Involvement of N6 and N3 polyunsaturated faty acids on the lipidic profile in central nervous system of the animals of experience

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Abstract

Introduction: N-3 and N-6 polyunsaturated fatty acids has many involvements in activities within or entering in regulating various physiological processes and in certain pathologies. Among systemic physiological effects in which they are involved we mention the central nervous system development and recall of the retina, regulating plasma lipid levels, cardiovascular and immune system functions, regulating the activity of insulin.

Material and methods: The experiment there were used 60 male Wistar rats , weight 180 ± 20 grams, procured from the animal farm of the Department of Pathophysiology, University of Medicine and Pharmacy "Gr.T. Popa", Iaşi.

Male Wistar rats were divided into two study groups: normal control animals (M) and test animals.

Test group was further divided into three groups - each group being composed of 15 animals.

Administration of the substances was made for 36 weeks (nine months), after which the animals were evaluated and subsequently sacrificed.

Results: Following statistical analysis, we determined the following:

• Averages of AGP n3 were significantly higher in groups 2 ($p < 0.001$) and 3 ($p < 0.001$) compared with group 4

• Averages ratio n6: n3 in nerve cell membrane were significantly lower in groups 2 ($p < 0.001$) and 3 ($p < 0.001$) compared with group 4

Discussions: Our experiment demonstrates that increased amounts of polyunsaturated fatty acids in the membranes of nerve cells which can justify the positive evolution of animals in assessing the performance of concomitant behavioral tests.

Conclusions: This study brings new light on the importance of the existence of a balance between PUFA intake and daily diet.

Keywords: PUFA, central nervous system, lipids profile, mass spectrometry, Eicosapentaenoic acid, Docosahexaenoic acid

N-3 and N-6 polyunsaturated fatty acids (PUFAs) have many involvements in activities within or entering in regulating various physiological processes and in certain pathologies. Among systemic physiological effects in which they are involved we mention the central nervous system development and recall of the retina, regulating plasma lipid levels, cardiovascular and immune system functions, regulating the activity of insulin (12, 26). At the level of cellular functions, n-3 and n-6 PUFAs has affects over the cellular membrane composition and function, the synthesis of eicosanoids and thus a role in cell signaling, regulation of gene expression and regulation of cell junctions (12, 24).

Recent research has shown that the deficit of long chain n-3 PUFAs is associated with memory loss and decreased cognitive functions. Many studies have found a correlation between changes in quantities of long chain n-3 and n-6 PUFAs, and changes into the profile of these essential fatty acids in neuronal membranes (13, 18). It was suspected that α-linolenic acid (18:3 n-3) controls neuronal membrane composition, theory untested so far. Changes into the relative quantities of free fatty acids on neuronal membrane level may be a key element in the physiological role of membranes (6, 25).

Changes into the composition of long chain PUFA diet had effect on function and membrane fluidity because they have a role in reducing serum cholesterol, cholesterol that has a role in controlling membrane fluidity and decrease microviscosity of the nervous tissue (10, 13, 24).

Identification of predisposing risk factors for both the deficit of the central nervous system development and neurodegenerative pathologies developing increasing incidence in the last period, especially modulation of protective factors through nutritional supplements can make an outstanding attitude in order to find a therapeutic method to prevent, delay or improve the central nervous system diseases and their possible complications (11, 26).

Material and methods

The experiment there were used 60 male Wistar rats, weight 180 ± 20 grams, procured from the animal farm of the Department of Pathophysiology, University of Medicine and Pharmacy “Gr T. Popa”, Iaşi.

Male Wistar rats were divided into two study groups: normal control animals (M) and test animals (T) (14, 16).

Test group was further divided into three groups - each group being composed of 15 animals, compared with the type of substance administered as follows:

- Group 1 - male white Wistar rats, aceticysteine (ACC) intraperitoneal administration of 35 mg/100 g animal;
- Group 2 - male white Wistar rats intraperitoneal administration of PUFA 50 mg/100 g animal;
- Group 3 - male white Wistar rats, ACC intraperitoneal administration of 35 mg/100 g animal and PUFA 50 mg/100 g animal;

Control group contained one group of 15 animals that received only normal diet without further intervention.

-Group 4 - male white Wistar rats, normal diet; (6, 12, 24)

Administration of the substances was made for 36 weeks (nine months), after which the animals were evaluated and subsequently sacrificed. The animals were evaluated through the Morris radial maze and multiple T maze.

The Morris water maze is a large round tub of opaque water (made white with powdered milk) divided by eight radial arms and a hidden platform located 1-2 cm under the water's surface in one of the arms. The rat was placed on in the center of the maze. The rat has to swim in the maze’s arms until it finds the other platform to stand on. We measured how long it takes for a rat to find hidden platform (12, 16).

The multiple T-maze is a complex maze made of many T-junctions. Performance in the multiple T-maze is easy to measure because each intersection is identical and has a clear right or wrong answer. Multiple T-mazes were used to answer questions of place vs. response learning and cognitive maps. We recorded the time needed for the rat to find the end of the maze (12, 16).

In both tests we found significant improvement in learning the mazes for the rats that received n3 PUFAs via intraperitoneal administration.

Also we monitored biochemical (glycemic profile, lipids profile and oxidative stress parameters such as Malondialdehyde, Glutathione Peroxidase, and Total Antioxidant Capacity) and zoometrical (weight, length) parameters which showed significant variations for the animals that received PUFA n3 treatment.

Brain lipid profile of Wistar rats was carried out using mass spectrophotometry liquid chromatography. Results were obtained using Agilent 6500 Series system Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC / MS (4, 5, 24).

The samples were separated on a reverse phase column Zorbax SB-C18 (4.6 x 150 mm, 5 mm). Mobile phase consists of H2O with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) filtered and degassed before use (2, 6, 16).

The following gradient program was used: 95% to 100% solvent B in 10 minutes, maintaining 100% B up to 25 minutes, then return to 95% B, the total registration was 60 minutes. Were injected 10 ml of the sample (dissolved in isopropanol: acetonitrile 1:1) at a rate of 1ml/minut (18, 19 , 26).

UV-VIS DAD detector was monitored between 190-900nm. LC system was directly connected to the electrospray ionization source (ESI) of mass spectrometry. Terms of the Q / TOF MS selected were: ESI in positive mode, drying gas flow (N2) 7L/min , gas temperature 325 º C, nebuliser pressure 35 psig, capillary voltage 4000 V, 200 V fragmentation voltage, the compounds have been investigated on the m / z 100-1500 (16, 17).

Results

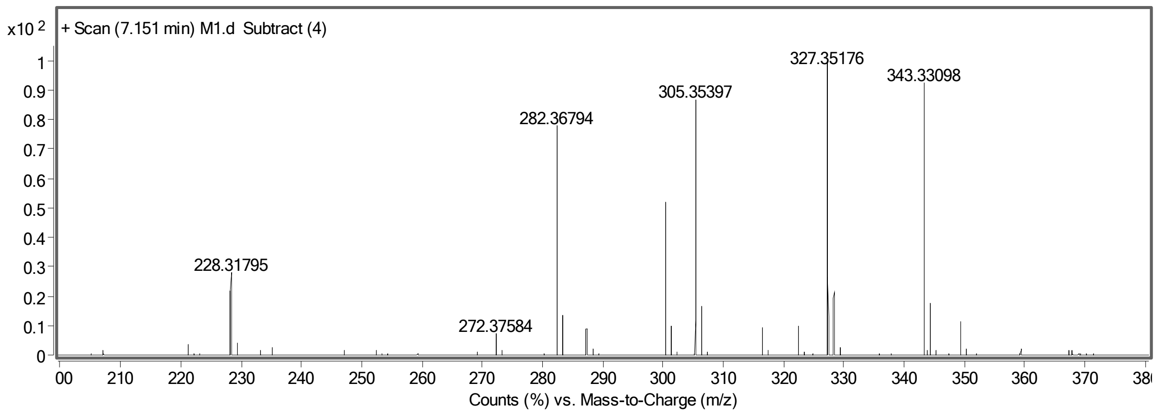

Figure 1 Mass spectrum of type of formed ions - AG in the brain

Table I

Interpretation of mass spectrum for the types of formed ions

P1_7,151 min	**X**		**DAD**
m/z	Abund	Charge	
228.31795	33198		9-Tetradecenoic acid / $[M^{+}H^{+}]^{+}$
272.37584	8676		Palmitoleic acid sau cis-6-Hexadecenoic acid / $[M^{+}NH^{4+}]^{+}$
282.36794	91847	1	Oleic acid sau Elaidic acid / M
283.37284	16569	1	Oleic acid sau Elaidic acid / $[M^{+}H^{+}]^{+}$
287.32845	11087		
300.39192	62818	1	Linoleic acid /$[M^{+}NH^{4+}]^{+}$ + (H) ori Stearidonic acid / $[M^{+}Na^{+}]^{+}$ + (H)
301.39546	11648	1	Gamma-linolenic acid, GLA or α-Linolenic acid / $[M^{+}Na^{+}]^{+}$ or Oleic acid or Elaidic acid / $[M^{+}NH^{4+}]^{+}$
305.35397	105383	1	Arachidonic acid / [$[M^{+}H^{+}]^{+}$ori Oleic acid sau Elaidic acid / $[M^{+}Na^{+}]^{+}$
306.35547	20256	1	Dihomo-γ-linolenic acid / M
316.43518	11386		
322.39191	11848		Eicosapentaenoic acid / Eicosapentaenoic acid
327.35176	117434	1	Arachidonic acid / $[M^{+}Na^{+}]^{+}$
328.35426	25140	1	Docosahexaenoic acid / M
343.33098	109164	1	
344.33403	20977	1	
349.35166	13684		
403.46611	7813		

Table II

Membrane polyunsaturated fatty acid composition

Brain	**% from the total quantity of fat acids**			
	Group 1	Group 2	Group 3	Group 4
n6 PUFAs	30.6	37.2	34.1	39.2
Linoleic acid	10.1	12.4	10.2	13.8
Arahidonic acid	17.2	20.8	20.1	21.2
n3 PUFAs	8.6	10.9	11.2	9.2
α linoleic acid	2.0	2.2	2.3	2.5
Eicosapentaenoic acid	1.8	2.8	2.8	1.8
Docosahexaenoic acid	4.8	5.9	6.1	4.9
N3: N6 report	3.6	3.4	3.0	4.2

Following statistical analysis, we determined the following:

• averages of AGP n3 were significantly higher in groups 2 ($p < 0.001$) and 3 ($p < 0.001$) compared with group 4 (19)

• averages ratio n6: n3 in nerve cell membrane were significantly lower in groups 2 ($p < 0.001$) and 3 ($p < 0.001$) compared with group 4 (1, 3).

Discussions

Approximately 20% of the dry weight of brain is represented by the long-chain polyunsaturated fatty acids which are incorporated into the phospholipids, substances with a major role for the structural integrity of neuronal membrane, membrane fluidity, and other associated membranes functions, such as receptors, enzymes, ion channels and eicosanoid-type functions (4, 12, 13, 24).

The beneficial effects of long-chain polyunsaturated fatty acids are directly related to their action on neuronal membrane composition and fluidity of the central nervous system and the supplementation in elongated and desaturate derivatives with subsequent activation of brain conversion and storage has proven to be effective (23, 24). Thus α-linolenic acid (18:3 n-3) is a source of acetate for de novo synthesis of palmitic acid and α-linolenic acid (18:3 n-3), essential for membrane integrity (6, 8, 9).

From our study we have observed that animal subjects from group 4 (control) had increased levels of n6 PUFAs in the brain tissue compared to groups 1 (ACC), 2 (PUFA) and 3 (ACC+PUFA). The lowest brain tissue levels of n6 PUFAS were recorded in group (ACC), these results may be explained by the anti-inflammatory effect of acetylcysteine (26).

The data we have obtained has similar behavior for the linolenic and Arachidonic acids in the brain tissue (the most common n6 PUFAs in the brain) and this is consistent with data obtained by other studies (3, 4, 11). The levels of n6 PUFAs registered by group 3 (ACC+PUFA) are higher than in group 2 (PUFA), observation that contradict in some matter by findings of other researchers (20, 21) and can be attributed to different absorption of test substances when administrated simultaneous via peritoneum.

The highest concentration of n3 PUFAs (known for their anti-inflammatory, atherosclerosis prevention and membrane fluidity increase effects) in the brain tissue were recorded in group 3 (ACC+PUFA) as expected. These findings are similar to the data encountered in other studies (11, 14, 21).

The most significant increase for a single fatty acid was recorded for the docosahexaenoic acid (DHA), in amount of more than 20% and also significant increase levels of eicosapentaenoic acid (EPA) were recorded for groups 2 (PUFA) and 3 (ACC+PUFA) but no change in group 1 (ACC). The peritoneal administration of salmon oil, rich source of PUFAs lead to increase neuronal deposits of DHA an EPA as shown by similar studies (20, 21, 25).

The use of new and improved methods of measuring of lipids in tissues and cells created a new, cutting edge, research domain called lipidomics. Lipidomics may be defined as the large-scale study of pathways and networks of cellular lipids in biological systems[The word "lipidome" is used to describe the complete lipid profile within a cell, tissue or organism. Lipidomics is a relatively recent research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes (27).

Lipidomics research involves the identification and quantization of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Investigators in lipidomics examine the structures, functions, interactions, and dynamics of cellular lipids and the changes that occur during perturbation of the system. In lipidomic research, a vast amount of information quantitatively describing the spatial and temporal alterations in the content and composition of different lipid molecular species is accrued after perturbation of a cell through changes in its physiological or pathological state. Information obtained from these studies facilitates mechanistic insights into changes in cellular function. Therefore, lipidomic studies play an essential role in defining the biochemical mechanisms of lipid-related disease processes through identifying alterations in cellular lipid metabolism, trafficking and homeostasis (27).

Analysis of brain lipid composition is a new and important subject of study but the number of studies at present time is scarce due to the expensive methods and procedures applied to biological material and reduced availability of specific measuring equipment. Mass spectroscopy combined with liquid chromatography aimed at the n3 and n 6 PUFAs lipid fractions of the brain represent the leading wave in the scientific approach to the understanding the basic foundations and the superior functions of the brain.

During our research we have also investigated the learning performance of the animal subjects in two different devices (radial Morris maze and the multiple T) and the data we acquired shown that the rats performance improvement was consistent with the increase of brain tissue levels of n3 PUFAs for groups 2 (PUFA) and 3 (ACC+PUFA). The shortest average time needed for the rats to complete both mazes was recorded by group 3 (ACC+PUFA) and the longest for group 4 (control). Group 2 (PUFA) also had average times close to group 3 but the difference was not statistically significant. Moderate improvement was recorded also in group 1 rats (ACC).

Our experiment it demonstrates increased amounts of polyunsaturated fatty acids in the membranes of nerve cells which can justify the positive evolution of animals in assessing the performance of concomitant behavioral tests (5, 22).

Also included in our study were the blood lipid profile and oxidative stress evaluation. Cholesterol and triglyceride blood levels were significantly decreased in groups 2 (PUFA) and group 3 (ACC+PUFA) and oxidative stress markers (MDA, GPx and TAC) also registered significant lower levels for these groups. The zoometric parameters recorded, length and weight had average values higher for groups 1 (ACC), 2 (PUFA) and 3 (ACC+PUFA) compared to group 4 (control).

Conclusions

Peritoneal administration of salmon oil rich in PUFAs determined an increase in brain tissue deposits of DHA and EPA, fact demonstrated trough the mass spectroscopy combine with liquid chromatography.

Mass spectroscopy combined with liquid chromatography is a valuable and relevant mean to the assess brain lipid concentration, but it is time consuming and expensive.

The brain n6 PUFA concentration decreased in animals that received only acetylcysteine via peritoneum, which can indicate a hipolipemiant effect; also blood levels of cholesterol and triglycerides were lower.

The increased measured levels of n3 PUFAs in brain tissue overlapped the increased performance measured in the previous behavioral experiment

This research makes an important contribution to the study of polyunsaturated fatty acids and the importance of placing them in the daily diet. Thus, by improving the diet with polyunsaturated fatty acids we can improve cognitive performance and helps prevent or ameliorate certain neurological diseases such as Parkinson or Alzheimer disease compared with normal diet, low in these acids.

The need for widespread use of

polyunsaturated fatty acids is proven by many scientific experiments and the results obtained in experimental animals.

This study brings new light on the importance of the existence of a balance between PUFA intake and daily diet.

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