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Free fatty acid patterns in rat brain synaptosomes following linseed dietary supplementation

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In this study, we report changes in the free fatty acid (FFA) content in rat brain synaptosomes following linseed dietary supplementation. Male Wistar rats at the age of three months were fed a standard chow diet supplemented with linseed at a dose of 3 g/day for three weeks. Afterwards, the rats were sacrificed by decapitation, the brain synaptosomal fraction was isolated and lipids were extracted. The FFA content was measured by gas-liquid chromatography.

In the brains of rats fed linseed, we found 2.7-fold increase of the total FFA. The most notable effect was observed for linoleic acid. However, arachidonic acid (AA) had the highest percentage in the synaptosomal FFA pool and it accounted for 35.39% of the total FFA. In general, the FFA composition was dominated by long-chain polyunsaturated free fatty acids.

Key words: free fatty acids, synaptosomes, rat brain, dietary linseed.

Introduction

Polyunsaturated fatty acids (PUFA) are an important component of the brain cellular membranes. PUFA have effects on diverse physiological processes, but they are crucial in multiple aspects of the neuronal development and function [3].

The majority of membrane PUFA are synthesized from linoleic acid (LA, $C_{18:2}$ n-6) and α-linolenic acid (ALA, $C_{18:3}$ n-3) through a series of elongation and desaturation reactions [17]. α-Linolenic and linoleic acids have been identified as essential fatty acids and they must be provided by the diet. Dietary sources of LA and ALA are vegetable oils, seeds, and some vegetables.

Linseed is a rich source of PUFA, including mainly LA and ALA. The percentage contribution of both these acids is around 73% [9]. Linseed has a high nutritional value and it is easily accessible, which makes it a beneficial rat diet supplement.

The brain synaptosomal membranes are especially enriched in PUFA. The unique lipid environment is essential for the development and regulation of the synaptic func-

tions. 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 follow up the influence of dietary linseed on the FFA content of rat brain synaptosomes.

Material and Methods

Three-month-old male Wistar rats 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 linseed at a dose of 3 g/day for three weeks. Afterwards, the rats were sacrificed by decapitation.

The animal experiments were performed in accordance with the animal protection guidelines approved by the Ethics Committee for Experimental Animal Use at IEMPAM, BAS.

The brain synaptosomal fraction was isolated as described by Venkov [18] using discontinuous two-step sucrose gradient. Lipids were extracted according to the method of Kates [19] using the following eluates: chloroform:methanol 1:2 (v/v) and chloroform:methanol:water 1:2:0.8 (v/v/v).

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 a gas-liquid chromatographic analysis. A gas chromatograph with a flame ionization detector and connected with Trio Vector computing integrator was used. The analysis was performed by injecting 5 µl of the sample into a SE-35 column. The temperature was programmed from 85 °C to 205 °C (2.5 °C/min). Nitrogen was used as a carrier gas at a flow-rate of 40 ml/min.

Results are reported as mean values ± SD and statistically analyzed by Student's *t*-test.

Results and Discussion

Various dietary oils intake has been documented to alter the brain membrane fatty acid composition [6, 7, 12]. It has been reported that these changes generally reflect the respective fatty acid pattern of the dietary fat [8]. 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 FFA pool and membrane phospholipid composition.

Regarding the synaptic functions, PUFA have been shown to have impact on the neuronal membrane fluidity, the activity and thermodynamic properties of the membrane-associated enzymes, the number and affinity of receptors, the function of the neuronal membrane ionic channels, the production of neurotransmitters and brain peptides, the initiation, consolidation, and assembly of the synaptic contacts during synaptogenesis [16]. Furthermore, PUFA have beneficial effect on learning and memory ability and synaptic plasticity [5, 14]. It has been shown that a simultaneous deficiency in LA and ALA affects the learning capacities of rodents [15].

In the present study, we examined the effect of linseed dietary supplementation on the FFA content of rat brain synaptosomes. The control FFA pool was enriched in arachidonic acid (AA, $C_{20:4}$ n-6), LA, palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$). The arachidonic acid represented 64.12% of the total FFA in the synaptosomes. Most probably

the high percentage could be related to the biological role of AA as a main precursor of prostaglandins, considered as modulators in the synaptic processes.

Feeding linseed resulted in significant increase in the total FFA (2.7-fold, from 6.449±0,1 to 17.621±0.07 mg/g dry lipid residue/ml, $p<0.001$). The changes in the amount of the individual FFA following dietary linseed supplementation are shown in Fig. 1. The most notable effect was observed for LA, whose concentration increased 2.8-fold (from 0.801±0,06 to 2.241±0.02 mg/g/ml, $p<0.001$) though it represented 12.72% of the total FFA. It has been shown that increasing dietary intake of the n-3 PUFA decreases the desaturation of LA, and thus, the production of arachidonic acid [4, 10]. Nevertheless, we estimated elevated content of arachidonic acid which comprised of 35.39% of the total FFA. 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: palmitoleic acid ($C_{16:1}$ n-7) – 8.26%, oleic acid ($C_{18:1}$ n-9) – 7.1%, ALA – 18.01%, eicosadienoic acid ($C_{20:2}$ n-6) – 5.81%, DHA – 18.95%. Other studies have also reported an increased content of ALA and long-chain PUFA following dietary linseed supplementation [1, 2].

It is known that mammalian tissues contain four families of PUFA (n-3, n-6, n-7 and n-9). Among all PUFA, only those of n-3 and n-6 classes are essential to the diet, because the 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 can be synthesized from LA. The parent fatty acid of the n-3 series is ALA. Moreover, the n–6 and n–3 fatty

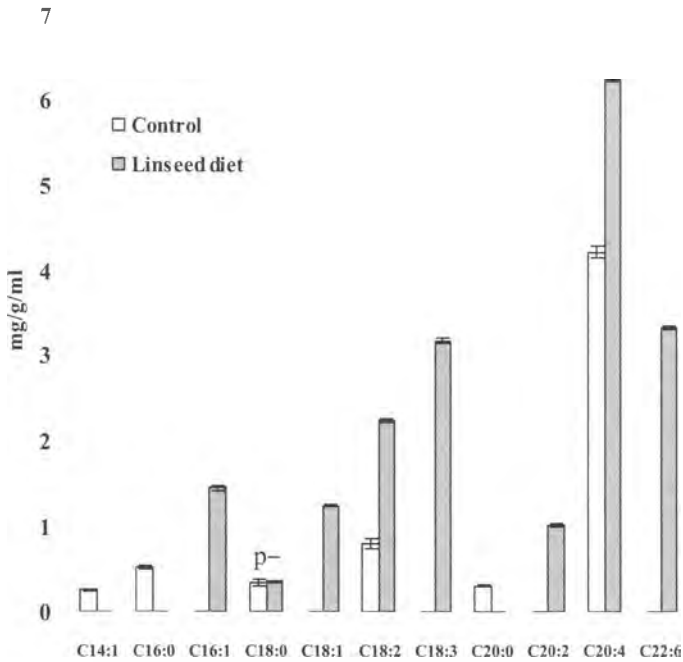

Fig. 1. Free fatty acid composition of the rat brain synaptosomal subcellular fraction following linseed dietary supplementation. Values are expressed in mg/g dry lipid residue/ml. $p<0.001$.

acids "compete" for the same enzymes for desaturation and elongation. Dietary studies on rats and other animals have shown that ALA is a strong suppressor of n-6 fatty acid metabolism [11].

However, the nutritional importance of the n-3 to n-6 fatty acid ratio in the diet has aroused a 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 serotoninergic and catecholaminergic neurotransmission, as well as prostaglandin formation [8]. Our findings in controls indicated that the n-6 series predominated among the polyenoic acids in the synaptosomal FFA pool. Feeding linseed resulted in enhanced n-3 fatty acids synthesis, though the n-6 FFA had a prevalence (n-3/n-6=0.69). In contrast, we have previously shown a higher content of the n-3 FFA in comparison to the n-6 FFA in a whole brain homogenate [13].

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

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