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Aging affects the response of female rats to a hypercaloric diet

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ABSTRACT

Metabolic syndrome is a major risk factor for the development of cardiovascular diseases and diabetes, among other conditions. Studies have shown that aging and metabolic syndrome share several metabolic alterations, and that aged individuals, in particular females, are at an increased risk of developing metabolic disorders. Although several studies have investigated the effects of hypercaloric diets in the development of obesity and metabolic syndrome in young animals, few studies have investigated these parameters in aged animals, especially in females. Therefore, the aim of this study was to investigate the effects of a hypercaloric diet in metabolic parameters of young and aged female rats, including its effects on lipid and glycemic profile and on liver lipid content. When compared to young animals, the aged rats presented increased serum levels of triglycerides and decreased serum levels of HDL cholesterol and glycemia, as well as increased hepatic levels of triglycerides and total cholesterol. The hypercaloric diet increased food intake, body weight gain and adiposity index, leading both young and aged animals to a dyslipidemia, represented by increased serum levels of triglycerides. The hypercaloric diet increased the glycemia and the HOMA index only in the young animals. On the other hand, the diet increased the frequency of hepatocellular microvacuolar degeneration only in the aged animals. In summary, it was observed that the females from different ages respond differently to hypercaloric diet intake: while the aged animals were more resistant to the changes in the glycemic profile, they were more susceptible to the hepatic damage caused by this diet.

1. Introduction

Obesity is a multifactorial disease characterized by an excessive accumulation of body fat that emerged as a leading health concern over the past century, being a result of the increased consumption of high calorie foods associated with sedentarism (Heymsfield and Wadden, 2017). Obesity is one of the major risk factors for the development of noncommunicable diseases (NCD), which are currently the major cause of death in the world. Currently, more than two thirds of the USA population and > 50% of the Brazilian population are overweight or obese (Ministério da Saúde – Brasil, 2016; Heymsfield and Wadden, 2017), being at an increased risk of developing NCD. In Brazil, In most of the countries, the prevalence of obesity is higher in women than in men (Finucane et al., 2011).

Metabolic syndrome is a major risk factor for the development of NCD, being characterized by a group of changes including large waistline, high levels of triglycerides, low levels of HDL cholesterol, high blood pressure and hyperglycemia, that increase the risk of cardiovascular diseases and diabetes, among other diseases (Onat, 2011). Several studies have associated obesity with metabolic syndrome, suggesting that the increased visceral and ectopic fat deposition exerts a key role in the development of metabolic syndrome and insulin resistance (Almeda-Valdes et al., 2017; Armani et al., 2017).

Studies have shown that aging and metabolic syndrome share several features, including increased abdominal adiposity, hypertension, dyslipidemia and hyperglycemia, and that aged individuals are at an increased risk of developing metabolic disorders (Dominguez and Barbagallo, 2016; Armani et al., 2017; Roos et al., 2017). In this context, it is known that the prevalence of obesity and dyslipidemia is higher in aged women due to the declining levels of estrogens and other

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hormones during menopause (Lizcano and Guzmán, 2014). Although several studies have investigated the effects of hypercaloric diets in the development of obesity and metabolic syndrome in young animals (Akiyama et al., 1996; Hoefel et al., 2011; Goularte et al., 2012; Cecconello et al., 2015), few studies have investigated the differences between the metabolic parameters (e.g. lipid and glycemic profile) of young and aged animals, especially females, after the exposure to hypercaloric diets. Therefore, the aim of this study was to investigate the effects of a hypercaloric diet in metabolic parameters of young and aged female rats, including its effects on lipid and glycemic profile and on liver lipid content.

2. Material and methods

2.1. Animals

Female Wistar rats of 2 months (young) or 15 months (aged) in the beginning of the experiment, born and reared in the Center for Laboratory Animal Reproduction and Research (CREAL) at Universidade Federal do Rio Grande do Sul (UFRGS), Brazil, were used in these experiments. After rearing, they were housed in polypropylene cages (40 cm × 33 cm × 17 cm), 4 per cage, under standard environmental conditions (room temperature of 22 ± 2 °C and a 12 h (light) – 12 h (dark) cycle from 0600 to 1800 h). All rats had free access to food and water. Our experimental protocol was carried out in accordance with the National Institute of Health *Guide for the Care and Use of Laboratory Animals*, following approval by the Ethical Commission for the Use of Animals (CEUA) of UFRGS. All possible efforts were made to minimize the animal suffering and to reduce the number of animals used to obtain reliable scientific data.

2.2. Diet

Young and aged female rats were divided in 4 experimental groups (N = 11–16/group) and were fed with a control diet, represented by a standard rat chow (2,95 kcal/g – 55% carbohydrate, 22% protein, 4,5% lipid) (NUVILAB, Curitiba, Brazil), or with a hypercaloric pelletized diet produced in our laboratory (3,44 kcal/g – 68,9% carbohydrate, 18,4% protein, 5,1% lipid) (for more details, please check Zanini et al., 2017) for 21 weeks. Rats had free access to food and water, and the animals fed with the hypercaloric diet also had access to the control diet.

2.3. Food intake, body weight gain and adiposity index

The chow was weighted before being offered to the animals and, 24 h after, the remaining chow was weighted for the determination of the food intake. The animals were weighted in the first and in the last day of the experiment, as well as each 2 weeks during the experiment, for the evaluation of the body weight gain during this period. Moreover, the adiposity index was calculated by dividing visceral fat weight by body weight.

2.4. Estrous cycle, euthanasia and tissue collection

Nineteen weeks after the beginning of the diet, vaginal smears of female rats were analyzed daily for the control of the estrous cycle. After 21 weeks since the beginning of the diet, the animals were killed by decapitation during the diestrous phase of the estrous cycle, and the trunk blood, visceral adipose tissue and liver were collected for further analysis.

2.5. Serum dosages

Serum levels of glucose, total cholesterol, HDL cholesterol and triglycerides were evaluated by the use of enzymatic kits (Labtest, Lagoa Santa, Brazil) following the manufacturer's instructions. Moreover, serum levels of insulin were determined by ELISA (Abcam, Cambridge, MA, USA), also following the instructions of the manufacturer. The homeostasis model assessment (HOMA), an index of insulin resistance, was calculated through the following equation: HOMA = glucose (mM) × insulin (lUI/mL) / 22.5.

2.6. Liver lipid content

For the evaluation of the liver lipid content, liver samples were homogenized in saline solution (20:1 proportion) and the levels of triglycerides and HDL cholesterol were evaluated by the use of enzymatic kits (Labtest, Lagoa Santa, Brazil). For the determination of HDL cholesterol, it was added 200 µl of trichloroacetic acid (50%). Then, after a centrifugation (2000 rpm for 10 min), the precipitated was left drying in the exhaust hood for a day. Then, it was added 800 µl of chloroform/methanol (2:1) and the supernatant was collected and put in a glass bottle for drying for 3 days. Later, it was added 500 µl of isopropanol and the samples were mixed in a vortex and used for the determination of HDL cholesterol by an enzymatic kit as previous mentioned.

2.7. Liver histopathological analysis

For the histopathological analysis, liver samples were fixed in 10% buffered formalin, for 48 h, then dehydrated with ethanol xylene mixtures, and embedded with paraffin wax. Sections (3 µm) were stained with hematoxylin and eosin and the histological evaluation was performed by optical microscopy with multi-observer microscopy.

2.8. Statistical analysis

Statistical analysis was carried out using the software GraphPad Prism 5.0 (La Jolla, CA, USA). The data from the histopathological analysis were analyzed by simple frequency (percentage of animals with the presence of hepatocellular microvacuolar degeneration in relation to the total number of animals in the group). All the other data were analyzed by a Two-way Analysis of Variance (Two-way ANOVA) followed by the test of Tukey-Kramer when appropriated. All the results are presented as mean ± standard error mean. The level for statistical significance was set at $P < 0.05$.

3. Results

3.1. Hypercaloric diet increased food intake, body weight gain and adiposity index

The animals exposed to the hypercaloric diet presented a higher food intake in grams ($F_{diet(3,51)} = 31.82$, $P < 0.0001$) and in calories ($F_{diet(3,51)} = 181.5$, $P < 0.0001$), independent of age, therefore leading to an increased weight gain in comparison to the control groups ($F_{diet(3,51)} = 25.90$, $P < 0.0001$) (Fig. 1A, B and C). Although the hypercaloric diet was able to increase the weight gain in both young and old animals, these values were higher for the young animals ($F_{age(3,51)} = 37.44$, $P < 0.0001$). Moreover, it was observed an increased adiposity index in the animals that received the hypercaloric diet in relation to those that were fed with the standard diet ($F_{diet(3,51)} = 29.29$, $P < 0.0001$) (Fig. 1D).

3.2. Diet and aging effects in the glycemic and lipid profile of female rats

Aging was associated with decreased serum levels of glucose ($F_{age(3,31)} = 4.791$, $P = 0.0363$), while the hypercaloric diet increased the glycemia of young animals only ($F_{int(3,31)} = 4.838$, $P = 0.035$) (Fig. 2A). No differences were found in the serum levels of insulin (Fig. 2B). Regarding HOMA index, it was found an interaction showing that young animals exposed to the hypercaloric diet presented a higher index in comparison to all other groups ($F_{int(3,27)} = 5.863$, $P = 0.0225$)