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CROSS-GENERATIONAL *TRANS* FAT INTAKE FACILITATES MANIA-LIKE BEHAVIOR: OXIDATIVE AND MOLECULAR MARKERS IN BRAIN CORTEX

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Abstract—Since that fast food consumption have raised concerns about people's health, we evaluated the influence of *trans* fat consumption on behavioral, biochemical and molecular changes in the brain-cortex of second generation rats exposed to a model of mania. Two successive generations of female rats were supplemented with soybean oil (SO, rich in n-6 FA, control group), fish oil (FO, rich in n-3 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA) from pregnancy, lactation to adulthood, when male rats from 2nd generation received amphetamine (AMPH-4 mg/kg-i.p., once a day, for 14 days) treatment. AMPH increased locomotor index in all animals, which was higher in the HVF group. While the FO group showed increased n-3 polyunsaturated fatty acid (PUFA) incorporation and reduced n-6/n-3 PUFA ratio, HVF allowed *trans* fatty acid (TFA) incorporation and increased n-6/n-3 PUFA ratio in the brain-cortex. In fact, the FO group showed minor AMPH-induced hyperactivity, decreased reactive species (RS) generation per se, causing no changes in protein carbonyl (PC) levels and dopamine transporter (DAT). FO supplementation showed molecular changes, since proBDNF was increased per se and reduced by AMPH, decreasing the brain-derived neurotrophic factor (BDNF) level following drug treatment. Conversely, HVF was related to increased hyperactivity, higher PC level per se and higher AMPH-induced PC level, reflecting on DAT, whose levels were decreased per se as well as in AMPH-treated groups. In addition, while HVF increased BDNF-mRNA per se, AMPH reduced this value, acting on BDNF, whose level was lower in the same AMPH-treated experimental group. ProBDNF level was influenced by HVF supplementation, but it was not sufficient to modify BDNF level. These findings reinforce that prolonged consumption of *trans* fat allows TFA incorporation in the cortex, facilitating hyperactive behavior, oxidative damages and molecular changes. Our study is a warning about cross-generational consumption of processed food, since high *trans* fat may facilitate the development of neuropsychiatric conditions, including bipolar disorder (BD).

Key words: *trans* fat, oxidative damage, DAT, BDNF, animal model of mania, bipolar disorder.

INTRODUCTION

Mania is a cardinal feature of bipolar disorder (BD), a debilitating psychiatric condition which can cause disabling impairment in social and occupational functions (Magalhaes et al., 2012). Many hypotheses have been postulated to explain the exact neurochemical mechanism underlying this pathophysiology, which has been related to different signaling pathways, abnormalities in neural plasticity and the body's neurochemical systems (Schloesser et al., 2008; Martinowich et al., 2009). More recently, some studies have reported the involvement of oxidative stress (OS) in the pathogenesis of mania, holding oxidative damage to lipid and proteins as possible factors related to neuronal and glial impairment in BD (Kunz et al., 2008; Andreazza et al., 2010; Steckert et al., 2010) because OS happens in situations in which the generation of free radicals exceeds the antioxidant defense capacity, thus causing damage to cellular proteins, DNA and lipids, thereby affecting cellular function (Cochrane, 1991; De Vasconcelos et al., 2005; Patki et al., 2009).

Intrauterine and early postnatal growth are characterized by a very fast deposition of fatty acids

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Abbreviations: AA, arachidonic acid; AMPH, amphetamine; BD, bipolar disorder; BDNF, brain-derived neurotrophic factor; DAT, dopamine transporter; DCF, dichlorofluorescein; DCHF-DA, 2′, 7′-dichlorofluorescein diacetate; DHA, docosahexaenoic acid; DNPH, dinitrophenylhydrazine; EFAs, essential fatty acids; FO, fish oil; HVF, hydrogenated vegetable fat; OS, oxidative stress; PC, protein carbonyl; PGE3, prostaglandins of series 3; PUFA, polyunsaturated fatty acids; PVDF, polyvinylidene difluoride; RS, reactive species; RT-PCR, real-time polymerase chain reaction; TBS, Tris-buffered saline; TFA, Trans fatty acid; SO, soybean oil.

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(FA) in the fetal tissues. In fact, the fetus and the newborn require large amounts of essential fatty acids (EFAs) that cannot be synthesized "de novo" by mammals, and therefore must be supplied from maternal diet and provided by placental transfer or breastmilk. Among these, long-chain polyunsaturated fatty acids (LC-PUFAs) belonging to the n-3 and n-6 series, such as docosahexaenoic acid (DHA, 22:6 n-3) and n-6 FA arachidonic acid (AA, 20:4 n-6), respectively, are rapidly incorporated in the retina and brain nervous tissue during the brain's growth spurt, mainly from the last trimester of pregnancy up to 2 years of age in humans (Dobbing and Sands, 1973; Martinez, 1992; Clandinin et al., 1980a,b). So these early development periods are critical for the brain and visual functions (Martinez, 1992). In addition n-3 and n-6 FA may influence brain function throughout life by modifying neuronal membrane fluidity, membrane activity-bound enzymes, number and affinity of receptors, function of neuronal membrane ionic channels, and production of neurotransmitters and brain peptides (Yehuda, 2003).

Given the importance of EFA to the fetus and newborn, concerns arose about the widespread changes in eating habits and the increased consumption of n-6 and TFA to the detriment of n-3 FA, altering the n-6/n3 ratio (Simopoulos, 2006). Dietary TFA are derived mainly from hydrogenated vegetable oils (Koletzko and Müller, 1990; Lichtenstein, 1995; Wolff et al., 1998; Stender et al., 2008), whose consumption has been steadily increasing since the 20th century and now accounts for 1.7–8% of the world dietary fat intake (Osso et al., 2008) through processed and fast food (Allison et al., 1999; Van de Vijver et al., 2000; Stender et al., 2008). It is well documented that dietary TFA can be quickly incorporated into membrane phospholipids, thus decreasing membrane fluidity and altering the biochemical properties as well the functionality of their proteins (Grandgirard et al., 1998; Morgado et al., 1998; Larqué et al., 2003). Considering the growing consumption of processed food in Western countries, reports have associated its chronic intake to the pathophysiology of neurological and psychiatric disorders such as hyperactivity, autism, schizophrenia and BD (Cott, 1999; Richardson and Ross, 2000; De Leon et al., 2002; Hamazaki et al., 2009).

Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family. It is initially synthesized as a precursor protein (prepro-BDNF) in the endoplasmic reticulum. Following cleavage of the signal peptide, proBDNF is transported to the Golgi apparatus for sorting into either constitutive or regulated secretory vesicles. BDNF is known to exert diverse influences on neural plasticity, such as neuronal survival, neuritic growth, neuronal differentiation, synaptic plasticity, and nerve repair (Dechant and Neumann, 2002). This neurotrophin has been found throughout the brain, with particular abundance in the hippocampus and cortex, which are brain areas thought to be critical for the control of mood, emotion, and cognition (Ernfors et al., 1990). Lower serum BDNF levels have been observed in both manic and depressive phases of BD compared with control and euthymic states, and showed a negative correlation with the severity of manic episode (Cunha et al., 2006; Machado-Vieira et al., 2007). On the other hand, Barbosa et al. (2010) reported increased BDNF during bipolar manic state in comparison to healthy people. So BDNF changes in the peripheral blood and their relationship with BD are not always consistent, besides depending on the state, phenotype, brain region and cell type involved (Matrisciano et al., 2009; Young, 2009). Consistent with these brain functions, BDNF was suggested to exert an important role in the pathophysiology and treatment of unipolar and BD (Post, 2007; Kapczinski et al., 2008).

Recent experimental studies of our group showed that *trans* fat supplementation from post-natal (Trevizol et al., 2011) and trans-natal development until adulthood (Trevizol et al., 2013) in rats were interestingly related to behavioral impairments and oxidative damage in brain tissues, while fish oil (FO), rich in n-3 FA, was found to be beneficial in the same parameters related to animal model of mania. This study was designed to comparatively evaluate the effect of FO versus *trans* fat supplementation on behavioral changes and oxidative and molecular parameters in the brain cortex of second generation rats exposed to a mania animal model.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures were approved by the Ethics Committee of Animal Use (CEUA) of the Federal University of Santa Maria. Animals were kept in Plexiglas cages with free access to food (Purina®) and water *ad libitum* in a room with controlled temperature (23 °C ± 1) and on a 12-h light/dark cycle throughout the experimental period. One week before mating, female adult *Wistar* rats (n = 8) were orally supplemented (3 g/kg) (Kuhn et al., 2013; Pase et al., 2013; Trevizol et al., 2013) with soybean oil Camera®, Ijuí, Brazil (SO-C, isocaloric control group), FO Herbarium® Curitiba, Brazil (FO, rich in n-3 FA) or hydrogenated vegetable fat (HVF) Primor®, Ijuí, Brazil (HVF, rich in *trans* fatty acids), and maintained under the same supplementation during pregnancy and lactation. The FA profile of each supplemented fat (SO, FO and HVF) was determined as described previously (Trevizol et al., 2013) and shown in Table 1. One female pup of each litter was maintained on the same supplementation until adulthood, when they were mated. These dams were kept on the same original supplementa-

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

	SO	FO	HVF
Σ SFA	18.1	46.1	25.9
Σ MUFA	26.0	25.3	43.3
Σ n-6 PUFA	50.2	1.7	17.9
Σ n-3 PUFA	5.5	26.9	0.5
Σ TFA	0.1	n.d.	19.8
n-6/n-3 ratio	9.2	0.1	37.3

SFA: saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: polyunsaturated, fatty acids; TFA: trans fatty acids.