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Negative effects of sucrose ingestion in testis weight and epididymal fat of adult Wistar rats

Efeitos negativos da ingestão de sacarose no peso testicular e na gordura epididimal de ratos Wistar adultos

Efectos negativos de la ingestión de sacarosa en el peso testicular y en la grasa epididimal de ratas Wistar adultas

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Abstract
Purpose: Evaluate the impact of chronic sucrose ingestion on body, testis and epididymal fat weights of male wistar rats, in addition to quantifying the influence on murinometric measurements and food intake. Methods: 30 male wistar rats were divided into control group (n = 15), which received water, and sucrose group (n = 15), in which water was replaced with a sucrose-enriched solution for 4, 8 and 12 weeks. Both groups were subdivided according to their intervention period: 4, 8 or 12 weeks. In the end, a total bilateral orchiectomy was performed. Testis and epididymal fat were weighted, and nasoanal length, GIS, Lee index and BMI were measured. Results: There was no significant difference in body weight between control and sucrose groups. However, there was a significant difference in epididymal fat weight and testicular mass. The S4 had higher resulting epididymal fat mass, 7,49g, while C4 showed 4,85 g. Testicular weight was significantly decreased in S8 rats (3,21g) when compared to C8 (6,82g). Conclusion: Chronic sucrose ingestion is capable of elevating fat mass and reducing testicular weight, without affecting body weight, besides elevating BMI and Lee index.
Keywords: Obesity; Sucrose; Rats, Wistar.

Resumo
Objetivo: Avaliar os efeitos da ingestão crônica de sacarose sobre peso corporal, testicular e de gordura epididimal em ratos Wistar machos, bem como seu impacto em medidas murinométricas e ingestão alimentar. Métodos: 30 ratos foram divididos em grupo controle (água) e grupo sacarose (solução enriquecida), ambos avaliados por 4, 8 e 12 semanas. Após o período de intervenção, realizou-se orquiectomia bilateral para pesagem dos testículos e da gordura

epididimal, além da aferição do comprimento nasoanal, GIS, índice de Lee e IMC. Resultados: O peso corporal não apresentou diferenças significativas entre os grupos. Contudo, observaram-se aumentos na gordura epididimal e redução do peso testicular. O grupo S4 apresentou maior massa epididimal (7,49 g) em relação ao C4 (4,85 g). Já o peso testicular foi significativamente menor em S8 (3,21 g) comparado a C8 (6,82 g). Conclusão: A ingestão crônica de sacarose aumenta a deposição de gordura epididimal e reduz o peso testicular, sem alterar o peso corporal, além de elevar o IMC e o índice de Lee.
Palavras-chave: Obesidade; Sacarose; Ratos Wistar.

Resumen
Objetivo: Evaluar los efectos de la ingestión crónica de sacarosa sobre el peso corporal, testicular y de grasa epididimal en ratas Wistar machos, así como su impacto en medidas murinométricas e ingesta alimentaria. Métodos: 30 ratas fueron divididas en grupo control (agua) y grupo sacarosa (solución enriquecida), ambos evaluados durante 4, 8 y 12 semanas. Al finalizar, se realizó una orquiectomía bilateral para pesar testículos y grasa epididimal, además de medir longitud nasoanal, GIS, índice de Lee e IMC. Resultados: No se observaron diferencias significativas en el peso corporal entre los grupos. Sin embargo, se evidenció aumento de grasa epididimal y reducción del peso testicular. El grupo S4 mostró mayor grasa epididimal (7,49 g) en comparación con C4 (4,85 g). El peso testicular fue significativamente menor en S8 (3,21 g) frente a C8 (6,82 g). Conclusión: La ingestión crónica de sacarosa incrementa la grasa epididimal y reduce el peso testicular, sin modificar el peso corporal, además de aumentar el IMC y el índice de Lee.
Palabras clave: Obesidad; Sacarosa; Ratas Wistar.

1. Introduction

The changes that occurred in the diet of the Western population contributed to a new nutritional parameter and the development of several diseases such as obesity. The advent of industrialized products accelerated the process, as food became tastier with high added sugar, fat, and salt (Adolph et al., 2024). In addition, there has been a change in previously practiced healthy habits, and the population has become more sedentary, which is harmful, as physical activity provides energy expenditure and decreases fat mass, and its lack contributes to the maintenance of obesity (Alves et al., 2022).

In this sense, obesity constitutes a disease that is characterized by excessive accumulation of body fat and can be understood as a problem with multifactorial causes. In addition to the points mentioned above, such as diet and sedentary lifestyle, it can also be influenced by genetics and emotional disorders (Cantanhede et al., 2021). Obesity is a risk factor for the development of other comorbidities, such as type II diabetes mellitus, by interfering with glucose and insulin homeostasis. As a consequence, there is greater production of glucose by the liver and lower uptake by tissues. Furthermore, cardiovascular diseases are prevalent, especially coronary atherosclerotic disease, which can culminate in heart tissue infarction (Araújo et al., 2022).

The impact on male health is also an important point to be considered. The repercussions on the testicles can result in the inability to reproduce and in erectile dysfunction. Some studies suggest that the endocrine disorders caused by obesity can influence the hormones involved in gametogenesis and thus affect testicular functionality. Consequently, there is a reduction in sperm count, a decrease in testosterone levels—the main hormone responsible for male reproduction—and an increase in estradiol levels. From this, knowing the main causes of infertility becomes essential in order to solve the problem (Ameratunga et al., 2023).

Thus, this study aims to evaluate the impact of chronic sucrose ingestion on body, testis, and epididymal fat weights of male Wistar rats, in addition to quantifying the influence on murinometric measurements and food intake.

2. Methodology

An experimental, quantitative, and laboratory study was carried out (Pereira et al., 2018), using descriptive statistics with data classes, mean values, and standard deviation (Shitsuka et al., 2014), and statistical analysis (Costa Neto & Bekman,

2009). This study followed the Brazilian law of use and care of animals (Law 11.794/08) and complied with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines recommendations. The project was approved in advance by the Animal Use and Care Committee of the Universidade Estadual do Pará (UEPA), under protocol number 18/2021.

Experimental design

Thirty male Wistar rats (*Rattus norvegicus)*, 60 days old, obtained from the Evandro Chagas Institute were used, having mean weight of 260-350g. They were housed in individual cages appropriately labeled, in a controlled environment with standard industrial rat chow and water ad libitum. The study was conducted in the Experimental Surgery Laboratory of UEPA.

The animals were randomly assigned into two groups: control (CG, n = 15) only bilateral orchiectomy; and sucrose (SG, n = 15), submitted to oral ingestion of a sucrose enriched solution prior to bilateral orchiectomy. Each group was subdivided into three subgroups: long, intermediate and short groups, in which the surgery was performed 4, 8 and 12 weeks after the start of the study, respectively. No animal was excluded from the research.

The sucrose enriched solution (300 g/l) was available for SG rats *ad libitum* during the experiment and was made twice a week, following the protocols established by Malaffaia et al. (2013) and Ahmed et al. (2019). The period of sucrose administration was 4, 8 or 12 weeks before the orchiectomy.

All authors were aware of the allocation of experimental units into groups at different moments of the experiment (during allocation, conducting the experiment, assessing outcomes and analyzing data).

Surgical assessments

The procedures were performed after proper analgesia and sedation, which consisted of intraperitoneal administration of ketamine (90 mg/kg) and xylazine (10 mg/kg). The animals were immobilized in the dorsal decubitus and the abdominal area was shaved. To access the abdominal cavity, a median laparotomy was made, followed by a bilateral total orchiectomy, whereas the testis were exposed by traction of the spermatic funicle. The testis were weighted, as well the epididymal fat of both sides. The animals were euthanized at the end of the surgical procedure by intravenous lethal injection.

Murinometric parameters

The body weight (g) was measured weekly and was always held on the same day of the week and at the same time. The values obtained were used to calculate the total weight gain (TWG) of each animal during the experiment. Testis and epididymal fat weights (g) were obtained with a precision scale, measured at the end of the surgical procedure. Nasoanal length (cm) was measured with millimeter paper after the anesthetic administration. Furthermore, the following variables were calculated: IGS (%), which represents how much of the body weight testis correspond; lee index, that indicates obesity when higher then 0,300 (Bernardis, 1970); and body mass index (g/cm2), considered normal among 0,46-0,68 g/cm2 according to Noleti et al. (2007).

Food intake

Quantification of food intake (g) was done weekly by subtracting the standard weight of food (320 g) from the remainder. At the end of the weighing, the amount of standard feed was reached. The mean and standard deviation of the five animals in each group were used for the final analysis.

Statistical analysis

The data obtained (Total Weight Gain, Resulting testicular weight, Resulting epididymal fat weight, Lee Index, Gonadosomatic Index, Body Mass Index, Nasoanal length, Food intake per week) were submitted to statistical analysis for normality evaluation by the Shapiro-Wilk test primarily. Subsequently, the Student's T test or the Mann-Whitney test was applied for non-parametric samples and the Analysis of Variance (ANOVA) was followed by Tukey's post-test for parametric samples according to variance and normality. SPSS software was used for analysis, adopting a 5% level of significance. All the data were expressed as means ± standard deviations.

3. Results

No animal died during the period of sucrose ingestion or before the end of surgical procedures. The results obtained are presented in Table 1.

Table 1 – Descriptive statistics data according the groups.

	GROUPS						P VALUE
	C12	**C8**	**C4**	**S12**	**S8**	**S4**	
TWG (g)	199,8 ± 31,2	150,6 ±28,91	79 ± 14,62	175,2 ± 28,39	169,4 ±36,80	80 ± 34,56	0,694
Resulting testicular weight (g)	3,48 ± 0,14	3,66 ± 0,40^{A}	3,10 ± 0,25	3,44 ± 0,18	3,42 ± 0,32^{A}	3,09 ± 0,37	0,000
Resulting epididymal fat weight (g)	11,10 ± 1,64	7,69 ± 2,65	4,85 ± 0,54^{A}	13,04 ± 1,32	12,53 ± 5,97	7,49 ± 1,87^{A}	0,024
Lee Index	0,32 ± 0,013	0,31 ± 0,008	0,32 ± 0,014	0,31 ± 0,008	0,316 ± 0,011	0,306 ± 0,005	0,004
GSI	0,63 ± 0,024	0,78 ± 0,04	0,82 ± 0,09	0,66 ± 0,06	0,72 ± 0,08	0,79 ± 0,08	0,400
BMI (g/cm^2)	0,89 ± 0,082	0,77 ± 0,064	0,76 ± 0,065	0,83 ± 0,04	0,80 ± 0,073	0,69 ± 0,019	0,160
Nasoanal length	24,6 ± 1,19	20,42 ± 0,49	22,5 ± 1,16	24,86 ± 0,89	24,2 ± 0,91	23,36 ± 0,50	0,836
Food intake per week (g)	187,5 ± 16,61^{B}	175,4 ± 14,29^{B}	171,1 ± 24,14^{A}	82,7 ± 10,22^{B}	92,9 ± 11,28^{B}	128,15 ± 14,069^{A}	0,008

C12 - Long Control Group; C8 - Intermediate Control Group; C4 - Short Control Group; S12 - Long Sucrose Group; S8 - Intermediate Sucrose Group; S4 - Short Sucrose Group; TWG - Total Weight Gain; GSI - Gonadosomatic Index; BMI - Body Mass Index.
Source: Meireles et al. (2025).

Total weight gain (TWG) of each group was higher in the control groups compared to the Sucrose groups corresponding to the intervention time. However, when observing S8 and C8, the S8, which was submitted to the obesity induction process, presented a higher TWG compared to the GCI, both evaluated after 8 weeks of intervention.

Sucrose was responsible for significantly reducing ($p = 0.009$) the resulting testicular weight when comparing the control and intermediate sucrose groups, only among those submitted to the intervention for 8 weeks. Although, the Sucrose groups (S12, S8, S4) had values of resulting epididymal fat, obtained by the sum of the adipose tissue located in each

epididymis, higher than those found in the control groups (C12, C8, C4), respectively, being significant only when comparing short groups (C4 x S4, p = 0,009).

Most animals developed obesity in the study (Lee index > 0.300). Only 04 animals had normal values, all with Lee index= 0.300, belonging to the groups C8 (01), S12 (01) and S4 (02). The average GSI/group was calculated, there was a decrease both in the sucrose and in the control group, associated with the intervention time in the animals, verifying a decrease in the intermediate and control groups. There was no difference between those who received or not the sucrose-rich solution (p > 0.05), but the total average for the sucrose group (S12, S8, S4) was approximately 0.72%, a little lower than the groups without intervention (0.74%).

The control group obtained a median BMI = 0.8 g/cm^2, while in the sucrose group the median BMI was equal to 0.77 g/cm^2. All animals were considered obese in this study, with a BMI growth pattern proportional to the intervention time. No significant differences were observed between control and sucrose (p > 0.05).

When comparing the nasoanal length between the control and sucrose groups evaluated in the same period, we found similar means (p = 0.103) with a lower value associated with the C8 group.

The mean food intake of each control and sucrose group with their respective temporal categorization showed a statistically significant difference, this has been verified by the Mann-Whitney U test. Among the analyses, the sucrose groups had lower values compared to the control groups.

4. Discussion

4.1 Food and Appetite

High sugar intake is associated with weight gain and contributes to obesity and cardiovascular abnormalities (Malik et al., 2013; Makarem et al., 2018). Sedentary lifestyles and diets high in ultra-processed foods, rich in sugar, fat, and salt but low in protein, vitamins, and fiber, promote the consumption of energy-dense, hyper-palatable foods that disrupt gut homeostasis and reduce satiety (Monteiro et al., 2019; Luiten et al., 2016; Zinöcker et al., 2018).

During the experiment, rats receiving sucrose solution exhibited reduced standard chow intake, consistent with the findings of Souza Cruz et al. (2020), who reported decreased voluntary feed intake due to the high caloric content of sucrose-rich solution (Souza Cruz et al., 2020). Additionally, sucrose reduced feed efficiency, indicating that its metabolism alters weight-regulating processes and produces adverse nutritional effects. In a separate study, chronic ingestion of sucrose solution for 40 days led to a compensatory reduction in dry food intake due to consuming a high percentage of calories as sucrose. Consequently, intervention rats did not gain more weight than controls, despite developing leptin resistance independent of body fat mass (Harris, 2018).

Similarly, Malita et al. (2022) demonstrated that in flies, injection of neuropeptide F (NPF) markedly reduces sugar intake. Comparable effects occur in humans, where neuropeptide Y (NPY) infusion suppresses food intake. Specialized enteroendocrine cells in adult female *Drosophila* detect sugar and release NPF, an ortholog of mammalian NPY, suppressing sugar appetite. This mechanism highlights a hormonal pathway by which animals regulate nutrient-specific intake (Malita et al., 2022).

Thus, research has shown that high energy intake leads to glucose intolerance, emphasizing that both excessive consumption of beverages enriched with sucrose and excessive intake of carbohydrates and solid diets with high fat content are harmful, considering the availability of glucose (Rahman et al., 2017; Souza Cruz et al., 2020).

Given this, Yunker et al. (2021) analyzed the effects of sucrose intake compared to glucose intake on insulin and hormones involved in appetite regulation and peripheral glucose. As a result, sucrose, compared to glucose ingestion, causes a

reduction in circulating levels of glucose, insulin, GLP-1, and PYY, in addition to inducing a lower satiety response in individuals with obesity, suggesting that obesity itself may potentiate the obesogenic metabolic effects of sucrose (Yunker et al., 2021).

Sex also influences neural regulation of appetite. Yunker et al. (2021) examined neural reactivity to high-calorie foods and found that women may be particularly sensitive to both sucralose and sucrose (Yunker et al., 2021). This increased sensitivity is reflected in higher blood oxygen level dependent (BOLD) signals, especially in the medial frontal cortex (MFC) and orbitofrontal cortex (OFC), which mediate conditioned motivation to eat. Similarly, Frank et al. (2010) investigated gender differences in neural activation after ingesting palatable foods in fasted versus fed states. Women showed higher BOLD signals in response to high-energy foods when fasted and reduced signals when fed, particularly in reward-related neural areas, suggesting sex-specific neural responses influenced by energy status (Frank et al., 2010).

4.2 Murinometric Measurements

In the present study, there was no significant difference in body weight between the groups. However, there was a significant difference in epididymal fat mass. Thus, sucrose was responsible for higher epididymal fat mass in all groups, with $p < 0.05$ only in short groups (C4 × S4). These results are supported by other studies with similar findings, in which animals experienced increased fat deposition without changes in body weight (Matias et al., 2018). This may be due to increased resistance to obesity, with accelerated metabolic enzyme production that contributes to an inhibitory state of lipogenesis in the early stages, mainly in models of obesity induced by high sugar intake (Matias et al., 2018; Melo et al., 2019). In addition, feeding a hypercaloric diet rich in lipids to Wistar rats shows more pronounced effects on metabolic disorders than a sugar-only diet, such as in weight gain and body fat parameters (Kobi et al., 2023; Gasparini et al., 2021).

Similarly, Bacelar et al. (2019) observed comparable results when comparing rats fed a commercial diet with those receiving a high-fat, high-calorie diet. The control group consumed more food daily, leading to greater weight gain but lower epididymal fat. Reduced food intake in rats consuming high-calorie substances, such as sucrose in the present study, may explain lower weight gain despite increased epididymal adipogenesis (Bacelar et al., 2019).

Furthermore, another study demonstrated the impact of a high-sucrose diet on body composition. Rats receiving a sucrose-rich solution instead of water showed marked increases in visceral, retroperitoneal, and epididymal fat (Souza Cruz et al., 2020). This fat accumulation is closely associated with insulin resistance, type 2 diabetes, and cardiovascular diseases. Moreover, high-sucrose diets may reduce sirtuin levels, a protein family essential for regulating energy metabolism and adipose tissue degradation (Nikroo et al., 2020). Consequently, suppression of these metabolic regulators exacerbates fat deposition and impairs the body's ability to utilize fat as an energy source.

From the results, it was noticed that the sucrose intermediate group had the greatest loss of testicular weight in comparison with its respective control group. One possible explanation is the interaction of obesity-induced hypogonadism. Hohl et al. (2023) described mechanisms by which obesity-induced insulin resistance affects male gonadal physiology, noting that testosterone production by Leydig cells is inversely proportional to insulin resistance (Hohl et al., 2023). Additionally, high-fat diets may reduce follicle-stimulating hormone (FSH) levels, impairing Sertoli cell proliferation and nutrient support via hypothalamic–pituitary–gonadal axis dysregulation, ultimately diminishing testicular germ cell development (Zhang et al., 2023).

Leptin, an energy reserve signal produced by adipose tissue, may also contribute to testicular dysfunction. Leydig cells express leptin receptors, which inhibit their activity. Thus, increased adipose tissue in obesity elevates leptin levels, reducing testosterone production (Caprio et al., 1999; De León-Ramírez et al., 2021). Beyond endocrine effects, elevated leptin

and insulin can promote testicular cell apoptosis through reactive oxygen species (ROS) and suppression of the NRF2 antioxidant pathway (Falvo et al., 2023; Li et al., 2023). These mechanisms support Elmorsy et al. (2024), who reported morphological and histological testicular alterations, including decreased testicular weight (Elmorsy et al., 2024).

4.3 Impact on Male Health

The rising global consumption of sugar-sweetened beverages has increased concern regarding their potential dietary impacts on male fertility. Excessive sugar intake is associated with multiple conditions, such as metabolic syndrome, insulin resistance, accelerated aging, and obesity (Win et al., 2025).

Oxidative stress related to high sugar consumption plays an essential role by causing lipid peroxidation of sperm membranes and DNA fragmentation, affecting sperm health and resulting in lower sperm concentration and viability (Agarwal et al., 2020). Additionally, sugar-sweetened beverages impair spermatogenesis through hormonal pathways by disturbing hypothalamic–pituitary signaling, leading to the absence of compensatory increases in FSH in cases of lower sperm counts and reducing the inhibin-B/FSH ratio (Nassan et al., 2021).

The literature reveals that daily intake of more than 200 mL of sugar-sweetened beverages is associated with impairments in male reproductive parameters, including reduced testicular motility, concentration, and total sperm count, when compared to non-consumers (Nassan et al., 2021). These findings are corroborated by data from Hatch et al. (2019), who observed that consuming seven or more 12-ounce servings (≈355 mL per serving) of sugar-sweetened beverages per week was linked to a significant decrease in fecundability in both males and females. Importantly, this negative association was most pronounced among male consumers of sugar-sweetened sodas. Conversely, the study found no consistent relationship between the consumption of diet sodas and fecundability outcomes (Hatch et al., 2019).

In one study, Wistar rats received a 30% sucrose solution from the postnatal period to adulthood, showing histological alterations in the testes and increased inflammation in epididymal tissue, evidencing that early sugar consumption may lead to infertility in adulthood (León-Ramírez et al., 2021). Similarly, the study by Guimarães et al. (2021) corroborated this association with infertility, based on the administration of a 20% sucrose solution for 12 weeks (Guimarães et al., 2021).

Furthermore, limiting the intake of sugary foods is crucial to reduce the negative health impacts on male well-being. A balanced diet rich in antioxidants is essential to support optimal functioning of the male reproductive system. Including foods high in vitamins C and E, folate, zinc, and omega-3 fatty acids plays a significant role in mitigating DNA damage and combating oxidative stress caused by the consumption of ultra-processed and high-sugar foods (Win et al., 2025).

5. Conclusion

In conclusion, chronic sucrose ingestion is capable of elevating fat mass and reducing testicular weight, without affecting body weight, besides elevating BMI and Lee index. Further investigation on sucrose influence on appetite and hunger may explain why animals receiving sucrose did not experience a higher body mass gain. Moreover, the reduction in testicular weight and increase in adiposity highlight possible endocrine and reproductive repercussions, reinforcing the harmful metabolic effects of sucrose. These findings emphasize the need for preventive strategies and deeper studies on the link between diet, obesity, and male fertility, particularly regarding hormonal imbalance, metabolic disruption, and long-term reproductive capacity, which may have significant translational relevance to human health. Additionally, understanding these mechanisms may contribute to the development of therapeutic interventions, nutritional guidelines, and public health policies aimed at reducing the negative impact of excessive sucrose consumption across populations.

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