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Obesity and chronic stress are able to desynchronize the temporal pattern of serum levels of leptin and triglycerides

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ABSTRACT

Disruption of the circadian system can lead to metabolic dysfunction as a response to environmental alterations. This study assessed the effects of the association between obesity and chronic stress on the temporal pattern of serum levels of adipogenic markers and corticosterone in rats. We evaluated weekly weight, delta weight, Lee index, and weight fractions of adipose tissue (mesenteric, MAT; subcutaneous, SAT; and pericardial, PAT) to control for hypercaloric diet-induced obesity model efficacy. Wistar rats were divided into four groups: standard chow (C), hypercaloric diet (HD), stress plus standard chow (S), and stress plus hypercaloric diet (SHD), and analyzed at three time points: ZT0, ZT12, and ZT18. Stressed animals were subjected to chronic stress for 1 h per day, 5 days per week, during 80 days. The chronic exposure to a hypercaloric diet was an effective model for the induction of obesity and metabolic syndrome, increasing delta weight, Lee index, weight fractions of adipose tissue, and triglycerides and leptin levels. We confirmed the presence of a temporal pattern in the release of triglycerides, corticosterone, leptin, and adiponectin in naïve animals. Chronic stress reduced delta weight, MAT weight, and levels of triglycerides, total cholesterol, and leptin. There were interactions between chronic stress and obesity and serum total cholesterol levels, between time points and obesity and adiponectin and corticosterone levels, and between time points and chronic stress and serum leptin levels. In conclusion, both parameters were able to desynchronize the temporal pattern of leptin and triglyceride release, which could contribute to the development of metabolic diseases such as obesity and metabolic syndrome.

1. Introduction

The pathogenesis of chronic diseases such as obesity and metabolic syndrome is related to genetic and environmental factors, including dietary habits and exposure to stress [8]. Worldwide changes in diet composition, alongside a more sedentary way of life, may contribute to the incidence and severity of these diseases [1]. Frequent hypercaloric diet intake has been reported as a determinant of an increase in adiposity [32]. While the causes of obesity are complex, the increasing availability of highly palatable foods rich in fat has played a key role [69]. Feeding behaviors that trigger obesity include frequent consumption of fast food meals; snacking [57]; and intake of sweetened beverages [24] and high-calorie foods, such as high-fat, low-fiber foods [40]. Obesity is characterized by excessive buildup of adipose tissue and has been linked to the development of cardiovascular and metabolic diseases, such as dyslipidemia, hypertension, impaired glucose tolerance, hyperinsulinemia, and type II diabetes mellitus [19].

Modern society is associated with a widespread availability of comfort foods and a high level of perceived stress [14]. The increasing stress of daily life can cause physiological and

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neuroendocrine changes [55], which are associated with an increased food intake and adipogenesis [63]. The stress response is related to activation of the hypothalamic–pituitary–adrenal (HPA) axis, with consequent release of hormones such as corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoids (GCs) [5]. The HPA axis is a key hormonal system that has a well-established temporal pattern. Therefore, HPA axis dysfunction [29,57] and the disruption of circadian rhythms [24] are implicated in the pathogenesis of eating disorders such as obesity [25] and metabolic syndrome [25], which can lead to abdominal obesity, associated with a high waist-to-hip ratio and body mass index (BMI) [68].

Chronic metabolic dysfunction is linked to circadian and metabolic consequences, including altered energy balance [41], elevated BMI [56], altered plasma lipid metabolism and adiposity [34], and an increased risk of cardiovascular disease [60]. Lifestyle changes such as an excessive caloric intake are known to contribute to the growth of obesity and metabolic syndrome, not only in industrialized nations but in developing countries as well [67].

Furthermore, stress is also among the main exogenous regulators of circadian rhythms [2,31]. Under the influence of stress, this pattern is altered and the homeostasis of stress-related neuroendocrine function is disrupted, with an adverse impact on health [2,30]. Alterations in circulating lipid and glucocorticoid levels are also involved in the physiological processes of adipose tissue, including adipogenesis and lipid metabolism [46]. Likewise, chronic activation of the stress axis may be linked to the risk of metabolic disorders and altered energy homeostasis [2,30].

In recent years, white adipose tissue has been recognized as playing a fundamental role in many pathological states including obesity, diabetes, and metabolic syndrome. It is an endocrine organ, producing adipokines such as adiponectin and leptin [75]. These peptide hormones play an important role in regulating energy homeostasis, with adiponectin controlling fat metabolism and insulin sensitivity, and leptin suppressing food intake and stimulating energy expenditure [75]. However, previous studies have demonstrated that diet and feeding time have an impact on secretion rhythms [26]. An interesting issue is that diet composition [35] and feeding time have also been shown to modulate the circadian clock [49]. Furthermore, there is a significant correlation between the circadian clock, development of obesity, and circulating lipid parameters [31]. Similarly, oscillations in glucocorticoid secretion have been observed [35]. Curiously, in obese humans, disruption of 24-h profiles of leptin and adiponectin was observed compared with healthy lean subjects [47]. Thus, adipokines, glucocorticoids, and lipid profile are expressed in a circadian manner in humans [43] and rats [35]. Therefore, it is tempting to speculate that the regulation of adipokines, glucocorticoids, and lipids may play an important role in metabolic homeostasis.

Considering the strong interactions between diet types and stress exposure, and that many physiological activities (such as feeding and energy expenditure) are adjusted to circadian rhythms, the present study was designed to assess the effects of the association between obesity and chronic stress on the temporal pattern of serum levels of adipogenic markers (adiponectin, leptin, triglycerides [TG], total cholesterol [TC]) and corticosterone (to control for the chronic stress model) in rats. Additionally, we evaluated the weekly weight, the delta weight, the Lee index, and the weight of specific adipose tissue fractions (mesenteric, MAT; subcutaneous, SAT; and pericardial, PAT) to assess the efficacy of the hypercaloric diet-induced obesity model.

2. Methods

2.1. Animals

A total of 98 naïve adult male Wistar rats (60 days old; weighing 200–250 g) were used. Rats were randomized by weight and housed in polypropylene home cages (49 cm × 34 cm × 16 cm) with sawdust-covered floors. The animals were kept on a standard 12:12 light–dark cycle (lights on at 0700 h, *Zeitgeber* time [ZT0], and lights out at 1900 h [ZT12]), in a climate-controlled environment (22 ± 2 °C), with water and chow available *ad libitum* (cafeteria diet and/or standard chow). The *Zeitgeber* time was used as a reference to detect the rhythmicity of the variables of interest. All experiments and procedures were approved by the Institutional Animal Care and Use Committee (GPPG-HCPA protocol No. 100383) and were compliant with Brazilian guidelines involving the use of animals in research (Law No. 11,794). The experimental protocol complied with the ethical and methodological standards of the Journal for Laboratory Animal Research [58]. Animal handling and all experiments were performed in accordance with international guidelines for animal welfare and measures were taken to minimize animal pain and discomfort. The experiment used the smallest number of animals necessary to produce reliable scientific data.

2.2. Experimental design

The rats were allowed to acclimate to the study environment for 1 week before the start of the experiment. Animals were then divided into the following four groups: standard chow (C), hypercaloric diet (HD), stress plus standard chow (S), and stress plus hypercaloric diet (SHD), according to chronic stress exposure and the type of diet used (cafeteria diet and/or standard chow). The animals were weighed weekly, and food intake was recorded daily. The experiment was performed during 80 days. The animals were housed in groups of four animals per cage.

2.3. Stress procedure

The animals were subjected to a chronic restraint stress model [20], using a plastic tube (25 cm × 7 cm) fixed with adhesive tape on the outside to avoid discomfort but limiting the movements of the animal; the frontal part of the tube was open to allow breathing [20]. The animals were exposed daily to 1 h of stress in the morning (between 0900 h and 1200 h), 5 days a week for 80 days [20] (no stress on weekends). After the stress procedure, the animals were returned to their home cages. Control animals were kept in their home cages throughout the experimental period. The apparatus was ventilated to avoid physical compression, hyperthermia, and sweating.

2.4. Experimental diets

The Nuvilab CR-1 standard rat chow (NUVITAL®, Curitiba, PR, Brazil) provides a total energy content of 2.93 kcal/g (information provided by the manufacturer), and is composed of 55% carbohydrates, 22% protein, 4.5% lipids, and other constituents (fiber and vitamins). The cafeteria diet (palatable high-calorie diet) consists of approximately 60% carbohydrates, 20% lipids, 15% protein, and 5% other constituents (sodium, calcium, vitamins, preservatives, minerals, *etc.*), providing a total of 4.186 kcal/g and 0.42 kcal/mL (calculated based on information provided by the manufacturer on the package label). The cafeteria diet was chosen because it mimics modern patterns of human food consumption and has been used successfully in experimental studies to induce obesity in lean animals [22,38]. This particular diet was adapted from the