±0,37	11,02±0,16	10,23±0,23	0,195
18:1n9DMA	2,76±0,08	2,82±0,02	2,64±0,04	0,368
20:1n9	1,39±0,08	1,55±0,05$^{\#\#}$	1,25±0,04	0,028
PUFA				
20:4n6 (ARA)	11,67±0,24	11,55±0,13	11,93±0,49	0,721
22:4n6	4,71±0,16	5,08±0,06	5,15±0,18	0,086
22:5n6	0,31±0,005**§§	1,28±0,02$^{\#\#}$	0,85±0,05	0,000
22:6n3 (DHA)	22,56±0,43**§§	20,17±0,27	19,56±0,86	0,003

Means±S.E.M.s are shown; ***P*<0,01 versus Placebo; §§*P*<0,01 versus Control; $^{\#}$*P*<0,05; $^{\#\#}$*P*<0,01 versus Control.

In the case of adults (13 months old rats, Table 7.), the effect of maternal supplementation on brain fatty acid composition has disappeared.

Table 7. Fatty acid content of PE from 13 months old rat brain

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	3,96±0,24	4,95±0,42	4,39±0,17	0,107
16:0DMA	3,47±0,37*§§	4,42±0,22	4,74±0,21	0,021
18:0	13,04±0,26	14,10±0,53	13,52±0,75	0,336
18:0DMA	6,25±0,86**§§	9,17±0,54	9,75±0,57	0,006
MUFA				
18:1n7	2,35±0,05	2,23±0,13	2,32±0,09	0,662
18:1n7DMA	3,12±0,43	3,80±0,30	4,35±0,40	0,110
18:1n9	13,96±0,27	15,20±0,81	14,18±0,36	0,266
18:1n9DMA	2,60±0,36**§§	3,79±0,20	3,87±0,23	0,010
20:1n9	3,49±0,18	3,34±0,28	3,07±0,17	0,482
PUFA				
20:4n6 (ARA)	10,88±0,15	10,64±0,47	11,00±0,18	0,746
22:4n6	5,70±0,18	5,07±0,27	5,35±0,08	0,118
22:5n6	0,41±0,01	0,43±0,02	0,48±0,03	0,156
22:6n3 (DHA)	17,74±0,35	15,12±1,11	16,73±0,35	0,065

Means±S.E.M.s are shown; **P*<0,05; ***P*<0,01 versus Placebo; §*P*<0,05; §§*P*<0,01 versus Control

The dietary groups did not differ in PUFAs, MUFAs, or SFAs content in the brain. Interestingly, the amount of all three DMA- plasmalogen derivates- decreased in the Supplement group compared to the Placebo and the Control groups.
In the case of the last age group, the very old rats (age of 26 months) (Table 8.) the only one difference was observed between Placebo and Control groups in the level of 18:1n9DMA. The level of 18:1n9DMA was higher in the Control group than in the Placebo group.

Table 8 Fatty acid content of PE from 26 months old rat brain

Fatty acids	Supplement	Placebo	Control	Anova *P*
SFA				
16:0	8,26±1,80	9,51±1,91	7,21±1,01	0,727
16:0DMA	5,04±0,33	4,36±0,64	5,12±0,42	0,501
18:0	17,07±1,58	18,64±1,91	15,92±0,80	0,590
18:0DMA	8,15±0,49	6,46±0,77	8,27±0,48	0,106
MUFA				
18:1n7	2,28±0,16	2,45±0,18	2,18±0,2	0,628
18:1n7DMA	5,40±0,33	4,21±0,69	5,09±0,38	0,224
18:1n9	13,35±0,79	12,97±0,58	13,62±0,71	0,856
18:1n9DMA	4,02±0,25	3,44±0,27$^{\#}$	4,80±0,49	0,045
20:1n9	2,86±0,22	2,90±0,20	3,45±0,09	0,190
PUFA				
20:4n6 (ARA)	10,11±0,68	9,67±0,71	9,72±0,08	0,858
22:4n6	4,77±0,30	5,19±0,32	5,24±0,15	0,489
22:5n6	0,73±0,11	0,59±0,05	0,70±0,04	0,535
22:6n3 (DHA)	13,38±0,71	12,87±0,61	13,92±0,84	0,668

Means±S.E.M.s are shown; $^{\#}P$<0,05 versus Control.

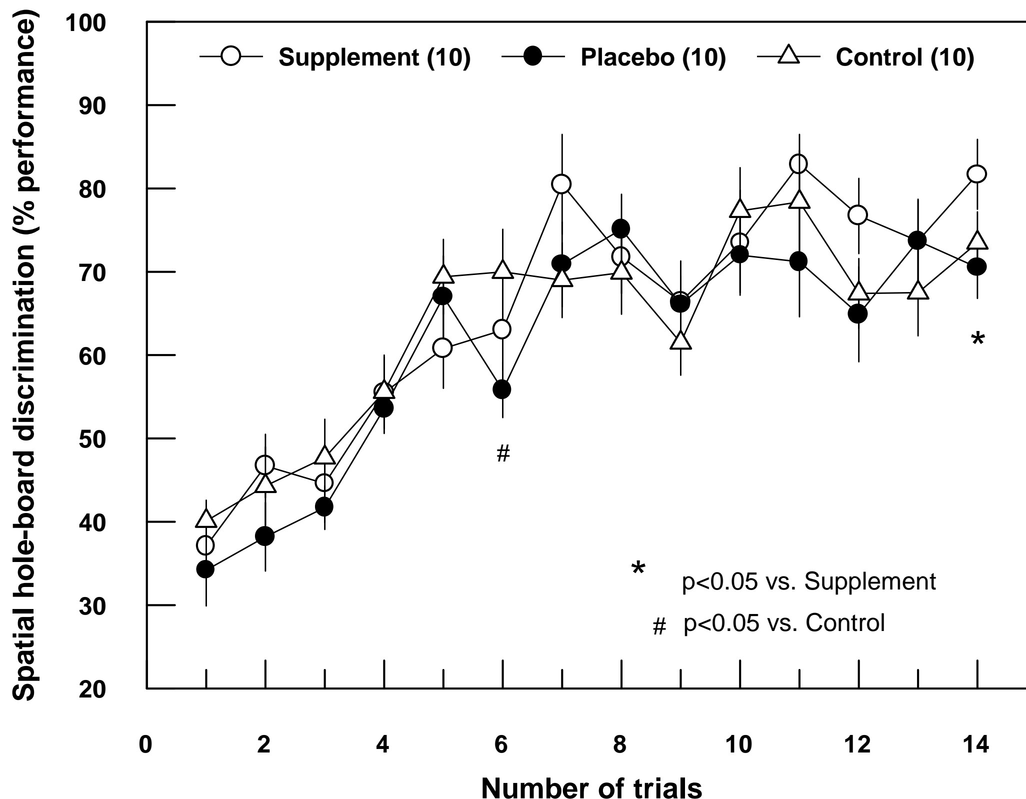
Examining the results from the point of view of aging, the effect of maternal supplementation was subsided after weaning. After using multivariate two-way ANOVA analysis, it has been revealed that the ratio of all fatty acids in the brain changes significantly during aging (*P*=0,000). Briefly, the content of both main SFAs, 16:0 and 18:0 gradually decreased from birth till adulthood, but increased in the old rats. The level of all types of MUFAs was increasing during aging, mainly between birth and adulthood; however slight reduction was measured in the 26 months old rats. The most marked changes were obtained in the level of 18:1n9. The amount of oleic acid increased with 286%, 271% and 299% in the Supplement, Placebo and Control groups, respectively. It seemed that the brain was not able to retain the high amount of various PUFAs. ARA, which is the most important n-6 fatty acid, showed an age-dependent, dramatic decrease till weaning (34%, 39% and 34% in the Supplement, Placebo and Control groups), but only little after it (only 14%, 17% and 19% in the

Supplement, Placebo and Control groups from the age of 35 days till the age of 26 months). Level of DHA was diminishing continuously during aging (47%, 34% and 32% in the Supplement, Placebo and Control groups. DPA appeared to substitute the lost ARA and mainly DHA, the level of DPA increased in the old rats again. But the decreasing ARA and DHA were replaced by MUFAs in adulthood, and MUFAs and SFAs in old rats.

Spatial discrimination learning task. There was no considerable difference between the dietary groups in adulthood in learning performance (Fig. 4). However, the Placebo group tended to perform worse than the other two groups, but significant differences were observed at only two trials on day 6 and 14. Supplement and Control groups did not differ from each other. There was no alteration obtained in the learning performance from old rats either (data not shown).

Fig. 4

Spatial discrimination performance in hole-board of 12-mo old female rats



Discussion of the late effect of maternal LC-PUFA supplementation.

In this experiment, we tried to prove that maternal n-3 supplementation had a late effect on mental performance of the offspring in rats. Similar tendencies were found in humans: infants whose mothers received n-3 supplemented diet, performed better at the age of 4 months (3) and age of 4 years (57) in the proper cognitive tests. In our present study, we showed that the LC-PUFA deficient group performed poorer compared to the Supplement and the Control group in spatial discrimination test in adulthood (at the age of 13 months). However at this age the fatty acid composition of the brain of rats from all three groups did not altered. Fatty acid alteration due to diet was observed only in the first two ages, in 7 days old and 35 days old rats. This period is important for brain development and brain requires large amount of DHA in this period (7,51). We concluded that lower level of DHA during the brain development had negative effect on learning performance in adulthood. It was well known that depletion of DHA through two generations caused decline in both brain DHA content and cognitive function (13). We showed it was enough to reduce the DHA intake during pregnancy and lactation, the first generation already exhibited failure in learning. However, this difference disappeared in old rats (data not shown here). The molecular mechanism of action of DHA during brain development is not clear. It may related with enhancing effect on NGF, or triggering gene expression, although gene expression evoked by DHA has not been investigated in infant rat brain yet, only in young (77) or old rats (125).On the other hand, there was no significant difference in the learning performance between the Supplement and the Control groups. It indicated that DHA supplemented diet had no beneficial effect on learning performance in adulthood. DHA supplementation after weaning was also unaffected on the learning performance in young rats (152). It was also revealed that brain was not able to retain the extra accumulated DHA during aging. The level of DHA was decreasing continuously during aging, but most rapidly in the Supplement group. There was a large drop in the level of ARA before weaning, but there was only a little loss after it. DTA did not change markedly during aging, while DPA was decreasing from infant till adulthood, but it seemed DPA tried to replace the lost DHA in old rats. Altogether, PUFAs were decreasing during aging. It may have been the consequence of age related impairment of Δ6 desaturase activity (15), or increased oxidative stress (69-71) in aged rat brain. MUFAs replaced PUFAs in the aging brain till adulthood, while SFAs in old rats. It indicated that activity of Δ9 desaturase might have been impaired in old rats.

3. **Results of the study of short term fish oil administration in old rats.**

Fatty acid content of PE and PC in old rats. Table 9. shows the effect of one month diet on brain PE fatty acid composition (% of total fatty acid).

Table 9. Fatty acid composition of PE in old rat brain

Fish oil	Control	Fish oil
16:0DMA	5,33±0,38	4,65±0,55
16:0	5,75±1,56	5,52±0,13
16:1n7	0,73±0,29	0,63±0,15
18:0DMA	9,15±0,35	9,29±1,14
18:1n-9DMA	3,53±0,32	3,30±0,87
18:1n-7DMA	5,44±0,53	5,59±1,28
18:0	13,81±0,74	13,83±0,81
18:1n9	13,22±0,76	13,72±0,56
18:1n7	5,44±0,53	5,59±1,28
18:2n-6	0,83±0,22	0,61±0,20
20:1n-9	3,39±0,33	3,69±0,62
20:4n-6	10,92±1,05	9,32±0,57
22:4n-6	5,60±0,38	5,05±0,35
22:6n-3	15,97±0,56*	17,21±0,54

Means±S.E.M.s are shown; *$P<0,05$.

Due to the high amount of DHA in the diet, the level of DHA in the brain elevated in the Fish oil group from 15,97% to 17,21% ($P<0,05$). This was the only significant alteration. There was no difference in fatty acid composition of PC in the brain (Table 10.).

Table 10. Fatty acid composition of PC in old rat brain

Fatty acids	Control	Fish oil
16:0	37,33±4,86	38,81±2,54
18:0	14,41±2,05	14,57±1,63
18:1n-9	27,04±2,54	25,06±3,66
18:2n-6	1,38±0,44	1,50±0,36
20:1n-9	1,24±0,36	1,21±0,16
20:4n-6	5,65±0,81	4,78±0,50
22:4n-6	0,73±0,86	0,60±0,70
22:6n-3	3,60±0,68	3,72±0,32

Means±S.E.M.s are shown.

Spatial learning performance. Fig. 5. shows that there was no difference in Morris water maze task between the two groups. It seemed that despite increased level of DHA in PE, the learning performance was not improved.

Aging is accompanied by the loss of DHA from the brain, first of all from PE (26,39). The loss of DHA supposed to be related to compromised brain function, memory and learning. We speculated that restoring the lost DHA by dietary supplementation would improve learning

performance. It took a relatively short time, only one month, to elevate DHA in PE in the brain. However, the learning performance was not improved.

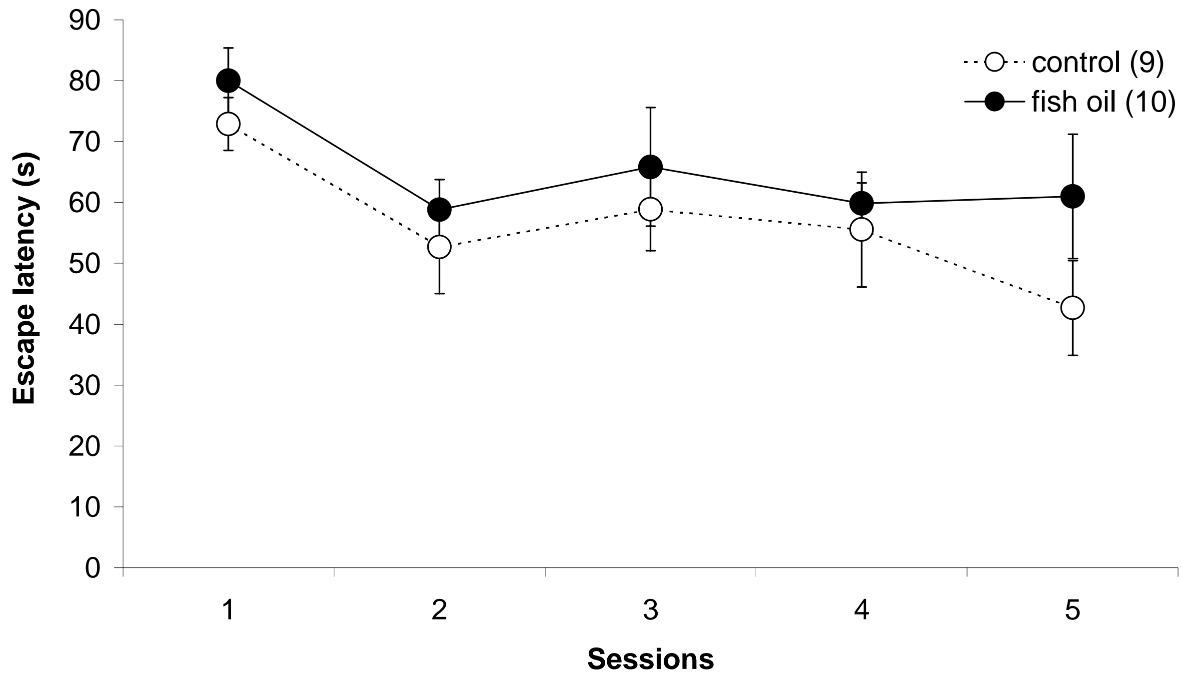


Fig. 5. *Morris water maze performance in old rats.*

Discussion of the study of short term fish oil administration in old rats.

The level of DHA was significantly higher in the fish oil group than in the control group, but the difference was little, only 8%. Longer time of feeding may have increased DHA more and it may have enhanced learning performance. The elevation of DHA was not accompanied by a significant reduction of any other fatty acids. The n-6 fatty acids showed a tendency for lower concentration, but the level of SFA and MUFA remained unchanged. We showed in the previous experiment, that SFAs and MUFAs replaced PUFAs in neural membrane in old rats (26 months) causing more rigid membrane and altered membrane function (163). Short term fish oil administration may have not been sufficient to make the membrane more fluid. Beside DHA, ARA concentration also decreases in old rats. The restoration of ARA improved the ability to sustain long-term potentiation, and glutamate release (99). Raising the ARA content of neural membrane might be beneficial in learning as well (81). The 1:4 ratio of LNA and

LA was found beneficial in younger rats (158). In this study, short term DHA intake failed to increase learning performance in old rats. Instead, a longer feeding regime or an ideal dietary DHA/ARA ratio may improve learning capacity.

4. **Results of the long term LC-PUFA feeding in SHR rats.**

Systolic blood pressure. Fig. 6. shows the beneficial effect of n-3 PUFAs on high blood pressure. Experimental diets (Diet 1, Diet 2) reduced systolic blood pressure (with around 50 and 30 mmHg, respectively) in adult (53-55 weeks) SHR rats. Blood pressure was unaffected by the experimental diets in the normotensive cases. Dietary effect on blood pressure faded with aging (79 weeks).

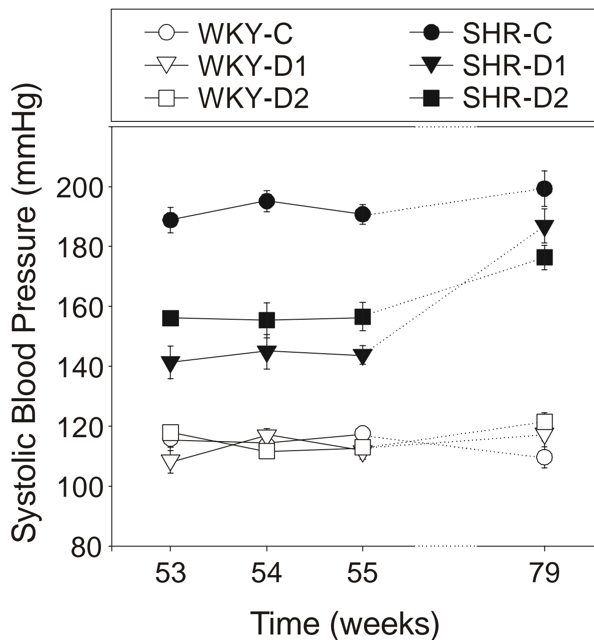


Fig. 6. *The systolic blood pressure in different experimental groups. Abbreviations: WKY: Wistar-Kyoto rat strain, SHR: spontaneously hypertensive rat strain, C: control diet, D1: diet 1, D2: diet 2.*

Learning test. Spatial learning in the holeboard (Fig. 7.) revealed that the experimental diets had a positive effect on memory score in WKY rats [F(diet)=3,727* in the first session, F(diet)=2,078 in the repeated session], but post hoc analysis showed that only Diet 2 group differed significantly from the controls. Blood pressure did not modify learning score [F(bp)=3,094 in the first session, F(bp)=1,668 in the repeated session]. Both experimental diets were unaffected in SHRs.

Fatty acid composition. Table 11-13. summarize the changes in fatty acid composition of neural membranes caused by diet and hypertension. Emphasizing the main changes: the experimental diets reduced the level of certain SFAs (16:0 and 18:0) in PC and PI, but there was no effect of blood pressure on SFA content. In case of MUFAs, the amount of 18 and 20

carbonic atom numbered fatty acids were decreased in hypertensive rats on control diet, however the experimental diets elevated the MUFA concentration. Both types of PUFAs decreased significantly in SHRs compared to WKY rats, mainly in PI. On the other

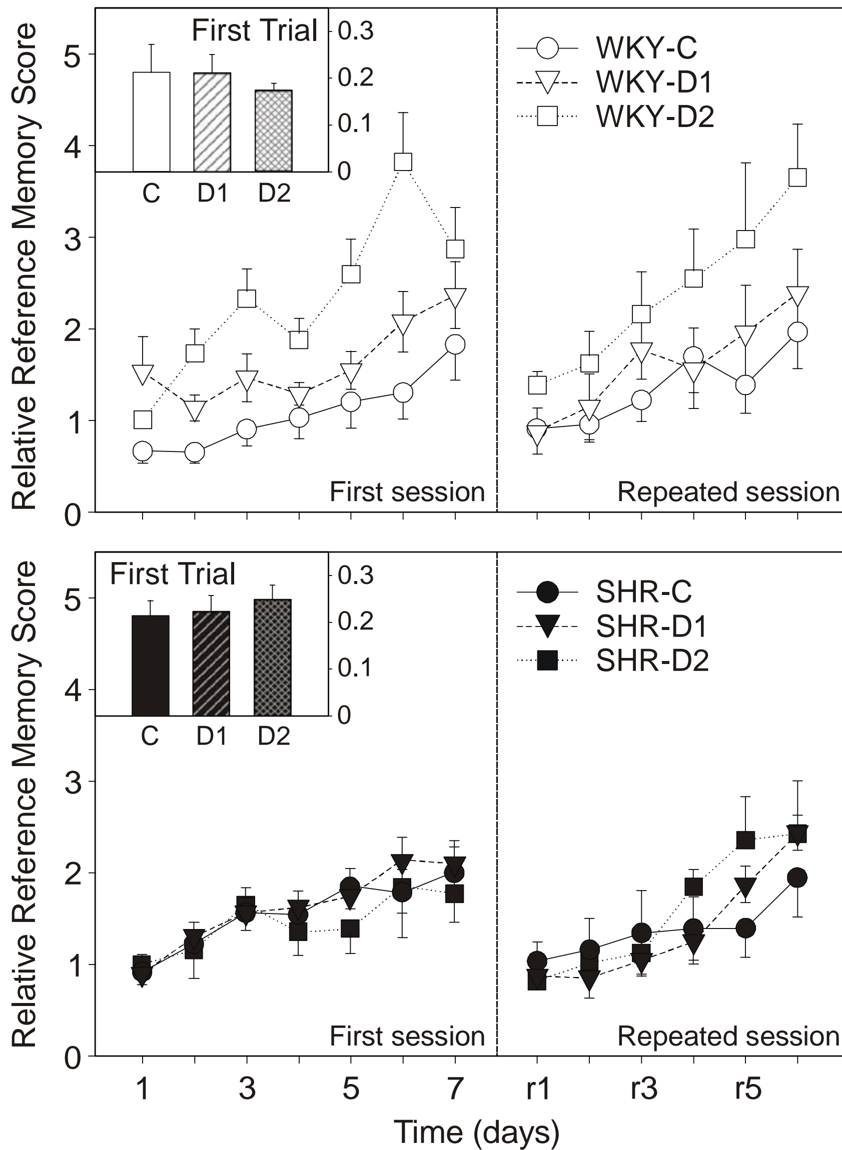


Fig. 7. *Memory score in the holeboard spatial discrimination test.*

hand, both PUFA enriched diets alleviated this reduction. The (n-6)/(n-3) ratio seemed the most sensitive to both diet and hypertension. Both experimental diets reduced the (n-6)/(n-3) ratio, while blood pressure had opposite effect on this ratio.. Fig 8. represents the correlation between blood pressure and (n-6)/(n-3) in PE. A positive correlation can be observed between the two parameters. Furthermore, (n-6)/(n-3) ratio correlated directly with learning score in the holeboard test. We found strong negative correlations (Fig. 9.)

Table 11. Fatty acid concentration of brain structural phospholipids in WKY.

FA Types	Fatty Acids	Phospholipid Family											
		PE			PS			PC			PI		
		WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2
DMA	16:0	5.16±0.76	5.72±0.74	4.81±1.20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	6.56±1.17	7.31±1.22	6.30±1.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	5.38±1.00	6.47±1.15	5.30±1.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	2.92±0.44	2.86±0.46	2.45±0.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	total DMA	20.02±3.29	22.37±3.54	18.86±4.94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	16:0	n.d.	n.d.	n.d	11.45±4.25	4.90±1.34	5.03±2.12	35.88±2.56	37.42±1.61	36.27±2.11	23.05±5.91	15.84±4.45	19.80±2.13
	18:0	14.31±3.07	11.21±1.23	12.32±1.36	33.40±4.25	36.06±1.48	35.89±1.53	17.67±2.22	13.99±1.18	15.33±2.15	28.15±6.72	26.23±5.05	32.00±3.24
	total SFA	14.31±3.07	11.20±1.23	12.32±1.36	44.84±7.14	40.96±1.65	40.92±1.87	53.55±2.25	51.41±1.79	51.60±3.30	11.49±4.54	42.06±9.04	51.80±4.01
MUFA	16:1 n-7	0.95±0.74		2.11±0.70	n.d.	n.d.	n.d.	1.93±0.31	1.46±0.26	1.10±0.50	3.34±0.20	2.79±0.94	1.79±0.16
	18:1 n-7	3.85±0.40	4.38±0.29	4.26±0.22	n.d.	n.d.	n.d.	6.89±0.94	7.89±0.70	7.75±0.62	2.71±0.41	3.24±0.44	2.84±0.70
	18:1 n-9	17.26±1.19	17.52±0.80	17.05±0.48	20.37±2.86	27.29±2.66	27.12±3.85	22.94±1.76	24.36±1.25	24.04±1.50	13.39±3.30	9.08±2.38	9.41±2.52
	20:1 n-7	0.64±0.59	1.13±0.09	1.15±0.12	n.d.	n.d.	n.d.	0.36±0.51	1.00±0.08	0.89±0.37	n.d.	n.d.	n.d.
	20:1 n-9	4.96±0.73	4.58±0.35	4.86±0.39	3.10±0.88	3.89±0.38	4.06±0.52	1.82±0.30	1.60±0.08	1.75±0.16	n.d.	n.d.	n.d.
	total MUFA	35.96±3.51	38.44±2.07	37.17±1.99	23.47±3.29	31.18±2.90	31.18±4.34	33.94±2.79	36.31±2.20	35.52±2.22	18.28±3.85	15.11±2.33	12.49±1.99
PUFA	18:2 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.06±0.71	1.04±0.27	1.08±0.57	5.49±1.55	2.63±1.60	2.77±1.06
	20:4 n-6 (ARA)	**6.91±1.17**	**7.22±0.37**	**7.43±0.31**	**2.40±0.71**	**3.52±0.25**	**3.88±0.66**	**3.90±0.52**	**4.29±0.38**	**4.31±0.34**	**8.89±1.92**	**20.51±4.47**	**20.00±5.95**
	22:4 n-6	3.25±0.50	3.11±0.22	3.49±0.20	1.68±0.46	2.27±0.23	2.39±0.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22:6 n-3 (DHA)	**11.76±1.72**	**14.14±0.84**	**13.81±0.54**	**9.62±2.60**	**14.73±0.68**	**14.07±1.69**	**3.90±0.60**	**4.47±0.16**	**4.35±0.36**	**0.83±1.45**	**3.41±0.73**	**4.29±1.33**
	total PUFA	**21.92±3.27**	**24.48±1.34**	**24.73±0.96**	**13.70±3.71**	**20.52±1.01**	**20.34±2.48**	**7.80±1.11**	**8.76±0.51**	**8.66±0.69**	**15.21±3.49**	**26.56±4.81**	**26.60±6.16**
	(n-6)/(n-3)	**0.86±0.05**	**0.73±0.03**	**0.79±0.02**	**0.43±0.04**	**0.39±0.02**	**0.45±0.03**	**1.30±0.25**	**1.19±0.05**	**1.24±0.13**	**11.49±4.54**	**7.02±1.83**	**5.36±1.12**
Rest		7.80±7.97	3.51±5.77	6.91±6.69	17.99±5.93	7.34±3.87	7.57±5.23	4.72±4.28	3.53±3.16	4.23±3.15	15.31±10.63	16.28±13.99	9.11±7.67

Table 12. Fatty acid concentration of brain structural phospholipids in SHR.

FA Types	Fatty Acids	Phospholipid Family											
		PE			PS			PC			PI		
		SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2
DMA	16:0	5.77±0.82	6.06±0.67	5.64±0.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	7.02±0.58	6.88±0.94	6.07±0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	4.65±0.86	5.35±0.84	4.96±1.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	2.62±0.45	2.51±0.39	2.33±0.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	total DMA	20.07±2.43	20.80±2.73	19.00±3.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	16:0	n.d	n.d	n.d	11.75±5.58	7.48±0.51	6.94±2.20	36.80±2.27	35.87±2.98	33.66±2.81	18.67±3.95	19.03±3.76	17.98±4.35
	18:0	12.53±2.16	13.60±1.02	11.90±1.55	34.42±3.90	37.04±4.70	35.05±2.49	16.49±1.85	15.51±0.70	16.03±1.38	29.89±11.37	27.45±5.69	30.12±6.82
	total SFA	12.53±2.16	13.60±1.02	11.90±1.55	46.17±8.01	44.51±5.09	41.99±3.72	53.29±2.92	51.39±2.85	49.68±1.98	48.56±15.30	46.48±9.13	48.10±10.54
MUFA	16:1 n-7	1.12±0.22	1.14±0.30	1.56±0.80	n.d.	n.d.	n.d.	1.39±0.62	1.78±0.58	1.53±0.32	2.91±1.31	3.90±1.33	2.41±1.38
	18:1 n-7	3.23±0.29	3.78±0.20	3.68±0.16	n.d.	n.d.	n.d.	5.98±0.55	6.07±0.56	6.01±0.24	1.95±0.79	2.73±0.47	2.58±0.52
	18:1 n-9	16.60±1.58	16.88±0.41	17.82±0.72	18.09±2.64	24.49±2.25	22.32±2.02	23.71±2.08	23.01±1.57	23.96±1.03	9.31±4.42	9.17±1.34	7.63±2.27
	20:1 n-7	0.64±0.28	0.91±0.10	0.93±0.10	n.d.	n.d.	n.d.	0.56±0.25	0.71±0.40	0.73±0.06	n.d.	n.d.	n.d.
	20:1 n-9	5.23±0.73	4.63±0.38	5.45±0.52	2.85±0.74	3.14±0.39	3.05±0.46	1.60±0.15	1.62±0.68	1.55±0.12	n.d.	n.d.	n.d.
	total MUFA	34.10±4.06	35.18±2.04	36.71±2.22	20.94±3.11	27.64±2.34	25.38±2.45	33.23±2.14	33.18±2.50	33.77±1.05	14.17±5.78	15.80±1.30	12.62±2.97
PUFA	18:2 n-6	n.d	n.d	n.d	n.d	n.d	n.d	1.08±0.50	1.41±0.32	1.37±0.23	3.45±1.92	2.62±1.27	2.50±1.08
	20:4 n-6 (ARA)	**7.13±0.70**	**6.68±0.42**	**7.40±0.58**	**2.02±0.59**	**3.13±0.47**	**3.21±0.54**	**4.06±0.57**	**3.45±0.15**	**4.12±0.26**	**5.54±1.67**	**11.22±3.67**	**13.85±5.15**
	22:4 n-6	3.38±0.35	2.83±0.23	3.41±0.32	1.69±0.49	1.61±0.85	2.03±0.27	n.d	n.d	n.d	n.d	n.d	n.d
	22:6 n-3 (DHA)	**11.61±1.40**	**12.70±0.77**	**13.00±0.97**	**8.35±2.81**	**13.99±2.54**	**12.26±1.92**	**3.38±0.49**	**3.21±0.28**	**3.99±0.26**	**0.39±0.37**	**1.19±0.75**	**2.41±0.85**
	total PUFA	**22.12±2.36**	**22.20±1.31**	**23.79±1.82**	**12.06±3.86**	**18.73±2.68**	**17.49±2.61**	**7.44±1.03**	**6.66±0.35**	**8.10±0.49**	**9.37±2.26**	**15.04±3.64**	**18.75±5.65**
	(n-6)/(n-3)	**0.91±0.05**	**0.75±0.03**	**0.83±0.03**	**0.45±0.03**	**0.35±0.07**	**0.43±0.03**	**1.54±0.22**	**1.53±0.21**	**1.38±0.08**	**11.75±2.74**	**10.22±3.16**	**7.04±1.05**
Rest		11.19±7.29	8.22±5.03	8.59±5.55	20.84±7.23	9.13±8.97	15.14±5.01	6.03±2.88	8.78±5.24	8.45±2.62	27.90±7.82	22.69±10.54	20.52±10.07

Table 13. Statistical F values of fatty acid concentration analysis in brain structural phospholipids (Multivariate analysis).

FA Types	Fatty Acids	Phospholipid Family											
		PE			PS			PC			PI		
		diet effect	bp effect	interaction	diet effect	bp effect	interaction	diet effect	bp effect	interaction	diet effect	bp effect	interaction
Overall Effect		8.472**	40.821**	1.469	6.547**	4.951**	0.751	4.463**	23.438**	3.149**	5.450**	8.741**	2.048*
DMA	16:0	2.601	4.557*	0.344	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	3.288*	0.052	0.886	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	3.037	5.152*	0.479	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	1.197	3.144	0.238	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	total DMA	2.533	0.229	0.334	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	16:0	n.d.	n.d.	n.d.	14.069**	2.521	0.629	2.203	2.346	2.182	0.863	0.015	1.732
	18:0	2.240	0.015	5.314*	2.419	0.148	0.366	7.505**	0.510	2.611	0.906	0.380	0.526
	total SFA	2.240	0.015	5.314*	2.288	1.510	0.232	4.907*	0.958	0.653	1.518	0.040	0.699
MUFA	16:1 n-7	7.786**	2.116	1.652	n.d.	n.d.	n.d.	2.911	0.303	5.450**	2.496	0.683	1.415
	18:1 n-7	18.801**	62.161**	0.025	n.d.	n.d.	n.d.	3.080	62.868**	2.367	5.667**	2.663	2.112
	18:1 n-9	1.197	0.428	3.147	25.597**	15.684**	0.845	0.737	0.240	1.840	4.313*	2.364	2.371
	20:1 n-7	9.995**	3.384	0.829	n.d.	n.d.	n.d.	6.899**	0.848	2.368	n.d.	n.d.	n.d.
	20:1 n-9	5.259**	3.960	1.045	4.417*	13.247**	1.496	0.406	2.238	0.770	n.d.	n.d.	n.d.
	total MUFA	2.589	5.769*	1.073	23.546**	17.574**	1.049	1.286	8.272**	1.166	5.185**	1.295	2.312
PUFA	18:2 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.553	2.746	0.580	5.025*	3.699	1.633
	20:4 n-6 (ARA)	2.305	0.400	0.256	24.864**	8.064**	0.307	3.132	6.164*	6.381**	17.458**	10.467**	3.068
	22:4 n-6	9.571**	0.669	1.726	4.804*	5.899*	1.981	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22:6 n-3 (DHA)	13.417**	6.507*	1.400	25.692**	3.742	0.208	7.552**	40.361**	6.113**	12.194**	20.608**	1.418
	total PUFA	5.240**	3.076	1.570	24.549**	5.584*	0.179	4.904*	21.061**	6.383**	18.233**	34.797**	1.432
	(n-6)/(n-3)	65.645**	11.041**	0.688	14.019**	1.258	3.028	1.834	23.992**	1.331	12.672**	4.140*	1.035
Rest		3.016	8.002**	0.255	13.302**	4.753*	0.873	0.302	11.428**	1.209	1.390	9.391**	0.354

Abbreviations: ARA: arachidonic acid, C: control diet, D1: Diet 1, D2: Diet 2, DHA: docosahexaenoic acid, DMA: dimethilacetale (plasmalogen-derived), FA: fatty acid, MUFA: monounsaturated fatty acids, n.d.: non-detected, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, p values: *≤0.05, **≤0.01.

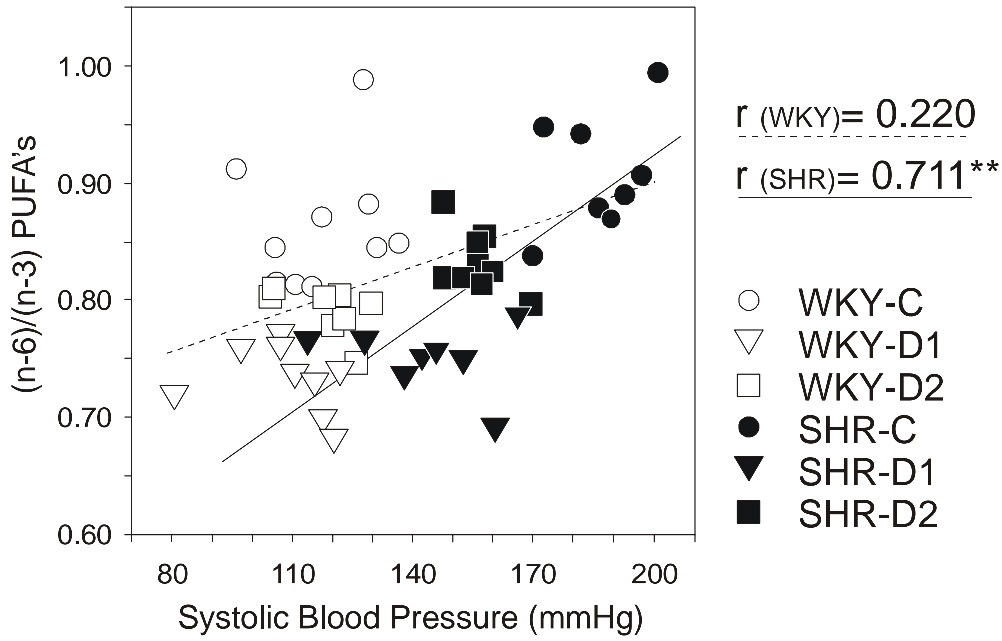


Fig. 8. *Correlation of systolic blood systolic blood press on week 53 and (n-6)/(n-3) ratio in PE*

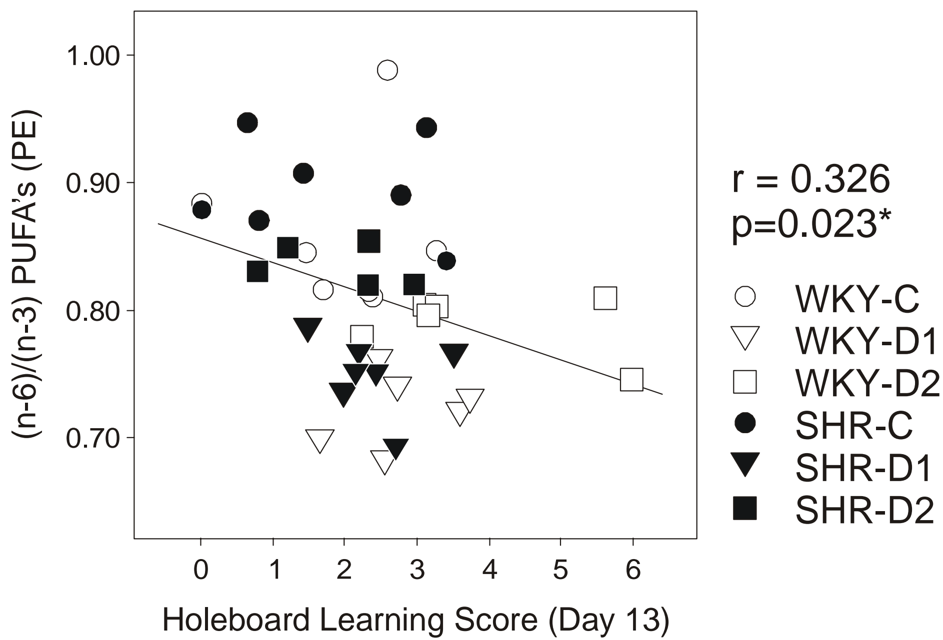


Fig. 9. *Correlation between holeboard learning score on day 13 and (n-6)/(n-3) ratio in PE.*

Discussion of the long term LC-PUFA feeding in SHR rats.

Our results support previous studies about the antihypertensive effect of n-3 fatty acid in SHR rats (59,160). This effect was profound in adulthood, but disappeared by aging. The possible mechanisms responsible for the blood-pressure lowering effect of the diets are the following. Thromboxane A_2 (TXA_2), which forms from ARA, is responsible for vasoconstriction and high blood pressure. DHA and EPA inhibit the formation of TXA_2 in different ways. (a) Both n-3 fatty acids inhibit ARA synthesis, and compete with ARA for in the incorporation into PLs (116), causing lower amount of ARA in platelets, (b) EPA competes with ARA in thromboxane synthesis, and shifts the synthesis toward the formation of TXA_3, which is not vasoactive (40,47).

Both dietary treatment and hypertension had deep impact on brain fatty acid content. PUFAs and MUFAs were found to be decreased in SHR rats, and positive correlation was found between (n-6)/(n-3) ratio and blood pressure. Decline in fatty acid transport through the BBB can be one of the possible explanation. Although, it has not been proven in the case of fatty acids, but the transport of certain amino acids through the BBB was affected by blood pressure (145). Furthermore, PUFA and MUFA metabolism may be impaired in SHR rats. Similar to liver (117), the activity of desaturases may be decreased due to high blood pressure. Increased lipid peroxidation in high blood pressure can damage PUFAs and MUFAs (66,120), what may lead to the loss of these fatty acids from the membrane.

Diet as opposed to blood pressure, increased both PUFAs and MUFAs content in the brain. DHA seemed to be raised the highest, this was the main reason of reduced (n-6)/(n-3) ratio.

The diets had positive effect on spatial discrimination test only in normotensive rats. It was interesting to note that Diet 2 was effective only, which contained extra additives besides n-3 PUFAs. However neither of the two experimental diets affected learning in the SHR rats when SHR groups were compared. Nevertheless, correlating individual data from SHR and WKY groups, it was revealed that (n-6)/(n-3) ratio in PE correlates negatively to learning score. It was possible that group comparison was not able to show such slide changing due to individual variations, while correlation seemed to be more sensitive. That is why we concluded, that long term dietary n-3 PUFA supplementation, which reduced (n-6)/(n-3) ratio, was beneficial for both WKY and SHR rats.

Summary

Dietary DHA supplementation was used in different ages to prove the beneficial effect of DHA on the brain function in rats. There are two main susceptible ages for the DHA supplementation: fetal age, the time of brain development and aging, when the brain looses higher amount of DHA. We focused on these two periods in our experiments.

1. Using different types of semi-synthetic diets we showed that LC-PUFAs, mainly DHA had neuroprotective effect on NMDA excitotoxicity in 14 days old rats. We found more neurons survived in the nucleus basalis magnocellularis (NBM) and less axonal degeneration in the cortex after NMDA injection into the NBM, in the pups whose mother received LC-PUFA supplementation during pregnancy and lactation, compared to control (enriched in n-6 PUFAs, but poor in n-3 PUFAs) and placebo (lack of both types of LC-PUFAs) groups. The level of DHA was elevated in all phospholipid classes in the supplemented group compared to the control and placebo groups. In conclusion, developmental DHA treatment increased the viability of cholinergic neurons.
2. Furthermore in studying the latent functional consequences, we have found that DHA deficiency during brain development had long-term effect on cognitive function of rats. The dams received the same diet as above during pregnancy and lactation. After weaning the pups received the same control diet. The placebo group performed worse in spatial discrimination learning task at the age of 1 year, than control and supplemented groups. However, the brain fatty acid profiles in the three groups have been restored by this age.
3. Related to aging, short-term fish oil supplementation did not improve learning performance in 24 moths old rats. After 1 month of fish oil treatment, the level of DHA in the brain increased in old rats, compared to controls. However, the fish oil group did not perform better in the Morris water maze task. It indicated, that restoration of the level of DHA was not enough to improve learning performance.
4. LC-PUFA administration from 4 to 80 weeks of age, and hypertension had huge impact on brain LC-PUFA status and on learning performance (hole-board spatial discrimination test) in old spontaneous hypertensive rats (SHR). Hypertension decreased the level of LC-PUFAs and the ratio of n-3/n-6 PUFAs in the brain, while long term LC-PUFA feeding increased the level of LC-PUFAs and the ratio of n-3/n-6 PUFAs. We found strong positive correlation between blood pressure and the ratio of n-6/n-3 PUFAs, and strong negative correlation between learning performance and the ratio of n-6/n-3 PUFAs.

References

1. Abeywardena, M.Y., and Head, R.J. (2001) *Cardiovasc. Res.* **52,** 361-371.
2. Abraham, S., Matthes K.J., and Chaikoff I.L. (1959) *Biochim. Biophis. Acta.* **36,** 556-558.
3. Agostoni, C., Trojan, S., Bellù, R., Riva, E., and Giovanni, M. (1995) *Pediatr. Res.* 38, 262-266.
4. Aki, T., Shimada, Y., Inagaki, K., Higashimoto, H., Kawamoto, S., Shigeta, S., Ono, K., and Suzuki O. (1999) *Biochem. Biophys. Res. Commun.* **255,** 575-579.
5. Alberghina, M., Lupo, G., and Anfuso, C.D. (1994) *Neurosci. Lett.* **171,** 133-136.
6. Anderson, G.J. (1994) *J. Lipid. Res.* **35,** 105-111.
7. Anderson., G.J., Hohimer, A.R., and Willeke, G.B. (1993) *Life Sci.* **53,** 1089-1098.
8. Araki, W., and Wurtman, R.J. (1997) *Proc. Natl. Acad. Sci. USA*. **94,** 11946-11950.
9. Ayala, S., Gaspar, G., Brenner, R.R., Peluffo, R.O., and Kunau, W.H. (1973) *J. Lipid Res.* **14,** 296-305.
10. Bao, D.Q., Mori, T.A., Burke, V., Puddey, I.B., and Beilin L.J. (1998) *Hypertension*. **32,** 710-717.
11. Batchelor, P.E., Armstrong, D.M., Blaker, S.N., and Gage, F.H. (1989) *J. Comp. Neurol.* **284,** 187-204.
12. Bellenger-Germain, S., Poisson, J.P., and Narce, M. (2002) *Lipids.* **37,** 561-567.
13. Bruce, G., Wainer, B.H., and Hersh, L.B. (1985) *J. Neurochem.* **45,** 611-620.
14. Bourre, J.M., Durand, G., Pascal, G., and Youyou, A. (1989) *J. Nutr.* **119,** 15-22.
15. Bourre, J.M., Piciotti, M., and Dumont, O. (1990) *Lipids.* **25,** 354-356.
16. Carrie, I., Smirnova, M., Clement M, De, J.D., Frances, H., and Bourre, J.M. (2002) *Nutr. Neurosci.* **5,** 42-52.
17. Chichester, C.O., and Rodgers, R.L. (1987) *J. Cardiovasc. Pharmacol.* **10 (Suppl 9)** 21-26.
18. Cho, H.P., Nakamura, M.T., and Clarke, J. (1999) *J. Biol. Chem.* **274,** 471-474.
19. Cho, H.P., Nakamura, M.T., and Clarke, J. (1999) *J. Biol. Chem.* **274,** 37335-37339.
20. Christensen, E., Woldseth, B., Hagve, T.A., Poll-The, B.T., Wanders, R.J.A., Sprecher, H., Stokke, O., and Christopherson, B.O. (1993) *Scand. J. Clin. Lab. Invest.* **53 (Suppl 215)** 61-74.
21. Cobiac, L., Clifton, P.M., Abbey, M., Belling, G.B., and Nestel, P.J. (1991) *Am. J. Clin. Nutr.* **53,** 1210-1216.

22. Coyle, J.T., and Puttfarcken, P. (1993) *Science*. **262,** 689-695.
23. Crawford, M.A., Costeloe, K., Ghebremeskel, K., Phylaktos A., Skirvin, L., and Stacey, F. (1997) *Am. J. Clin. Nutr.* **66 (Suppl 4),** 1032-1041.
24. Delachambre, M.C., Narce, M., Asdrubal, P., and Poisson, J.P. (1998) *Lipids.* **33,** 795-801.
25. De la Torre, J.C., and Mussivand, T. (1993) *Neurol. Res.* **15,** 146-153.
26. Delion, S., Chalon, S., Guilloteau, D., Besnard, J.C., and Durand, G. (1996) *J. Neurochem.* **66,** 1582-1592.
27. de Wilde, M.C., Farkas, E., Gerrits, M., Kiliaan, A.J., and Luiten, P.G.M. (2002) *Brain Res.* **947,** 166-173.
28. Douma, B.R., van der Zee, E.A., and Luiten, P.G.M. (1998) *Behav. Neurosci.* **112,** 496-501.
29. Dunbar, L.M., and Bailey, J.M. (1975) *J. Biol. Chem.* **250,** 1152-1153.
30. Dyerberg, J., Leaf, A., and Galli, C. (1995) *J. Am. Coll. Nutr.* **14,** 213-214.
31. Eckert, G.P., Cairns, N.J., Maras, A., Gattaz, W.F., and Muller, W.E. (2000) *Dement. Geriatr. Cogn. Disord.* **11,** 181-186.
32. Eckert, G.P., Cairns, N.J., and Muller, W.E. (1999) *J. Neural. Transm.* **106,** 757-761.
33. Eckert, G.P., Kirsch, C., and Muller, W.E. (2001) *Neuroreport.* **12,** 883-887.
34. Eijkenboom, M., and van der Staay, F.J. (1999) *Neuroscience.* **91,** 1299-1313.
35. Engler, M.M., Bellenger-Germain, S.H., Engler, M.B., Narce, M., Poisson, J.P. (2000) *Lipids.* **35,** 1011-1105.
36. Estilaei, M.R., Matson, G.B., Payne, G.S., Leach, M.O., Fein, G., and Meyerhoff, D.J. (2001) *Alcohol Clin. Exp. Res.* **25,** 89-97.
37. Farkas, E., de Wilde, M.C., Kiliaan, A.J., Meijer, J., Keijser, J.N., and Luiten, P.G.M. (2002) *Brain Res.* **954,** 32-41.
38. Farooqui, A.A., Yang, H.C., Rosenberger, T.A., and Horrocks, L.A. (1997) *J. Neurochem.* **69,** 889-901.
39. Favreliere, S., Stadelmann-Ingrand, S., Huguet, F., De Javel, D., Piriou, A., and Tallineau, C. (2000) *Neurobiol. Aging.* **21,** 653-660.
40. Fischer S., and Weber, P.C. (1983) *Biochem. Biophys. Res. Comm.* **116,** 1091-1099.
41. Flaten, H., Hostmark, A.T., Kierulf, P., Lystad, E., Trygg, K., Bjerkedal, T., and Osland, A. (1990) *Am. J. Clin. Nutr.* **52,** 300-306.

42. Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957) *J. Biol. Chem.* **226,** 497-509.
43. Fujishima, M., Ibayashi, S., Fujii, K., and Mori, S. (1995) *Hypertens. Res.* **18,** 111-117.
44. Gabbita, S.P., Butterfield, D.A., Hensley, K., Shaw, W., and Carney J.M. (1997) *Free. Radic. Biol. Med.* **23,** 191-201.
45. Galli, C., Trzeciak, H.I., and Paoletti, R. (1971) *Biochim. Biophys. Acta.* **248,** 449-454.
46. Gamoh, S., Hashimoto, M., Hossain, S., and Masamura, S. (2001) *Clin. Exp. Pharmacol. Physiol.* **28,** 266-270.
47. Garg, M.L., Sebokova, E., and Thompson A.B.R. (1998) *Biochem. J.* **249,** 351-356.
48. Gasull, T., Sarri, E., DeGregorio-Rocasolano, N., and Trullas, R. (2003) *J. Neurosci.* **23,** 4100-4107.
49. Ginsberg, L., Xuereb, J.H., and Gershfeld, N.L. (1998) *J. Neurochem.* **70,** 2533-2538.
50. Green, P., Glozman S., Kamensky, B., and Yavin E. (1999) *J. Lipid Res.* **40,** 960-966.
51. Green, P., and Yavin, E. (1996) *Lipids.* **31,** 859-865.
52. Hagve, T.A., Narce, M., Tjonneland, S.E., Odden, N., Liabo, J., and Poisson, J.P. (2001) *Scand. J. Clin. Lab. Invest.* **61,** 151-159.
53. Hamazaki, T., Urakaze, M., Makuta, M., Ozawa, A., Soda, Y., Tatsumi, H., Yano, S., and Kumagai, A. (1987) *Lipids.* **22,** 994-998.
54. Haubner, L.Y., Stockard, J.E., Saste, M.D., Benford, V.J., Phelps, C.P., Chen, L.T., Barness, L., Wiener, D., and Carver, J.D. (2002) *Brain Res. Bull.* **58,** 1-5.
55. Hedreen, J.C., Bacon, S.J., and Price, D.L. (1985) *J. Histochem. Cytochem.* **33,** 134-140.
56. Helland, I.B., Saugstad, O.D., Smith, L., Saarem, K., Solvoll, K., Ganes, T., and Drevon, D.A. (2001) *Pediatrics.* **105,** 82-92.
57. Helland, I.B., Smith, L., Sareem, K., Saugstad, O.D., and Drevon, C.A. (2003) *Pediatrics.* **111,** 39-44.
58. Horrocks, L.A. and Yeo, Y.K. (1999) *Pharmacol. Res.* **40,** 211-225.
59. Howe, P.R.C., Rogers, P.F., and Lungerhausen, Y. (1991) *Prostaglandins Leukot. Essent. Fatty Acids.* **44,** 113-117.
60. Huster, D., Arnold, K., and Gawrisch, K. (1998) *Biochemistry.* **37,** 17299-17308.
61. Ilincheta da Boschero, M.G., Roque, M.E., Salvador, G.A., and Giusto, N.M. (2000) *Exp. Gerontol.* **35,** 653-668.
62. Ikemoto, A., Kobayashi, T., Watanabe, S., and Okuyama, H. (1997) *Neurochem. Res.* **22,** 671-678.

63. Ikemoto, A., Nitta, A., Furukawa, S., Ohishi, M., Nakamura A., Fujii, Y., and Okuyama, H. (2000) *Neurosci. Lett.* **285,** 99-102.
64. Ikemoto, A., Ohishi, M., Sato, Y., Hata, N., Misawa, Y., Fujii, Y., and Okuyama, H. (2001) *J. Lipid Res.* **42,** 1655-1663.
65. Innis S.M. (1993) *Can. J. Physiol. Pharmacol.* **72,** 1483-1492.
66. Ito, H., Torii, M., and Suzuki, T. (1993) *J. Biochem.* **25,** 1801-1805.
67. Jamil H., Utal, A.K., and Vance, D.E. (1992) *J. Biol. Chem.* **267,** 1752-1760.
68. Ji, L.L., Leewenburgh, C., Leichweis, S., Gore, M., Fiebig, R., and Hollander, J. (1998) *Ann. NY. Acad. Sci.* **854,** 102-117.
69. Joseph, J.A., Denisova, N.A., Fisher, D., Shukitt-Hale, B., Bickford P., and Prior, R. (1998) *Ann. NY. Acad. Sci.* **854,** 268-276.
70. Joseph, J.A., Denisova, N.A., Fisher, D., Bickford P., and Cao, G. (1998) **16,** 747-755.
71. Joseph, J.A., Denisova, N.A., Bielinski, D., Fisher, D.R., and Shukitt-Hale, B. (2000) *Mech. Ageing Dev.* **116,** 141-153.
72. Kennedy, E.P., and Weiss S.B. (1956) *J. Biol. Chem.* **222,** 193-214.
73. Kim, H.Y., and Edsall, L. (1999) *Lipids.* **34,** 249-250.
74. Kimura, S., Minami, M., Saito, H., Kobayashi, T., and Okuyama, H. (1995) *Clin. Exp. Pharmacol. Physiol.* **22 (Suppl. 1),** 308-309.
75. Kimura, S., Saito, H., Minami, M., Togashi, H., Nakamura, N., Ueno, K., Shimamura, K., Nemoto, M., and Parvez, H. (2002) *Neurotoxicol. Teratol.* **24,** 683-693.
76. Kiss, J., Shooter, E.M. and Patel, A.J. (1993) *Neuroscience.* **57,** 297-305.
77. Kitajka K., Puskas, L.G., Zvara, A., Hackler, L., Jr., Barcelo-Coblijn, G., Yeo, Y.K., and Farkas, T. (2002) *Proc. Natl. Acad. Sci. USA.* **99,** 2619-2624.
78. Klenk, E., Mohrhauer, H., and Hoppe-Seylers Z. (1960) *Physiol. Chem.* **320,** 218-232.
79. Koenig, B.J., Strey, H.H., and Gawrisch, K. (1997) *Biophys. J.* **73,** 1954-1966.
80. Koh, S., and Loy, R. (1989) *J. Neurosci.* **9,** 2999-3017.
81. Kotani, S., Nakazawa, H., Tokimasa T. *et al.* (2003) *Neurosci. Res.* **46,** 453-461.
82. Knutzon, D.S., Thurmond, J.M., Huang, Y.S., Chaudhary, S., Bobik, E.G., Chan, G.M., Kirchner, S.J., and Mukerji, P. (1989) *J. Biol. Chem.* **273,** 29360-29366.
83. Kumar, V.B., Buddhiraju, M., Alshaher, M., Flood, J.F., and Morley, J.E. (1999) *Life Sci.* **65,** 1657-1662.

84. Lanting, C.I., Fidler, V., Huisman, M., Touwen, B.C., and Boersma E.R. (1994) *Lancet.* **344,** 1319-1322.
85. Leonard, A.E., Kelder, B. Bobik, E.G., Chuang, L.T., Parker-Barnes, J.M., Thurmond, J.M., Kroeger, P.E., Kopchick, J.L., Huang, Y.S., and Mukerji, P. (2000) *Biochem. J.* **347,** 719-724.
86. Lehman, J., Nagy, J.I., Atmadja, S., and Fibiger, H.C. (1980) *Neuroscience.* **5,** 1161-1174.
87. Leonard, A.E., Pereira, S.L., Sprecher, H., and Huang Y.S. (2004) *Prog. Lipid Res.* **43,** 36-54.
88. Lim, S.Y., and Suzuki, H. (2000) *Int. J. Vitam. Nutr. Res*. **70,** 251-259.
89. Lim, S.Y., and Suzuki, H. (2000) *J. Nutr.* **130,** 1629-1632.
90. Lim, S.Y., and Suzuki, H. (2002) *Int. J. Vitam. Nutr. Res.* **72,** 77-84.
91. Litman, B.J., and Mitchell, D.C. (1996) *Lipids.* **31,** 193-197.
92. Lorenz, R., Spengler, U., Fischer, S., Duhm, J., and Weber, P.C. (1983) *Circulation.* **67,** 504-511.
93. Lucas, A., Morley, R., Cole, T.J., Lister, G., and Leeson-Payne, C. (1992) *Lancet.* **339,** 261-264.
94. Luiten, P.G.M., Douma, B.R.K., Van der Zee, E.A., and Nyakas, C. (1995) *Neurodegeneration.* **4,** 307-314.
95. Luiten, P.G.M., Gaykema, R.P.A., Traber, J., and Spencer, D.G., Jr. (1987) *Brain Res.* **413,** 229-250.
96. Maeda, M., Doi, O., and Akamatsu, Y. (1978) *Biochim. Biophys. Acta.* **530,** 152-164.
97. Matthes, K.L., Abraham, S., and Chaikoff, I.L. (1960) *J. Biol. Chem.* **235,** 2560-2568.
98. Mattson, M.P., Duan, W., Lee, J., and Guo, Z. (2001) *Mech. Aging Dev.* **122,** 757-778.
99. McGahon, B.M., Clements, M.P., and Lynch, M.A. (1997) *Neuroscience.* **81,** 9-16.
100. McGahon, B.M., Lynch, M.A. (1996) *Neuroscience.* **3,** 847-855.
101. McGahon, B.M., Martin, D.S., Horrobin, D.F., and Lynch, M.A. (1999) *Neuroscience.* **94,** 305-314.
102. McGahon, B.M., Murray, C.A., Horrobin, D.F., and Lynch, M.A. (1999) *Neurobiol. Aging.* **20,** 643-653.
103. Mead J.F. (1968) *Progress in the Chemistry of Fats and Other Lipids.* **Vol IX,** 159-192.
104. Meldrum, B., and Garthwaite, J. (1990) *Trends. Pharmacol Sci.* **11,** 379-387.

105. Michaelson, L.V., Napier, J.A., Lewis, M., Griffits, G., Lazarus, C.M., and Stobart, A.K. (1998) *FEBBS Lett.* **439,** 215-218.

106. Mills, D.E., Summers, M.R., and Ward, R.P. (1985) *Lipids.* **20,** 573-577.

107. Mitchell, D.C., and Litman, B.J. (1998) *Biophys. J.* **74,** 879-891,

108. Moon, Y.A., Shah, N.A., Mohapatra, S., Warrington, J.A., and Horton, J.D. (2001) *J. Biol. Chem.* **276,** 45358-66.

109. Moore, S.A., Hurt, E., Yoder, E., Sprecher, H., and Spector, A.A. (1995) *J. Lipid Res.***36,** 2433-2443.

110. Mori, T.A., Bao, D.Q., Burke, V, Puddey, L.J., and Beilin, L.J. (1999) *Hypertension.* **34,** 253-260.

111. Moriguchi, T., Greiner, R.S., and Salem, N. Jr. (2000) *J. Neurochem.* **75,** 2563-2575.

112. Morris, M.C., Sacks, F., and Rosner, B. (1993) *Circulation.* **88,** 523-533.

113. Murphy, M.G., Moak, C.M., and Rao, B.G. (1987) *Biochem. Pharmacol.* **36,** 4079-4084.

114. Nakamura-Palacios, E.M., Caldas, C.K., Fiorini, A., Chagas, K.D., Chagas, K.N., and Vasquez, E.C. (1996) *Behav. Brain Res.* **74,** 217-227.

115. Napier, J.A., Hey, S.J., Lacey, D.J., and Shewry, P.R. (1998) *Biochem. J.* **330,** 215-218.

116. Narce, M., Frenoux, J.M., Dardel, V., Foucher, C., Germain, S., Delachambre, M.C., and Poisson, J.P. (1997) *Biochimie.* **79,** 135-138.

117. Narce, M., and Poisson, J.P. (1995) *Prostaglandins Leukot. Essent. Fatty Acids.* **53,** 59-63.

118. Nyakas, C., Buwalda, B., Kramers, R.J.K., Traber, J., and Luiten, P.G.M. (1994) *Neuroscience.* **59,** 541-559.

119. Nouvelot, A., Delbart, C., and Bourre, J.M. (1986) *Ann. Nutr. Metab.* **30,** 316-323.

120. Ohtsuki, T., Matsumoto, M., Suzuki, N., Taniguchi, N., and Kamada, T. (1995) *Am. J. Physiol.* **268,** 1418-1421.

121. Paine, B.J., Makrides, M., and Gibson, R.A. (1999) *J. Pediatr. Child. Health.* **35,** 82-85.

122. Pawlosky, R.J., Hibbeln, J.R., Novotny, J.A., and Salem, N., Jr. (2000) *J. Neurochem.* **42,** 1257-1265.

123. Pawlosky, R.J., Ward, G., and Salem, N., Jr. (1996) *Lipids.* **31,** 103-107.

124. Pu, L., Igbavboa, U., Wood, W.G., Roths, J.B., Kier, A.B., and Spener F. (1999) *Mol. Cell. Biochem.* **198,** 69-78.

125. Puskas, L.G., Kitajka, K., Nyakas, C., Barcelo-Coblijn, G., and Farkas, T. (2003) *Proc. Natl. Acad. Sci. U S A.* **18,** 1580-1585.
126. Raygada, M., Cho, E., and Hilakivi-Clarke L. (1998) *J. Nutr.* **128,** 2505-2511.
127. Rogan, W.J., and Dladen, B.C. (1993) *Early Hum. Dev.* **31,** 181-193.
128. Rothman, S.M., and Olney, J.W. (1995) *Trends. Neurosci.* **18,** 57-58.
129. Salem, N. Jr. (1989) *Curr. Top. Nutr. Dis.* **22,** 109-228.
130. Salem, N., Jr., Litman, B., Kim, H-Y., and Gawrisch, K. (2001) *Lipids* **36,** 945-959.
131. Salem, N., Jr., Moriguchi, T., Greiner, R.S., McBride, K., Ahmad, A., Catalan, J.N., and Slotnick, B. (2001) *J. Mol. Neurosci.* **16,** 299-307.
132. Salvati, S., Attori, L., Avellino, C., Di Biase, A., and Sanchez, M. (2000) *Dev. Neurosci.* **22,** 481-487.
133. Sattler, R., and Tymianski, M. (2000) *J. Mol. Med.* **78,** 3-13.
134. Savelli, J.L., Narce, M., Fustier, V., and Poisson, J.P. (2002) *Prostaglandins Leukot. Essent. Fatty Acids.* **66,** 541-547.
135. Scott, B.L., and Bazan, N.G. (1989) *Proc. Natl. Acad. Sci. USA.* **86,** 2903-2907.
136. Sheaff Greiner, R.C., Zhang, Q., Goodman, K.J., Giussani, D.A., Nathanielsz, P.W., and Brenna, J.T. (1996) *J. Lipid. Res.* **39,** 286-292.
137. Sinclair, A.J. (1975) *Lipids.* **10,** 175-184.
138. Sprecher, H. (2000) *Biochim. Biophys. Acta.* **1486,** 219-231.
139. Strosznajder, J., Chalimoniuk, M., Strosznajder, R.P., Albanese, V., and Alberghina, M., (1996) *Neurosci. Lett.* **209,** 145-148.
140. Strosznajder, J., Samochocki, M., and Duran, M. (1994) *J. Neurochem.* **62,** 1048-1054.
141. Stuiver, B.T., Douma, B.R.K., Bakker, R., Nyakas, C., and Luiten, P.G.M. (1996) *Neurodegeneration.* **5,** 153-159.
142. Su, H.M., Bernardo, L., Mirmiran, M., Ma, X.H., Corso, T.N., Nathanielsz, P.W., and Brenna, J.T. (1999) *Pediatr. Res.* **45,** 87-93.
143. Suzuki, H., Park, S.J., Tamura, M., and Ando, S. (1998) *Mech. Ageing Dev.* **101,** 119-128.
144. Takeuchi, T., Fukumoto, Y., and Harada, E. (2002) *Behav. Brain. Res.* **131,** 193-203.
145. Tang, J.P., Xu, Z.Q., Douglas, F.L., Rakhit, A., and Melethil, S. (1993) *Life. Sci.* **53,** PL417-420.
146. Tapiero, H., Nguyen Ba, G., Couvreur, P., and Tew, K.D. (2002) *Biomed. Pharmcother.* **56,** 215-222.

147. Terracina, L., Brunetti, M., Avellini, L., de Medio, G.E., Trovarelli, G., and Gaiti, A. (1992) *Mol. Cell. Biochem.* **115,** 35-42.

148. Ulmann, l., Mimouni, V., Roux, S., Porsolt, R., and Poisson, J.P. (2001) *Prostaglandins Leukot. Essent. Fatty Acids.* **64,** 189-195.

149. van der Zee, E.A., Compaan, J.C., de Boer, M., and Luiten, P.G.M. (1992) *J. Neurosci.* **12,** 4808-4815.

150. Van der Zee, E.A., and Luiten, P.G.M. (1999) *Prog. Neurobiol.* **58,** 409-471.

151. Voss, A., Reinhardt, M., Sankarrapa, S., and Sprecher, H. (1991) *J. Biol. Chem.* **266,** 19995-20000.

152. Wainwright, P.E., Xing, H.C., Ward, G.R., Huang, Y.S., Bobik, E., Auestad, N., and Montalto, M. (1999) *J. Nutr.* **129,** 1079-1089.

153. Wakil, S.J., Stoops, J.K., and Joshi V.C. (1983) *Annu. Rev. Biochem.* **52,** 537-579.

154. Watts, J.L., and Browse J. (1999) *Arch. Biochem. Biophys.* **362,** 175-182.

155. Wing, L.M., Nestel, P.J., Chalmers, J.P., Rouse, I., West, M.J., Bune, A.J., Tonkin, A.L., and Russel, A.E. (1990) *J. Hypertens.* **8,** 339-343.

156. Yavin, E., Brand, and A., Green, P. (2001) *Nutr. Neurosi.* **5,** 149-157.

157. Yehuda, S., and Carasso, R.L. (1993) *Proc. Natl. Acad. Sci. USA.* **90,** 10345-10349.

158. Yehuda, S., Rabinovitz, S., Carasso, R.L., and Mostofsky, D.I. (1998) *Peptides.* **19,** 407-419

159. Yehuda, S., Rabinovitz, S., Carasso, R.L., and Mostofsky, D.I. (2000) *Int. J. Neurosci.* **101,** 73-87.

160. Yin, K., Chu, Z.M., and Beilin, L.S. (1991) *Br. J. Pharmacol.* **102,** 991-997.

161. Yonekubu A., Honda, S., Okano, M., Takahashi, K., and Yamamoto, Y. (1993) *J. Nutr.* **123,** 1703-1708.

162. Youdim, K.A., Martin, A., and Joseph J.A. (2000) *Int. J. Dev. Neurosci.* **18,** 383-399.

163. Zachowski, A. (1993) *Biochem.J.* **294,** 1-14.

Acknowledgement

I am very grateful to the following persons for contributing in my Ph.D. project during the last couple of years:

Prof. Dr. Tibor Farkas my supervisor, who introduced me to the word of lipids. He was the most influential person in my life. He made huge impact on me as a scientist and a man as well. It is very hard to sustain his inheritance.

Prof. Dr. Csaba Nyakas professor at University of Semmelweis, was my main co-author in my papers. He taught me the immunostaining methods in Groningen. He was the best collaborating partner who I have ever worked with.

Prof. Dr. Penke Botond my tutor, who got my career started. He rescued me from the College of Food Industry and always supported me during my Ph.D. period and after it.

Prof. Dr. László Vígh the head Institute of Biochemistry, who let me work in his institute and helped me after Tibor Farkas's death.

Dr. Eszter Farkas at the Department of Anatomy, made the linguistic correction of my thesis. She was also co-author in one of my paper. I learnt so much from her. Last, but not least she cooked for me in Groningen.

Dr. Zsuzsa Penke at the Department of Animal Physiology, made the learning tasks for us.

And my dearest collegians I have ever met: Judit Baunoch, Gwendolyn Barcelo, and Erika Zukic. They created that friendly environment in which it was fantastic to work.

Thank you all!