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Isolation during the prepubertal period associated with chronic access to palatable diets: Effects on plasma lipid profile and liver oxidative stress

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HIGHLIGHTS

- Isolation stress during early life leads to oxidative imbalance in the liver.
- These effects were accentuated with a high-fat diet.
- The high-fat diet increased abdominal fat and plasma glucose and leptin levels.
- The high-fat diet increased plasma cholinesterase activity.

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ABSTRACT

Pre-puberty is a critical period for the final maturation of the neural circuits that control energy homeostasis, as external stimuli such as exposure to diets and stress may influence the adaptive responses with long-term repercussions. Our aim is to investigate the effects of isolation stress during early life and of chronic access to palatable diets, rich in sugar or fat, on the metabolic profile (glycemia, plasma lipids, leptin and cholinesterase activity) and oxidative stress parameters in the livers of adult male rats. We observed changes mainly in animals that received the high-fat diet (increased body weight and abdominal fat in adults, as well as increased plasma glucose, and cholinesterase activity), and most of these effects were further increased by exposure to stress. High-fat diet also affected the rats' lipid profile (increased cholesterol, LDL-cholesterol and triglycerides); these effects were more marked in stressed animals. Additionally, exposure to stress led to an oxidative imbalance in the liver, by increasing production of reactive species, as well as the activity of antioxidant enzymes (superoxide dismutase and catalase); these effects were accentuated with the high-fat diet (which also caused a severe reduction in glutathione peroxidase activity). Taken together, these results show that the pre-pubertal period constitutes a critical window for stressful interventions during development, leading to alterations in metabolic parameters and increased oxidative stress during adulthood that may be more pronounced in animals that receive a high-fat diet.

1. Introduction

The consumption of diets rich in sugar and fat, along with a sedentary lifestyle, is associated with increased obesity prevalence [1]. Levels of obesity have been increasing in children and adolescents, creating concern, as exposure to diets rich in calories during this period of development could modify the maturation of neuronal circuits and lead to dysfunction or diseases during adulthood [2]. In addition, it has been suggested that environmental factors, such as exposure to stress, are strongly implicated in this higher prevalence of obesity [3].

A stressor is defined as a challenge to the organism that can potentially disrupt homeostasis and, therefore, requires a physiological response. During development, when the plastic capacity is maximal, these adjustments become more important. The childhood and adolescence are critical periods for the maturation of the neural circuits that control energy homeostasis and stress responses [4]. Early life events, such as childhood stress, may have long term effects on behavior and metabolism [5,6]. In these periods, one of the most potent stressors, in both humans and animals, is social isolation [7–9], which can lead to behavioral, anatomical and neurochemical changes that may remain during adulthood [8,10].

Exposure to stress induces a variety of responses, including activation of the sympatho-adrenomedullar system, release of catecholamines, and activation of the hypothalamic–pituitary–adrenal (HPA) axis, culminating in the release of glucocorticoids (GCs) [11]. The

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metabolic effects of the GCs include increased plasma glucose due to gluconeogenesis and glycogen degradation, as well as inhibition of glucose uptake in some tissues, mobilization of amino acids from extrahepatic tissues, stimulation of lipolysis in adipose tissue and increased metabolic rate [12,13]. Animal studies show that stress may both increase and decrease food ingestion depending on the duration and intensity of the stress [14–18]. In human and animal studies, many factors have been implied in these effects related to stress and feeding behavior, including autonomic nervous system activation [12], effects of hormones related to the stress response, such as CRH and glucocorticoids [1,19], leptin [20,21], and/or stress activation of neural systems involved in the cognitive, rewarding, and emotional aspects of ingestive behavior [12]. Stress exposure may increase food intake and insulin levels, facilitating the development of obesity and the metabolic syndrome [22,23]. Conversely, the regulation of the HPA axis will depend on the type of palatable food consumed: Studies using rodents have shown that diets high in calories and sugar reduce this axis response to stress [24], while diets rich in fat enhance stress-induced levels of glucocorticoids [25,26].

Additionally, studies in animals and humans, as well as in tissue cultures, have reported that stress exposure and elevated GCs levels increase the generation of reactive oxygen species (ROS) [27–30]. When there is an imbalance between antioxidant defenses and oxidative species, oxidative stress occurs [31], leading to damage to cell structures like proteins, lipids, membranes and DNA, which have been observed both in humans [32,33] and in rodents [34,35]. Moreover, some studies using animal models have presented evidence that the presence of ROS and excessive intake of fatty foods can lead to breaks in cellular DNA [36–40]. In this context, the consequences of stress exposure in animals with *ad libitum* access to palatable diets require a better understanding.

Since the pre-pubertal period is critical for development, being important to the stress response and for the emergence of eating disorders, the aim of our study is to verify whether stress by social isolation during the pre-puberty period in animals with chronic access to palatable diets until adulthood may alter oxidative stress parameters in the liver, and metabolic profiles such as plasma lipids, plasma glucose and leptin. Serum cholinesterase activity was also measured, since relationships between the activity of this enzyme and hyperlipidemia, diabetes, and obesity have been reported [41].

2. Material and methods

2.1. Experimental subjects

All animal proceedings were performed in strict accordance with the recommendations of the Brazilian Society for Neurosciences (SBNeC) and Brazilian Law on the use of animals (Federal Law 11.794/2008), and were approved by the Institutional Ethical Committee. All efforts were made to minimize animal suffering, as well as to reduce the number of animals used.

Animals were housed in home cages made of Plexiglas (65 × 25 × 15 cm) with the floor covered with sawdust, and were maintained on a standard 12 h dark/light cycle (lights on between 7:00 h and 19:00 h), temperature of 22 ± 2 °C. On postnatal day (PND) 21, sixty-three Wistar rats were weaned. Only male pups were used from each litter, and these pups were divided into six groups, in such a way that only one animal per litter was used in each group. Male pups were weighed at PND 21 and distributed into 3 groups, according to the diet that they received: (1) receiving standard lab chow (44.3% carbohydrate, 22% protein and 4% fat); (2) receiving both chow and a diet with a high content of simple carbohydrate [42] and (3) receiving chow and a high-fat diet (25% carbohydrate, 28% protein and 42% fat). Therefore, animals from these last two groups could choose the diet they consumed from the two diets available. Half of the animals on each diet were housed in groups of 4; the other half were stressed by isolation (one animal in a smaller home cage, 27 × 17 × 12 cm) [43], in such a way that six groups were obtained; controls receiving chow (CC), controls receiving chow and high-carbohydrate diet (HCC), controls receiving chow and high-fat diet (HFC), isolated animals receiving chow (IC), isolated animals receiving chow and high-carbohydrate diet (HCI), and isolated animals receiving chow and high-fat diet (HFI). The isolation stress occurred between postnatal days 21 and 28. On PND 28, isolated animals were returned to regular home cages (65 × 25 × 15 cm) in groups of four. During 40 days, beginning on PND 21, amounts of palatable diets and standard lab chow were offered *ad libitum*. At postnatal day 60, the animals were killed by decapitation and biochemical evaluations were performed.

2.2. Diets

Studies have shown that diets rich in simple carbohydrates or in fat have distinct effects on HPA axis response to stress [24–26]. Therefore, we used a standard chow, a diet rich in sugar and a fat-enriched diet. The nutritional compositions of each diet used are displayed in Tables 1 and 2. The high-carbohydrate diet (HCD) used in this study was enriched in simple carbohydrates, and made with condensed milk, sucrose, vitamins and a salt mix, powder standard lab chow, purified soy protein, soy oil, water, methionine and lysine. The nutritional content of this diet is similar to that of a standard lab chow, however most carbohydrates in the palatable diet were sucrose [42]; in contrast, the standard lab chow contained carbohydrates obtained mainly from starch.

The high-fat diet (HFD) used in the study was enriched with fat (42%) from lard and soy oil. In addition, this diet contained vitamins and a salt mixture, purified soy protein, methionine, lysine and starch [adapted from 44]. This ratio soy oil/lard has a larger amount of saturated and monounsaturated fatty acids, to reproduce the consumption of fat in the western diets, that have higher percentage of these types of fat, such "fast foods". However, we added 1.6% (w:w) of soy oil to provide a minimal amount of n3 fatty acids for an adequate ratio of n6:n3 fats [45].

2.3. Food consumption

Previously weighed quantities of standard lab chow and palatable diets were offered and the remaining amounts of pellets were measured each day to evaluate consumption. The food consumption was measured per cage and the amount of food consumed was divided by the number of animals per cage to determine mean consumption per animal. To verify the amount of kilocalories consumed per animal, we multiplied the amount of food ingested by the caloric content per gram of chow or diets. The standard lab chow has a caloric content of 3.01 kcal/g, whereas the high-carbohydrate diet has a caloric content

Table 1
Nutritional composition/100 g of the food used in the studies performed. HCD: high carbohydrate diet; HFD: high-fat diet.

Diet	Energy (kcal)	Total protein (g)	Total carbohydrate (g)	Total fat (g)
Standard chow[a]	301.2	22	44.3 (from starch)	4 (0.62 from saturated and 3.4 from unsaturated fat)
HCD[b]	346.3	27	40 (13.3 from starch and 26.7 from sucrose and lactose)	8.7 (3.04 from saturated and 5.6 from unsaturated fat)
HFD[c]	588	28	25 (12.5 from starch and 12.5 from sucrose)	42 (16 from saturated and 26 from unsaturated fat)

[a] Nuvilab®.
[b] Souza CG, et al. Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. Life Sci, 2007, 81:198-203.
[c] Adapted from Ziegler DR, et al. A ketogenic diet increases protein phosphorylation in brain slices of rats. J Nutr 2002, 132:483-487.