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Chronic consumption of *trans* fat can facilitate the development of hyperactive behavior in rats

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HIGHLIGHTS

- Consumption of foods rich in *trans* fatty acids (TFA) is growing in Western countries.
- TFA for 10 months and across two generations induced active coping in forced swimming task.
- TFA was associated with increased locomotion before and after amphetamine administration.
- TFA across two generations increased locomotor and exploratory activities after environmental stress.
- Processed foods in early life may facilitate the development of hyperactive-like symptoms.

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ABSTRACT

In recent decades, the increased consumption of processed foods, which are rich in hydrogenated vegetable fat (HVF), has led to a decreased consumption of fish and oilseed, rich in omega-3 fatty acids. This eating habit provides an increased intake of *trans* fatty acids (TFA), which may be related to neuropsychiatric conditions, including inattention and hyperactivity. In this study, we evaluated the potential connection between prolonged *trans* fat consumption and development of hyperactivity-like symptoms in rats using different behavioral paradigms. *Trans* fat intake for 10 months (Experiment 1), as well as during pregnancy and lactation across two sequential generations of rats, (Experiment 4) induced active coping in the forced swimming task (FST). In addition, HVF supplementation was associated with increased locomotion before and after amphetamine (AMPH) administration (Experiment 2). Similarly, HVF supplementation during pregnancy and lactation were associated with increased locomotion in both young and adult rats (Experiment 3). Furthermore, *trans* fat intake across two sequential generations increased locomotor and exploratory activities following stressors (Experiment 4). From these results, we suggest that chronic consumption of *trans* fat is able to enhance impulsiveness and reactivity to novelty, facilitating hyperactive behaviors.

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is a serious neuropsychiatric condition that affects about 3–7% of school-aged children [1] and approximately 4% of adults [2,3]. ADHD is characterized by a cross-situational pattern of inattention, hyperactivity, and/or impulsivity, interfering with appropriate social and/or academic functioning [4]. Studies have investigated the association between reduced intake of n − 3 PUFA and inattention, hyperactivity and behavioral disorders [5,6]. Some authors have proposed that ADHD may be related to deficiencies in the conversion of EFA to its long chain derivatives (LC-PUFA) [7], which are particularly important in the neural membrane structure, exerting beneficial influences on signals transduction of the normal brain, thus affecting emotional functions [8,9].

In animal models of ADHD, locomotor hyperactivity is the main outcome measured and several reports have revealed an increase in locomotion in animals fed n − 3 PUFA deficient diets [10–12]. Interestingly, the exposure of mice to n − 3 PUFA deficient diet during pregnancy was able to increase locomotor activity in the offspring [13]. Blood biochemical evidence has suggested that a relative deficiency in n − 3 PUFA (in serum,

Abbreviations: ADHD, attention-deficit hyperactivity disorder; AMPH, amphetamine; AS, acute stress; DHA, docosahexaenoic acid; EFA, essential fatty acids; FA, fatty acids; FST, forced swimming task; HVF, hydrogenated vegetable fat; LC-PUFA, long chain polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; OFT, open-field task; SO, soybean oil; TFA, *trans* fatty acids.

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plasma and cell membrane) may underlie some of the behavioral and learning problems central to ADHD [14–16]. Likewise, spontaneously hypertensive rats exhibit locomotor hyperactivity together with low levels of DHA in plasma and brain membranes [17,18], signalizing the involvement of brain FA content in the development of hyperactive symptoms.

Characteristic of modern society, changes in dietary habits such as a high consumption of processed foods, especially fast food rich in *trans* fatty acids (TFA) [19], can be considered a risk factor for the development of central nervous system diseases [20–22]. In recent decades, there has been an increased presence of hydrogenated vegetable fat and saturated fat in foods [23] accompanied by a significant reduction in the consumption of foods rich in essential fatty acids (EFA) [24]. Regular consumption of TFA may eventually result in a loss of EFA, with unpredictable impacts on human health, because TFA derivatives may be incorporated into membrane phospholipids [25] and alter membrane fluidity, plasticity and neurotransmission [26,27]. *Trans* fat intake has also been linked to cognitive dysfunction [28,29], changes in dopaminergic neurotransmission [30], addiction [31], mania [32], movement disorders [25] and higher sensitivity to stress and anxiety [33].

So far, most studies about hyperactivity have been focused on dietary omega-3 fatty acids deficiencies [34–36], but little is known about the influence of long-term chronic consumption of TFA, especially in western countries, on the development of hyperactive behaviors. Our hypothesis of a link between regular consumption of *trans* fat and hyperactivity-like behavior emerged from unexpected behavioral findings from several research studies using different experimental paradigms, whose initial aim was not hyperactivity itself. These behavioral observations were so significant that we thought they could not be ignored and deserved a separate report.

2. Material and methods

The experiments were conducted with male Wistar rats from the central animal breeding facility of the Universidade Federal de Santa Maria (UFSM), RS-Brazil, 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. All the experimental protocols were approved by the Animal Ethical Committee of this university (24/2010; 23/2010; 004/2012), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

2.1. Experiment 1

The trial was conducted with 20 male Wistar rats weighing 40–60 g at the beginning of the treatments. Immediately after weaning (21 days of age), rats were randomly assigned to two experimental groups (n = 10) and the diets started. Dietary supplementation consisted in the incorporation (20%) [25,37] of soybean oil (SO) (rich in polyunsaturated fatty acids-PUFA) or hydrogenated vegetable fat (HVF) (rich in *trans*-monounsaturated and saturated-TFA) in standard chow, Supralab® (Alisul alimentos LTDA, São Leopoldo, RS Brazil) as purified pelletted rodent diet and stored at refrigeration temperature (Tables 1 and 2). SO was considered as a control group (C-SO), mainly because it contains adequate levels of PUFA, n − 6/n − 3 ratio within acceptable limits [38–40], and by its elevated consumption worldwide [25,37]. The two experimental groups (C-SO and HVF) were isocaloric in order to prevent metabolic differences between animals of different experimental groups [41,42] from interfering with the antioxidant defense system [43] and dopamine and serotonin neurotransmission [44]. The consumption of the diets was monitored every other day. Both experimental groups (C-SO and HVF) were submitted to behavioral observations in the forced swimming task (FST) from ten months of dietary consumption.

Table 1
Composition of the diet.

Ingredient	Amount (g/kg diet)
Casein	180
Cornstarch	460
Sucrose	230
Cellulose	20
Fat[a]	20
Mineral mix[b]	50
Vitamin mix[c]	10

[a] Represented by soybean oil or hydrogenated vegetable fat.
[b] Composition (g/kg): sucrose 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4.7H2O$, 100; NaCl, 60; MgO, 40; $FeSO_4.7H_2O$, 8; $ZnSO_4.7H_2O$, 7; $MnSO_4.H_2O$, 2; $CuSO_4.5H_2O$, 1; $Na_2SiO_7.3H_2O$, 0.5; $AlK(SO_4)_2.12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4.6H_2O$, 0.1; H_2BO_3, 0.1; $CoSO_4.7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)6Mo_7O_{24}.4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01.
[c] Composition (g/kg): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL-a-tocopheryl acetate, 20; phylloquinone, 0.1; thiamin HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanobalamin, 2.5; cholin HCl, 200; DL-methionin, 200; *p*-aminobenzoic acid, 5; inositol, 10.

Table 2
Fatty acid composition of diets enriched with different fats (% of total fatty acids identified).

Fatty acids	Soybean oil	Hydrogenated vegetable fat
∑ SFA	19.36	25.83
∑ MUFA	28.56	59.10
∑ n − 3 FA	4.72	0.67
∑ n − 6 FA	46.47	13.69
∑ PUFA	51.19	14.36
∑ *trans* FA	0.61	16.51

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

2.2. Experiment 2

A trial was conducted with 32 male Wistar rats weighing 30–40 g at the beginning of the study. Twenty-one-day-old rats were assigned to two experimental groups (n = 16): 0.1% soybean oil (SO) and 0.1% hydrogenated vegetable fat (HVF) (Table 3). SO and HVF were incorporated to tap water as a homogenous 1% Tween suspension [45], which was prepared daily and offered to the animals in place of drinking water in dark bottles. The consumption was monitored daily and no differences between the experimental groups were observed (data not shown). After 8 weeks of *ad libitum* intake of FA, half of each experimental group (n = 8) received a single daily injection of amphetamine (4 mg/kg/ip) for 8 consecutive days [46]. Two hours after the last AMPH administration, the locomotor activity was evaluated in the open field task (OFT).

Table 3
Fatty acid composition of different dietary supplementation (% of total fatty acids identified).

Fatty acids	Soybean oil	Hydrogenated vegetable fat
∑ SFA	18.07	25.94
∑ MUFA	26.03	43.35
∑ n − 6 FA	50.25	17.89
∑ n − 3 FA	5.48	0.48
∑ PUFA	55.73	18.37
∑ *trans* FA	0.15	19.79

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.