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Body development and serum parameters of sprague-dawley rats fed a diet enriched with hydrogenated vegetable fat and sugar

Desenvolvimento corporal e parâmetros séricos de ratos sprague-dawley alimentados uma dieta enriquecida com gordura hidrogenada de vegetais e açúcar

Desarrollo corporal y serios parámetros de las ratas sprague-dawley que alimentan una dieta enriquecida con grasas vegetales y azúcares hidrogenadas

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Abstract
Hypercaloric and hyperlipidic diets are often used in obesity research to induce excess weight and dyslipidemia in rats. In this experiment, Sprague-Dawley rats received a hypercaloric diet that was enriched with hydrogenated vegetable fat and sugar but hypoproteic and nutrient deficient. The rats' body development and serum parameters were evaluated. Nine rats were fed a standard diet, while 27 rats were fed a hypercaloric diet prepared by substituting 15% of the standard diet with hydrogenated vegetable fat and 10% with sugar. Feed and water were provided ad libitum for 63 days. Between 35 and 98 days of age, the rats' naso-anal length, body weight, and Lee index were measured weekly. At the end of the experimental period, blood samples were obtained to determine the serum levels of total cholesterol, triacylglycerol, and glucose. It was observed that the rats fed the hypercaloric diet containing hydrogenated vegetable fat and sugar exhibited less body development and did not develop either dyslipidemia or obesity although they exhibited increased serum glucose concentration.
Keywords: Glucose; Diet; Cholesterol; Triglycerides.

Resumo
Dietas hipercalóricas e hiperlipídicas são frequentemente utilizadas em pesquisas sobre obesidade para induzir excesso de peso e dislipidemia em ratos. Neste experimento, ratos Sprague-Dawley receberam uma dieta hipercalórica que foi enriquecida com gordura vegetal hidrogenada e açúcar, porém, hipoproteica e deficiente em nutrientes. O desenvolvimento corporal e parâmetros séricos dos ratos foram avaliados. Nove ratos foram alimentados com uma dieta padrão, enquanto 27 ratos foram alimentados com uma dieta hipercalórica preparada substituindo-se 15% da dieta padrão por gordura vegetal hidrogenada e 10% por açúcar. A ração e a água foram fornecidas ad libitum por 63 dias. Entre 35 e 98 dias de idade, o comprimento naso-anal dos ratos, o peso corporal e o índice de Lee foram medidos semanalmente. Ao final do período experimental, amostras de sangue foram obtidas para determinar os níveis séricos de colesterol total, triacilglicerol e glicose. Observou-se que os ratos alimentados com a dieta hipercalórica contendo gordura vegetal hidrogenada e açúcar apresentaram menor desenvolvimento corporal e não desenvolveram nem dislipidemia nem obesidade, embora tenham exibido aumento da concentração sérica de glicose.ude the abstract in Portuguese
Palavras-chave: Glicose; Dieta; Colesterol; Triglicerídeos.

Resumen
Las dietas hipercalóricas e hiperlipídicas se utilizan a menudo en la investigación sobre la obesidad para inducir sobrepeso y dislipidemia en ratas. En este experimento, las ratas Sprague-Dawley recibieron una dieta alta en calorías que estaba enriquecida con grasa vegetal hidrogenada y azúcar, sin embargo, hipoproteica y deficiente en nutrientes. Se evaluaron el desarrollo corporal y los parámetros séricos de las ratas. Nueve ratas fueron alimentadas con una dieta estándar, mientras que 27 ratas fueron alimentadas con una dieta alta en calorías preparada reemplazando el 15% de la dieta estándar con grasa vegetal hidrogenada y el 10% con azúcar. Se proporcionó alimento y agua ad libitum durante 63 días. Entre los 35 y los 98 días de edad, se midieron semanalmente la longitud nasoanal de las ratas, el peso corporal y el índice de Lee. Al final del período experimental, se obtuvieron muestras de sangre para determinar los niveles séricos de colesterol total, triacilglicerol y glucosa. Se observó que las ratas alimentadas con la dieta alta en calorías que contenía grasas vegetales hidrogenadas y azúcar mostraron un menor desarrollo corporal y no desarrollaron ni dislipidemia ni obesidad, aunque sí mostraron un aumento en la concentración de glucosa sérica.
Palabras clave: Glucosa; Dieta; Colesterol; Triglicéridos.

1. Introduction

Obesity is a chronic disease with a multifactorial etiology characterized by excessive accumulation of fat in the body. The main cause of excessive fat accumulation is an imbalance between the number of calories consumed and expended (Serra-Majem, L., & Bautista-Castaño, 2013; Tobore., 2020). In experiments on obesity, rodents are commonly used owing to the genetic similarity between these animals and humans (Farias et al., 2020). Since excess feeding is an important factor that triggers overweight and obesity in humans (Popa et al., 2020), hypercaloric and hyperlipidic diets have been used to induce excess weight and dyslipidemia in rats (Malafaia et al., 2013; Almeida et al., 2015; Da Silva, et al., 2020). Therefore, in this experiment, Sprague-Dawley rats were fed a hypercaloric diet enriched with hydrogenated vegetable fat and sugar but hyperproteic and nutrient deficient. Therefore, the aim was evaluating the effects of hypercaloric diet enriched with hydrogenated vegetable fat on Sprague-Dawley rats' body development and serum parameters.

2. Methodology

Experimental procedures

All procedures used in this study were approved by the Ethics Committee on Animal Use of the Universidade José do Rosário Vellano (UNIFENAS), under the protocol number 14A/2016. Thirty-six male Sprague-Dawley rats (Rattus norvegicus) that were 35 days old and weighed approximately 85 ± 2 g were obtained from the vivarium of the UNIFENAS. They were housed in collective cages (3 animals per cage) in a room with controlled temperature and a 12-hour-light/12-hour-dark schedule. Animals were divided into two groups, with nine rats being fed a standard diet (control group; Nuvilab CR-1®; 348.5 kcal/100 g, 56% carbohydrates, 22% proteins, 4% lipids, 8% fiber, and 10% mineral-vitamin premix) and the other 27 rats received a hypercaloric diet (432.38 kcal/100 g, 52% carbohydrates, 16.5% proteins, 18% lipids, 6% fiber and 7.5% mineral-vitamin premix). For the preparation of the hypercaloric diet, the standard diet was initially ground in an industrial blender and posteriorly, 15% and 10% of the standard diet were replaced by hydrogenated vegetable fat and sugar (sucrose), respectively. Both groups received food and water ad libitum for 63 days.

Evaluated parameters

The weight gain and growth curves were plotted using weekly measurements of weight and naso-anal length, respectively, from age 35 to 98 days. The Lee index was calculated by dividing the cubic root of the weight in grams by the naso-anal length in centimeters multiplied by 1000 (Lee, 1929). At the end of the experimental period, and after a 12-hour fasting period, blood samples were obtained by retro-orbital venous plexus puncture in animals anesthetized with xylazine (6 mg/kg) and ketamine (40 mg/kg) (Bayer AS/Parke-Davis, USA). Subsequently, the blood was centrifuged (3,000 rpm at 4°C for 15 minutes), and the serum levels of total cholesterol, triacylglycerol, and glucose were determined by enzymatic

colorimetric methods using commercial kits (Labtest Diagnóstica S.A., Brazil).

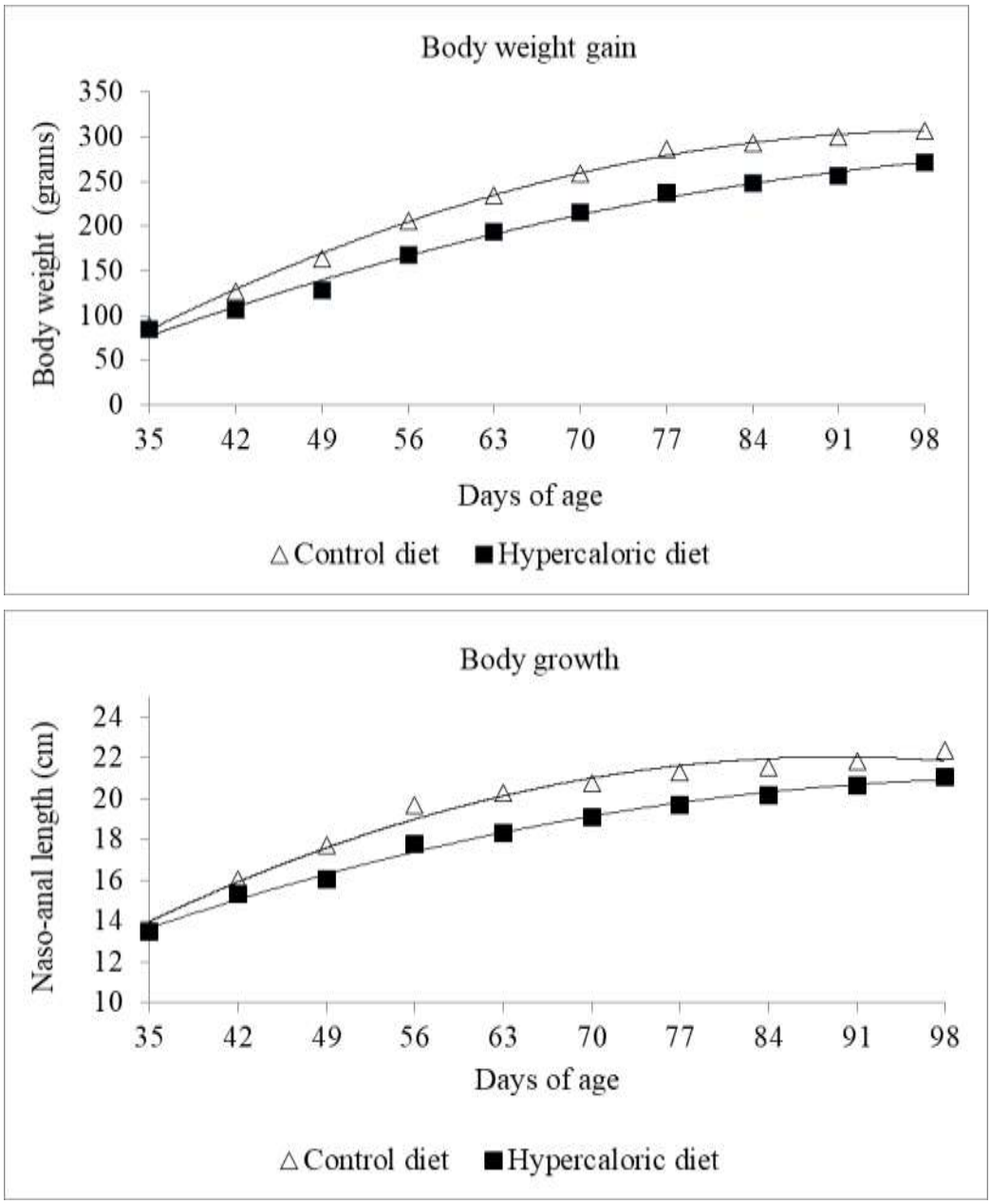
Statistical analysis

On the weight gain and growth graphs, for rats fed the control diet, each point represents the mean of the nine animals. For rats fed the hypercaloric diet, each point represents the mean of the 27 animals. For the serum parameters, the data were subjected to variance analysis using the statistical software SAS (SAS Institute Inc, Cary, 2011), and the significance of the diet effect on these parameters was evaluated by the F test at 5% probability.

3. Results and Discussion

The weight gain and growth of the rats in both groups can be explained by quadratic curves (Figure 1). However, during the whole experimental period, it was observed that the weight and naso-anal length of the control group rats were higher than the values measured in the rats that consumed the hypercaloric diet.

Figure 1: Body weight gain and growth of Sprague-Dawley rats.



Source: Authors (2021).

Weight gain and growth of Sprague-Dawley rats fed a control diet or a hypercaloric diet from 35 to 98 days of age. For rats fed the control diet, each point represents the mean of the nine animals. For rats fed the hypercaloric diet, each point represents the mean of the 27 animals. Effect of the control diet on weight gain: y = -0.0528x2 + 10.559x - 221.01 (R2 = 0.998). Effect of the hypercaloric diet on weight gain: y = -0.0281x2 + 6.821x – 127.31 (R2 = 0.994). Effect of the control diet on growth: y = -0.0027x2 + 0.486x + 0.28 (R2 = 0.981). Effect of the hypercaloric diet on growth: y = -0.0015x2 + 0.311x + 4.58 (R2 = 0.994).

During the entire experimental period, the Lee index of the rats remained constant ($P > 0.05$) at 0.3 for rats consuming both diets. In addition, the type of diet did not change the serum levels of total cholesterol or triacylglycerol ($P > 0.05$) (Table 1). However, rats fed a hypercaloric diet showed a higher serum glucose concentration ($P < 0.05$) than that of animals fed the control diet (Table 1).

Table 1: Serum concentrations of total cholesterol, triacylglycerol, and glucose of Sprague-Dawley rats fed a control diet or a hypercaloric diet from 35 to 98 days of age.

	Control diet	Hypercaloric diet	CV (%)	P value
Total cholesterol (mg/dL)	87.84[a]	88.47[a]	7.44	P > 0.05
Triacylglycerol (mg/dL)	81.07[a]	85.10[a]	8.92	P > 0.05
Glucose (mg/dL)	113.41[b]	130.54[a]	10.18	P < 0.05

Means followed by different letters in the line differ by F test (P < 0.05). CV: coefficient of variation. For rats fed the control diet, each data point represents the mean the nine animals. For rats fed the hypercaloric diet, each data point represents the mean of the 27 animals. Source: Authors (2021).

The hypercaloric diet was an unbalanced diet containing fewer proteins, vitamins, and minerals. Food intake was not recorded because the rats were raised in polypropylene boxes. However, during the daily management, it was possible to observe visually that the rats fed the hypercaloric diet consumed less than did the control group. According to Moura et al. (2014), the metabolizable energy value of the feed is a critical point in the nutrition of rodents because these animals stop their food intake when the daily energy demand is reached. In addition, Almeida et al. (2011) reported a lower food intake for Wistar rats fed a hypercaloric diet.

Therefore, although hydrogenated vegetable fat and sugar (sucrose) are palatable, the highest energy value of the hypercaloric diet seems to have acted as the main regulator of the feed intake. Thus, the lower weight gain and lower growth of the rats fed the hypercaloric diet is likely due to the fact that these rats did not consume sufficient food to meet their nutritional requirements (protein, fiber, minerals, and vitamins).

The Lee index can be used as a fast and accurate way to determine obesity in rats, and values greater than 0.3 indicate obesity (Malafaia et al., 2013). Throughout the entirety of the present study, the Lee index remained constant at 0.3, demonstrating that the rats fed the hypercaloric diet did not develop obesity. The similarity of the Lee index between the two evaluated groups can be explained by the fact that the rats fed the hypercaloric diet had both a lower weight and a lower naso-anal length. In a recent study (Araújo et al., 2017), rats that consumed a high-fat diet containing 20% pork fat for 60 days became obese, showing a Lee index higher than 0.3; however, both the control diet and the diet containing pork fat were isoproteinic and isoenergetic.

Compared to the control group, rats fed the hypercaloric diet did not have higher serum levels of total cholesterol and triacylglycerol. A similar result was reported by Almeida et al. (2015), who fed Wistar rats a hyperlipidic diet consisting of 25% chocolate, 25% roasted peanuts, 12.5% biscuit, and 37.5% standard rat feed. On the other hand, the hypercaloric diet evaluated in the present experiment increased the rats' serum glucose concentration. According to Kubant et al. (2015), the addition of hydrogenated vegetable fat to the rat diet does not affect blood glucose concentration. Therefore, in the present experiment, the increased glycemia can be justified by the inclusion of sucrose in the feed.

4. Conclusion

Sprague-Dawley rats fed a hypercaloric diet containing hydrogenated vegetable fat and sugar and reduced protein and other nutrients exhibited less body development and did not develop either dyslipidemia or obesity although they exhibited increased serum glucose concentrations. The use of the model of diet in Sprague-Dawley rats has shown to be effective for the study of the physiopathology of complications associated with dyslipidemia or obesity to human, pet and production animals. The induction of dyslipidemia or obesity in rats via consumption of high-calorie diet, this case, not was observed. However,

our results, showed that the diet affec the body development. Therefore, other studies aimed at understanding hypercaloric and/or hyperlipidic diets for Sprague-Dawley rats could be performed.

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