Body development and serum parameters of sprague-dawley rats fed a diet enriched with hydrogenated vegetable fat and sugar in English in bold left aligned

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 hipercaleóricas 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 hipercaleórica 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 hipercaleórica 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 hipercaleórica 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.

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. Introducción
Obesidad es una enfermedad crónica con una etiología multifactorial caracterizada por una acumulación excesiva de grasa en el cuerpo. El principal causante de la acumulación excesiva de grasa es el desequilibrio entre el número de calorías consumidas y expandidas (Serra-Majem, L., & Bautista-Castaño, 2013; Tobore., 2020). En experimentos de obesidad, los roedores son comúnmente utilizados debido a la semejanza genética entre estos animales y los humanos (Farias et al., 2020). Ya que el exceso de alimentación es un factor importante que desencadena sobrepeso y obesidad en humanos (Popa et al., 2020), dietas hipercalóricas y hiperlipídicas se han utilizado para inducir sobrepeso y dislipidemia en ratas (Malafaia et al., 2013; Almeida et al., 2015; Da Silva, et al., 2020). Por lo tanto, en este experimento, castores Sprague-Dawley fueron alimentados con una dieta hipercalórica enriquecida con grasa hidrogenada de origen vegetal y azúcar, pero hipoproteica y deficiente en nutrientes. El objetivo era evaluar los efectos de una dieta hipercalórica enriquecida con grasa hidrogenada de origen vegetal en el desarrollo corporal y los parámetros séricos de los castores Sprague-Dawley.

2. Metodología

Experimental procedures
Todos los procedimientos utilizados en este estudio fueron aprobados por el Comité Ético en el Uso de Animales de la Universidad José do Rosário Vellano (UNIFENAS), bajo el protocolo número 14A/2016. Trescientos sexenta y seis ratas Sprague-Dawley (Rattus norvegicus) que eran 35 días viejas y pesaban aproximadamente 85 ± 2 g fueron obtenidas del vivarium de la UNIFENAS. Estas fueron alojadas en jaulas colectivas (3 animales por jaula) en una sala con temperatura controlada y un horario de luz/decrepimiento de 12/12 horas. Los animales fueron divididos en dos grupos, con nueve ratas en cada uno. El grupo control fue alimentado con la dieta estándar (Nuvilab CR-1®, 348.5 kcal/100 g, 56% carbohidratos, 22% proteínas, 8% lípidos, 8% fibra y 10% mineral-vitamin premix) y el grupo experimental fue alimentado con una dieta hipercalórica enriquecida con grasa hidrogenada de origen vegetal (432.38 kcal/100 g, 52% carbohidratos, 16.5% proteínas, 18% lípidos, 6% fibra y 7.5% mineral-vitamin premix). Para la preparación de la dieta hipercalórica, se dividió la dieta estándar en dos partes iguales, con 15% de la dieta estándar reemplazada por grasa hidrogenada de origen vegetal y 10% por azúcar (sucro). Ambos grupos quedaron libres de alimentación y agua durante 63 días.

Evaluado parameters
La ganancia de peso y el crecimiento se representaron utilizando medidas semanales de peso y longitud nasoanal, respectivamente, desde las edades de 35 a 98 días. El índice de Lee fue calculado dividiendo la raíz cúbica del peso en gramos por la longitud nasoanal en centímetros multiplicado por 1000 (Lee, 1929). Al final del período experimental, y después de un horario de fastigio de 12 horas, se obtuvieron muestras de sangre en ratas anestesiadas con xilazina (6 mg/kg) y ketamina (40 mg/kg) (Bayer AS/Parke-Davis, USA). Luego, la sangre fue centrífugada (3,000 rpm a 4°C por 15 minutos), y los niveles séricos de colesterol total, triacilglicerol y glucosa fueron determinados por enzimático.
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.
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: \( y = -0.0528x^2 + 10.559x - 221.01 \) (\( R^2 = 0.998 \)). Effect of the hypercaloric diet on weight gain: \( y = -0.0281x^2 + 6.821x - 127.31 \) (\( R^2 = 0.994 \)). Effect of the control diet on growth: \( y = -0.0027x^2 + 0.486x + 0.28 \) (\( R^2 = 0.981 \)). Effect of the hypercaloric diet on growth: \( y = -0.0015x^2 + 0.311x + 4.58 \) (\( R^2 = 0.994 \)).

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) | 87.84a       | 88.47a            | 7.44   | P > 0.05|
| Triacylglycerol (mg/dL)   | 81.07a       | 85.10a            | 8.92   | P > 0.05|
| Glucose (mg/dL)              | 113.41b      | 130.54a           | 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 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 affect 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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