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Obesity and malnutrition similarly alter the renin–angiotensin system and inflammation in mice and human adipose☆,☆☆

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Abstract

The main goal of the present study was to evaluate the metabolic profile, inflammatory markers and the gene expression of the renin–angiotensin system (RAS) components in the visceral adipose tissue of eutrophic, obese and malnourished individuals and mice models of obesity and food restriction. Male Swiss mice were divided into eight groups and fed different levels of food restriction (20%, 40%, or 60%) using standard or high-fat diet. Metabolic profile and adipose tissues were assessed. The expression of AGT (Angiotensinogen), ACE (Angiotensin-converting enzyme), ACE2 (Angiotensin-converting enzyme 2), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in the mice epididymal adipose tissue and the human visceral adipose tissue was assessed. The main findings showed reduced body weight, improved metabolism, decreased adipose tissues weight and reduced adipocyte area in mice submitted to food restriction. Diminished expression of IL-6, TNF-α, AGT, AT1 and ACE was detected in the 20% and 40% food restriction animal groups, although they were increased in the 60% malnourished group. Increased expression of IL-6, TNF-α, AGT and ACE in obese and malnourished individuals was observed. Adipocytes size was increased in obese individuals and reduced in malnutrition. In conclusion, we found that food restriction of 20% and 40% improved the metabolic profile, ameliorated the inflammatory status and down-regulated the RAS in mice. Severe 60% food restriction (malnutrition), however, stimulated a proinflammatory state and increased AGT and ACE expression in the adipose tissue of mice. A similar profile was observed in the adipose tissue of obese and malnourished humans, supporting the critical role of inflammation and RAS as mediators of metabolic disorders.

Keywords: Food restriction; Malnutrition; Obesity; Inflammatory mediators; Renin–angiotensin system

☆ Conflict of interests: The authors have nothing to disclose.

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1. Introduction

The increasing prevalence of obesity [1,2] is a matter of great concern worldwide. Obesity is considered a risk factor for cardiovascular diseases, and it is one of the key features of the metabolic syndrome (MetS) [3]. The main comorbidities of MetS include hypertension, dyslipidemia, stroke, type 2 diabetes mellitus and some types of cancer [4]. Obesity is characterized by the accumulation of body fat resulting from an imbalance between food intake and energy expenditure [3,5]. Recently, obesity has been described as a proinflammatory state associated with elevation of tissue and circulating levels of proinflammatory enzymes, procoagulant factors, cytokines and chemokines, demonstrating that the adipose tissue modulates not only its biology but also the reproductive and endocrine systems, immunity, inflammation and insulin sensitivity [6]. There are evidence suggesting that the white adipose tissue (WAT) becomes hypertrophied due to macrophage infiltration that secretes proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and some interleukins, such as interleukin-6 (IL-6) [7].

A growing number of studies describe the importance of the renin–angiotensin system (RAS) in regulating the metabolism and the development of cardiovascular and inflammatory diseases [8–10]. Various components of the RAS have been identified in the adipose tissue [11]. Recent studies have shown that RAS significantly modulates the metabolism and endocrine function of adipocytes [12]. Further RAS components may be modulated according to different obesity degrees [13].

Caloric restriction, on the other hand, is characterized by a low-caloric diet regimen without causing malnutrition. Experimental studies in rodents and rhesus monkeys showed that partial caloric restriction increases longevity and prevents or delays the occurrence of chronic diseases such as diabetes, atherosclerosis, cardiomyopathy, tumors, autoimmune diseases, and renal and respiratory problems [14–19]. Some evidence have shown that the maximum positive effect occurs with restrictions of 55% to 60% compared to baseline intake [20,21]. Clinical studies further showed that weight loss diminishes the inflammatory status in obesity and subsequent comorbidities by decreasing the number of circulating inflammatory molecules [22]. However, a significant loss in body weight can induce a state of malnutrition. Malnourished individuals may present inflammatory, hypermetabolic and hypercatabolic conditions, in addition to reduced albumin levels [23–26].

We, therefore, hypothesize that different levels of food restriction, associated or not with a high-fat diet, may differently modulate adipose tissue inflammatory state and RAS regulation, preventing metabolic alterations as observed in obesity. Thus, the purpose of the present study was to evaluate the metabolic profile and expression of inflammatory markers and RAS components in adipose tissue of mice submitted to different food restriction degrees and to validate such profile in the visceral adipose tissue of eutrophic, obese and malnourished humans.

2. Methods

2.1. Animal study

The experiment was conducted with 64 Swiss mice (male, 4 weeks old) divided into 8 groups (n=8 each) and fed with the following respective experimental diets for 8 weeks. The groups were divided into the following: standard diet (ST) *ad libitum*, ST-20% food restriction, ST-40% food restriction, ST-60% food restriction, high-fat diet (HFD) *ad libitum*, HFD-20% food restriction, HFD-40% food restriction and HFD-60% food restriction. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (CEEBEA - Universidade Estadual de Montes Claros). The animals were maintained under controlled light and temperature conditions.

2.2. Food restriction protocol and diet

The mice groups fed with standard and high-fat diet given *ad libitum* had their food intake measured on a daily basis. From the food intake of these groups, the food restriction of 20%, 40% and 60% was then calculated. Standard diet (Purina; Labina) used for regular mice maintenance is composed of 66% carbohydrate, 23% protein and 11% fat, representing a total of 3.95 kcal per 1 g of diet. The high-fat diet was composed of cornstarch (40.57%), casein (14%), dextrinized starch (15.5%), sucrose (10%), soybean oil (10%), cellulose fiber (5%), mineral mix AIN-93M (3.5%), vitamin mix AIN-93 (1%), L-cysteine (0.18%), choline bitartrate (0.25%) and *tert*-butylhydroquinone (0.0008%), (24% of carbohydrate, 15% of protein, and 61% of fat), representing a total of 5.28 kcal per 1 g of diet [27]. The high-fat diet was prepared according to the standards of the Official Analytical Chemists Association as described previously [28,29]. All of the high-fat diet components were purchased from Rhoster LTDA (São Paulo, SP, Brazil).

2.3. Glucose tolerance and insulin sensitivity tests (GTT and IST)

For the GTT, D-glucose (2 mg/g of body weight) was intraperitoneally injected into overnight-fasted mice. Glucose levels from tail blood samples were monitored at 0, 15, 30, 60 and 120 min after injection. ISTs were performed with the animals in the fed state after intraperitoneal injection of insulin (0.75 U/kg body weight), where tail's blood samples were taken at the time points 0, 15, 30 and 60 min after injection for the measurement of blood glucose levels.

2.4. Measurements of body weight, food intake and tissue collection

Food intake and body weight in mice were measured every day during the treatment period. Overnight-fasted mice were killed by decapitation and blood, and WAT (epididymal, retroperitoneal and mesenteric) samples were collected, weighed and frozen in dry ice and stored at −80°C for posterior analysis.

2.5. Human study

Samples of the visceral adipose tissue were collected from patients divided into the following groups: control group (eutrophic patients group), obese patients group and malnourished patients group. The control group was composed of adult patients, clinically healthy, who submitted to abdominal surgical procedures due to esthetic reasons. The obese patients were selected during the screening procedure for bariatric surgery. The bariatric procedures consisted of Roux-en-Y gastric bypass, and the biopsies of the WAT were performed during surgery. Malnourished patients (being excluded patients with cancer in this study) composed the third group. All malnourished patients were categorized using previous criteria. The biopsies were obtained during gastrointestinal surgery. During the surgeries, samples of visceral WAT and serum were collected, immediately frozen and stored at −80°C. Additional information of the individuals is presented in Table 1. All procedures performed in studies involving human participants were in agreement with the ethical standards of the institutional committee (Plataforma Brazil, 85742/13-07-2012) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.