

Effects of Fish Oil on the Central Nervous System: A New Potential Antidepressant?

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In the last 100 years major depression has increased worldwide. In this study we provided coconut fat (CF, rich in saturated fatty acids) or fish oil (FO, rich in n-3 polyunsaturated fatty acids) to female rats throughout pregnancy and lactation and then to their offspring post-weaning and examined lipid brain profile and the possible effect of FO as antidepressant agent in the offspring in adulthood (F1). Rats were submitted to forced swimming test, elevated plus maze, Morris water maze and open field. Peroxidation rate in the cerebral cortex and hippocampus were measured. Docosahexaenoic acid (DHA) concentration in dam's milk, eicosapentaenoic acid (EPA) and DHA concentration in hippocampus and cerebral cortex from F1 rats FO supplemented increased significantly when compared to control (C) and CF rats. Arachidonic acid/EPA ratio in the cerebral cortex and hippocampus decreased in rats submitted to forced swimming test. Peroxidation rate were not different between the groups. Immobility time in the forced swimming test in FO group was reduced ($p < 0.01$) when compared to C and CF rats. We conclude that lifelong intake of FO was able to induce an antidepressant effect with EPA and DHA concentration increased in the cerebral cortex and hippocampus.

Keywords: Cerebral function; Depression; Fish oil; Forced swimming; Polyunsaturated fatty acids

INTRODUCTION

The brain has high concentration of complex lipids, which will determine the structural and functional properties of its cellular and subcellular membranes (Farooqui *et al.*, 2000; Das, 2003). Docosahexaenoic acid

(22:6n-3, DHA) and arachidonic acid (20:4n-6, AA) are the most important polyunsaturated fatty acids (PUFAs) in neuronal membranes, but the major PUFAs present is usually DHA (Broadhurst *et al.*, 2002). The precursors of these PUFAs are α -linolenic acid (18:3n-3, LNA) and linoleic acid (18:2n-6, LA), respectively, which cannot be synthesized *de novo* by mammals, and are therefore, considered as essential fatty acids. DHA and AA are incorporated by cell membranes during development and adult age, with brain and retina requesting more DHA than other tissues (Jiménez *et al.*, 1997). DHA plays an important role on brain metabolism influencing cognitive performance, visual acuity, neurotransmitters metabolism and neural development (Jiménez *et al.*, 1997; Carlson and Neuringer, 1999; Chalon *et al.*, 2001). Long-term deficiency in LNA leads to changes in brain fatty acid composition causing loss of learning ability (Innis, 2000) and during neuronal development could be responsible for memory impairment or neurological disease in adult (Moriguchi *et al.*, 2000; Youdim *et al.*, 2000). Modifications of the brain membrane fatty acid (FA) composition occur with supplementation of various dietary oils. If the lipid profiles in diet are not suitable to maintain cerebral needs, the cells will replace DHA by other PUFAs, changing brain functions (Fernstrom, 1999; Carlson, 2001).

Amount and type of FA in the diet has been reported to be involved with some disease development (Simopoulos, 2002). In fact, modern Western diet in the past 100 years has changed significantly,

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which lowered the ratio of omega-3 to omega-6 to about 1:25 (Simopoulos *et al.*, 1999). The correct balance of omega-3 and omega-6 fatty acids within phospholipids is ultimately essential for normal neuronal function. In addition, during the last 100 years, the prevalence of major depression has increased worldwide (Hibbeln, 1998). There are evidences that alterations in FA metabolism and the composition of phospholipids in the serum and membranes are involved in the development of major depression (Hibbeln *et al.*, 1996; Maes *et al.*, 1999). Epidemiological studies have shown that places with higher rates of omega-3 consumption, where fish is a core of the diet, have lower rates of depressive disorders (Tanskanen *et al.*, 2001). In countries such as Japan and Germany, prevalence rates of depression are 0.12 and 5%, respectively. This has been associated with the annual fish consumption diet which is close to 150 lbs per person in Japan and less than 30 lbs per person in Germany (Hibbeln, 1998). This author found a correlation of fish consumption in different countries with the prevalence of major depression showing a highly significant decrease of depression with increasing fish consumption. In addition, fish oil (FO) has been used as mood stabilizer or adjunctive therapy in depression, bipolar disorders and schizophrenia (Stoll *et al.*, 1999; Nemets *et al.*, 2002).

Most previous studies investigating the effect of dietary fatty acids on major depression have used human beings (Peet and Horrobin, 2002; Puri *et al.*, 2002). There are few reports on the effect of dietary fatty acids in laboratory animals on major depression. These studies have used young adult animals fed for a short period before or after induction of the depression (Hillakivi-Clarke *et al.*, 1996; Raygada *et al.*, 1998). A more likely scenario is that a dietary pattern will be lifelong and may exist from conception. We are not aware of any studies that have investigated the effect of lifelong consumption of particular fatty acids on susceptibility to depression in adulthood. Therefore, in this study we provided coconut fat (CF, rich in saturated fatty acids) or FO (rich in n-3 PUFA) to female rats throughout pregnancy and lactation and then to their offspring post-weaning and examined the possible effect as *antidepressant* agent in the offspring in adulthood.

MATERIAL AND METHODS

Animals and Study Design

All studies involving animals were approved by the University Federal of Paraná Committee of Animal Welfare. Female Wistar rats adults were kept in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) on a 12/12 h

TABLE I Fatty acid composition (%) of the diets given to rats

Fatty acids	Fish oil	Coconut fat
<i>Fatty acid composition (g/100 g total fatty acids)</i>		
Lauric acid 12:0	0.50	61.04
Miristic acid 14:0	11.45	18.93
Palmitic acid 16:0	14.19	5.57
Stearic acid 18:0	3.16	2.62
Oleic acid 18:1 (n-9)	8.69	7.04
Linoleic acid 18:2 (n-6)	0.20	2.86
Linolenic acid 18:3 (n-3)	1.74	0.50
Araquidonic acid 20:4 (n-6)	12.11	–
Eicosapentaenoic acid 20:5 (n-3)	26.01	1.42
Docosahexaenoic acid 22:6 (n-3)	20.19	–

light/dark cycle (lights on 07:00 h). The animals were divided into three groups. One received a normal chow diet (C) *ad libitum*, and the other two were fed chow but were also supplemented orally with either CF or FO, kindly donated by Herbarium Foundation (Curitiba, PR, Brazil). The FO used was a mixed marine triacylglycerol preparation containing 180 g eicosapentaenoic acid (EPA) and 120 g DHA per kg (Table I). The oils were supplemented at a level of 3 g/kg body weight per day and were provided as a single bolus using a pipette during 15 days, when they were 90 days old. Then they were mated with male Wistar rats that had been fed on normal laboratory chow. Throughout gestation and lactation the females continued to receive CF or FO, according to the same supplementation protocol. After weaning (age 21 days) the male offspring (F1) received the same diet and supplementation as their mothers, until adult age (90 days), when they were submitted to different behavior tests as described below.

Forced Swimming Test

The procedure was done as described by Bejjamini *et al.* (1998), a modified method of Porsolt *et al.* (1978). Rats were placed in glass aquarium ($20 \times 20 \times 40 \text{ cm}^3$) containing 15 cm deep water ($24 \pm 1^\circ\text{C}$) for 15 min followed by a 5 min retest session 24 h later. Immobility time was recorded during the test session. The rat was judged immobile wherever it stopped swimming and remained floating in the water, with its head just above water level. The water was changed for each rat after test session to avoid influence of alarm substance (Abel and Belitzke, 1990). Following the test, the animals were dried in a warm enclosure.

Morris Water Maze

A black circular tank (160 cm diameter \times 50 cm deep) was filled with water ($22 \pm 1^\circ\text{C}$) to a depth of 30 cm. A transparent acrylic platform ($10 \times 10 \text{ cm}^2$) was located in one of four quadrants, 1 cm below

the water surface. All tests were recorded by a video camera system located in the roof (Morris, 1984).

Place Learning

During six consecutive days the rats were submitted to four trials per day. For each trial, the rat was placed in water facing the tank wall at one of four possible starting locations (North, South, East or West). In the room, there were extra-maze cues, including posters on the walls, lights and the experimenter, who always stood at the same position. The rat used these cues to develop a spatial map and locate the hidden platform. The latency to find the platform was recorded. If the rat did not find the platform after 60 s, it was gently put on the platform and placed there for 20 s. After that, the rat was placed in a cage outside the water maze for 30 s and then placed in a new start point. This protocol was repeated for six days. On the seventh day, the platform was removed from the tank and did the probe trial. The rat was placed in the quadrant opposite to that the platform and it swam during the 60 s.

Working-memory

One week after the probe trial, animals were submitted to working-memory test during five consecutive days, with four trials per day (Dos Santos, 1999). The rat was placed in the tank facing the wall in one of four start points. Latency was recorded in each trial. If the rat did not find the platform after 60 s, it was gently put on the platform and placed there for 30 s. After that, rat was placed in a new start point. The hidden platform was placed in a different quadrant each day.

Elevated Plus Maze

The elevated plus maze consists of two opposite open arms and two opposite enclosed arms ($45 \times 15 \text{ cm}^2$). The arms are connected by a central square ($15 \times 15 \text{ cm}^2$), forming a plus shape. The maze is elevated 70 cm above the floor in a room. This test has been widely used to measure anxiety or exploratory behavior (File and Zangrossi, 1993; File and Gonzales, 1996). The rat was placed in the central square and its behavior was observed for 5 min.

Open-field

The open-field apparatus consists in a circular arena (1 m diameter), the floor is divided into central and peripheral units (Kelly, 1993). The rat was placed individually in the center of the open-field. The animal's behavior was evaluated during 5 min

and locomotion (number of units crossed) was recorded. After that, the open-field was washed with water-alcohol (10%) solution, to avoid bias due to odors or residues left by the rats tested earlier.

Fatty Acid Composition in the Diet, Milk, Cerebral Cortex and Hippocampus

Total lipids were extracted from diet, dam's milk and from cerebral cortex and hippocampus tissue of F1 generation using chloroform-methanol (2:1 vol/vol) according to Folch *et al.* (1957). Fatty acids were derivatized with 4-bromomethyl-7-coumarin and then separated on a Shimadzu LC-10A high performance liquid chromatograph using an octadecylsilica column ($25 \text{ cm} \times 4.6 \text{ mm i.d.}$; particle size $5 \mu\text{m}$). Fatty acids were resolved isocratically using a mobile phase of acetonitrile-water (gradient from 77:23 to 90:10 vol/vol) at a flow rate of 1 ml/min. Fatty acid derivatives were detected by fluorescence (325-nm excitation; 395-nm emission).

Determination of the Products of Lipid Peroxidation

Measurement of Stable Products of Lipid Peroxidation by Thiobarbituric Acid Assay

Aldehyde products of lipid peroxidation were measured using thiobarbituric acid (TBA), following method described by Brown and Kelly (1996). Briefly, brain tissue was extracted in phosphate buffer (0.1 M), pH 7.4. Then, 0.5 ml was mixed with 0.5 ml TBA (1% in NaOH 50 mM), 0.5 ml of HCl 25% and 0.045 ml of butylated hydroxytoluene (2% in methanol). The samples were then heated in a boiling water bath for 10 min. After cooling, 1.5 ml of butanol was added to the mixture and centrifuged at 3000 rpm (Eppendorf 5810 R) for 20 min and the absorbance of the supernatant was read at 532 nm. The concentration of malondialdehyde (MDA) was calculated from TBA-reactive material using the extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ given for the MDA-TBA adduct.

Lipid Hydroperoxide Measurement

The products of lipid peroxidation were measured as lipid hydroperoxides following the method described by Jiang *et al.* (1991). Briefly, brain tissue was homogenized in phosphate buffer (0.1 M), pH 7.4. One hundred microliters was added to 900 μl of the following reaction mixtures and incubated for 30 min at room temperature prior to measurement at 560 nm: 100 μM of xylenol orange, 25 mM of H_2SO_4 , 250 μM of FeSO_4 , and 4 mM of butylated hydroxytoluene, in 90% (v/v) methanol. The reaction is essentially complete after 15 min and the color is

stable overnight at room temperature. The extinction coefficient of the Fe³⁺-xylenol orange complex ($1.5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) was used to calculate oxidation of ferrous by hydroperoxide.

Statistical Analysis

Data are presented as mean \pm SEM or median \pm semi-interquartile range when indicated. Kruskal–Wallis ANOVA followed by *post-hoc* Dunn test evaluated the data of the forced swimming test. The place of learning and working memory in the Morris water maze were examined by two-way analysis of variance (ANOVA) followed by Duncan test. Elevated plus maze, open field, fatty acids composition and products of lipid peroxidation were analyzed by one-way ANOVA followed by *post-hoc* Newman–Keuls. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Fatty Acid Composition

Milk

The fatty acid composition of the DHA in the dam's milk differed according to diet (Table II). Dam's milk from rats fed with FO contained higher proportions of DHA than those from rats fed the control diet or supplemented with CF ($p < 0.001$). Unsaturation index in FO was about 10% higher when compared to C and CF groups.

Cerebral Cortex

The fatty acid composition of cerebral cortex lipids also differed according to diet (Table III). Cerebral

TABLE II Fatty acid composition of milk from rats fed a control (C) or supplemented with fish oil or coconut fat (CF)

Fatty acids	Control	Fish oil	Coconut fat
<i>Fatty acid (g/100 g total fatty acids)</i>			
12:0	12.2 \pm 0.74	12.2 \pm 0.01	13.0 \pm 0.62
14:0	13.1 \pm 1.17	12.8 \pm 0.95	14.6 \pm 1.16
16:0	19.3 \pm 1.17	18.6 \pm 0.86	19.5 \pm 1.17
16:1 (n-7)	1.8 \pm 0.06	1.7 \pm 0.10	1.4 \pm 0.06
18:0	4.1 \pm 0.19	4.6 \pm 0.33	3.9 \pm 0.28
18:1 (n-9)	15.5 \pm 0.53	12.1 \pm 0.5	12.7 \pm 0.35
18:2 (n-6)	27.9 \pm 0.73	29.2 \pm 1.7	26.9 \pm 1.87
18:3 (n-3)	2.8 \pm 0.09	2.9 \pm 0.10	2.9 \pm 0.16
20:4 (n-6)	2.2 \pm 0.23	2.0 \pm 0.18	2.2 \pm 0.02
20:5 (n-3)	1.3 \pm 0.13	1.8 \pm 0.13	1.5 \pm 0.11
22:6 (n-3)	0.7 \pm 0.06	2.1 \pm 0.003*	0.8 \pm 0.10
Unsaturation index	101	110.5	97.7

* $p < 0.05$ vs. C and CF groups. Data are presented as mean \pm SEM of 9 animals for each group.

TABLE III Fatty acid composition of cerebral cortex from rats fed a control (C) diet or supplemented with fish oil (FO) or coconut fat (CF)

Fatty acids	Control	Fish oil	Coconut fat
<i>Fatty acid (g/100 g total fatty acids)</i>			
12:0	5.41 \pm 0.84	1.61 \pm 0.09*	5.20 \pm 0.18
14:0	2.99 \pm 0.03	1.37 \pm 0.18	4.06 \pm 0.27
16:0	3.58 \pm 0.49	1.23 \pm 0.18*	7.04 \pm 0.44**
16:1 (n-7)	1.41 \pm 0.03	3.59 \pm 0.17*	1.24 \pm 0.36
18:0	3.56 \pm 0.52	0.60 \pm 0.018*	3.97 \pm 0.34
18:1 (n-9)	9.75 \pm 1.29	5.59 \pm 0.26*	16.46 \pm 1.07***
18:2 (n-6)	60.5 \pm 0.21	10.87 \pm .35*	49.6 \pm 3.19**
18:3 (n-3)	3.03 \pm 0.12	2.63 \pm 0.21	2.05 \pm 0.28
20:4 (n-6)	1.45 \pm 0.45	2.76 \pm 0.16	1.12 \pm 0.33
20:5 (n-3)	4.15 \pm 0.05	30.79 \pm 0.78*	4.50 \pm 0.11
22:6 (n-3)	4.30 \pm 0.03	38.95 \pm 0.79*	4.28 \pm 0.19
Unsaturation index	193.6	437.5	175.7

* $p \leq 0.05$ vs. C and CF groups; ** $p \leq 0.05$ vs. C group; *** $p \leq 0.05$ vs. C and FO groups. Data are presented as mean \pm SEM of 9 animals for each group.

cortex from animals supplemented with FO contained higher proportions of EPA and DHA than those from rats fed the control diet or supplemented with CF ($p < 0.001$). The AA–EPA ratio of cerebral cortex lipids decreased in the order C (0.35) > CF (0.25) > FO (0.09). The proportion of saturated fat (except 14:0) and linoleic acid were significantly lower in FO group when compared to C and CF animals. Unsaturation index in FO group was approximately 2.4-fold greater than C and CF groups.

Hippocampus

The fatty acid composition of hippocampus lipids also differed according to diet (Table IV). Hippocampus from animals supplemented with FO contained higher proportions of EPA and DHA

TABLE IV Fatty acid composition of hippocampus from rats fed a control (C) diet or supplemented with fish oil (FO) or coconut fat (CF)

Fatty acids	Control	Fish oil	Coconut fat
<i>Fatty acid (g/100 g total fatty acids)</i>			
12:0	3.31 \pm 0.4	2.87 \pm 0.2	2.87 \pm 0.05
14:0	5.53 \pm 0.1	1.06 \pm 0.1*	3.9 \pm 0.8***
16:0	26.89 \pm 0.5	1.8 \pm 0.39*	25.4 \pm 0.4
16:1 (n-7)	1.01 \pm 0.003	4.08 \pm 0.4*	0.89 \pm 0.04
18:0	31.55 \pm 0.1	0.83 \pm 0.05*	27.79 \pm 0.3***
18:1 (n-9)	8.17 \pm 0.01	3.85 \pm 0.3*	9.09 \pm 0.8
18:2 (n-6)	14.73 \pm 0.3	1.6 \pm 0.06*	23.07 \pm 0.7**
18:3 (n-3)	1.56 \pm 0.3	2.62 \pm 0.2	1.67 \pm 0.03
20:4 (n-6)	3.06 \pm 0.1	3.51 \pm 0.2	2.07 \pm 0.5
20:5 (n-3)	1.43 \pm 0.4	41.58 \pm 0.6*	1.37 \pm 0.5
22:6 (n-3)	2.02 \pm 0.001	36.19 \pm 0.7*	1.8 \pm 0.6
Unsaturation index	74.8	458.1	87.1

* $p < 0.001$ vs. C and CF groups; ** $p < 0.05$ vs. C group; *** $p < 0.05$ vs. C and FO groups. Data are presented as mean \pm SEM of 9 animals for each group.

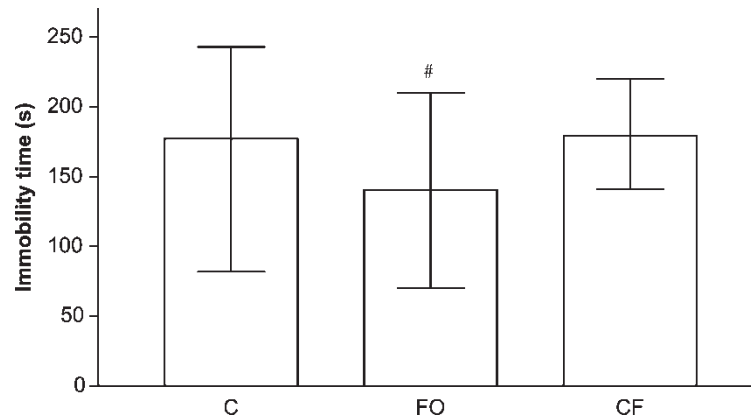


FIGURE 1 Immobility time in rats fed a control diet (C) or supplemented orally with fish oil (FO) or coconut fat (CF), submitted to the forced swimming test. Data are presented as median \pm semi-interquartile range ($n = 19$ for each group). $p < 0.05$ vs. C and CF group.

than those from rats fed the control diet or supplemented with CF ($p < 0.001$). The AA-EPA ratio of hippocampus lipids decreased in the order C (2.13) > CF (1.15) > FO (0.08). The proportion of saturated fat (except 12:0) and linoleic acid were significantly lower in FO group when compared to C and CF animals. Unsaturation index in FO group was approximately 5.6-fold greater than C and CF groups.

Determination of the Products of Lipid Peroxidation

The TBARs and lipid hydroperoxides content in the cerebral cortex and hippocampus was not different between the groups ($p > 0.05$), which was about 5.0 and 4.5 $\mu\text{mol}/\text{mg}$ protein and 133 and 117 mmol/mg protein, respectively.

Forced Swimming Test

Supplementation with FO significantly decreased immobility time ($H(2) = 11.52$; $p < 0.05$; Dunn $p < 0.001$) when compared to control animals or supplemented with CF (Fig. 1).

Morris Water Maze

On Morris water maze no differences were found in latency time between the three groups on place learning test (Fig. 2, $F(10.275) = 0.88$; $p > 0.2$) or working memory test (Fig. 3, $F(6.81) = 0.57$; $p > 0.2$). In probe trial, no difference was found in time spent on target quadrant (data not shown).

Elevated Plus-maze

There was no difference between the groups in the elevated plus-maze (Table V) either in visiting time in the open arm ($F(2.54) = 0.654$; $p = 0.5$) or in the closed arm ($F(2.54) = 0.49$; $p = 0.61$), or in the percentage of

entries in the open arm ($F(2.54) = 1.09$; $p = 0.34$) and in the closed arm ($F(2.54) = 1.14$; $p = 0.32$) and in the total entries on closed arms ($F(2.54) = 0.49$; $p > 0.2$).

Open Field

There was no difference between the groups in the motor activity (Fig. 4) evaluated by the number of squares crossed ANOVA ($F(2.54) = 2.09$; $p = 0.13$).

DISCUSSION

In this study, we examined the effect of lifelong supplementation of the diet with CF or FO on brain fatty acid profile and its action as an antidepressant agent in F1 rats.

The increase in our knowledge about the role of FA as a mediator and modulator of central nervous system (CNS) activity and peptides is becoming understood. Depression is a serious disorder in

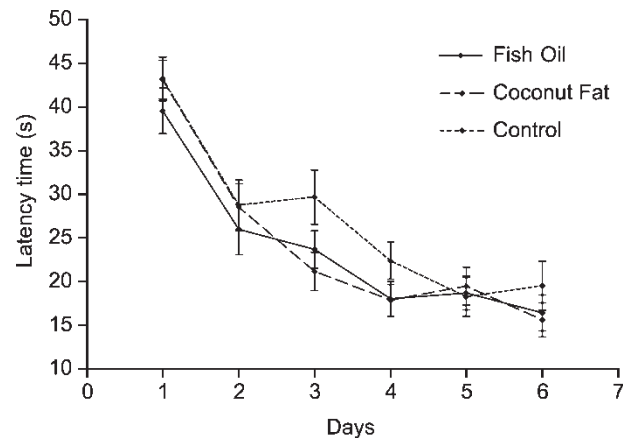


FIGURE 2 Place learning performance in the Morris water maze as measured by latency time to find the hidden platform. Each point represents mean group performance (\pm SEM) in four trials on days 1–6 ($n = 19$ rats in each diet group).

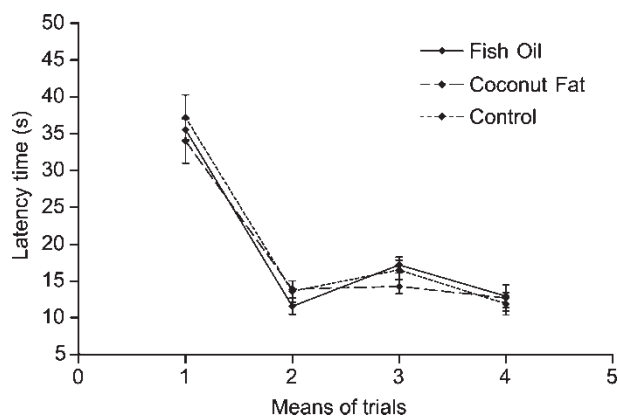


FIGURE 3 Working memory performance in the Morris water maze was measured by latency time to find the hidden platform. Each point represents mean group performance (\pm SEM) in four trials on five days ($n = 19$ rats in each diet group).

western society with prevalence as high as 21% of the general population (Wong and Licinio, 2001). It has been suggested that part of the reason for this can be traced to major dietary changes which happened over the past century (Adams *et al.*, 1996; Simopoulos, 2002). During this time there has been a large increase in the intake of saturated fats and n-6 vegetable oils at the expense of n-3 oils. Researchers have postulated that the sharp raises in depression and other neurological disorders are closely associated to the increase in n-6 vegetable oils (Hillakivi-Clarke *et al.*, 1996).

The concentration of DHA in dam's milk supplemented with FO (3.0 g/kg/day) was significantly increased (3-fold) when compared to control and CF groups (Table II). Probably, this was caused by the amount of DHA and EPA present in the diets (Table I). Yonekubo *et al.* (1993) provided 10 g/kg/day of FO in the diet and got an increase around 12-fold higher as compared to control groups. These results show that offspring, from conception to suckling phase, were exposed to an environment rich in DHA which is known to be essential for the development of neural tissues during early postnatal life (Carlson, 2001).

Several works have reported that the FO intake affect FA composition of tissues (Bourre *et al.*, 1997; Srinivasarao *et al.*, 1997). In fact, cerebral cortex and hippocampus (Tables III and IV, respectively) from

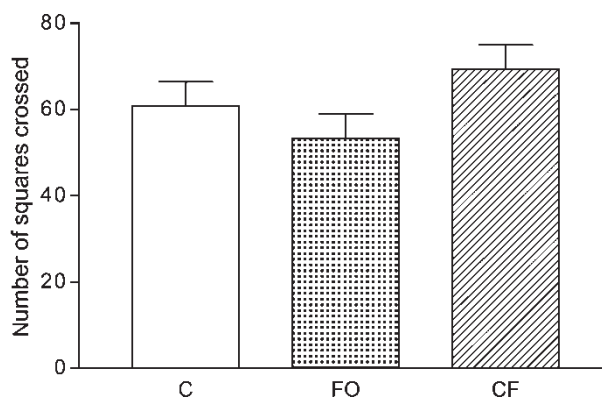


FIGURE 4 Locomotor activity in the open field was measured by the number of squares crossed and recorded during 5 min. Values are means \pm SEM of 19 animals per group.

rats supplemented with FO, from conception to adult age, had an increase in EPA and DHA concentrations of several fold higher than that of control and CF supplemented. Neuronal membrane (n-6) FA, in particular AA, was replaced with competing (n-3) FA in brain tissues examined. Also EPA concentration in the hippocampus was 35% greater than in the cerebral cortex in FO supplemented rats, showing that incorporation rate in both areas are different. These findings suggest that a high level of EPA and DHA can be achieved in those areas and perhaps in others by intake of 3.0 g/kg/day lifelong.

The incorporation of highly unsaturated FA into tissue phospholipids may enhance peroxidation of cellular membranes. Suárez *et al.* (1996) reported that dietary supplementation with a 10% long-chain PUFAs for 4 weeks increased susceptibility of weanling rat tissue lipids to *in vitro* lipid peroxidation. These results are in contrast with ours described here when a 3 g/kg/day FO-supplementation lifelong was given to rats. Despite the great increase in the unsaturation index in cerebral cortex and hippocampus (Tables III and IV) there was no difference between the groups concerning to lipid peroxidation rate. We believe that the activities of antioxidant enzymes in brain tissues were not affected by supplementation with FO which may explain the lipid peroxidation results meaning that there is no lipid peroxidation susceptibility in those tissues.

TABLE V Anxiety in elevated plus maze

	Elevated plus maze				
	Total number of entries in closed arms	Percent of entries in open arms	Percent of entries in closed arms	Percent of time spent in open arms	Percent of time spent in closed arms
Control	14 \pm 1.3	51.84 \pm 5.2	48.16 \pm 5.2	57.06 \pm 5.22	51.54 \pm 6.1
Fish oil	15.2 \pm 1.2	53.67 \pm 4.3	41.59 \pm 3.8	48.41 \pm 6.14	44.45 \pm 5.5
Coconut fat	11.7 \pm 1.3	60.53 \pm 3.3	39.47 \pm 3.3	55.72 \pm 5.98	44.28 \pm 5.9

Percentages of numbers and time spent in open and closed arms during 5-min observation. Data are presented as mean \pm SEM of 19 animals per group.

Many studies in animal models of depression investigating drugs treatment and criteria for their evaluation have been established, but few studies have approached alteration in the diet and depression (Wainwright *et al.*, 1994; Raygada *et al.*, 1998).

Our results show that rats chronically supplemented with FO for one generation decreased significantly immobility time when compared to control and CF groups suggesting that FO was able to cause an antidepressant effect in rats (Fig. 1). The forced swimming test is used to evaluate an antidepressant effect of a substance preclinically (Porsolt *et al.*, 1978). However, this test alone can not be used to exclude other factors that could cause a false positive test. Hence we submit the rats to tasks to assess possible behavioral alterations such as anxiety, motor activity and learning which could interfere in the results found in forced swimming test. Anxiety can trigger the behavior despair. Animals with increased motor activity would show a reduced immobility time. An impairment learning means that the animals will not remember the training session which happens 24 h earlier, leading to increased mobility and consequently reduced immobility time. In the open field (Fig. 4), water maze (Figs. 2 and 3) and elevated plus maze tests (Table V) all groups present the same behavior, showing that FO supplementation has no effect on this behaviors. Other studies found the same result, which corroborate our data (Wainwright *et al.*, 1994; Wainright *et al.*, 1999; Arterburn *et al.*, 2000; Carrié *et al.*, 2000; Wilde *et al.*, 2002). These behavior tests were important because they give support to suggest that the results shown in forced swimming test were caused by FO effect upon CNS.

Cerebral cortex and hippocampus are areas involved in learning and depression, respectively (Spedding *et al.*, 2003). Morphologic and morphometric analysis of the hippocampus in depressed patients have revealed structural alterations (Sheline 2000). FO supplementation increased significantly EPA and DHA concentration in both areas. Work in humans reported that lower concentration of long chain n-3 PUFAs in cellular membranes is associated with the appearance of depression and that n-3 PUFAs supplementation would be efficient to alleviate the depression symptoms (Hibbeln and Salen, 1995; Edwards *et al.*, 1998; Peet *et al.*, 1998). Alterations in phospholipids and cholesterol of the cell membranes in the brain may induce changes in the fluidity/viscosity and consequently, in many neurotransmitter systems thought to be related to depression development, e.g. serotonin (Engelberg, 1992; Yadid *et al.*, 2000) and noradrenaline (Anand and Charney, 2000). There are findings that AA-EPA ratio in serum cholesteryl and phospholipids are higher in major depression subjects (Adams *et al.*, 1996; Maes *et al.*, 1996). Our results show that

the AA-EPA ratio in the cerebral cortex and hippocampus (Tables III and IV, respectively) was decreased in rats submitted to forced swimming test (Fig. 1).

The mechanisms by which FO induce antidepressant effect are unknown and were not investigated here. However, we suggest that FO may increase number of serotonin receptors and the level of receptor binding because it has been shown that α -linolenic acid (18:3 n-3), the parent acid of EPA and DHA, is able to do it (Bergelson, 1995; Rego and Oliveira, 1995; Delion *et al.*, 1996;). In addition, one may take into consideration the content of DHA and its relation to the molecular activity of Na^+K^+ ATPase in the membrane bilayer. Up to 60% of energy consumption in the brain is linked to the Na^+K^+ ATPase enzyme. This may represent a fundamental relationship, underlying metabolic activity, but it may also represent the link between reduced levels of PUFAs and neurological dysfunction as the majority of energy consumption in the brain is linked to the Na^+K^+ ATPase enzyme (Turner *et al.*, 2003). Both hypotheses must be tested.

The results presented herein led us to conclude that the lifelong intake of FO was able to induce an antidepressant effect with EPA and DHA concentration increased in the cerebral cortex and hippocampus.

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