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EFFECT OF HYPERLIPIDEMIC DIET ON WISTAR RATS HYPERLIPIDEMIC DIET ON WISTAR RATS

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ABSTRACT

Obesity is defined as a chronic inflammatory disease characterized by accumulation of fat in the body. The increase in body weight through the consumption of saturated fats can trigger the onset of chronic non-communicable diseases, metabolic syndrome and insulin resistance. Thus, this study aimed to evaluate the feeding behavior and the physiological changes in fatty tissues of rats after hyperlipidemic diet. 8 Wistar rats were divided into two groups: Control Group, with a standard diet and Intervention Group, with a hyperlipidemic diet. During 16 weeks of treatment, the food intake and weight gain of the animals were evaluated. After this period, the white retroperitoneal, epididymal, mesenteric, perirenal and omental adipose tissues were weighed for posterior quantitative analysis. Regarding food intake, the Control Group showed significantly greater difference when compared to the Intervention Group. However, there was no statistical difference in the average weight gain of each group during the study. In the Intervention Group, the hyperlipidemic diet promoted increase in the retroperitoneal, epididymal, mesenteric and perirenal adipose tissues. There was no difference in relation to the omental adipose tissue. It was concluded that the hyperlipidemic diet was able to induce obesity and may lead to the onset of chronic non-communicable diseases.

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INTRODUCTION

Obesity is a chronic inflammatory disease with a major impact on global public health, characterized by excessive accumulation of adipose tissue in the body, a fact attributed to an imbalance in energy intake and expenditure over a prolonged period (White *et al.,* 2013). The etiological mechanisms of this disease are related to multifactorial causes of genetic, environmental, social, economic, cultural, nutritional, metabolic, psychological, neurological and endocrine backgrounds (Gonnelli *et al.,* 2014).

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It is also added that this disease is considered an epidemic, since it affects about 52.5 % of the Brazilian population, with an increase of 10% in the last decade (Brasil, 2014). Increased body weight may trigger diseases such as type II diabetes mellitus, systemic arterial hypertension (Gonzalez *et al.,* 2010), cardiovascular diseases and some types of neoplasias (Gonnelli *et al.,* 2014). Since the adipose tissue is a dynamic organ, the distribution of fat in the deposits, such as visceral, omental, epididymal fat, may be more important than the total adipose tissue for the risk of developing diseases associated with obesity. Consequently, the accumulation of saturated fat in peripheral tissues reflects the development of the metabolic syndrome (Bjorndal *et al.,* 2011; Hermsdorff and Monteiro, 2004). These phenomena are explained by the consumption of fatty foods, such as junk food and industrialized food, in addition to the sedentary lifestyle (França *et al.,* 2012). This type of feeding is highly energetic, highly palatable and when consumed for an extended period of time, results in obesity in humans and experimental animals (Cabeço *et al.,* 2010). The trial with animal experimentation is of great importance in research aimed at combating CNCDs and in the development of therapeutic innovations in the public health scenario, given the metabolic similarity with humans, as well as the easy control and monitoring of food intake (Fundação Oswaldo Cruz, 2006). In view of the above, the present work had as objective to evaluate the feeding behavior and the physiological changes in fatty tissues resulting from the consumption of hyperlipidemic diet inducing obesity in Wistar rats.

MATERIALS AND METHODS

It is a research of laboratory experimentation, of exploratory character. For the development of this work, eight male rats of the species *Rattus novergicus*, variety *albinus*, of the Wistar line, aged 60 days and with an initial average weight ranging from 200 to 240 grams were obtained from the Bioterium of the Universidade Católica Dom Bosco (UCDB), and kept in the Laboratory of Experimental Nutrition. The animals were randomly divided into 2 groups with 4 animals each, the first being: Control Group (CG: standard diet recommended by AIN93 - American Institute of Nutrition) and the second: Intervention Group (IG: hyperlipidemic diet). Both experimental diets were started simultaneously. For 16 weeks, the animals were kept in individual cages with drinking water supply, diet (standard or hyperlipidemic) *ad libitum*, photoperiod of 12 hours light and 12 hours dark and temperature maintained at 22 ± 2 °C with humidity of 55 ± 10 %. The animals were subjected to immobilization and/or containment in case of drug application and during the experimental stages. For the extraction of biological materials (omental, epididymal, retroperitoneal, perirenal and mesenteric fats), intraperitoneal (ip) administration was used, with application in the lower quadrant of the abdomen of the animal, using ketamine (40 to 80 mg/kg) and xylazine (5-10 mg/kg). The experiment was carried out in accordance with the Brazilian legislation on the scientific use of animals (Law No. 11.794, of October 8, 2008). The protocol was approved by the Committee on Ethics in the Use of Animals (CEUA) of the Bioterium of the Universidade Católica Dom Bosco - MS, protocol No. 001/2015.

Composition of the Standard Diet

The CG animals were maintained with standard feed (Probiotério MP 77 Moinho Primos S.A., São Paulo - SP, Brazil), a chemical composition balanced in macronutrients, containing 32.16 % proteins, 58.42 % carbohydrates, 9.40 % lipids and 10.00 % fibers, mix of minerals and vitamins, with 2,869.50 kcal/kg.

Composition of the Hyperlipidemic Diet

The IG animals were maintained with purified unbalanced diet (PregSoluções Biociências, Jaú - SP, Brazil), 6.73 % with soybean oil and lard (50.47 %), with caloric distribution of 10.47 % proteins, 31.36 % carbohydrates and 57.20 % lipids and 5,350.00 kcal/kg.

Food Intake Evaluation: To determine the food intake, the diets offered were added to the feeders three times a week, using the Rest-Intake index (Luca *et al.,* 1996).

Weight Control Evaluation: The weight control of the animals was performed once a week, in a semi-analytical balance, and expressed in grams (g). The weight gain was evaluated weekly by means of the difference between the weighings.

Food Efficiency: Food efficiency evaluates the animal's ability to convert the energy consumed in body weight, being given through the food efficiency coefficient (FEC) and the coefficient of weight gain by calorie consumption (CWGCC). The FEC is the relationship between the weight gain per quantity of food eaten. FEC = (FW-IW) / TF, where: FW: final weight (g) of the animal during the follow-up period, IW: body weight of the animal at the beginning of the experiment, in grams, and TF: total amount of food ingested in the period, in grams. Coefficient of weight gain per calorie consumption: CWGCC = (FW-IW) / kcal ingested; where FW: final weight (g) of the animal during the follow-up period, IW: body weight of the animal at the beginning of the experiment, in grams, and Kcal: caloric value of the diet ingested (Campbell, 1963).

Statistical Analysis

All measurements were performed in triplicates, and the data obtained were analyzed with the GraphPad Prism 5.01 program (Graph Pad Software, Inc., USA), and compared by analysis of mean and standard deviation using the independent t-student test with significance level of 5 %.

RESULTS

It was observed during the experimental period that the food intake of the animals that received standard diet (CG) was significantly higher when compared to those who received a hyperlipidemic diet (IG) ($p<0.0001$) (Figure 1a). On the other hand, the average weight gain did not present a significant difference ($p>0.05$) (Figure 1b). Regarding the measurement of the visceral fat regions, there was a significantly higher difference in the animals that had hyperlipidemic diet when compared to the CG (Figure 1c), a fact demonstrated by the mesenteric ($p= 0.03$), retroperitoneal ($p= 0.003$), epididymal ($p= 0.0003$) and perirenal ($p= 0.0007$) fats of the IG animals (Figure 1d).

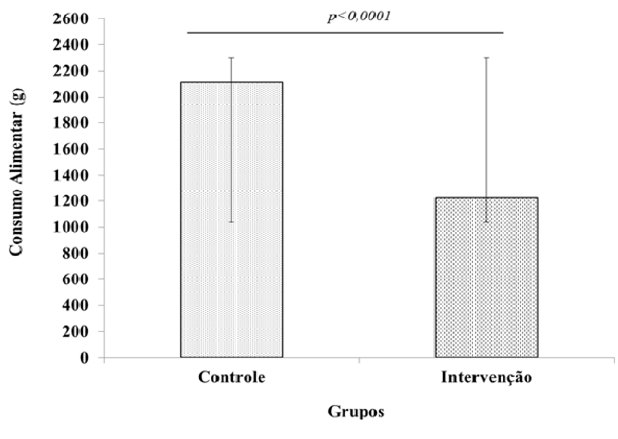


Figure 1. Mean ± standard deviation of the food intake (g) of animals fed with standard diet (control) and hyperlipidemic diet (intervention). *Significant difference by independent t-student test

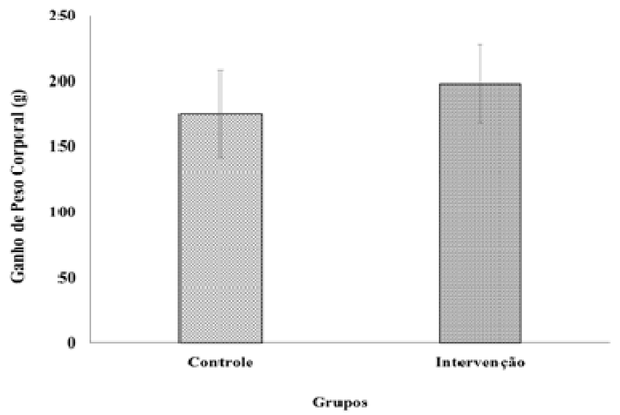
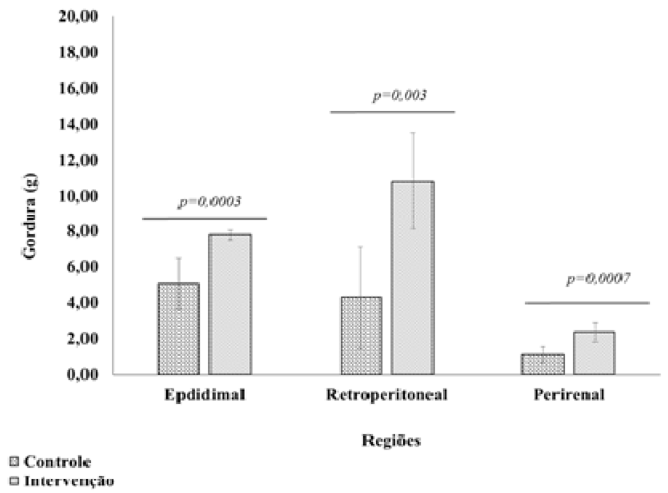


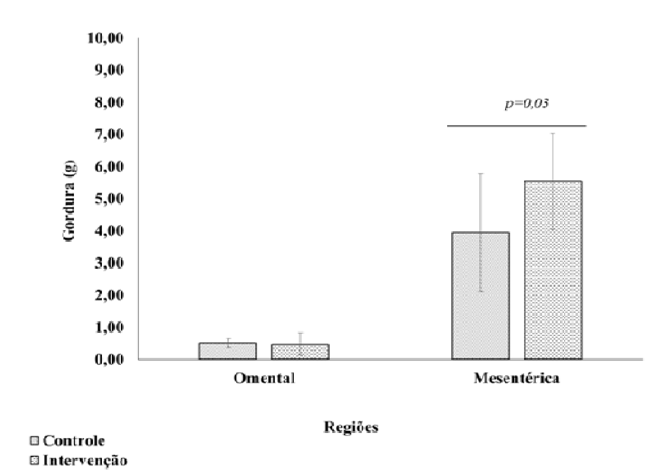
Figure 2. Mean ± standard deviation of the body weight gain (g) of animals fed with standard diet (control) and hyperlipidemic diet (intervention). Independent t-student test

The evaluation of the biological value of the diets, presented by the calculations of the food efficiency coefficient (FEC) and the coefficient of weight gain per calorie consumption (CWGCC), showed that the hyperlipidemic diet was efficient in promoting weight gain in the animals when compared with the standard diet (FEC: 0.16 and 0.084, CWGCC: 0.031 and 0.029, respectively).



*Significant difference by independent t-student test.

Figure 3. Mean ± standard deviation of the epididymal, retroperitoneal, perirenal fat regions (g) of animals fed with standard diet (control) and hyperlipidemic diet (intervention)



*p-value in the independent t-student test.

Figure 4. Mean ± standard deviation of the omental and mesenteric fat regions (g) of animals fed with standard diet (control) and hyperlipidemic diet (intervention)

DISCUSSION

The animal model has been used for the progress of research on the nutritional imbalance of hyperlipidemic/hyperenergetic diets in the exogenous development of obesity (Lladó *et al.,* 2002). The reduction of food intake in the group of animals treated with hyperlipidemyc diet, evidenced in the present study, corroborates the findings of Campanella *et al*. (2014) and Santos *et al*. (2010), who observed a reduction from 30.86 g to 19.96 g over the course of four weeks; and from 29.27 g to 22.72 g, respectively. A fact attributed to the increase in satiety with lower feed efficiency and higher metabolic efficiency, due to the high concentrations of glucose and triglycerides (Bernardes *et al.,* 2004). This observation becomes relevant because, in this study, the reduction of food intake in the hyperlipidemic diet group may be related to the energetic increase (5,350.00 kcal/kg), when compared to the standard diet (2,869.50 kcal/kg). In this research, there was no statistical difference regarding the weight gain of the animals. In contrast, Panveloski-Costa *et al*. (2011) observed an increase in the body weight of sedentary obese rats submitted to hyperlipidemic diet, when compared to the standard diet group. It is known that fat as an energy substrate offers 9 kcal/g of energy, therefore it provides a positive balance and, consequently, accumulation in the form of triglycerides. Thus, when not mobilized to meet the need of the body, fat can be associated with physical inactivity, leading to weight gain and body fat (Seth *et al.,* 2012; Moura *et al.,* 2012). Hypercaloric and hyperlipidemic diets allow the accumulation of adipose tissue in the abdominal region (Shadid, Koutsari and Jensen, 2007) and, as shown in the present study for the IG, higher fat levels in the retroperitoneal, epididymal, perirenal and mesenteric regions. These results confirm that the hyperlipidemic diet was efficient in developing obesity in Wistar rats, in the same way as in the study by Panveloski-Costa *et al*. (2011).

Estrela *et al.* (2015) found in his study with Wistar rats fed with cafeteria and standard diet, at the 8th week of experimentation, results similar to those of this study. Therefore, these data indicate that the hyperlipidemic diet offered was efficient in inducing obesity in the animals. However, there was no significant difference between the analyzed groups regarding omental fat; once this tissue is in low quantity, it may potentiate the inflammatory process in obesity. According to Giacchetti *et al*. (2002), this tissue secretes higher concentrations of adipokines linked to pro-inflammatory processes such as resistin, angiotensin I, PAI-1 (Plasminogen activator inhibitor-1), CRP (C-Reactive Protein), IL-6 (interleukin 6). The fats analyzed in the present study are classified as white fat and are considered as an active endocrine organ and therefore release anorexigenic hormones such as adipokines, leptin, adiponectin and are related to the inflammatory process (Coelho *et al.,* 2016). In this case, emphasis is given to one of the functions of leptin that is related to reduction of food intake and increased energy expenditure due to action on α-MHC (Myosin heavy chain) neuropeptide cells located in the arcuate nucleus of the hypothalamus, in the central nervous system, their structure being similar to that of cytokines, such as interleukin-2 (Ottaviani, Malagoni and Franceschi, 2011). One of the first studies on the relationship between obesity and chronic inflammation was described in 1993, after observing the elevation in the expression of pro-inflammatory cytokines such as interleukin-1b (IL-1b), and tumor necrosis factor in the adipose tissue of obese mice (Hotamisligil, Shargill and Spiegelman, 1993). It is known that some fatty deposits are more linked to risk factors for disease than others; in particular, deposits of visceral, omental and mesenteric fat represent a risk for the development of cardiovascular disease and type II diabetes mellitus (Phillips, Jing and Heymsfield, 2003). In general, visceral omental adipose tissue is the most active, i.e., more sensitive to lipolysis Arner (2003), and the exacerbated increase in lipolysis results in high plasma concentrations of non-esterified fatty acids, resulting in peripheral insulin resistance (Cheik *et al.,* 2006). Several studies have shown that the intake of a hypercaloric diet is associated with insulin resistance in animals (Lessard *et al.,* 2007), being associated with reduced GLUT4 glucose transporter expression and/or impairment in the insulin signaling pathway in the skeletal muscle and adipose tissue (Valerio *et al.,* 2006).

According to Hermsdorff *et al*. (2004), the omental and mesenteric tissues are of greater interest as to the adipose deposition in the abdomen, the mesenteric tissue being the fat more deeply inserted around the intestine. This varies according to the age, nutrition, sex and energy homeostasis of individual adipose tissues. In the present study, it was observed that the IG presented larger epididymal fat when compared to the CG, corroborating the findings of Chen and Farese (2002), who observed during a 16-week study that the animals fed with a diet of 16% lipids presented epididymal adipocyte area 2.5 times greater than that of the CG. Regarding retroperitoneal fat, Shadid *et al*. (2007) observed that the group that received hyperlipidemic diet had a higher value of this fat in relation to the group that consumed a standard diet, as well as an increase in omental fat. In the study by Wang *et al*. (2012), when analyzing rats for 8 weeks, they observed that consumption of hyperlipidemic diet increased perirenal fat and adiponectin levels. In addition, this type of fat plays an important role in the pathogenesis of systemic arterial hypertension (SAH), since this fat accumulates and encompasses the kidneys, especially in obese individuals, a condition that can impair renal function.

Conclusion

The intake of hyperlipidemic/hyperenergetic diet in adult rats increased body mass, although the IG had a lower dietary intake than the CG, which demonstrates the efficiency of the hyperlipidemic diet. There was weight gain in the CG and IG, and consequently increase of the epididymal, retroperitoneal, perirenal, mesenteric fats, however, there was no expressive increase of the omental fat; notwithstanding, the latter suffers stimulation of the lipolysis and contributes to the increase in the liberation of free fatty acids, and the presence of this fat in the abdominal region is one of the characteristics of CNCDs.

Abbreviation

CNCD: chronic non-communicable diseases; GC: Control Group; IG: Intervention Group; Universidade Católica Dom Bosco (UCDB); AIN93: American Institute of Nutrition; ip: intraperitoneal; CEUA: Committee on Ethics in the Use of Animals; SP: São Paulo; g: Grams; FEC: food efficiency coefficient; CWGCC: calorie consumption; FW: final weight; IW: body weight of the animal at the beginning of the experiment; TF: total amount of food ingested in the period; USA: United States of America; PAI-1: Plasminogen activator inhibitor-1; CRP: C-Reactive Protein; IL-6: interleukin 6; α-MHC: Myosin heavy chain; IL-1b: interleukin-1b; GLUT4: glucose transporter expression 4; SAH: systemic arterial hypertension;

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