

## SYSTEMATIZATION OF OFFERING A DIET FOR INDUCING OBESITY IN ADULT RATS

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### ABSTRACT

**Aim:** To systematize the offering of foods which were highly palatable and rich in calories and to investigate the effects on morphometric and metabolic parameters of adult Wistar rats. **Methods:** The study used 15 male Wistar rats, aged 45 days old, divided into a control group and a cafeteria diet group. To the control group was offered a commercial diet ad libitum, while to the cafeteria diet group was offered the commercial diet supplemented each day with four items selected from a list of 28 foods. The consumption of foods from the cafeteria diet was checked daily, and the two groups' consumption of water and feed was checked weekly. After seven weeks, the morphometric parameters were checked and animals were sacrificed for collection of blood and the deposit of perigonadal fat. **Results:** The mean weight of the cafeteria diet group was higher than that of the control group, as was abdominal circumference, deposit of perigonadal fat, body mass index and the Lee index. Furthermore, animals from the cafeteria diet group presented high blood levels of triglycerides; also, changes were not observed in levels of cholesterol and glucose. Animals from the cafeteria diet group ingested more calories, carbohydrates and fats than animals in the control group. **Conclusion:** This study showed that a protocol of offering a cafeteria diet formulated based in foods typically consumed by the Brazilian population can be used for inducing obesity in rats, requiring a relatively short time period for obtaining results.

**Key words:** Obesity. Overnutrition. Triglycerides. Body Mass Index.

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### RESUMO

Sistematização do oferecimento de uma dieta para indução de obesidade em ratos adultos

**Objetivo:** sistematizar um protocolo de dieta de cafeteria para indução de obesidade em roedores a partir da ingestão de alimentos altamente palatáveis, ricos em gorduras e carboidratos. **Método:** foram utilizados ratos Wistar com 45 dias de vida, divididos aleatoriamente em grupo controle (GC) e grupo dieta de cafeteria (GDC). Ao GC foi oferecido dieta comercial balanceada para roedores à vontade e ao GDC foi oferecido a dieta comercial balanceada à vontade suplementada diariamente com quatro itens selecionados de uma lista de 28 alimentos. O consumo dos alimentos da dieta de cafeteria foi verificado diariamente e o consumo de água e ração dos dois grupos verificado semanalmente. Ao final das sete semanas foram verificados os parâmetros morfométricos, a seguir, os animais foram sacrificados para coleta de sangue e gordura perigonadal. **Resultados:** o peso médio do GDC foi significativamente maior do que do GC, assim como a circunferência abdominal e o depósito de gordura perigonadal. Adicionalmente, os animais do GDC apresentaram níveis sanguíneos significativamente elevados de triglicerídeos comparados aos animais do GC, entretanto, não foi observada alteração nos níveis de colesterol e glicose. Os animais do GDC ingeriram significativamente mais calorias, carboidratos e gorduras os animais do GC. **Conclusão:** este estudo mostrou que um protocolo de oferecimento de dieta de cafeteria formulado com base em alimentos tipicamente consumidos pela população brasileira, pode ser utilizado para indução de obesidade em roedores, necessitando de um período de tempo relativamente curto para obtenção de resultados.

**Palavras-chave:** Obesidade, Hipernutrição, Triglicerídeos, Índice de Massa Corporal.

## INTRODUCTION

The dietary pattern and the selection of foods are determined by various factors such as availability, price accessibility, and diet-related, religious and cultural considerations. However, the main factor in food selection is the pleasure of eating (Zandstra and collaborators, 2002).

One of the factors that determine the pleasure provided by food is its nutrient content. Foods that are rich in sugars, fats and proteins are preferred to those that have low levels of these nutrients (Nielsen and Popkin, 2003; Zandstra, El-Deredy, 2011).

People appreciate variety in their diet, and select foods which differ in flavor or texture from those consumed recently (Brondel and collaborators, 2007).

The modern diet in the developed countries was projected to exploit human characteristics, and consists of a wide variety of foods which differ in their flavors and textures and which are rich in fat, sugar and proteins.

Additionally, the increase in availability and variety and the low cost of the foods makes them accessible to most the population in developed and developing countries, which contributes to the increase of the prevalence of persons who are overweight or obese (Berthoud, Lenard and Shin, 2011).

Obesity and overweight are considered a worldwide epidemic. In the United States, the Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention, 2011) showed that between 2007 and 2009 there was an increase of 1.1% in the prevalence of obesity in adults, that is, approximately 2.4 million more obese people.

In Brazil, the tendency for increase in the population's weight is also present. According to data from the Brazilian Ministry of Health's Vigitel study (Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico: Surveillance of Risk and Protective Factors for Chronic Diseases through Telephone Surveying), the number of Brazilians who are overweight went from 42.5% into 2006, to 52.8% in 2012. In the same period, the proportion of Brazilians who are obese increased from 11.4% to 15.8% (Brasil, 2014).

The increase in the prevalence of obesity is a problem worldwide, because it

predicts an increase of various illnesses, mainly cardiovascular diseases, diabetes mellitus and cancer, which are the main causes of health expenses, incapacity and death.

The undertaking of animal models for overweight and obesity is useful for studying the effect of the increase in body weight on specific health conditions, such as cardiovascular diseases and diabetes mellitus, as well as the effects of treatments for these conditions.

One of the ways of inducing obesity in animals – particularly in rodents – is the cafeteria diet, also known as the Western diet. This type of diet has high palatability and a raised level of carbohydrates, most of which are simple carbohydrates, arising from the use of refined cereals; large quantities of fat, principally saturated fats, and low levels of proteins, dietary fiber and micronutrients (Bayol and collaborators, 2010).

Moreover, this model has great similarity with the genesis of obesity in humans (Tschop and Heiman, 2002).

In the literature, it is possible to find various protocols for the cafeteria diet for inducing obesity in rodents (Rosini, Silva and De Moraes, 2012).

Some protocols, however, may not be shown to be efficacious, depending on the animal's strain and age. Protocols of studies with Wistar rats are very common due to the characteristics of this strain, such as the size of the structures and the ease of handling during the experimental period.

As a result, the need arises to investigate different possibilities regarding the diets for inducing obesity, and its physiological disorders, particularly in this animal model. In addition to this, it is important to standardize one model of obesity induced by the cafeteria diet which depicts the modern dietary context, and which offers a variety of foods with high energy value and which is easy to access for the majority of the population, thus possessing greater ecological value.

This study's objective, therefore, was to systematize the offering of different foods, highly palatable and rich in fats and carbohydrates, and to investigate the effects of this diet on the morphometric and metabolic parameters of adult Wistar rats.

## MATERIAL AND METHODS

### Animals

Experiments were performed in 15 male Wistar rats, 45 days old, with initial weights of approximately 250 grams (g), supplied by the Animal Colony of Ribeirão Preto Campus of the University of São Paulo (USP) and afterwards kept in the maintenance vivarium of the Physiology Laboratory of the Ribeirão Preto School of Nursing. The animals were kept on a ventilated shelf (Alesco) with a light/darkness cycle of 12 hours, and a

temperature of  $\pm 24^{\circ}\text{C}$ . Three animals were kept in each cage, as, due to rats being social animals, keeping them in individual cages for seven weeks could cause stress and interfere in their ingestion of food (Animal Research Review Panel, 2002).

This study was undertaken in accordance with the ethical principles in animal experimentation, and was approved by the Ethics Committee in the Use of Animals (CEUA) of the Ribeirão Preto Campus of the University of São Paulo (Protocol number 13.1.835.53.0).

**Table 1** - Food components of the cafeteria diet, macronutrient and dietary fiber content and day of the week they were offered.

Day of the week	Food	Calories (Kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Dietary fiber (g)
Sunday	Toast	36.6	6.6	0.6	0.7	0.3
	Marshmallow	33.5	8.0	0.5	0.0	0.0
	Peanut candy	52.0	4.8	1.8	2.9	0.3
	Salami	40.1	0.4	2.5	3.3	0.0
Monday	Arrowroot cookies	43.3	7.0	1.0	1.2	0.3
	White chocolate	55.6	5.6	0.6	3.0	0.0
	Mozzarella cheese	32.0	0.0	2.6	2.4	0.0
	Sugar-frosted corn flakes	37.6	8.3	0.5	0.2	0.3
Tuesday	Cookies with chocolate filling	48.0	7.0	0.6	1.9	0.0
	Processed cheese	29.0	0.4	1.0	2.6	0.0
	Almonds	61.9	0.7	2.2	5.6	1.2
	Coconut fudge	45.3	9.3	0.3	0.6	0.0
Wednesday	Brazil nuts	69.8	1.2	1.3	5.5	0.1
	Soft cheese	38.0	0.0	2.3	3.2	0.0
	Milk chocolate	54.3	5.8	0.5	3.1	0.3
	Peanut butter	61.0	2.8	1.4	5.0	0.5
Thursday	White bread	24.0	4.8	0.7	0.3	0.3
	Chocolate-flavored corn cereal	38.0	8.3	0.6	0.5	0.3
	Semi hard cheese	38.6	0.0	2.5	3.2	0.0
	Chocolate finger biscuits	47.7	7.7	0.4	1.7	0.2
Friday	Chocolate Swiss roll	40.6	6.0	0.6	1.6	0.2
	Cream crackers	45.3	7.0	1.2	1.4	0.3
	Mortadella	22.0	1.7	1.4	0.3	0.3
	Local brand M&Ms	46.8	7.6	0.3	1.7	0.0
Saturday	Sponge cake with filling	39.5	5.8	0.4	1.6	0.1
	Bacon	37.0	0.0	1.5	3.4	0.0
	Chocolate wafer biscuits	52.0	6.6	0.4	2.6	0.0
	Peanuts	59.3	1.2	2.4	5.5	0.9

### Diet

Animals were randomly divided in two experimental groups; the control group, containing six animals, and the cafeteria diet

group, containing nine animals. The control group was offered a balanced commercial diet for rodents (Nuvilab® CR1 Nuvitall Nutrientes S/A, Brazil) ad libitum, while the cafeteria diet group was offered the balanced commercial

diet plus four items selected from a list of 28 foods, which was called the cafeteria diet. The cafeteria diet offered was established in accordance with the study by Pini (2012) (Pini, 2012), being made up of 28 foods selected according to nutritional value and availability in the market.

Each day, four foods from the list were offered, over the 24 hour period, with weekly rotation of the foods in order to avoid monotony. The cafeteria diet was offered daily at 18:00 (dark phase of the cycle) in metal bowls with diameters of nine centimeters placed on the floor of the box. Foods that made up the cafeteria diet, the macronutrient and dietary fiber content and the day of the week on which they were offered are stated in Table 1.

The diet was offered for the period of seven weeks. The protocol for offering was standardized so that animals (three per cage) would receive increasing quantities of each food, these being 10 g in the first week, 20 g in the second week, 30 g in the third week and 40 g in the fourth week. From the fifth to the seventh week, the animals received 50 g of each food. Foods were duly weighed prior to being made available and after having been offered for 24 hours, the difference between what was offered and what was removed from the cage after meticulous checking of the wood shavings was considered as the consumption.

For calculating consumption per rat, the total consumption of each food per cage was divided by the number of rats contained in the cage. The quantity of nutrients ingested was calculated according to the quantity of food consumed and the nutritional information of the foods. Furthermore, the consumption of water by animals was checked; for this calculation, the weekly water consumption per cage was divided by the number of animals in the cage.

The nutritional values of the cafeteria diet foods were taken from the nutritional information offered by the manufacturers on the products' labels. The standard commercial diet for rodents has the following composition: gross energy: 3976 kilocalories/ kilograms (kcal/kg), carbohydrate: 57%, gross protein: 22%, lipids: 4% and gross fiber: 5,7% (Gonçalez, 2004).

### **Morphometric and metabolic parameters**

Rats were weighed weekly in order to determine the gain in body weight. At the end of the seven weeks, the animals were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg), administered intraperitoneally.

Following that, the nose-rump length and abdominal circumference were measured. The abdominal circumference was measured half way between the iliac crest and the costal margin (Noveli and collaborators, 2007).

The body mass index (BMI) was calculated based on the relationship between body weight (g)/square of the nose-rump length (cm<sup>2</sup>) (Noveli and collaborators, 2007).

The Lee index was calculated by the cube root of the body weight (g<sup>3</sup>)/nose-rump length (cm) (Noveli and collaborators, 2007).

Differently from what is proposed for human beings, for rats, BMI does not have classificatory values; it is considered that animals with higher rates are those which present a greater deposit of fat.

Animals were next decapitated and blood samples collected and centrifuged for 20 minutes at 2.000 g at 4°C. Concentrations of glucose, total cholesterol and triglycerides were determined by the colorimetric method, using a commercial kit of the Labtest® brand (Minas Gerais, Brazil). The perigonadal fat deposit was removed totally and weighed.

### **Statistical analysis**

The STATISTICA software, version 8.0 was used for statistical analysis. Analysis of central tendency (mean) and dispersion (standard error of the mean - SEM) was undertaken for the variables. The normality of the distribution was ascertained using the Kolmogorov-Smirnov test. Variance analysis (ANOVA) was undertaken using the Newman-Keuls post hoc repeated measures test or comparison of the means of two groups, using the non-paired t-test when applicable. The values were considered significant when  $p < 0.05$ .

## RESULTS

### Morphometric and metabolic parameters

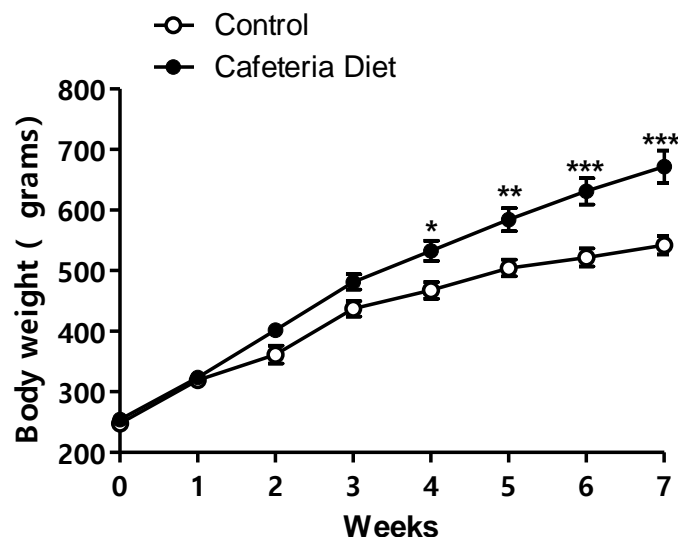
During the study period, the mean weight gain of the rats which received the cafeteria diet was 417.6 g (variation from 290 to 575 g), compared with the mean gain of 294.1 g of the group which received commercial feed (variation from 250 to 351 g). At the end of the experimental period, the mean weight of the cafeteria diet group was  $671.6 \pm 79.8$  g while that of the control group was  $542 \pm 40$  g (Figure 1).

One variance analysis (ANOVA) for repeated measures was undertaken regarding the bodyweight of the rats, taking into consideration the group (control v. cafeteria diet) as a factor between groups, and weeks (seven weeks) as an intra-animal factor. The analysis revealed statistically significant effects for group ( $F(1,14)=11.209$ ,  $p=0.004$ ), with the cafeteria diet group presenting a greater weight gain than the control group, for weeks

( $F(7,98)=413.66$ ,  $p=0.001$ ), and an interaction between group and weeks ( $F(7,98)=13.123$ ,  $p=0.001$ ).

Paired comparisons undertaken for the factor of weeks showed that the weight gain was constant over the period. Paired comparisons between groups and weeks showed that the cafeteria diet presented greater weight gain than the control group from the fourth week onward (Figure 1).

In addition to greater bodyweight, the animals of the cafeteria diet group also presented alterations in abdominal circumference, deposits of perigonadal fat, body mass index and Lee index, which were significantly greater than those in the control group. In relation to the metabolic parameters, the animals of the cafeteria diet group presented significantly higher blood levels of triglycerides in comparison with animals from the control group, however, change was not observed in blood levels of cholesterol and glucose (Table 2).



**Legend:** Data presented as means  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , compared to the control group, Newman-Keuls post hoc test.

**Figure 1** - Mean body weight of animals fed on commercial feed (Control) or cafeteria diet for seven weeks.

**Table 2** - Morphometric and metabolic parameters of rats fed with a standard rodent chow (control) or cafeteria diet for seven weeks.

Variable	Control	Cafeteria Diet	P value
<b>Morphometric parameters</b>			
Abdominal circumference (cm)	20.7 ± 1.27	23.2 ± 2.12	0.018
Deposits of perigonadal fat (g)	9.24 ± 0.84	23.31 ± 6.68	0.000
Body mass index	0.84 ± 0.01	1.09 ± 0.13	0.000
Lee index	0.32 ± 0.0	0.35 ± 0.02	0.001
<b>Biochemical parameters</b>			
Triglycerides (mg/dl)	75.73 ± 19.72	148.71 ± 45.28	0.000
Cholesterol (mg/dl)	64.31 ± 11.36	68.33 ± 21.17	0.658
Glucose (mg/dl)	85.60 ± 14.83	88.25 ± 14.52	0.725

**Legend:** \* Data presented as means ± SEM. Cafeteria diet group compared to Control group. Non-paired t-test.

### Dietary preference, ingestion of feed and water

In relation to the ingestion of food, animals of the cafeteria diet group ingested a significantly lower quantity of commercial feed than animals in the control group during the experimental period ( $216.9 \pm 22,3$  v  $1654.2 \pm 16.6$  grams, per animal), demonstrating a preference for the consumption of highly palatable foods. The analysis revealed statistically significant effects for group ( $F(1.14)=6,900.9$ ,  $p=0.000$ ), for weeks ( $F(6.84)=140.98$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.84)=173.02$ ,  $p=0.000$ ).

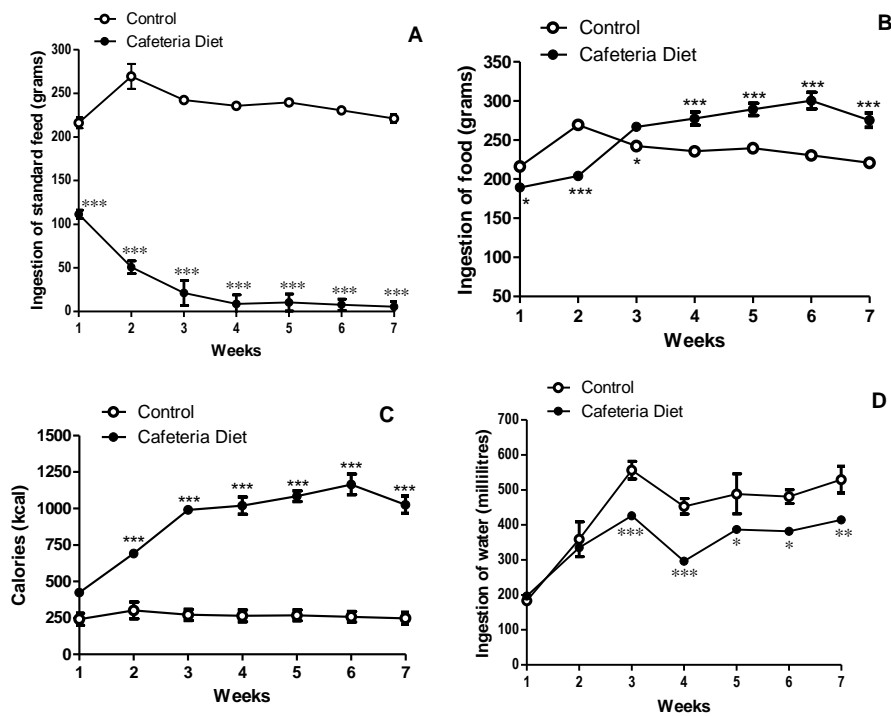
Until the second week, animals from the cafeteria diet group ingested a lower total quantity of food (adding the ingestion of commercial feed and foods that made up the cafeteria diet). From the third week onward, this behavior was altered and the animals ingested greater quantities of food until the end

of the experimental period. The analysis revealed statistically significant effects ( $F(1.14)=10.916$ ,  $p=0.005$ ), for weeks ( $F(6.84)=51.226$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.84)=65.043$ ,  $p=0.000$ ).

Therefore, animals of the cafeteria diet group presented greater ingestion of calories in comparison with animals of the control group. The analysis revealed statistically significant effects for group ( $F(1.30)=156.08$ ,  $p=0.001$ ), for weeks ( $F(6.18)=37.093$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.18)=36.976$ ,  $p=0.000$ ).

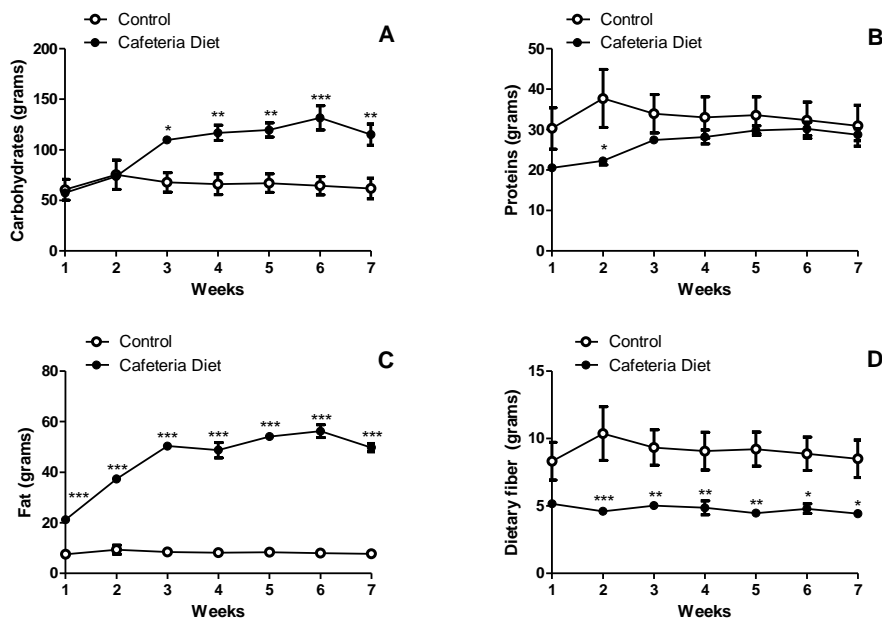
A lower ingestion of water was also observed among animals fed with the cafeteria diet (Figure 2).

The analysis revealed statistically significant effects for group ( $F(1.14)=10.700$ ,  $p=0.005$ ), for weeks ( $F(6.84)=127.84$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.84)=11.062$ ,  $p=0.000$ ).



**Legend:** Ingestion of standard feed (A), total ingestion of food (only feed for the control group and, for the cafeteria diet group, the sum of the ingestion of standard feed and foods from the cafeteria diet) (B), total ingestion of calories (C), ingestion of water (D) of animals fed with standard feed for rodents (Control) or cafeteria diet for seven weeks. Data presented as means  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , compared with the control group, Newman-Keuls post hoc test.

**Figure 2 - Dietary preference, ingestion of feed and water.**



**Legenda:** Ingestion of carbohydrates (A), proteins (B), fats (C) and dietary fiber (D) of rats fed with commercial feed (Control) or cafeteria diet for seven weeks. Data presented as means  $\pm$  SEM. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , compared with the control group, Newman-Keuls post hoc test.

**Figure 3 - Ingestion of macronutrients and dietary fiber.**

### **Ingestion of macronutrients and dietary fiber**

Due to the composition of the diet, animals of the cafeteria diet group presented greater ingestion of carbohydrates and fats in comparison with animals of the control group. The analysis revealed statistically significant effects for group ( $F(1.30)=12.646$ ,  $p=0.037$ ), for weeks ( $F(6.18)=16.014$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.18)=17.315$ ,  $p=0.000$ ).

The ingestion of protein by the cafeteria diet group was inferior to that of the control group only in the first week, there being no difference in the six subsequent weeks. The analysis did not reveal statistically significant effects for group ( $F(1.30)=2.4684$ ,  $p=0.214$ ), however, there was effect for weeks ( $F(6.18)=9.4900$ ,  $p=0.000$ ), and an interaction between group and weeks ( $F(6.18)=12.608$ ,  $p=0.000$ ).

On the other hand, animals of the cafeteria diet group ingested significantly less dietary fiber than animals of the control group during the entire period, a fact that occurred due to the composition of the foods offered. The analysis revealed statistically significant effects for group ( $F(1.30)=15.694$ ,  $p=0.029$ ), for weeks ( $F(6.18)=3.2652$ ,  $p=0.023$ ), and an interaction between group and weeks ( $F(6.18)=4.8588$ ,  $p=0.004$ ). The graphical representation of the ingestion of macronutrients and dietary fiber can be seen in Figure 3.

### **DISCUSSION**

In this study, it was demonstrated that Wistar rats prefer foods that are highly palatable and high in energy – the same ones ingested by human beings – to balanced feed for rodents. Therefore, these animals presented greater ingestion of food, gained more bodyweight, exhibited a greater abdominal circumference, deposit of perigonadal fat, and a high concentration of blood triglycerides in comparison with animals that ingested only commercial feed.

Animals which received the cafeteria diet presented a greater weight gain than those fed only on standard feed from the fourth week of the offering of the diet, and continued to increase their weight rapidly, reaching a mean increase of 62.2%, comparing the initial

weight with the weight at the end of seven weeks.

The increase of bodyweight can be explained by the ingestion of greater quantities of fats and carbohydrates from the foods which make up the cafeteria diet, resulting in greater intake of calories. The hyperphagia observed in animals of the cafeteria diet group was induced by offering varied foods with high energy density (Shafat, Murra, Rumsey, 2009).

One important aspect of the cafeteria diet is that it must be able to change the animals' metabolic parameters. In this study, a significant increase was observed in the blood level of triglycerides; however, the cafeteria diet offered for seven weeks was not able to alter the blood level of glucose and total cholesterol.

Other studies with Wistar rats fed on the cafeteria diet (Naderali and collaborators, 2001) or the hypercaloric diet (De Moraes and collaborators, 2007) for a period of time similar to that of this study also did not observe changes in blood glucose and cholesterol. However, the alteration of the circulating levels of triglycerides is a favorable point for the cafeteria diet protocol used in the present study, given that the triglycerides are related to the increase of the production of superoxide anions and a consequent reduction in the availability of nitric oxide, an important endogenous vasodilator (Naderali et al., 2001).

In addition to this, the increase in the blood levels of triglycerides, a consequence of the consumption of a hypercaloric diet, is correlated with impairment in the relaxant response to acetylcholine in rats' aortic and mesenteric arteries (De Moraes, 2007).

In this study, animals which received the cafeteria diet ingested a greater quantity of foods than animals which received the balanced feed for rodents, suggesting that the variety of more palatable foods contained in the cafeteria diet stimulated the ingestion of greater quantities of foods. The variety of foods may have contributed to the reduction of sensory satiety (Rolls, Van Duijvenvoorde and Rowe, 1983).

Rats that received the cafeteria diet were able to alternate between foods, maintaining the palatability and increasing the possibility of eating a greater number of snacks. On the other hand, rats that received only feed reached sensory satiety more quickly.



One important aspect of this study is that rats that received the cafeteria diet were able to choose between a highly palatable diet, rich in carbohydrates and fats, and the commercial balanced feed for rodents. As they were offered greater quantities of the cafeteria diet, rats' ingestion of feed reduced significantly.

Animals preferred (in the sense that they ate more when they were given the opportunity) the cafeteria diet to the feed for rodents, in the same way that humans also judged the cafeteria foods as more pleasant, which approximates this model of obesity to the genesis of obesity in humans.

In relation to nutritional ingestion in terms of macronutrients, animals of the cafeteria diet group ingested significantly more calories than those of the control group, because of ingesting significantly greater quantities of carbohydrates and fats. No difference was observed in the ingestion of proteins; however, the cafeteria diet offered lower quantities of dietary fiber.

The fact that rats of the cafeteria diet group initially ingested a lower quantity of food (feed and cafeteria diet) may be related to a compensatory mechanism of the organism in relation to the high energy level of the cafeteria diet; however, the continuous availability of highly palatable and calorie-rich foods resulted in a state of energy conditioning in those animals, that is, they preferred the foods rich in calories to the balanced feed for rodents (Zandstra and El-Deredy, 2011; Shafat, Murra and Rumsey, 2009).

The lower ingestion of water by the group that received the cafeteria diet, in comparison with animals that ingested only the feed, has already been observed in another study (Rogers and Blundell, 1984) and may be explained by the higher level of humidity in the cafeteria diet in relation to feed – for example, fresh bread may contain up to 38% humidity.

Animals' age at the start of the experimental protocol may have interfered in the gain in body mass. Young animals have a different metabolism and accumulate less adipose tissue due to the metabolic characteristics appropriate to the age (Bayol and collaborators, 2010), it also being advisable that older animals – of approximately 100 days old – should be subjected to diet-induced obesity (Rolls, Van Duijvenvoorde and Rowe, 1983).

However, the systematized protocol in this study was able to induce alteration of bodyweight even in young animals, which makes it useful considering the need to study the effects of obesity and its comorbidities in the early phases of life, mainly due to the epidemic of obesity ascertained in children and young people.

In this study, alterations of glycemia and cholesterolemia were not observed. In addition to evaluating total cholesterol, it would be important also to evaluate the fractions of cholesterol Low Density Lipoproteins (LDL) and High Density Lipoproteins (HDL), given that high levels of LDL are related to the appearance of coronary heart disease. As a result, using older animals or offering the cafeteria diet for longer periods can intensify the harm caused to the organism through the excess of weight, and may bring more robust results.

This study corroborates the premise that unlimited access to a highly palatable diet reinforces the hedonism of the food and eventually results in obesity. However, when one transports this theory to the human being, one must take into account that not all people exposed to a variable palatable diet become obese; therefore, behavioral, genetic and epigenetic factors must also be taken into account (Berthoud, Lenard and Shin, 2011).

## CONCLUSIONS

In conclusion, the protocol of offering the cafeteria diet, formulated based in foods typically consumed by the Brazilian population, was efficacious for inducing obesity in young rodents, a relatively short period of time being necessary for obtaining results – in this case, the changes were ascertained in 7 weeks.

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## AUTHORS' CONTRIBUTIONS

Pereira R and Stabile AM were the principal investigators, contributed to the study design, conduction of the experiments, data analyses, interpretation of the findings, and wrote the manuscript; Batalhão ME conducted the experiments; Cárnio EC provided the structural support; Moraes C and Bertazone TMA contributed to interpretation of the findings and reviewed the manuscript; Pini RTB, Costa TMB and Vales LDMF contributed to the study design and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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#### **Conflict of interest**

Authors declare no financial or personal conflict of interest regarding this article.

#### **Statement of the Research Ethics Committee**

This study was undertaken in accordance with the ethical principles in animal experimentation, and was approved by the Ethics Committee in the Use of Animals (CEUA) of the Ribeirão Preto Campus of the University of São Paulo (Protocol number 13.1.835.53.0).

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