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**EFFECT OF LINSEED DIETARY
SUPPLEMENTATION ON FREE FATTY ACID
AND PHOSPHOLIPID CONTENT IN RAT
BRAIN**

**EFEKAT SEMENA LANA KAO DIJETALNE
DOPUNE NA SLOBODNE MASNE KISELINE
I SADRŽAJ FOSFOLIPIDA
U MOZGU PACOVA**

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Abstract

The aim of the present investigation was to establish the changes of rat brain free fatty acids (FFA) and phospholipids in linseed-supplemented diet.

Male Wistar rats at the age of three months were used in the experiment. Animals were fed a standard chow diet supplemented with linseed at a dose of 3g/day for three weeks. A 10% brain homogenate was prepared and lipids were extracted. The phospholipid and FFA content was measured by thin-layer chromatography, gas-liquid chromatography and spectrophotometrically.

In the brains of rats fed dietary linseed, we found 1.8-fold increase of total FFA and increases of the individual FFA. The content of total phospholipids increased 1.14-fold. In general, the free fatty acid and phospholipid composition was dominated by long-chain polyunsaturated free fatty acids (PUFA), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylcholine (PC).

Ključne reči

fosfolipidi, slobodne masne kiseline,
seme lana, mozak pacova.

Key words

phospholipids, free fatty acids, dietary
linseed, rat brain.

INTRODUCTION

Brain maintains a unique lipid environment that is essential for normal brain function. Polyunsaturated free fatty acids are major components of brain cell membranes. PUFA have effects on diverse physiological processes impacting normal health and chronic disease, but they are crucial in multiple aspects of neuronal development and function [1]. They are involved in modification of neuronal membrane fluidity, membrane activity-bound enzymes, the number and affinity of receptors, the function of neuronal membrane ionic channels, and of the production of neurotransmitters and brain peptides [2]. PUFA have also been shown to modulate gene expression and to improve learning and memory ability.

Beneficial effects of PUFA have been proposed for a wide range of central nervous system diseases, including depression, bipolar disorder, Alzheimer's disease, dementia, and schizophrenia [3, 4, 5]. PUFA have also been shown to reduce the risk of ischemic stroke [6].

The majority of membrane PUFA are synthesized from linoleic acid (LA, C18:2 n-6) and α -linolenic acid (ALA, C18:3 n-3), which act as precursors for the synthesis of longer-chain PUFA through a series of elongation and desaturation reactions [7]. α -Linolenic and linoleic acids have been identified as essential fatty acids, because they can not be synthesized de novo and they must be provided in the diet. Dietary sources of LA and ALA are vegetable oils, seeds, and some vegetables.

Linseed is a rich source of PUFA, including primarily LA and ALA. The percentage contribution of both these acids is around 73% [8]. Linseed contains approximately 40% oil, which is known to be one of the major commercial sources that have significant amounts of ALA beside rapeseed oil and soybean oil. The content of ALA attains 23% in linseeds and 53% in linseed oil. Moreover, linseed has high nutritional value and it is easily accessible, which makes it a beneficial rat diet supplement.

As brain lipid composition can be modulated through dietary sources, it is of great interest to study how it is affected by PUFA dietary supplementation. Our experiment was conducted to investigate the influence of dietary linseed on free fatty acid and phospholipid content in rat brain.

MATERIAL AND METHODS

Experimental animals and dietary interventions

Twenty-five male Wistar rats at the age of three months, each weighing 190-220 g, were used in the experiment. Animals were divided into control group (n=5) and experimental group (n=20). The control group was fed a standard chow diet. The experimental group diet was supplemented with ground linseed at a dose of 3g/day. Three weeks later, rats were deprived of food for 24 hours, lightly anesthetized with diethyl ether and sacrificed by decapitation.

The experiments were performed in accordance with the guide for the care and use of laboratory animals.

Brain phospholipid and FFA analysis

A 10% brain homogenate was prepared in ice-cold 0.32 M sucrose according to the method described by Venkov [9]. Lipids were extracted according to the method of Kates [10] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v).

Total phospholipids were measured spectrophotometrically at 820 nm [11]. All major phospholipid classes were separated by thin-layer chromatography using eluate from chloroform:methanol:water 65:25:4 (v/v/v). Perkin-Elmer scanning spectrophotometer was used to estimate the concentration of migrated spots.

The FFA content was determined by gas-liquid chromatography. The fatty acids were converted to fatty acyl methylesters (FAME) by addition of methanol and 25% hydrochloric acid. The FAME were extracted by petroleum ether, then concentrated in a rotary vacuum evaporator and subjected to gas-liquid chromatographic analysis. A gas chromatograph with flame ionization detector and connected with Trio Vector computing integrator was used. The analysis was performed by injecting 5 µl of the sample into SE-35 column. The temperature was programmed from 85°C to 205°C (2.5°C/min). Nitrogen was used as carrier gas at a flow-rate of 40 ml/min.

Statistical analysis

Data were analyzed with Student's t-test. Values are expressed as mean ± SD.

RESULTS AND DISCUSSION

Modifications of the brain membrane fatty acid composition have been documented with supplementation of various dietary oils [12, 13, 14]. It has been reported that these changes generally reflect the respective fatty acid pattern of

the dietary fat [15, 16]. As vegetable oils are the major sources of PUFA, dietary oils intake should significantly increase ALA, eicosapentaenoic acid (EPA, C_{20:5} n-3), docosapentaenoic acid (DPA, C_{22:5} n-3) and docosahexaenoic acid (DHA, C_{22:6} n-3) in the free fatty acid pool and membrane phospholipid composition. The increased PUFA content in membrane phospholipids has been shown to affect cell membrane properties such as fluidity, flexibility, permeability and the activity of membrane bound enzymes. The degree of fatty acid's desaturation determines its 3-dimensional structure and it appears to be an important factor affecting the membrane behaviour [16]. Besides, it has been demonstrated that not only unsaturation but also the position of a double bond has a pronounced effect on membrane properties. It was found that lipid species with double bond located close to the center of the chain are characterized by the lowest phase transition temperature which increases the disorder in the membrane hydrocarbon region [17]. The replacement of even a single double bond in these PUFA is sufficient to exert a profound effect on the physical properties of the membrane [18]. In addition, the introduction of a double bond into one of the acyl chains has been found to reduce permeation of water into the bilayer [19].

In the present study, we examined the effect of linseed dietary supplementation on the rat brain FFA and phospholipid content. First of all, the FFA and phospholipid content in control rat brain was established. The fatty acid analysis showed that palmitic acid (C_{16:0}), stearic acid (C_{18:0}) and arachidonic acid (C_{20:4}, AA) were the major components of the FFA pool (Fig. 1). Among the phospholipid classes, PS, PE and PC were the most prominent and they together accounted for 76% of total phospholipids in the brain homogenate (Fig. 2).

Feeding linseed resulted in significant increase of total FFA (1.8-fold, from 11.432±0.19 to 20.641±0.08 mg/g dry lipid residue/ml, p<0.001) and all individual FFA in brain (Fig. 1). The most notable effect was observed for linoleic acid, whose concentration increased 18-fold (from 0.06±0.01 to 1.093±0.07 mg/g/ml, p<0.001). In spite of this, it represented only 5% of total FFA. Stearic acid, arachidonic acid and docosahexaenoic acid had the largest percentage of the total FFA and their estimated concentrations were 4.273±0.04 mg/g/ml, 0.05<p<0.02; 5.407±0.06 mg/g/ml, p<0.001; 4.559±0.06 mg/g/ml, respectively. Studies in the literature consider concentrations of arachidonic acid in animal tissues as a parameter of linoleic acid desaturation [20, 21]. Besides the FFA pool size, the composition of the FFA pool was also modified by linseed supplementation. The latter was comprised of mono- and polyunsaturated FFA, some of which were absent in controls (C_{16:1}, C_{18:1}, C_{18:3}, C_{22:6}). These findings indicate that the FFA pattern resembles those of linseed regarding the high content of LA and ALA, but FFA pool also contained considerable amounts of FFA not present in the linseed FFA composition.

The variations of results could be due to different nutritional conditions of the animals. It is well established that the activity of Δ6- and Δ5-desaturase – key enzymes in the regulation of unsaturated FFA biosynthesis, is highly dependent on several nutritional and hormonal factors, particularly on the ingestion of linoleic acid [22].

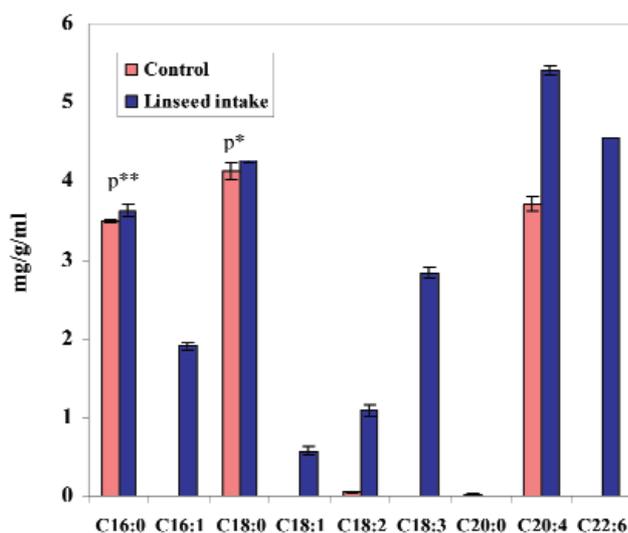


Fig. 1. Changes of the individual FFA in rat brain after linseed-supplemented diet. Values are expressed in mg/g dry lipid residue/ml. $p < 0.001$; p^{**} indicates $0.02 < p < 0.01$; p^* indicates $0.05 < p < 0.02$.

It is known that mammalian tissues contain four families of PUFA (n-3, n-6, n-7 and n-9) designated according to the number of carbon atoms from the terminal methyl group to the first carbon of the first double bond. Among all PUFA, only those of n-3 and n-6 classes are essential to the diet, because mammals lack the enzymes necessary to insert a cis double bond at the n-6 or the n-3 position of a fatty acid. These fatty acid families are not convertible and have very different biochemical roles. The long chain n-6 fatty acids (arachidonic acid, etc.) can be synthesized from LA. The parent fatty acid of the n-3 series (EPA, DPA, DHA, etc.) is ALA. Moreover, the n-6 and n-3 fatty acids “compete” for the same enzymes for desaturation and elongation. Dietary studies on rats and other animals have shown that ALA is strong suppressor of n-6 fatty acid metabolism [23].

Recently, the nutritional importance of the n-3 to n-6 fatty acid ratio in the diet has aroused great interest. It is reported that the ratio is important to avoid imbalance of membrane fluidity. Studies in animal models also demonstrate that the ratio influences various aspects of serotonergic and catecholaminergic neurotransmission, as well as prostaglandin formation [16]. Boudreau et al. [24] indicate that the dietary n-3 to n-6 fatty acid ratio may be more important than the absolute amount of dietary n-3 fatty acids in the inhibition of arachidonic acid metabolism. Our observations showed that n-3 to n-6 fatty acid ratio increased to 1.14 and the ratio of PUFA to saturated fatty acids increased to 1.76 with linseed supplement. These results indicate the general tendency to synthesize long-chain n-3 PUFA [25, 26, 27] although the ratios values are lower than those reported in the literature.

Furthermore, we also examined the changes in phospholipid content. Adding linseed to rat diet produced a higher content of total phospholipids in the brain – 1,14 times the control value (from 19.199 ± 0.24 to 21.797 ± 0.11 mg/g/ml, $p < 0.001$). Among the individual phospholipids, sphingomyelin (SM), PS, PC and PE showed a slight increase (between 1.05- and 1.2-fold, $p < 0.001$) (Fig. 2). Their concentrations rose to 1.075 ± 0.05 , 3.566 ± 0.03 , 6.095 ± 0.06 and

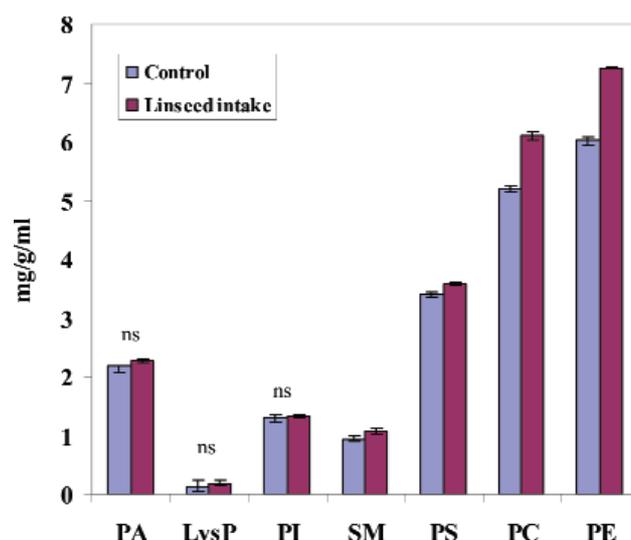


Fig. 2. Changes of the individual phospholipids in rat brain after linseed-supplemented diet. Values are expressed in mg/g dry lipid residue/ml. $p < 0.001$; ns indicates no significant difference.

7.266 ± 0.02 mg/g/ml, respectively. As we have established in controls, PS, PC and PE were still the predominant classes in the phospholipid composition and they together accounted for 78% of total phospholipids. However, no statistically significant changes were observed in the level of phosphatidic acid (PA), lysophospholipids (LysP) and phosphatidylinositol (PI). A review of the literature shows that most of the studies are focused on the changes in PUFA composition of membrane phospholipids. It probably is prompted because of the crucial impact of PUFA on membrane permeability, for example by modifying the relative activity of Ca^{2+} - Mg^{2+} -ATPase, voltage dependence of inactivation of Na^+ current, Na^+ - Ca^{2+} exchanger activity, etc. [28].

In conclusion, alterations in the FFA pool composition and in the phospholipid content were observed in response to linseed dietary supplementation. There was a tendency to synthesize high amounts of long-chain PUFA which indicates that both ratios n-3 to n-6 PUFA and PUFA to saturated FFA can be modulated by dietary intake. This would be beneficial for further nutritional implications.

Apstrakt

Cilj ovog istraživanja je da se utvrde promene slobodnih masnih kiselina i fosfolipida u mozgu pacova kod dijete sa dodatkom semena lana.

U eksperimentu su korišćeni Wistar pacovi muškog pola starosti tri meseca. Hranjeni su standardno, sa dodatkom semena lana u dozi od 3gr/die tokom tri nedelje. Lipidi su ekstrahovani iz 10% homogenata nervnog tkiva. Sadržaj fosfolipida i slobodnih masnih kiselina je određen hromatografijom na tankom sloju, gasno-tečnom hromatografijom i spektrofotometrijski.

U mozgu pacova koji su hranjeni semenom lana našli smo 1,8 puta povećanu ukupnu količinu slobodnih masnih kiselina i porast pojedinačnih slobodnih masnih kiselina. Sadržaj ukupnih fosfolipida porastao je 1,14 puta. Uopšteno, sastav slobodnih masnih kiselina i fosfolipida dominantno je bio u vidu dugo-lančanih višenezasićenih slobodnih masnih kiselina, fosfatidil-serina, fosfatidil-etanolamina i fosfatidil-holina.

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