Soft drink consumption reduces food intake in Wistar rats

Consumo de refrigerante reduz a ingestão de alimentos em ratos Wistar

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ABSTRACT

Aims: To evaluate the effect of caloric and non-caloric soft drink intake on food consumption, body weight and composition, and metabolic parameters in rats.

Methods: Controlled experimental study in which 30 male Wistar rats were divided into three groups and given food and beverage *ad libitum* during 17 weeks. The groups were as follows, according to the offered food: Control group – standard chow and water; Caloric soft drink group – standard chow, caloric soft drink, and water; and Non-caloric soft drink group – standard chow, non-caloric soft drink, and water.

Results: There was no statistical difference in total energy intake, body weight, and fat deposition between groups. However, the chow energy intake was 45% lower in the caloric soft drink group compared to the control and non-caloric soft drink groups (198.7±0.7 kJ vs. 349.4±2.0 and 373.0±1.3 kJ, respectively), with 46% of the energy provided by the soft drink. The caloric soft drink group consumed 22% more carbohydrate, especially sucrose, compared to the control group (p<0.05). Macronutrient intake was not different between the control and non-caloric soft drink groups, but the caloric soft drink group consumed less protein and lipids when compared to the other groups (3.5±1.0 g of protein vs. 6.2±0.1 and 6.7±0.1 g, respectively; 0.7±0.01 g of lipids vs. 1.3±0.02 g and 1.4±0.02 g, respectively). Consumption of non-caloric soft drinks increased total sodium intake and consumption of both soft drinks decreased water intake. Although body weight varied during the experiment, there was no significant difference between groups at the end of the experiment, and no difference in fat deposition, fasting glucose, insulin and leptin, insulin resistance index, and lipid profile.

Conclusions: The consumption of both types of soft drinks did not affect energy intake, body weight and composition, or metabolic parameters; however, it increased fluid intake and decreased water ingestion. Caloric soft drink intake influenced the amount and the quality of solid food consumed, compromising diet quality.

KEY WORDS: soft drinks; food consumption; macronutrients.

RESUMO

Objetivos: Avaliar o efeito do consumo de refrigerante calórico e não calórico sobre a ingestão alimentar, composição corporal, massa corporal e parâmetros metabólicos em ratos.

Métodos: Estudo experimental com grupo controle. Trinta ratos Wistar machos foram divididos em três grupos e receberam alimentos e bebidas *ad libitum*. Os grupos foram os seguintes, conforme o alimento oferecido: Grupo controle – ração padrão e água; Grupo refrigerante calórico – ração padrão, refrigerante calórico e água; e Grupo refrigerante não calórico – ração padrão, refrigerante não calórico e água.

Resultados: Não houve diferença estatística na ingestão total de energia, peso corporal e depósito adiposo entre os grupos. Entretanto, a ingestão de energia da ração foi 45% menor no Grupo refrigerante calórico comparado ao Grupo controle e ao Grupo refrigerante não calórico (198,7±0,7 kJ vs. 349,4±2,0 kJ e 373,0±1,3 kJ, respectivamente), sendo 46% da energia proveniente do refrigerante. O grupo refrigerante calórico consumiu 22% mais carboidrato, especialmente sacarose, comparado ao Grupo controle (p<0,05). A ingestão de macronutrientes não foi diferente entre o Grupo controle e o Grupo refrigerante não calórico, mas o Grupo refrigerante calórico consumiu menos proteína e lipídios que os outros dois (3,5±1,0 g de proteína vs. 6,2±0,1 e 6,7±0,1 g, respectivamente; 0,7±0,01 g de lipídios vs. 1,3±0,02 g e 1,4±0,02 g, respectivamente). O consumo de refrigerante não calórico aumentou a ingestão total de sódio e o consumo de ambos os refrigerantes diminuiu a ingestão de água. Embora a massa corporal tenha variado durante o experimento, não houve diferença significativa entre os grupos ao final do mesmo e, igualmente, não houve diferença no depósito adiposo, glicose, insulina e leptina em jejum, índice de resistência à insulina e perfil lipídico.

Conclusões: A ingestão de ambos os refrigerantes (calórico e não calórico) não afetou a ingestão de energia, composição e massa corporal e parâmetros metabólicos, entretanto aumentou a ingestão de fluidos e diminuiu a de água. A ingestão de refrigerante calórico influenciou a quantidade e qualidade de comida sólida consumida, comprometendo a qualidade da dieta.

DESCRITORES: refrigerantes; consumo alimentar; macronutrientes.

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Abbreviations: ANOVA, analysis of variance; CON, Control group; CS, Caloric soft drink group; NCS, Non-caloric soft drink group; ELISA, enzyme-linked immunosorbent assay; HDL-cholesterol, high-density lipoprotein; HOMA-IR, insulin resistance index; UFRGS, Universidade Federal do Rio Grande do Sul.

INTRODUCTION

Some epidemiological studies suggest that consumption of soft drinks may be associated with the obesity epidemic, development of type 2 diabetes mellitus, and metabolic syndrome [1]. Soft drink consumption, regarded as a public health problem [2-4], has risen in countries such as Japan, the United States, and Brazil [5-7]. In Brazil, according to data from the "Family Budget Survey," cola soft drinks were the top selling flavored carbonated beverages in 2008 and 2009, and the average per capita consumption has increased by approximately 92% over the past 6 years [8].

The association between diet quality and the consumption of both caloric and non-caloric soft drinks has been poorly explored, especially in controlled studies. Studies evaluating the effects of caloric soft drinks on eating habits are underway, and some of them suggest that caloric soft drinks influence the amount and quality of nutrient intake in humans, leading to a low-quality diet [2,7]. Besides the relationship between disease and diet quality, experimental studies have found that soft drink consumption is associated with a reduction in solid food intake [9-11].

The effect of the consumption of these beverages on food and energy intake is still controversial. Milei et al. [10] reported no change in food intake and total energy intake in male rats treated with non-caloric soft drinks while Swithers and Davidson [12] reported an increase in total energy intake in male rats given non-caloric beverages. Thus, the aim of this study was to evaluate the effect of caloric and non-caloric soft drink intake on food consumption, body weight and composition, and metabolic parameters in rats.

METHODS

Experimental Procedures

The sample size was calculated with the WinPepi v. 11.1 software. Significance was set at 0.05 with an 80% power using the study of Milei et al. [10] as reference. A sample of 30 animals was set to account for foreseeable failure to complete the protocol.

Twenty-one-day-old male Wistar rats were weaned and procured from the Laboratory of Animal Reproduction and Experimentation of the Federal University of Rio Grande do Sul (UFRGS). On the day of weaning, the animals were divided into three groups,and given food and beverage *ad libitum* for 17 weeks. The control group (CON, n=10) received standard chow and water; the caloric soft drink group (CS, n=10) received standard chow, water, and caloric soft drink; and the non-caloric soft drink group (NCS, n=10) received standard chow, water, and non-caloric soft drink. This time length was based on our previous experience with highly palatable diet in promoting changes in glucose, lipids, and body weight [13,14]. Moreover, we aimed to see the animals' metabolic responses to chronic soft drink intake from their childhood to adulthood.

The animals were kept in plastic boxes (3-4 animals per box) in an environment with a light-dark cycle (lights on at 7 a.m. and off at 7 p.m.) and controlled temperature (24-26 °C). Nuvilab CR-1 (NUVITAL®, Curitiba, PR, Brazil) rat and mouse chow, with an energy density of 2.95 kcal/g, 2.7 mg/g of sodium, and a macronutrient ratio of 55% carbohydrate, 22% protein, and 4.5% lipids, was used.

The soft drinks were purchased in local shops and the nutritional values were obtained from the manufacturer's website (Coca-Cola®). The nutritional composition of regular Coca-Cola® was 1.79 kJ/mL; 0.11 g/mL carbohydrate, 0.05 mg/mL of sodium; and that of Zero Coca-Cola® was 0.0 kJ/mL; 0.0 carbohydrate; 0.28 mg/mL of sodium.

The animals' food and drink intake was controlled daily by the staff between 2 p.m. and 3 p.m. and the daily consumption of food and drink was calculated by subtracting what was offered from what remained after 24 hours. This value was summed and divided by the number of animals in the box, resulting in individual intake estimates.

All the experimental procedures were performed in accordance with Act No. 11.794/2008 [15] and in accordance with the International Guiding Principles for Biomedical Research Involving Animals, developed by the Council for International Organizations of Medical Sciences. The experimental protocol, registered under no. 21811, was approved by the UFRGS Animal Ethics Committee.

Body composition and blood collection

Body weight was assessed at baseline, every three days, and at the end of the experiment. After 17 weeks,

the animals were euthanized by decapitation after a 12-hour fasting period.

Blood was collected in test tubes and settled for 30 minutes before centrifugation. It was subsequently separated into aliquots and stored at -80 °C until the completion of serum analysis. The intra-abdominal adipose tissue and the perigonadal adipose tissue were removed and weighed jointly in a semi-analytical balance (Shimadzu BL3200).
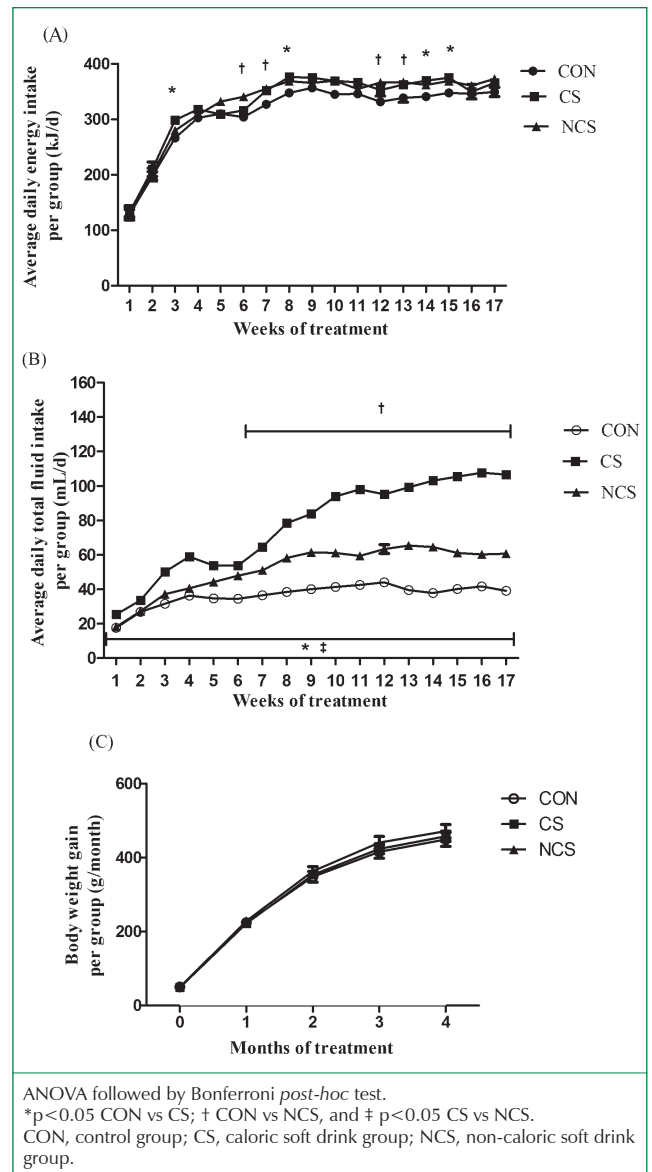
Serum biochemical analysis

Commercial kits were used to determine serum glucose concentration (Glucose PAP Liquiform, Labtest, Brazil) and lipid profile (Cholesterol Liquiform, HDL-cholesterol, Triglycerides Liquiform – Labtest, Brazil). Serum insulin and leptin were determined by ELISA (Enzyme-Linked Immunosorbent Assay).

Statistical Analysis

The distribution of variables was analyzed using the Shapiro-Wilk test and values were expressed as mean and standard error. The effects of each treatment on the intake of chow, nutrients, energy, water, total fluid, body weight, adipose tissue weight, glucose, insulin, insulin resistance index (HOMA-IR), leptin, total cholesterol, HDL-cholesterol, and triglycerides were analyzed at the end of the experiment by analysis of variance (ANOVA) followed by Bonferroni post-hoc test. A Student's t test was used to analyze caloric and non-caloric soft drink intake at the end of experiment. The interactions between time and type of treatment regarding energy, total fluid, and chow and nutrient intake over the 17 weeks were analyzed by repeated measures ANOVA followed by Bonferroni post-hoc test. The correlations were assessed by Pearson's correlation coefficients. All analyses were performed with the GraphPad Prism software, version 5.04 (GraphPad Software, Inc, CA, USA), and a $p < 0.05$ was regarded as statistically significant.

RESULTS

During the experiment, the average daily energy intake from chow and fluids was not different between groups (**p>0.05, Figure 1A**); however, there was a significant interaction between time and type of treatment, demonstrated by the difference in the initial and final values of energy intake [$F(288;32)=1.66$; $p<0.05$] (**Figure 1A**). Despite the similarity in total

ANOVA followed by Bonferroni *post-hoc* test.
*p<0.05 CON vs CS; † CON vs NCS, and ‡ p<0.05 CS vs NCS.
CON, control group; CS, caloric soft drink group; NCS, non-caloric soft drink group.

Figure 1. (**A**) Average daily energy intake per animal per group (kJ/day), (**B**) Average daily total fluid intake per animal per group (mL/day) and (**C**) Body weight gain per animal per group (g/month) during 17 weeks (4 months) of experiment.

energy consumption, the average daily energy intake from the chow was approximately 45% lower in the CS group compared to the CON and NCS groups ($p<0.05$, **Table 1**). The CS group had 46% of energy ingested from soft drinks. The CON and NCS groups showed no difference in energy intake ($p>0.05$, **Table 1**).

The average daily intake of carbohydrates, proteins, and lipids from the chow, and of total sodium, was significantly lower in the CS group compared to the CON and NCS groups ($p<0.05$, **Table 1**). However,

the CS group consumed around 10.2±0.2 g/day of carbohydrates (sucrose) from soft drinks, totaling 19.1 g carbohydrate/day and a 22% higher consumption of carbohydrates/day compared to the other groups (**Table 1**). The NCS group showed a higher consumption of sodium (p<0.05, **Table 1**) when compared to the CON and CS groups, and this was attributed to the sodium content inherent to artificial sweeteners present in non-caloric soft drinks.

The total daily intake of fluids (water and soft drinks) was influenced by time and type of treatment, demonstrated by the difference in the initial and final values of total fluid intake [F(288;32)=66.3; p<0.05] (**Figure 1B**). This intake was significantly higher in the CS group when compared to the CON and NCS groups and higher in the NCS group when compared to the CON group, especially from the 6th to the 17th weeks (p<0.05, **Figure 1B**). The CS group consumed significantly less water when compared to the CON and NCS groups (p<0.05, **Table 1**) and the NCS group consumed less water compared to the CON group (p<0.05, **Table 1**). Soft drink intake was responsible for the largest total fluid consumption in the CS group (three times more than in the CON group) and in the NCS group (two times more than in the CON group) (**Figure 1B**).

There was no significant difference in weight gain (**Figure 1C**) or weight of intra-abdominal and perigonadal adipose tissue (data not shown) between the groups at the end of the experiment.

The daily chow and nutrient consumption during the experiment is illustrated in **Figure 2**. There was a significant interaction between time and type of treatment, demonstrated by the difference in the initial and final values of daily chow intake [F(288;32)=19.67; p<0.05] (**Figure 2A**), carbohydrate intake [F(288;32)=4.47; p<0.05] (**Figure 2B**), protein intake [F(288;32)=19.61; p<0.05] (**Figure 2C**), and lipid intake [F(288;32)=20.01; p<0.05] (**Figure 2D**). From the beginning, the ingested amounts of chow, protein, and lipid were significantly lower in the CS group as compared to the CON and NCS groups (p<0.05, **Figure 2A, C** and **D**, respectively). However, the average daily intake of carbohydrates was significantly higher in the CS group as compared to the CON and NCS groups in the third and fourth weeks, and from the seventh week to the end of treatment, especially due to sucrose consumption from soft drinks (p<0.05, **Figure 2B**).

Fasting glucose, lipid profile, leptin, insulin, and HOMA-IR did not differ between groups at the end of the experiment (**Table 2**). Correlations between insulin and chow intake, insulin and energy intake, leptin and chow intake, and leptin and energy intake were tested; however, no significance was observed (data not shown).

Table 1. Average intake of standard chow, energy, nutrients, and fluids per animal at the end of the experiment (17th week)

	CON (n=10) Mean±SE	CS (n=10) Mean±SE	NCS (n=10) Mean±SE
Total energy (chow + soft drink) (kJ/day)	349.4±2.0	365.7±1.2	373.0±1.3
Standard chow energy (kJ/day)	349.4±2.0[a]	198.7±0.7[b]	373.0±1.3[a]
Soft drink energy (kJ/day)	–	166.9±0.7	–
Standard chow (g/day)	28.3±0.7[a]	16.1±0.4[b]	30.2±0.5[a]
Chow carbohydrate (g/day)	15.6±0.4[a]	8.9±0.2[b]	16.6±0.3[a]
Soft drink carbohydrate (g/day)	–	10.2±0.2	–
Chow protein (g/day)	6.2±0.1[a]	3.5±1.0[b]	6.7±0.1[a]
Chow lipids (g/day)	1.3±0.02[a]	0.7±0.01[b]	1.4±0.02[a]
Total Na (mg/day)	76.4±1.8[a]	48.1±1.1[b]	92.6±1.1[c]
Soft drink Na (mg/day)	–	4.6±1.1[a]	11.0±0.3[b]
Total water (mL/day)	39.1±1.0[a]	13.8±1.1[b]	21.5±1.0[c]
Total soft drink (mL/day)	–	92.811.2[a]	39.2±1.0[b]

ANOVA with Bonferroni post-hoc test. Student's t test was used to assess the amount of sodium in soft drinks.
[a,b,c] Different letters in the same row represent a significant difference between groups (p<0.05).
CON, control group; CS, caloric soft drink group; NCS, non-caloric soft drink group; SE, standard error.

ANOVA followed by Bonferroni *post-hoc* test.
*p<0.05 CON vs CS; † CON vs NCS, and ‡ p<0.05 CS vs NCS.
CON, control group; CS, caloric soft drink group; NCS, non-caloric soft drink group.
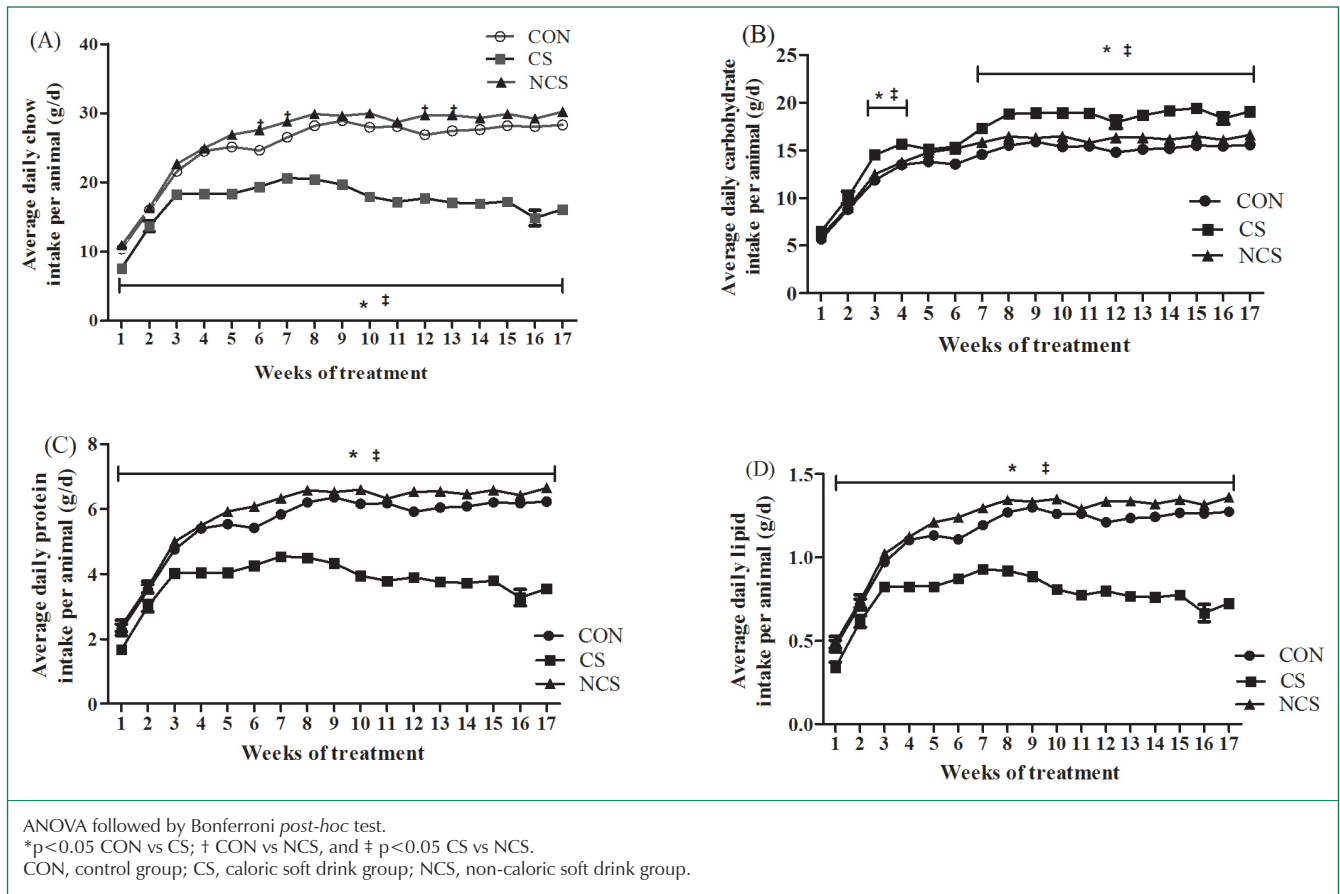
Figure 2. (**A**) Average daily standard chow intake per animal per group (g/day), (**B**) Average daily carbohydrate intake per animal per group (g/day), (**C**) Average daily protein intake per animal per group (g/day), and (**D**) Average daily lipid intake per animal per group (g/day) during 17 weeks (4 months) of experiment.

Table 2. Serum levels of glucose, insulin, leptin, HDL-cholesterol, total cholesterol, triglycerides, and HOMA-IR values at the end of the experiment (17th week)

	CON (n=10) Mean±SE	CS (n=10) Mean±SE	NCS (n=10) Mean±SE	p value*
Glucose (mg/dL)	110.3±3.7	110.9±2.9	111.9±4.7	0.986
Insulin (pmol/L)	2.6±0.3	3.0±0.3	3.3±0.3	0.183
HOMA-IR	0.10±0.04	0.14±0.04	0.15±0.04	0.191
Leptin (mg/dL)	5.7±0.7	6.3±0.5	5.3±0.7	0.592
HDL-c (mg/dL)	36.6±1.8	35.3±1.7	34.7±1.2	0.663
Total cholesterol (mg/dL)	55.3±2.0	49.4±1.9	51.0±1.5	0.080
Triglycerides (mg/dL)	84.9±6.1	106.8±5.6	81.2±13.2	0.115

*ANOVA with Bonferroni post-hoc test.
CON, control group; CS, caloric soft drink group; NCS, non-caloric soft drink group; SE, standard error; HOMA-IR, insulin resistance index; HDL-c, high-density lipoprotein.

DISCUSSION

The data presented in this study demonstrate that caloric soft drink intake resulted in a drastic reduction of solid food intake and in an increase in total fluid intake (largely due to soft drink consumption). This resulted in a lower intake of lipids and proteins and in a higher intake of simple carbohydrates.

On the other hand, the presence of non-caloric soft drinks did not influence total intake of solid foods at the

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end of treatment, but there was an increase in sodium intake and in total fluid consumption. However, this increase was smaller than that provided by caloric soft drink intake. Despite all these changes, there was no difference in daily energy intake, body composition, or metabolic parameters.

Similarly to the results found in this study, an experiment conducted with male rats for 6 months showed lower standard chow intake and increased fluid intake in animals treated with caloric soft drinks [10]. Likewise, other authors have reported a decrease in the ingestion of solid food in animals that received caloric soft drinks [9-12] and other sucrose-rich artificial beverages, and an increase in total fluid consumption due to the consumption of these beverages [16,17].

A mechanism which may be responsible for the excessive intake of and preference for sucrose-containing soft drinks involves the reward and dopaminergic systems, which contribute to increased intake of palatable foods, rich in sugar and/or fat. It is known that the sucrose present in caloric soft drinks is a highly palatable nutrient, capable of increasing the extracellular levels of dopamine in the nucleus accumbens (brain area related to the reward circuit) in proportion to the ingested concentration, thus favoring the continued intake of and preference for this nutrient [18,19].

Moreover, the compensation of excessive caloric soft drink intake by a lower chow intake can be attributed to regulatory mechanisms of energy homeostasis by insulin, both in the hypothalamus and in the amygdala, which are two important brain sites that regulate food intake [20]. Recent data have shown that insulin receptors in the brain play an important role in weight maintenance, appetite, and energy balance, regulating the required and consumed energy [21]. This hypothesis is strengthened by the data that indicate a reduction in chow intake by animals that drank non-caloric soft drinks; theoretically, they had no greater insulin spikes.

The reduction in chow intake can also be attributed to constant gastric distension caused by soft drink ingestion, due to gastrointestinal hormones (cholecystokinin and glucagon-like peptide 1), which act in the hypothalamus and stimulate the feeling of satiety. This factor mainly depends on the activation of mechanoreceptors in these areas of the gastrointestinal tract, which results in the transmission of signals to sensory fibers of the vagus nerve. Another mechanism proposed to explain the decrease in the intake of solid foods is the glucostatic theory, which postulates that an increase in glycemia followed by a subsequent increase in glucose utilization in glucose-sensitive brain regions (ventromedial hypothalamus) leads to a reduction in hunger and cessation of the desire to eat by inhibiting regions of the lateral hypothalamus, which induces the suppression of food intake [22-24].

In the present study, it was also observed that water intake was mainly replaced with caloric and non-caloric soft drinks. Water represented 13% of the total fluid intake in the CS group and 35% in the NCS group. The preference for soft drinks instead of water refers once again to the palatability of food and brain activations linked to pleasure (limbic system), although sucrose activates more brain regions, such as the left ventral striatum, the left dorsal caudate nucleus, the mesencephalon, and bilateral thalamus, than do sweeteners [25]. This reflects a greater consumption of caloric than non-caloric soft drinks.

The intake of caloric soft drinks by the CS group led to a higher consumption of carbohydrates, namely sucrose (approximately 53% of the total carbohydrate intake). Excessive consumption of sucrose from caloric soft drinks has been increasingly related to various health problems such as childhood obesity [26] and type 2 diabetes mellitus [27], which are strongly associated with the development of non-communicable chronic diseases. Based on this association, a recent study by Eyles et al. [28] suggests that raising taxes on the sale of this type of drink and subsidizing the production of fruits and vegetables would be a potential strategy to promote beneficial changes in the dietary pattern of the population.

When comparing the effect of caloric soft drink to non-caloric soft drink intake, the latter seems to be less harmful to food intake, and recent studies have suggested that there are inconsistent data in the current literature to confirm that non-caloric soft drink increases the risk of obesity and of chronic diseases or to recommend or refute them [29,30]. However, non-caloric soft drink is high in sodium, which is demonstrated in the present study by a 12% increase in total sodium intake only by the non-caloric soft drink group. High sodium intake is associated with high blood pressure and cardiovascular disease [31]. Even if non-caloric soft drinks do not contain sucrose and their association with obesity is questioned, it is suggested that its consumption be avoided due to the amount of sodium they provide.

A limitation of this study is the short treatment time. However, studies that compared the effects of consuming fluids with caloric or non-caloric sweeteners have heterogeneous treatment periods [9-12]. Unlike studies that use adult rats, our goal was to start

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treatment early in order to mimic children receiving soft drink in their childhood. We hypothesized that, during the animals' development (from weaning), the chance to rupture food intake and hormonal control mechanisms in adulthood could be higher. Besides, other studies of the same research group found alterations in glucose, lipids, and body composition after 4 months of treatment with high levels of sugar intake [13,14]. As opposed to other studies [9,10], the present data show no differences in body composition, weight, or biochemical parameters, which may be credited to the absence of difference in total energy intake between groups. Furthermore, previous studies conducted experiments for longer periods, which may explain the difference in the present results, which were obtained from experimental conditions over 17 weeks.

Although there were no differences in these variables, it is important to highlight the impact of soft drink consumption on the nutritional quality of the diet, even in a relatively short period of time. A low-quality diet is one of the factors associated with the large increase in non-communicable chronic diseases worldwide, which are the leading causes of death around the globe [2,7]. In this way, an improvement in diet quality is considered one of the most promising strategies to prevent these harms.

The ingestion of caloric and non-caloric soft drinks did not change daily energy intake, body weight and composition, or the metabolic parameters evaluated. However, the consumption of caloric soft drinks led to a lower intake of chow, protein, and lipids, and to a higher intake of carbohydrates and sucrose. Non-caloric soft drink consumption did not affect food intake, but increased total sodium intake. Both beverages led to an increase in fluid intake caused by increased soft drink intake. These changes in the pattern of food intake compromised the quality of the diet and turned out to be a risk factor for the development of non-communicable chronic diseases.

Conflicts of interest disclosure

The authors declare no potential conflicts of interest relevant to the content of this study.

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