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Effects of supplementation with fish oil and n-3 PUFAs enriched egg yolk phospholipids on anhedonic-like response and body weight in the rat chronic mild stress model of depression

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Polyunsaturated fatty acids play an important role in the human organism. They guarantee a normal function of nervous cells, influence neurotransmission, and build some elements of cellular membranes. Several reports indicate an association between a deficiency of polyunsaturated fatty acids and depression. The aim of this study was to examine the effects of diet supplemented with fish oil, which is rich in omega-3 polyunsaturated fatty acids (n-3 PUFAs) and n-3 PUFAs enriched phospholipids ("super lecithin") obtained from designed eggs on anhedonic-like response and body weight in the rat chronic mild stress (CMS) model of depression. The results showed that neither fish oil nor n-3 PUFAs enriched egg yolk phospholipids supplementation reversed disturbances caused by CMS, such as anhedonic-like state or reduction of body weight gain.

1. Introduction

Polyunsaturated fatty acids (PUFAs) belong to two families: omega 3 (n-3) that are synthetically derived from linoleic acid (LA) and omega 6 (n-6) which are derived from alpha-linolenic acid (ALA). LA and ALA are termed "essential" fatty acids because they are not synthesized in the human body and must be obtained with food. Diet rich in fish and seafood is the major source of n-3 PUFAs. The marine n-3 PUFAs primarily consist of eicosapentaenoic acid (EPA, C20:5n–3) and docosahexaenoic acid (DHA, C22:6n–3). In some mammalian tissues, EPA and DHA can be converted from ALA, ingredient of most of plant oils (e.g. flaxseed, canola, and soybean), by serial steps of desaturation, elongation, and β-oxidation (Davis-Bruno et al. 2011; Das 2006). However, the rate of conversion in humans is very slow, resulting in an estimated 2 to 10% of ALA being converted to DHA or EPA (Emken et al. 1994; Holub and Holub 2004). Thus, the major source of EPA and DHA is diet, mainly via fatty fish consumption. n-3 PUFAs, particularly DHA, play an essential role in the normal development and functioning of the central nervous system (CNS). DHA is necessary for the structure of brain cells membranes and regulates multiple processes, including membrane fluidity, neurotransmission (Chalon 2006), ion channel, enzyme regulation (Yehuda et al. 2002) and gene expression (Barcelo-Coblijn et al. 2003). Both DHA and EPA have anti-inflammatory (Calder and Grimble 2002) as well as antiexcitotoxic (Xiao et al. 1997) and antioxidant (Garrel et al. 2012) action in brain tissue. Epidemiological and biochemical studies indicate an association between low dietary intake of n-3 PUFAs and depression (Liperoti et al. 2009). It has been found that the patients with major depressive disorder (MDD) showed a number of fatty acid deficiencies in peripheral tissues such as blood red cells (Peet et al. 1998), plasma (Tiemeier et al. 2003) and adipose tissue (Mamalakis et al. 2004). Both genetic and environmental factors may be a reason of n-3 PUFAs deficiency in MDD patients (Ross 2007). A number of animal studies demonstrated that n-3 PUFA deficiency induced neurobiological alternations like decreased hippocampal of brain-derived neurotrophic factor (BDNF) gene expression (Rao et al. 2007), reduced monoaminoergic transmission (Chalon 2006), and augmented the hypothalamic-pituitary-adrenal axis (HPA) response to stress (Takeuchi 2003). These disturbances may be involved in the pathophysiology of mood disorders (Palazidou 2012). Epidemiological data and clinical trials suggest that supplementation with n-3 PUFAs may improve depression (Sublette et al. 2011; Mozurkewich et al. 2011), and enhance the efficacy of antidepressants (Peet and Horrobin 2002; Nemets et al. 2002). Fish is considered to be the primary source of n-3 PUFAs but many people eat only limited amount of that food. n-3 PUFAs-enriched eggs may provide an alternative method to increase the dietary intake of these fatty acids (Lewis et al. 2000).

2. Investigation and results

In this study we compared the effects of fish oil and n-3 PUFAs enriched phospholipids obtained from designed eggs on anhedonic-like response and body weight in the rat chronic mild stress (CMS) model of depression**.** The CMS model of depression is accepted as a valuable method for evaluating the efficacy of antidepressants in animals. In this model, prolonged exposure of experimental animals to a variety of mild stressors induces an anhedonic-like state (inability to experience pleasure), a core symptom of depression, which should be reversed by antidepressant drugs (Willner 1997).

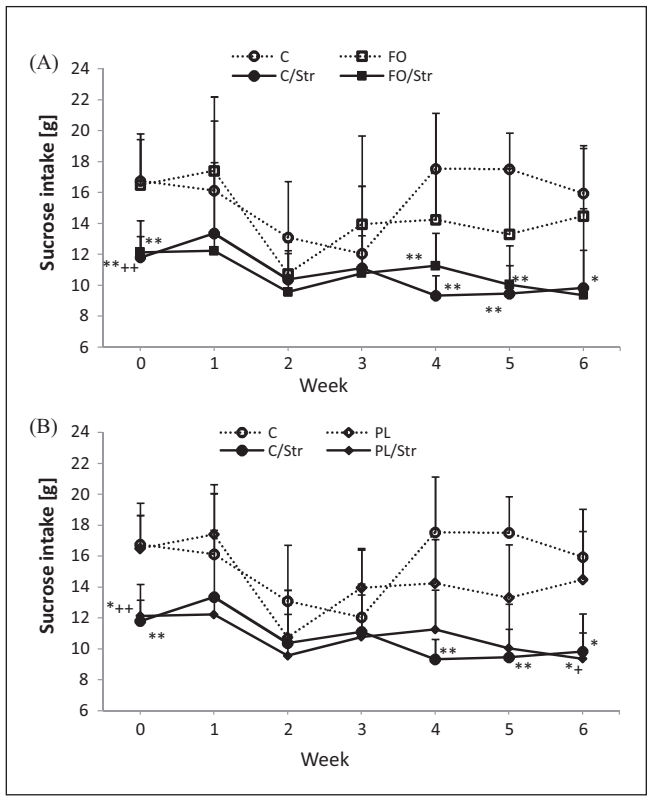

Fig. 1: Effects of fish oil (A) and n-3 PUFAs enriched phospholipids (B) on sucrose intake in Wistar Han male rats exposed to chronic mild stress. C = control, FO = fish oil, PL = n-3 PUFAs enriched phospholipids, Str = stressed rats. Data are expressed as mean ± SD (n = 9–11). Tukey's test: $^{*}P < 0.01$, $^{**}P < 0.001$ compared to the non-stressed control; $^{+}P < 0.05$, $^{++}P < 0.01$ compared to the non-stressed group receiving fish oil (FO) or n-3 PUFAs enriched phospholipids (PL)

In the initial baseline test, sucrose intake did not differ significantly among the groups (data not shown). After 3 weeks of the CMS procedure, sucrose intake in the stressed group was significantly reduced compared to the non-stressed group. Neither fish oil nor n-3 PUFAs enriched phospholipids supplementation reversed the decreased sucrose intake in rats exposed to CMS. Fish oil and n-3 PUFAs enriched phospholipids supplementation also did not significantly affect sucrose intake in non-stressed rats, except for the 5th week, when sucrose intake in supplemented rats was lower than in control ones (Fig. 1). Increased body weight in the stressed groups was significantly lower than that observed in the non-stressed groups. Both non-stressed and stressed groups that received a diet rich in fish oil and n-3 PUFAs enriched phospholipids, showed a higher weight increase compared to adequate control groups but the difference was not statistically significant (Fig. 2).

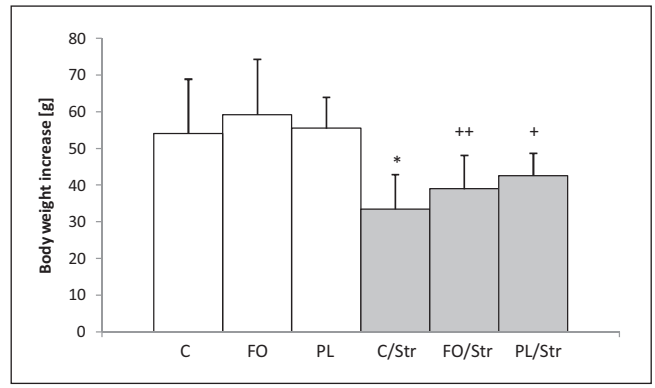

Fig. 2: Effects of fish oil and n-3 PUFAs enriched phospholipids on body weight in Wistar Han male rats exposed to chronic mild stress. C = control, FO = fish oil, PL = n-3 PUFAs enriched phospholipids, Str = stressed rats. Data are expressed as mean ± SD (n = 9–11). Tukey's test: $^{*}P < 0.001$ compared to the non-stressed control; $^{+}P < 0.05$, $^{++}P < 0.01$ compared to the non-stressed group receiving fish oil (FO) or n-3 PUFAs enriched phospholipids (PL)

3. Discussion

Earlier studies on rats in the forced swimming test proved that n-3 PUFAs induce an antidepressant-like effect (Huang et al. 2008; Lakhwani et al. 2007) and act synergistically with antidepressants, such as mirtazapine and fluoxetine (Laino et al. 2010). Influence on neurotransmitters and signal transduction in monoaminoergic systems that take part in mood regulation and affective disease pathogenesis is one of mechanisms which may explain n-3 PUFAs antidepressant activity. Experimental evidence from animal studies demonstrates a positive correlation between a chronic dietary n-3 PUFAs deficiency and abnormal activity of dopaminergic, noradrenergic, and serotoninergic systems (Delion et al. 1994, 1996). Furthermore, n-3 PUFAs deficiency decreases cerebral BDNF (Rao et al. 2007), that encourages synaptic plasticity, enhances neurotransmission, and has an antidepressant effect (Sen et al. 2008). Another possible mechanism is associated with the "cytokine hypothesis" of depression. Two major bioactive components of n-3 PUFAs (DHA and EPA) decrease the production of proinflammatory cytokines and eicosanoids derived from arachidonic acid, that may be involved in the development of depressive symptoms (Calder and Grimble 2002).

In the present study, we used the CMS rat model of depression, which reflexes one of the main symptoms of major depression, i.e., anhedonia (Willner 1997). The surveys revealed that prolonged supplementation with fish oil and n-3 PUFAs enriched phospholipids did not prevent from an anhedonic-like state provoked by long lasting stress. Our results support earlier observations that n-3 PUFAs did not inhibit behavioral disturbances caused by prolonged stress, including: physical condition degradation, body weight decrease or increased aggressiveness in mice (Vancassel et al. 2008). It is also important to note that in the discussed experiment, n-3 PUFAs supplementation reversed the stress induced-reduction of serotonin level the brain.

In our study, n-3 PUFAs enriched phospholipids were used as alternative source of n-3 PUFAs. Earlier, Carrie et al. (2000) reported that egg yolk phospholipids can reverse behavioral changes and restore a normal composition of fatty acids in mice brain induced by a diet deficient in n-3 PUFAs. However, egg yolk phospholipids are primarily an important source of the acetylcholine precursor choline (Magil et al. 1981). Acetylcholine has enormous significance in learning and memory processes, therefore choline-containing phospholipids, especially soybean lecithin, are commonly applied to improve memory functions.

According to the cholinergic-monoaminoergic theory, excessive supremacy of the cholinergic system over the adrenergic one may induce depressive symptoms (Janowsky et al. 1972). However, no conclusive evidence of a depression-inducing effect of lecithin or other acetylcholine precursors has been found. Depressive symptoms have been observed in some choline-treated patients with schizophrenia (Davis et al. 1979) or tardive dyskinesia (Tamminga et al. 1977). On the other hand, patients with Alzheimer's disease showed no significant change in the mean Geriatric Depression Scale score following treatment with lecithin (Vida et al. 1989). Also, a large population–based study did not reveal any correlation between blood serum choline level and depression (Bjelland et al. 2009).

In our study, n-3 PUFAs enriched phospholipid supplementation did not influence significantly sucrose solution consumption in

Table: Fatty acid composition of the supplements

Fatty acid*	Supplement	
	Fish oil	Egg yolk lecithin
C14:00	3.94	0.26
C16:00	14.73	3.56
C16:01	7.49	1.24
C18:00	2.34	11.0
C18:01	27.87	39.02
C18:02	1.21	9.06
C18:03	15.97	1.90
C20:03	–	0.11
C20:04	9.98	1.58
C20:05	7.48	0.21
C22:06	8.99	3.07
Total (n-3) PUFAs	32.40	5.17
Total (n-6) PUFAs	11.20	10.74
n-6/n-3 ratio	0.35	2.08
Total saturated	21.0	43.83
Total unsaturated	79.0	56.17
Total monounsaturated	35.4	40.26
Total polyunsaturated	43.6	15.92

* -In g/100 g total fatty acid

non-stressed rats, except for 5th week when sucrose consumption in supplemented rats was lower compared to the control group. Similar temporary drop of intake was found in rats fed with fish oil. This may imply that n-3 PUFAs enriched phospholipids do not induce depression in normal rats.

In pharmacotherapy, soybean phospholipids are used most often, and they include comparatively higher amounts of n-6 PUFAs than n-3 PUFAs (contrary to egg yolk phospholipids they do not contain DHA or EPA). Some reliable data suggest that n-6 PUFAs are also involved in depression. However, in contrast to n-3 PUFAs that have been inversely associated with depressive symptoms (Sublette et al. 2011; Mozurkewich et al. 2011; Hibbeln 1998), a positive correlation has been reported for n-6 PUFAs and the high ratio of n-6/n-3 (Mamalakis et al. 2006; Kiecolt-Glaser et al. 2007).

We have also found that 9 week CMS procedure reduced rats body weight increase. In both groups supplemented with fish oil or n-3 PUFAs enriched phospholipids and exposed to CMS, body weight increase was higher than in the stressed control group but the difference was not statistically significant.

The presented results demonstrate that fish oil and n-3 PUFAs enriched phospholipid supplementation does not reverse disturbances caused by CMS, such as anhedonic-like state or reduction of body weight gain.

4. Experimental

4.1. Supplements and diets

Fish oil was purchased from LYSI Hf (Reykjavik, Iceland). The phospholipid preparation (enriched with n-3 PUFAs) was obtained from Wroclaw University of Environmental and Life Sciences (Wroclaw, Poland). The pure phospholipid fraction, extracted from designed eggs (defined as the amount of substance insoluble in acetone) was 73%. The content of phosphatidylcholine in this fraction was 81.73%, and phosphatidylethanolamine −18.27%. The preparation also contained ovo-lipids, proteins and small amounts of vitamins (especially E and group B vitamins). The fatty acid compositions of supplements were estimated by Gas chromatography and shown in the Table. The test diet was prepared by supplementation a conventional laboratory diet (Labofeed H, Kcynia, Poland) with fish oil (3 g/100 g diet) or egg yolk phospholipids (5 g/100 g diet). LYSI's fish oil contained 32.4% w/w n-3 PUFAs; thus there were approximately 1.0 g of n-3 PUFAs per 100 g of the enriched food. The test diet was prepared every 2–3 days and stored at 4 °C.

4.2. Animals

The experiment was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Ethical Committee on the Animal Research of the Institute of Immunology and Experimental Therapy Polish Academy of Sciences in Wroclaw. Male Wistar-Crl:WI (Han) rats, approximately 2 months old, were purchased from the Experimental Medicine Centre of Medical University of Bialystok. Before the experimental period, the animals were acclimatized for 14 days. Rats were housed individually in chambers with a 12:12 h light-dark cycle (lights on at 7:00 a.m.) and a temperature maintained at 21 ± 2 °C with free access to food and water, except when the CMS procedure was required.

4.3. Chronic mild stress protocol

The stress protocol was based on the CMS procedure described by Papp et. al. (2003). Initially, the animals were trained to consume a palatable sucrose solution (1%). Training consisted of ten 1-h free access to a bottle with sucrose solution, in the home cage, following 14 h of food and water deprivation. Sucrose consumption was measured by weighing the bottles at the beginning and at the end of the test. During the stress period the sucrose consumption test was performed once a week, under similar conditions. On the basis of their sucrose intake in the final baseline test, the animals were divided into two matched groups. One group was exposed to nine weeks of chronic mild stressors whereas the other group was unstressed. The stress protocol included seven different stress conditions each lasting 10 to 14 h. Each week consisted of two periods of food deprivation, one period of water deprivation, one period of 45 cage tilt, two periods of intermittent illumination (lights on and off every 2 h), one period of soiled cage (200 ml water in sawdust bedding), one period of paired housing, three periods of low intensity stroboscopic illumination (150 flashes/min) and one period of no stress. The non-stressed animals were housed in a separate room and had no contact with the stressed animals. They were deprived of food and water for 14 h preceding each sucrose intake test, but otherwise food and water was freely available in the home cage. On the basis of their sucrose intake following initial 3 weeks of stress, both stressed and non-stressed animals were divided into 6 subgroups: a non-stressed group fed with standard laboratory chow (Control, C), a non-stressed group fed with diet containing 3% fish oil (FO), a non-stressed group fed with 5% n-3 PUFAs enriched phospholipids (PL), a stressed group fed with standard laboratory chow (stressed control, C/Str), a stressed group fed with diet containing 3% fish oil (FO/Str), and a stressed group fed with 5% n-3 PUFAs enriched phospholipids (PL/Str). All groups were then fed for subsequent 6 weeks. Body weight was measured at the start and the end of the 6-wk feeding trial.

4.4. Statistical analysis

In chronic mild stress test statistical analysis of the effect of the examined substances and time of their action was performed using two-way analysis of variance (ANOVA) with repeats. One-way analysis of variance and Tukey's test were used for specific comparisons between groups of rats in particular points of time. Influence of both CMS and examined substances on mean body weight increase in groups of rats was analyzed by two-way analysis of variance ANOVA and Tukey's test. Hypotheses were considered positively verified if $p < 0.05$. Data were expressed as the mean values ± SD.

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