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Comparative study between n-6, *trans* and n-3 fatty acids on repeated amphetamine exposure: A possible factor for the development of mania

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ABSTRACT

In the last decades, foods rich in omega-3 (ω-3) fatty acids (FA) have been replaced by omega-6 (ω-6) and *trans* FA, which are found in processed foods. The influence of ω-6 (soybean oil – SO), *trans* (hydrogenated vegetable fat – HVF) and ω-3 (fish oil – FO) fatty acids on locomotor and oxidative stress (OS) parameters were studied in an animal model of mania. Rats orally fed with SO, HVF and FO for 8 weeks received daily injections of amphetamine (AMPH — 4 mg/kg/mL-ip) for the last week of oral supplementation. HVF induced hyperactivity, increased the protein carbonyl levels in the cortex and decreased the mitochondrial viability in cortex and striatum. AMPH-treatment increased the locomotion and decreased the mitochondrial viability in all groups, but its neurotoxicity was higher in the HVF group. Similarly, AMPH administration increased the protein carbonyl levels in striatum and cortex of HVF-supplemented rats. AMPH reduced the vitamin-C plasmatic levels of SO and HVF-fed rats, whereas no change was observed in the FO group. Our findings suggest that *trans* fatty acids increased the oxidative damage per se and exacerbated the AMPH-induced effects. The impact of *trans* fatty acids consumption on neuronal diseases and its consequences in brain functions must be further evaluated.

1. Introduction

Trans fatty acids (TFA) in food attract the attention of the general public due to their potential adverse effects on human health. Although TFA from ruminant fats have been part of the human diet for thousands of years, the intake of TFAs increased enormously with the increasing use of hydrogenated vegetable fats (HVF) during the second part of the 20th century (Pfeuffer and Schrezenmeir, 2006).

The consumption of convenience food and fast food in the Western diet has increased during the last decades (Baggio and Bragagnolo, 2006). These foods often contain large amounts of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids of the omega-6 (ω-6) series (Baggio and Bragagnolo, 2006), as well as considerable amounts of TFA (Allison et al., 1999). These changes in the feeding habits increased the ratio of ω-6/ω-3 polyunsaturated fatty acids (PUFAs) intake, mainly as a consequence of intake reduction of ω-3 fatty acids (FA) (Ailhaud et al., 2006). From a dietary point of view, the consumption of TFA represents a loss of essential fatty acids intake that may have a hazardous impact on human health.

α-linolenic acid (LNA; 18:3n-3) and linoleic acid (LA, 18:2n-6) are essential fatty acids (EFAs) from the omega-3 and omega-6 series, respectively, and they play an important role in the biological membranes function (Sarsilmaz, 2003). LNA, eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and docosapentaenoic acid (DPA; 22:5n-3) are the most important ω-3 EFAs. They are abundantly found in fish oil (EPA and DHA) and in smaller amounts in vegetable oils (LNA and LA).

LNA can be converted to long-chain polyunsaturated fatty acids such as DHA and EPA, whereas LA can be converted to arachidonic acid (AA). Of particular nutritional importance, the human intake of these EFAs reflects the composition of neuronal cell membrane phospholipids (Haag, 2003; Yehuda et al., 2005) and modulates the brain physiological functions by modifying cell permeability, synaptic membrane fluidity (Jump, 2002), the number and affinity of receptors, as well as the activity of neurotransmitter systems (Yehuda et al., 2005), such as dopamine (DA) (Wainwright, 2002). In this sense, ω-3 FA supplementation can be considered as important in view of its scarcity in diets containing high levels of ω-6 (Simopoulos, 1999).

Preclinical and clinical studies have indicated that low intake of ω-3 FA may be associated with the pathophysiology of neurological and psychiatric disorders, including depression (Borsonelo et al., 2007), hyperactivity, schizophrenia and bipolar disorder (BD) (Cott, 1999;

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Hamazaki et al., 2009). Of potential importance for BD, phosphatidylinositol associated second messenger activity can be suppressed by ω-3 supplementation, indicating a molecular mechanism similar to that of lithium and valproate (Kinsella, 1990), two current drugs for BD treatments. BD affects about 1% of the world population (Belmaker, 2004), however its pathophysiology remains unknown. Clinically, BD management is complex and can be accomplished by pharmacological agents approved for the treatment of others mental disorders, such as antipsychotics and anticonvulsants (Gould et al., 2004).

Despite difficulties (Gould and Einat, 2007), the development of animal models has been an important tool for investigating factors that can be involved in BD (Einat et al., 2003), as well as new therapeutic perspectives for BD treatment (Lamberty et al., 2001).

The clinical diagnosis of BD includes manic symptoms, such as elation, irritability and hyperactivity. Therefore, psychostimulant-induced hyperactivity (McCormick and Ibrahim, 2007) is one of the most accepted animal models of mania and has been widely used for explaining the putative mechanisms involved in BD (Frey et al. 2006a, b; Lamberty et al., 2001; Machado-Vieira et al., 2004).

Amphetamine (AMPH) is a drug that increases neuronal release of dopamine (DA) and inhibits DA vesicular uptake (Brown et al., 2000) inducing manic symptoms in normal human volunteers and in BD subjects (Anand et al., 2000). That exacerbation of dopaminergic transmission can result in dopamine autoxidation and its monoamine oxidation deamination, increasing ROS production (Graham et al., 1978), which can be hazardous to cells and tissues. In fact, imaging studies of the human brain showed that AMPH can induce DA release in the striatum and reduce striatal DA transporter density (McCann et al., 1998), acting favorably to reactive oxygen species (ROS) generation.

Oxidative stress may be associated with an inhibition of the mitochondrial electron transport chain (Berman and Hastings, 1999), which can lead to cellular dysfunction. In this sense, it has been demonstrated that ROS generation plays a critical role in the pathophysiology of several neuropsychiatric disorders, particularly BD (Steckert et al., 2010).

So far, there are no comparative studies on the influence of the different dietary fatty acids on the development of neural disorders, particularly BD. Taking into account the potential modulation of dopamine neurotransmission by dietary FA, the objective of this study was to investigate the influence of the different fatty acids (ω-6, *trans* and ω-3 FA), found in soybean oil, hydrogenated vegetable fat and fish oil, on behavioral and oxidative parameters in an animal model of mania.

2. Materials and methods

2.1. Animals

A trial was conducted with 64 male Wistar rats weighing 30–40 g at the beginning of the study. Groups of four animals were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23 °C ± 1 °C) and on a 12 h-light/dark cycle with lights on at 7:00 a.m. The number of animals used was kept to a minimum, just enough to obtain relevant results, and they were maintained and used in accordance with the guidelines of the Brazilian Association for Laboratory Animal Science (COBEA), following international norms of care and animal maintenance.

2.2. Drugs and solutions

DL-amphetamine (Merck, Germany) was dissolved in saline solution (4 mg/mL), which was the vehicle.

2.3. Experimental design

The 21-day-old rats were divided into four groups (n = 16), namely: control (C), 0.1% soybean oil (SO), 0.1% hydrogenated vegetable fat (HVF) and 0.1% fish oil (FO). SO, HVF and FO were incorporated to tap water as a homogenous 1% Tween suspension (Barcelos et al., 2009), which was prepared daily and offered to the rats in place of drinking water in dark bottles. The control group received only the vehicle (water). The consumption of FO, HVF and SO was monitored daily and no differences between the three experimental groups were observed.

After 8 weeks of *ad libitum* oral supplementation of FAs, half the rats of each group (n = 8) received a single daily injection of 4 mg/kg/ip with AMPH for 7 days (Frey et al., 2006a). Two hours after the first and last AMPH administration, locomotor activity was determined in an open-field arena (42 × 42 × 28 cm) for five minutes (Broadhurst, 1960). Rats were then anesthetized with thiopental (50 mg/kg body weight ip) and euthanized by exsanguinations (blood was collected by cardiac puncture in heparinized tubes), and plasma obtained by centrifugation at 1310 G, 15 min for vitamin C. The brains were put on ice and cut coronally at the caudal border of the olfactory tubercle. The cortex and striatum were used for biochemical assays.

2.4. Fatty acids composition

Soybean oil, fish oil and hydrogenated vegetable fat were submitted to saponification in methanolic KOH solution and esterification in methanolic H_2SO_4 solution (Hartman and Lago, 1973). Methylated fatty acids were analyzed using an Agilent Technologies gas chromatograph (HP 6890) equipped with a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm) and flame ionization detector. The temperature of the injector port was set at 250 °C and the carrier gas was nitrogen (1.1 ml/min). After injection (1 μL, split ratio 50:1), the oven temperature was kept at 140 °C for 5 min and then raised to 240 °C at 4 °C/min and kept at this temperature for 12 min. Standard fatty acid methyl esters (Sigma, Saint Louis, USA) were subjected to the same conditions and the following retention times were used to identify the fatty acids. Results were expressed as percentage of total area of the identified fatty acids.

2.5. Measurement of carbonyl protein content

To determine protein carbonyl levels, striatal and cortical tissues were homogenized in 10 volumes (w/v) of 10 mM Tris–Hcl buffer, pH 7.4 and measured by the method described by Yan et al., 1995, with some modifications. Aliquots of 1 mL of homogenates were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (10 mM DNPH). After incubation at room temperature for 1 h in the dark, 0.5 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing 3% SDS), 2 mL of heptane (99.5%) and 2 mL of ethanol (99.8%) were added sequentially and mixed with vortex agitation for 40 s and centrifuged for 15 min. After that, the protein isolated from the interface was washed twice with 1 mL of ethyl acetate/ethanol 1:1 (v/v) and suspended in 1 mL of denaturing buffer. Each sample was measured at 370 nm against the corresponding HCL sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} according to Levine et al., 1990.

2.6. Plasmatic vitamin C determination

Plasma vitamin C (VIT C) was estimated as described by Galley et al., 1996 with some modifications (Jacques-Silva et al., 2001). This method produces an orange chromogen reacting with dinitrophenylhydrazine (DNPH) at 37 °C, measured spectrophotometrically at 520 nm. A standard curve using ascorbic acid was used to calculate the content of VIT C and expressed as μg VIT C/mL plasma.

2.7. Slices viability

The MTT assay is a mean of measuring the activity of living cells by assessing the activity of mitochondrial dehydrogenases. The viability of cortex and striatum slices was quantified by measuring the reduction of [3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-MTT to a dark violet formazan product by mitochondrial dehydrogenases (Mosmann, 1983). Slices (0.4 mm) of the brain areas of rats fed with the various FA supplementations and repeated treatment with vehicle or AMPH were prepared with a McIlwain chopper. MTT reduction assays were performed in plates containing 500 μL of phosphate buffer saline, and the reaction was started by adding MTT to a final concentration of 0.1 mg/mL. After 1 h of incubation at 37 °C, the medium was removed and the slices dissolved in dimethylsulfoxide (DMSO). The MTT reduction was measured spectrophotometrically by the difference in absorbance between 570 and 630 nm. Data were calculated as a percentage of values from control.

2.8. Statistical analysis

All the data were analyzed by two-way ANOVA 4(control/soybean oil/trans saturated fat/fish oil)×2 (vehicle/amphetamine) followed by Duncan's multiple range test, when appropriate. $P<0.05$ was regarded as statistically significant.

3. Results

3.1. Composition of the oils/fats used as dietary supplement

Soybean oil was used as a source of ω-6 fatty acids, hydrogenated vegetable fat as a source of *trans* fatty acids, and fish oil as a source of ω-3 fatty acids. The soybean oil used had a high content of ω-6 PUFA (about 50%), a low content of ω-3 PUFA, and a very low content of *trans* fatty acids. The hydrogenated vegetable fat used had about 20% *trans* fatty acids, 18% ω-6 PUFA and 0.5% ω-3 PUFA. The fish oil used had about 27% ω-3 PUFA, 1.7% ω-6 PUFA and undetectable levels of *trans* fatty acids (Table 1).

3.2. Locomotor activity

Two-way ANOVA revealed a significant main effect of diet supplementation ($F(3,56)=11.7$; $P<0.000$) and repeated AMPH administration ($F(1,56)=167.10$, $P<0.000$). Post-hoc comparisons by Duncan's multiple range test indicated that HVF intake caused a significant increase in locomotor activity in vehicle and AMPH-treated rats as compared to the control, SO or FO groups. Duncan's multiple range test indicated that AMPH caused a significant increase in locomotor activity in all supplementation groups (Fig. 1).

3.3. Carbonyl proteins in striatum and cortex

Two-way ANOVA of striatal carbonyl content revealed a significant main effect of diet supplementation ($F(3,56)=33.74$; $P<0.000$) and a significant main effect of repeated AMPH administration ($F(1,56)=4.70$, $P<0.05$). Post-hoc comparisons by Duncan's multiple range test indicated that striatal protein carbonyl levels were higher in control and HVF than in SO and FO vehicle treated groups. After repeated administration of AMPH, HVF consumption caused a significant increase in striatal carbonyl levels as compared to the other groups. AMPH administration tended to increase carbonyl levels in all supplementary treatments; however, the increase was significant only for the AMPH group supplemented with HVF when compared to the vehicle HVF group (Fig. 2A).

Two-way ANOVA of cortical carbonyl levels revealed a significant main effect of supplementation ($F(3,56)=14.04$; $P<0.000$) and significant main effect of AMPH ($F(1,56)=8.16$, $P<0.05$) and significant diets X AMPH interaction ($F(3,56)=3.23$; $P<0.05$). Post-hoc comparisons by Duncan's multiple range test indicated that ingestion of HVF caused an increase in carbonyl levels when compared to control and FO groups in vehicle treated groups. In AMPH-treated rats, cortical carbonyl levels were higher in the HVF-fed group than in the other groups. Post-hoc comparison also indicated that AMPH increased carbonyl levels in the control and HVF groups as compared to their respective vehicle groups (Fig. 2B).

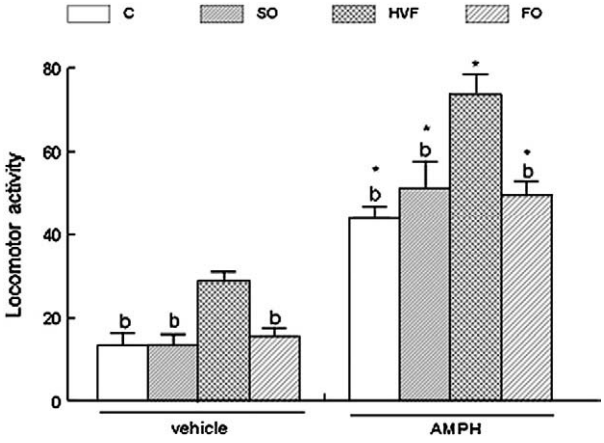

Fig. 1. Spontaneous locomotor activity and the behavioral responsiveness to amphetamine, in open-field (number of crossing responses) of rats supplemented with different diets (control-C, soybean oil-SO, hydrogenated vegetable fat-HVF or fish oil-FO) for 8 weeks (n=8). Behavioral evaluation was carried out 2 h after the last injection of seven daily injections of dl-amphetamine (4 mg/kg/mL-ip). Data are expressed as mean ± S.E.M. [b]Different from HVF within the same treatment ($P<0.05$); *Different from vehicle within the same supplementation ($P<0.001$).

3.4. Vitamin C plasmatic levels

Two-way ANOVA of plasmatic vitamin C showed only a significant main effect of repeated AMPH administration ($F(1,56)=17.61$; $P<0.001$). In fact, AMPH treatment decreased vitamin C levels in all dietary groups, except for the FO group (Fig. 3).

3.6. Mitochondrial dehydrogenases activity (MTT assay) in slices of brain tissues

Two-way ANOVA of MTT reduction by cortical brain slices revealed a significant main effect of supplementation ($F(3,56)=23.2$; $P<0.000$), of AMPH administration ($F(1,56)=514.2$, $P<0.000$) and a significant diet X AMPH interaction ($F(3,56)=17.85$; $P<0.000$). In vehicle-treated rats, MTT reduction was impaired in HVF-fed rats when compared to the other groups. Furthermore, MTT reduction was lower in cortical slices from SO than in slices from the control and FO groups. In AMPH-treated rats, MTT reduction was lower in the control and HVF-fed rats when compared to the FO group. Repeated administration of AMPH caused a marked decrease in cortical slices viability in all supplemented groups. Two-way ANOVA of MTT reduction by striatal brain slices revealed a significant main effect of

Table 1
Fatty acid composition (% of total identified FA) of the dietary supplementation.

	Soybean oil	Hydrogenated vegetable fat	Fish oil
Saturated	18.07	25.94	46.10
Monounsaturated (*cis*)	26.03	43.35	25.31
Trans	0.15	19.79	n.d.
PUFAs n-6 (*cis*)	50.25	17.89	1.70
PUFAs n-3 (*cis*)	5.48	0.48	26.87

PUFA: polyunsaturated fatty acids. n.d.: not detected.

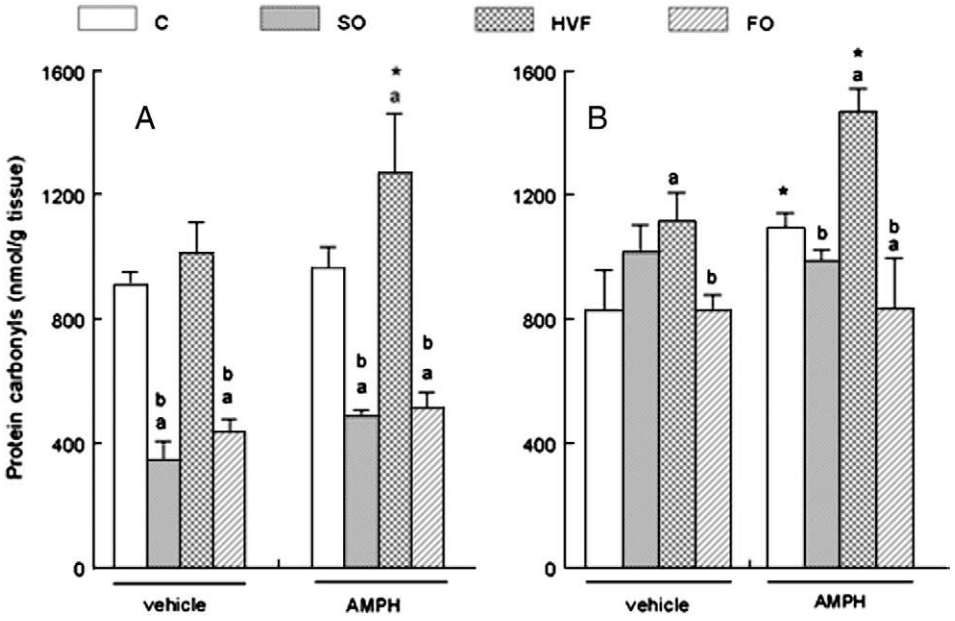

Fig. 2. Accumulation of protein carbonyl in striatum (A) and cortex (B) of rats supplemented with different diets (control-C, soybean oil-SO, hydrogenated vegetable fat-HVF or fish oil-FO) for 8 weeks (n = 8) and treated with seven daily injections of dl-AMPH (4 mg/kg/mL-ip). Data are expressed as mean ± S.E.M. The lowercase letters show significant differences between the diets within the same treatment; Asterisk shows significant difference between treatments within the same supplementation. [a]Different from control within the same treatment ($P<0.05$). [b]Different from HVF ($P<0.05$). *Different from vehicle ($P<0.001$).

diet ($F(3,56) = 21.2$; $P<0.000$), of AMPH treatment ($F(1,56) = 442.5$, $P<0.000$) and a significant supplementation X AMPH interaction ($F(3,56) = 40.0$; $P<0.000$). In vehicle-treated groups, post-hoc comparisons indicated that MTT reduction decreased in HVF and SO groups when compared to the control and FO groups. In AMPH-treated rats, MTT reduction in all supplementation groups was lower than in vehicle-treated groups. Furthermore, MTT reduction was decreased in the control and HVF groups when compared to the FO and SO groups (Table 2).

4. Discussion

In the present study we showed that HVF supplementation caused an increase of about 2 times in the locomotor activity, while SO and FO supplementation did not affect this behavioral parameter. Similarly, AMPH administration caused a 5.5-fold increase in crossings of HVF-treated rats against 3.9- and 3.71-fold increases in the rats supplemented with SO and FO, respectively. Different studies have indicated the beneficial effects of the ω-3 FA supplementation on BD symptoms (Turnbull et al., 2008; Marangell et al., 2006; Stoll et al., 1999). Here we are showing for the first time that HVF supplementation increased the locomotor activity in vehicle- and amphetamine-treated rats, which may indicate a greater susceptibility of HVF-fed animals to develop manic-like symptoms. AMPH increases the brain levels of dopamine, facilitating its auto-oxidation and dopamine quinones generation. In agreement with this, LaVoie & Hastings, 1999 demonstrated an increase in the binding of dopamine-quinones to cysteine residues on proteins following methamphetamine administration, which can impair the function of proteins. A recent animal study demonstrated that repeated AMPH administration increased protein carbonyl formation in rat brain, leading to the notion that repeated manic episodes may be associated with brain oxidative damage (Frey et al., 2006a, b). Here, it was observed an increase in protein carbonyl content in the cortex of rats supplemented with HVF, whereas carbonyl levels were similar in the

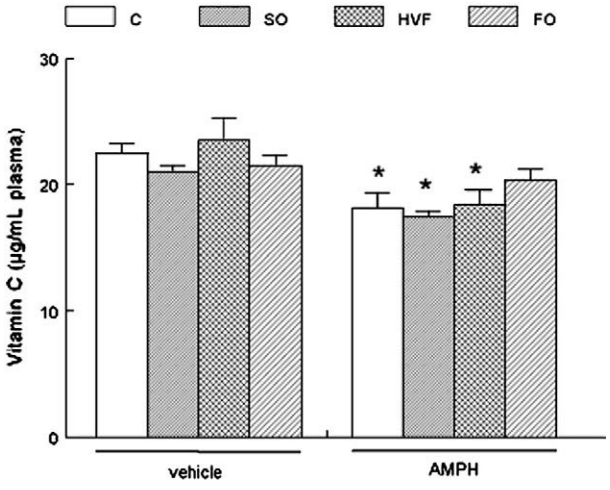

Fig. 3. Plasmatic vitamin C levels of rats supplemented with different diets (control-C, soybean oil-SO, hydrogenated vegetable fat-HVF or fish oil-FO) for 8 weeks (n = 8) and treated with seven daily injections of dl-AMPH (4 mg/kg/mL-ip). Data are expressed as mean ± S.E.M. [a]Different from control within the same treatment ($P<0.05$). [b]Different from HVF within the same treatment ($P<0.05$). *Different from vehicle within the same supplementation ($P<0.001$).

Table 2
Ex-vivo brain slices viability in cortex (A) and striatum (B) of rats supplemented with different diets (control-C, soybean oil-SO, hydrogenated vegetable fat-HVF or fish oil-FO) for 8 weeks (n = 8). Experiments were performed after seven daily injections of dl-amphetamine (4 mg/kg/mL-ip). The lowercase letters show significant differences between the diets within the same treatment; asterisk shows significant differences between the different treatments within the same supplementation.

	Striatum		Córtex	
	Vehicle	AMPH	Vehicle	AMPH
C	100	40.7 ± 2.2[*]	100	55 ± 2.0[*]
SO	84.8 ± 3.9[a,b,c]	62.3 ± 2.6[a,b,*]	85.3 ± 4.0[a,b,c]	59 ± 0.8[*]
HVF	68.2 ± 3.0[a]	53.7 ± 1.8[a,*]	73.4 ± 2.9[a]	55.7 ± 1.4[*]
FO	93 ± 0.9[b]	61.7 ± 0.7[a,b,*]	95.7 ± 0.6[b]	63 ± 0.9 [a,b,*]

[a] Different from C, ($P<0.05$).
[b] Different from HVF ($P<0.001$).
[c] Different from FO ($P<0.05$).
[*] Different from vehicle ($P<0.001$).

control, SO and FO groups. Repeated administration of AMPH increased carbonyl levels in striatum and cortex of HVF-supplemented animals. Of particular significance, FO supplementation tended to be associated with lower levels of protein carbonylation when compared to control and HVF-supplemented groups. This was particularly evident in striatum. The consumption of SO was found to decrease the striatal protein carbonylation levels.

In addition, FO supplementation shows some beneficial effects. In fact, it is plausible to suppose that the ω-3 fatty acids present in FO prevented the amphetamine-induced depletion of plasma VIT C levels, in contrast, fatty acids found in soybean oil and hydrogenated vegetable fat failed to do so. Taken together, the increase in protein carbonylation in rats supplemented with HVF indicates that *trans* fatty acids can increase cerebral susceptibility to oxidative damage, which can contribute to neuronal injury. Although the exact mechanism of AMPH-induced neurotoxicity is unknown, the involvement of oxidative stress has been strongly suggested (Brown and Yamamoto, 2003). In addition to induce dopamine accumulation in the extracellular medium, AMPH also increases the release of striatal glutamate (Stephans and Yamamoto, 1994). Glutamate triggers a cascade of events, starting with an increase in calcium influx (Tarazi and Baldessarini, 1999) that stimulates ROS production (here determined by an enhanced protein carbonylation) and impairs the mitochondrial function (here determined by a marked inhibition of MTT reduction by striatal and cortical slices).Of particular importance, we have observed that FO intake could partially counteract the neurotoxic effects caused by AMPH. Soy bean oil had a modest protective effect, whereas HVF generally exacerbated the neurotoxicity of AMPH, which reinforces the neuroprotective, antioxidant and anti-inflammatory effects of FO (Innis 2008; Farooqui et al., 2007).

In the literature, the biological effects and safety of *trans* fatty acids consumption are controversial (Wandall, 2008). In this sense, all the observations related to the consumption of *trans* fatty acids become very important, because they are consumed in rather large amounts in industrialized countries (2–8 g/day), corresponding to about 2.5% of the total energy (Allison et al., 1999). In this sense, omega-3 fatty acids supplementation becomes especially important because the majority of diets contain a great quantity of ω-6 and insufficient ω-3 fatty acids (Simopoulos, 1999). In fact, DHA is the precursor of neuroprotectin D1, a potent neuroprotective mediator which attenuates apoptotic processes by OS (Bazan, 2006, 2007).

In conclusion, this study suggests that the predominance of *trans* fatty acids in the diet may be associated with spontaneous or amphetamine-induced hyperactivity, which can indicate behavioral abnormalities linked with manic symptoms, found in psychiatric disorders. Consequently, further detailed investigations are urgently needed to establish the nutritional safety of *trans* fatty acids in humans. Furthermore, in view of the putative predictability of AMPH-induced hyperactivity as a model of mania, it becomes important to conduct a large epidemiological study to determine a possible link between *trans* fatty acid consumption and bipolar disorders in industrialized societies.

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References

Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. Prog Lipid Res 2006;45:203–36.

Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT. Estimated intakes of *trans* fatty and other fatty acids in the US population. J Am Diet Assoc 1999;99:166–74.

Anand A, Verhoeff P, Seneca N, Zoghbi SS, Seibyl JP, Charney DS, et al. Brain SPECT imaging of amphetamine-induced dopamine release in euthymic bipolar disorder patients. Am J Psychiatry 2000;157:1108–14.

Baggio SR, Bragagnolo N. The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products. Food Chem 2006;95:611–7.

Barcelos RCS, Benvegnú DM, Boufleur N, Reckziegel P, Muller LG, Pase C, et al. Effects of ω-3 essential fatty acids (ω -3 EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter. Neuro Res 2009;17:228–37.

Bazan NG. The onset of brain injury and neurodegeneration triggers the synthesis of docosanoid neuroprotective signaling. Cell Mol Neurobiol 2006;26:899–911.

Bazan NG. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Clin Nutr Metab Care 2007;10:136–41.

Belmaker RH. Bipolar disorder. N Engl J Med 2004;351:476–86.

Berman SB, Hastings TG. Dopamine oxidation alters mitochondrial respiration and induces permeability transition in brain mitochondria: implications for Parkinson's disease. J Neurochem 1999;73:1127–37.

Borsonelo EC, Galduróz JCF, Suchecki D, Calil HM. The influence of n-6 fatty acid supplemented diet on the effect of imipramine in an animal model of depression. Pharmacol Biochem Behav 2007;86:113–6.

Broadhurst PL. Experiments in Psychogenetics. In: Eysenk HJ, editor. Experiments in personality. London: Routledge & Kegan Paul; 1960. p. 76.

Brown JM, Yamamoto BK. Effects of amphetamine on mitochondrial function: Role of free radicals and oxidative stress. Pharmacol Ther 2003;99:45–53.

Brown JM, Hanson GR, Fleckenstein AE. Methamphetamine rapidly decreases vesicular dopamine uptake. J Neurochem 2000;74:2221–3.

Cott J. Omega-3 fatty acids and psychiatric disorders. Alternat Therap women's health 1999;1(13):97-104.

Einat H, Yuan P, Gould TD, Li J, Du J, Manji HK, et al. The role of the extracellular signal-regulated kinase signaling pathway in mood regulation. J Neurosc 2003;23(19):7311–6.

Farooqui AA, Ong WY, Horrocks LA, Chen P, Farooqui T. Comparison of biochemical effects of statins and fish oil in brain: the battle of the titans. Brain Res Rev 2007;56(2):443–71.

Frey BN, Martins MR, Petronilho FC, Dal-Pizzol F, Quevedo J, Kapezinski F. Increased oxidative stress after repeated amphetamine exposure: possible relevance as a model of mania. Bip Disord 2006a;8:275–80.

Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, et al. Changes in antioxidant defense enzymes after D-amphetamine exposure: implications as an animal model of mania. Neurochem Res 2006b;31:699–703.

Galley H, Davies MJ, Webster NR. Ascorbil radical formation in patients with sepsis: effects of ascorbate loading. Free Radic Biol Med 1996;20:139–43.

Gould TD, Einat H. Animal models of bipolar disorder and mood stabilizer efficacy: a critical need for improvement. Neurosc Biobehav Rev 2007;31:825–31.

Gould TD, Quiroz JA, Singh J, Zarate CA, Manji HK. Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. Molec Psychiatry 2004;9:734–55.

Graham DG, Tiffany SM, Bell Jr WR, Gutknecht WF. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. Mol Pharmacol 1978;14:644–53.

Haag M. Essential fatty acids and the brain. Can J Psychiatr 2003;48:195–203.

Hamazaki K, Choi KH, Kim HY. Phospholipid profile in the postmortem hippocampus of patients with schizophrenia and bipolar disorder: no changes in docosahexaenoic acid species. J Psychiatr Res 2009, doi:10.1016/j.jpsychires.2009.11.017.

Hartman L, Lago BC. A rapid preparation of fatty methyl esters from lipids. Lab Pract 1973;22:475–7.

Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res 2008;27:35–43.

Jacques-Silva MC, Nogueira CW, Broch LC, Flores EM, Rocha JB. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Toxicol Appl Pharmacol 2001;88:119–25.

Jump DB. Dietary polyunsaturated fatty acids and regulation of gene transcription. Curr Opin Lipidol 2002;13:155–64.

Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. J Parenter Enteral Nutr 1990;14:200–17.

Lamberty Y, Margineanu DG, Klitgaard H. Effect of the new antiepileptic drug levіracetam in an animal model of mania. Epilepsy Behav 2001;2:454–9.

LaVoie MJ, Hastings TG. Dopamine quinone formation and protein modification associated with the striatal neurotoxicity of methamphetamine: evidence against a role for extracellular dopamine. J Neurosci 1999;19:1484–91.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG. Determination of carbonyl content in oxidatively modified proteins. Meth Enzymol 1990;86:464–78.

Machado-Vieira R, Kapezinski F, Soares JC. Perspectives for the development of animal models of bipolar disorder. Prog Neuropsychopharmacol Biol 2004;28:209–24.
Marangell LB, Suppes T, Ketter TA, Dennehy EB, Zboyan H, Kertz B, et al. Omega-3 fatty acids in bipolar disorders. Clinical and research considerations. Prostagland Leukotrien Essent Fatty Acids 2006;75:315–32.
McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11 C]WIN-35, 458. J Neurosci 1998;18:8417–22.
McCormick CM, Ibrahim FN. Locomotor activity to nicotine and Fos immunoreactivity in the paraventricular nucleus of the hypothalamus in adolescent socially-stressed rats. Pharmacol Biochem Behav 2007;86:92-102.
Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Meth 1983;65:55–63.
Pfeuffer M, Schrezenmeir J. Impact of *trans* fatty acids of ruminant origin compared with those from partially hydrogenated vegetable oils on CHD risk. Internat Dairy J 2006;16:1383–8.
Sarsilmaz M. The regulatory role of dietary omega-3 essential fatty acids on oxidant/antioxidant balance in rat hippocampus. Neurosci Res Commun 2003;33:114–23.
Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999;70:560S–9S.
Steckert AV, Valvassori SS, Moretti M, Dal-Pizzol F, Quevedo J. Role of oxidative stress in the pathophysiology of bipolar disorder. Neurochem Res 2010, doi:10.1007/s11064-010-0195-2.
Stephans SE, Yamamoto BY. Effect of repeated metamphetamine administration on dopamine and glutamate efflux in rat prefrontal cortex. Brain Res 1994;700:99-106.
Stoll A, Severus WE, Freeman MP, Rueter S, Zboyan HA, Diamond E, et al. Omega 3 fatty acids in bipolar disorder. Arch Gen Psychiatry 1999;56:407–12.
Tarazi FI, Baldessarini RJ. Regional localization of dopamine and ionotropic glutamate receptor subtypes in striatolimbic brain regions. J Neurosci Res 1999;55:401–10.
Turnbull T, Cullen-Drill M, Smaldone A. Efficacy of omega-3 fatty acid supplementation on improvement of bipolar symptoms: a systematic review. Arch Psychiatry Nurs 2008;22:305–11.
Wainwright PE. Dietary essential fatty acids and brain function: a developmental perspective on mechanisms. Proc Nutr Soc 2002;61:61–9.
Wandall B. The controversy over trans fatty acids: effects early in life. Food Chem Toxicol 2008;46:3571–9.
Yan LJ, Traber MG, Packer L. Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal Biochem 1995;228:349–51.
Yehuda S, Rabinovitz S, Mostofsky DI. Essential Fatty Acids and Stress. In: Yehuda S, Mostofsky DI, editors. Nutrition, stress and medical disorders. Totowa, NJ: Humana Press; 2005. p. 99-100.