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EFFECT OF DIFFERENT HYPERCALORIC DIETS ON THE BIOCHEMICAL AND ANTHROPOMETRIC PARAMETERS OF WISTAR RATS

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ABSTRACT

Increase in the body weight, blood glucose, and abdominal fat, as well as dyslipidemias, are considered risk factors for various diseases, especially cardiovascular diseases. Thus, there is a growing search for new compounds that improve the parameters described above. In this sense, animal models that induce changes in lipid and glycemic profiles are necessary. The present study evaluated the effect of three diets on biochemical and anthropometric parameters of male Wistar rats according to G1 - treated with commercial rat food (Nuvilab®) and water *ad libitum*; G2 - treated with hypercaloric beverage and commercial rat food *ad libitum*; G3 - treated with hypercaloric rat food and water *ad libitum*; G4 - treated with hypercaloric rat food and drink *ad libitum*; G5 - treated with water and hypercaloric rat food supplemented with bacon *ad libitum*, and G6 - treated with hypercaloric drink and hypercaloric rat food added with bacon *ad libitum*. The diet in which condensed milk was mixed with water promoted an increase in glycaemia and triglycerides. In turn, the diet in which condensed milk has been integrated into the beverage and vegetable and animal fat in the diet promoted an increase in total cholesterol. No changes were observed in the weight gain and in the cholesterol fractions with the diets tested.

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INTRODUCTION

The incidence of cardiovascular and metabolic diseases in the world population has been increasing, and its higher prevalence in obese individuals has attracted the attention of health professionals and researchers. Changes in lifestyle, such as sedentarism and diet, cause chronic non-communicable diseases such as diabetes, metabolic syndrome, obesity and cardiovascular diseases, and this last one is among the leading causes of death in the modern world. In this context, hypertension, dyslipidemia, obesity and increased visceral fat contribute directly to the development of these diseases (Assunção *et al.*, 2017; Visser *et al.*, 2017; Anderson *et al.*., 2011). Obesity is a metabolic disease, characterized by excessive weight gain, increased adipose tissue, changes in carbohydrate and lipid metabolism, and the appearance of insulin resistance and autonomic dysfunction (Li t al. 2017; Stolarczyk, 2017). The number of overweight adult individuals has been increasing worldwide and in 2008 represented one and a half billion people, of whom over 30% were considered obese. At least 2.8 million people die each year in the world as result of overweight or obesity. The study of the mechanisms by which obesity induces physiological changes may be facilitated by the use of animal models.

There are different animal models, usually rodents, that develop obesity from genetic mutations. However, considering that the model should be as close as possible to the genesis of obesity in humans, the induction of this condition through consumption of highly palatable and high-energy foods seems to be the most appropriate (Cox, Church, 2011; McLaughlin *et al.*, 2017; Krishna *et al.*, 2017; WHO, 2017; Umer *et al.*, 2017; Rosini *et al.*., 2012). In this sense, the standardization of an animal model for the induction of dyslipidemias and obesity allows evaluating the efficacy of new drugs and natural compounds in the treatment of cardiovascular disorders. Thus, the present project had the objective of evaluating diets with the purpose of inducing alterations in the lipid, glycemic and obesity parameters in male Wistar rats.

METHODS

The study was approved by the Committee on Ethics in Animal Use of the University of Marília - CEUA / UNIMAR – Marília City– São Paulo State – Brazil

Animals

Forty-eight male Wistar rats, weighing approximately 180-220 g were obtained from the Center for Experimentation in Animal Models of the University of Marília – UNIMAR, Marília – São Paulo – Brazil. Animals were maintained on a 12-h light / dark cycle, temperature 22 ± 2°C and relative humidity of the air of 60 ± 5% until the end of the experimental protocol.

Preparation of the hypercaloric drink

The hyper-caloric drink was prepared by mixing 395 g of condensed milk in 500mL of water. According to the manufacturer, 20 g of the condensed milk contains 11 g of carbohydrates, 1.4 g of proteins and 1.8 g of lipids, totaling a caloric content of 85 grams. This product is popular in Brazil as a component of candies, candies and several other products.

Preparation of the hypercaloric rat food

The hypercaloric rat food was prepared from commercial rat food, previously ground, and then supplemented with lipids (20% of hydrogenated vegetable fat and 5% animal fat). The blend was modeled so that the final format was similar to the commercial rat food. After this pelletization, it was submitted to drying for 6 hours at 65ºC in a greenhouse with air circulation. Then the rad food was kept under refrigeration (5°C). In two experimental groups, the hypercaloric ration was added with *bacon*.

Experimental protocol

Animals were randomly divided into six groups (n = 8) and then submitted to a period of seven days for acclimatization in which they received water and commercial feed *ad libitum* After that, the animals were treated for 60 days according to:

G1 - treated with commercial rat food (Nuvilab®) and water *ad libitum*;
G2 - treated with hypercaloric beverage and commercial rat food *ad libitum*;
G3 - treated with hypercaloric rat food and water *ad libitum*;
G4 - treated with hypercaloric rat food and drink *ad libitum*;
G5 - treated with water, hypercaloric rat food and bacon *ad libitum*;
G6 - treated with hypercaloric drink, hypercaloric rat food and with bacon *ad libitum*.

The weight of each animal and the consumption (average per box) of water and feed was evaluated weekly. The percentage and specific rate of weight gain and Lee index were calculated. After 60 days of treatment, the animals were anesthetized with an overdose of thiopental (200mg/kg). Immediately after death, blood samples from the inferior vena cava were collected for evaluation of the blood glucose, triglycerides, total cholesterol, and fractions. Abdominal adipose tissue was also removed and weighted.

Statistical analysis

The results were expressed as mean ± standard error of the mean and submitted to the analysis of variance complemented with the Dunnet test, using the program GraphPad Prism 5. The results with values of $p < 0.05$ were considered significant.

RESULTS

Figure 1 shows the effect of the different types of diet on the percentage of weight gain of the animals. There was no statistically significant change in the weight gain in the animals treated with hypercaloric diet.

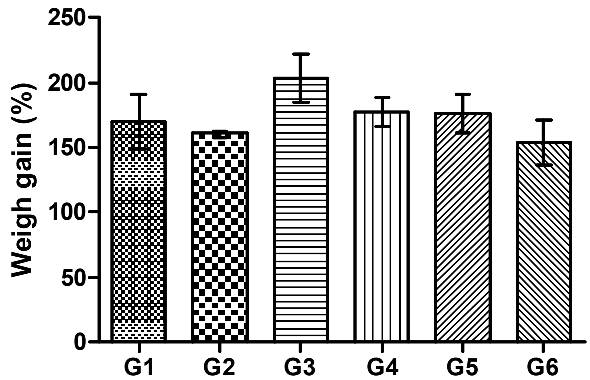

Figure 1. Effects of the different types of diet on the weight gain of the animals from G1-G6

Figure 2 shows the effect of different types of diet on visceral fat. G4 (hypercaloric drink and ration) and G6 (hypercaloric drink and ration supplemented with bacon) had an increase in visceral fat.

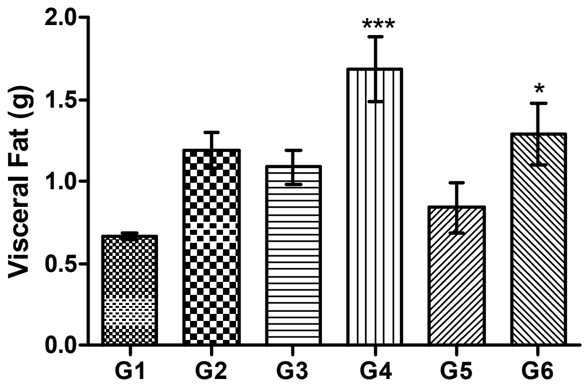

Figure 2. Effects of the different types of diet on the visceral fat of the animals from G1-G6

Figure 3 shows the effect of the different types of diets on the glycaemia of male Wistar rats. The consumption of hypercaloric drink (G2) significantly increased the glycaemia of the animals.

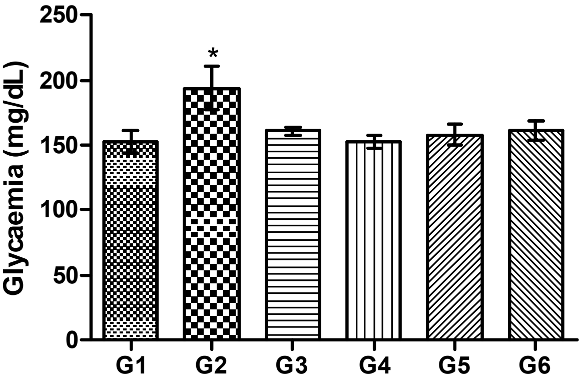

Figure 3. Effects of the different types of diet on the glycaemia of the animals from G1-G6

Figure 4 shows the effect of diets on the triglyceride levels of male Wistar rats. It was observed an increase of triglycerides with the consumption of hypercaloric drink (G2) and with the consumption of hypercaloric rat food supplemented with bacon (G5).

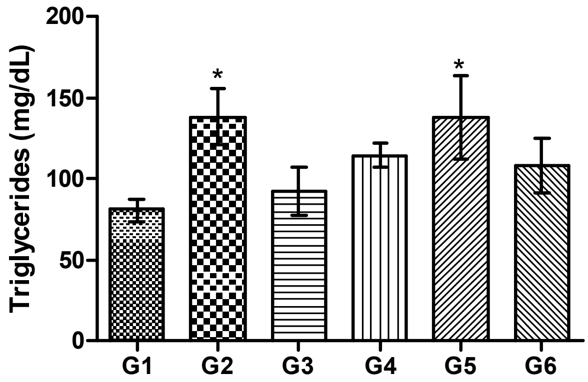

Figure 4. Effects of the different types of diet on the triglycerides of the animals from G1-G6

Figure 5 shows the effect of diets on total cholesterol in male Wistar rats. The consumption of hypercaloric rat food, both isolated (G5) and supplemented with bacon (G6) increased total cholesterol.

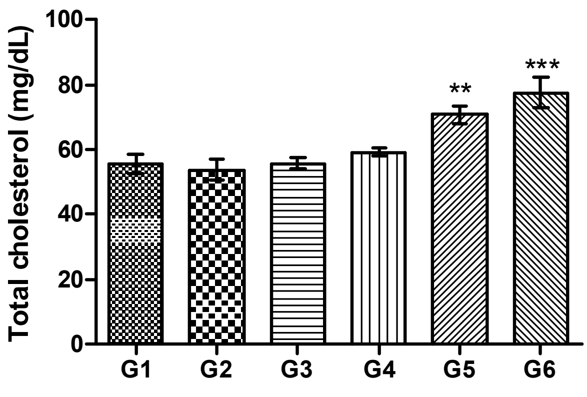

Figure 5. Effects of the different types of diet on the total cholesterol of the animals from G1-G6

The ingestion of hypercaloric diet did not produce changes in HDL-c and LDL-c (data not shown).

DISCUSSION

Our study evaluated three hypercaloric diets, and the results show that hypercaloric beverages (water added by condensed milk) led to an increase in glycaemia and triglycerides in male Wistar rats. In the same way, the intake of hypercaloric food (commercial ration added of animal and vegetal fat) caused an increase in the cholesterol and the abdominal fat. The adoption of hypercaloric or hyperlipidemic diets has been widely used as a model for inducing obesity in laboratory animals. These animal models are instrumental in research on obesity because of their high similarity to the human Genetics and in the metabolic responses. Obesity is usually the consequence of a positive energy balance generated by environmental factors, such as excessive consumption of high-calorie foods and sedentary lifestyle (Rastogi *et al.*, 2017; Ornellas *et al.*, 2017; Muredda *et al.*, 2017). Although there is not a perfect animal model for the study of cardiovascular comorbidities, Suman *et al.* (2016) have developed a model for induction of dyslipidemia, hypertension, and diabetes when using a low-fat diet with low doses of streptozotocin in Wistar rats. Dyslipidemia and insulin resistance, two important markers of cardiovascular disease, were induced in rats by administering an oil-rich emulsion plus fructose for six consecutive weeks (Munshi *et al.*., 2014).

Many other authors have evaluated the effect of different types of diet on the metabolism of animals (Blancas-Velazquez *et al.*, 2017, Santos *et al.*, 2017). Zanini *et al.* (2017) also evaluated the effects of a hypercaloric diet rats (female rats) on the metabolic profile and showed that those animals that used a hypercaloric diet showed increase food intake in grams and calories, resulting in higher weight gain when comparing to control group animals. Gao *et al.* (2017) studied the effects of a combined overconsumption of fat and sugar in mice and observed that this component might promote an overexpression on the inflammatory markers of obesity and leads to a neuronal imbalance in the control of energy metabolism. Similarly to our study, Pini *et al.* (2017) studied the effects of hyper caloric diet on the metabolism of male Wistar rats but used a cafeteria diet and a high-fat diet. They found no differences on the body weight but found an increase in the abdominal fat in the treated animals and concluded that this kind of diet might trigger metabolic dysfunctions.

Diepenbroek *et al.* (2017) investigated whether modifications on the insulin sensitivity is associated with high fat or high sugar diet and found that rats treated with a free-choice high-sugar, high-fat, fastly develop obesity and glucose intolerance. Furthermore, these animals were hyperphagic and presented an increase in the adiposity. They also found that overconsumption of sugar water slightly interfered in the insulin sensitivity. The standardized diets in this study were able to increase relevant biochemical parameters in the evaluation of cardiovascular problems. However, the increase was not possible with a single treatment since the increase in glycaemia and triglycerides was only obtained with the ingestion of the hypercaloric drink whereas the increase of cholesterol and abdominal fat was only possible with the intake of both the drink and the hypercaloric rat food. In conclusion, the present study standardized two diets that could be used to induce hyperglycemia, hypertriglyceridemia, and hypercholesterolemia in animal models of obesity and metabolic syndrome.

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