ABSTRACT

Objectives: To evaluate the effects of soft drink and/or cafeteria diet consumption on eating behavior and metabolic parameters in rats. Material and Methods: Two months male Wistar rats were treated for twelve weeks, divided into groups: 1) CON: standard chow and water (SCW); 2) CD: cafeteria diet and SCW; 3) CS: caloric soft drink and SCW; 4) NCS: non-caloric soft drink and SCW; 5) CD+CS: cafeteria diet, caloric soft drink and SCW; and 6) CD+NCS: cafeteria diet, non-caloric soft drink and SCW. Results: The cafeteria diet intake resulted in higher energy consumption (p<0.0001), a lipid consumption increase (p<0.0001), and a protein reduction intake (p<0.0001), which contributed to an increase in body weight (p<0.0001) compared to the controls. There was a correlation between caffeine and carbohydrate consumption in the CS, CD+CS, and CD+NCS groups, as well as between leptin and liposomatic index in the same groups. Conclusion: The animals fed with a cafeteria diet...
consumed more ultra-processed foods, resulting in greater weight gain, and visceral fat. The animals that received the cafeteria diet and non-caloric soda had less visceral fat compared to the other cafeteria diet groups but further studies are needed, as it is an unhealthy beverage.

Keywords: soft drink; food intake; feeding behavior; cafeteria diet.

RESUMO

Objetivos: Avaliar os efeitos do consumo de refrigerantes e/ou dieta de cafeteria no comportamento alimentar e parâmetros metabólicos em ratos. Material e Métodos: Ratios Wistar machos de dois meses foram tratados por doze semanas, divididos em grupos: 1) CON: ração padrão e água (RPA); 2) DC: dieta de cafeteria e RPA; 3) RC: refrigerante calórico e RPA; 4) RNC: refrigerante não-calórico e RPA; 5) DC+RC: dieta de cafeteria, refrigerante calórico e RPA; e 6) DC+RNC: dieta de cafeteria, refrigerante não-calórico e RPA. Resultados: A ingestão da dieta de cafeteria resultou em maior consumo de energia (p<0,0001), aumento do consumo de lipídios (p<0,0001) e redução na ingestão de proteínas (p<0,0001), contribuindo para o aumento do peso corporal (p<0,0001) comparado aos controles. Houve correlação entre consumo de cafeína e carboidrato nos grupos RC, DC+RC e DC+RNC, assim como entre leptina e índice lipossomático nos mesmos grupos. Conclusão: Os animais alimentados com dieta de cafeteria consumiram mais alimentos ultraprocessados, resultando em maior ganho de peso e gordura visceral. Os animais que receberam dieta de cafeteria e refrigerante não-calórico apresentaram menos gordura visceral em comparação aos outros grupos dieta de cafeteria, porém são necessários estudos mais aprofundados, por ser uma bebida não saudável.

Palavras-chave: refrigerante; ingestão de alimentos; comportamento alimentar; dieta de cafeteria.

INTRODUCTION

Consumption of soft drinks (caloric beverages) and highly palatable food is changing eating habits. These foods, also known as ultra-processed foods, contain a low nutritional value and because they are very palatable, are consumed excessively, resulting in high-calorie intake in the form of simple carbohydrates and fats, and reduced fiber, mineral, and vitamin consumption 1–3. Those who consume large amounts of ultra-processed food consume an insufficient quantity of fruits and vegetables, which contributes to the increase in obesity worldwide 3,4. Obesity is a multifactorial disease, which can be caused by excessive consumption of food and ultra-processed food with a marked increase in energy consumption 3,5,6. Overweight and obesity are the main risk factors for several chronic non-communicable diseases, including type 2 diabetes mellitus (DM2), heart disease, hypertension, asthma, arthritis, metabolic syndrome, and various types of cancers 7. Unfortunately, overweight and obesity are not just a concern in developed countries, but also in developing countries, especially in urban environments. According to the World Health Organization, in 2016, more than 1.9 billion adults were diagnosed as overweight, of which 650 million were obese 8.

The high rates of obesity and overweight presented above are closely related to dietary patterns centered on highly palatable foods. It is known that the act of eating has a complex involvement of pleasure and reward with motivations and behaviors involving several neural systems 9–11. Food dependency has been described in the literature, despite not being formally recognized by the Diagnostic and Statistical Manual of Mental Disorders-5 12. However, some studies have indicated changes in the brain reward circuit induced by excessive consumption of highly palatable foods, like those observed in substance use disorder. Regarding differences between foods, it has been proposed that addiction is particularly associated with ultra-processed foods 13. They have a high glycemic load (because they are rich in sugar and/or other refined carbohydrates), rich in fat, or both. The high palatability of these foods results from the sweet, salty, or umami flavor, which
is associated with high caloric density14. Excessive sugar intake does not seem to affect body weight but combining the intake of sweets with fat results in increased body weight15. Fat can be the nutrient that leads to excess weight, and the sweet taste can be the main contributor to the production of addictive behavior, including withdrawal syndrome16.

The cafeteria diet (composed of ultra-processed food) has been widely used as a model for inducing obesity in laboratory animals because of its great similarity with genesis and metabolic responses due to obesity in humans17–19. The animal model advantage concerning researches with humans is the possibility to estimate very closely the consumption of food and fluids of animals.

In a previous study20, the supply of a balanced diet and caloric or non-caloric soft drinks did not cause changes in total energy intake, body weight, and intra-abdominal and perigonadal fat deposition in rats during the 17 weeks of exposure. However, caloric soft drink intake decreased the consumption of standard chow, with a consequent reduction in consumption of nutrients such as vitamins and minerals. Interestingly, the non-caloric soft drink intake did not influence food consumption. Both caloric and non-caloric soft drinks increased the total liquid consumption with a strong decrease in water consumption. These results motivated the investigation into the feeding behavior of the animals in the face of exposure to caloric and non-caloric soft drinks and the offer of highly palatable solid foods. Due to the lack of evidence on the effects of the association of caloric and non-caloric soft drink intake with an ultra-processed and highly palatable diet, this study aimed to evaluate the effects of soft drink and/or cafeteria diet intake on the eating behavior and metabolic parameters in rats that were fed a cafeteria diet.

**MATERIAL AND METHODS**

*Experimental Procedures*

The sample size was calculated using WINPEPI v. 11.1 software. Significance was set at 0.05 with 80% power, using the Milagro et al. study as a reference21. A sample of 10 animals per group was set to account for foreseeable failure to complete the protocol. A total of sixty, 2 months old, male Wistar rats were provided by the Laboratory of Animal Reproduction and Experimentation of the Universidade Federal do Rio Grande do Sul. The rats were acclimatized to their environment for one week before the start of the experiment. The experiments and procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 13-0136), were compliant with Brazilian guidelines involving the use of animals in research (Federal Law 11,794/2008) and were performed in accordance with the International Guidelines for Animal Welfare. The minimum number of animals required to produce reliable scientific data was used, and efforts were made to minimize suffering and external sources of pain and discomfort.

All animals were kept in polypropylene boxes (2 animals/box) in a 12-hour dark-light cycle, with humidity between 70% and 80%, a temperature equal to 22 ± 2 °C, and were provided food and beverage *ad libitum* for 12 weeks. The animals were randomized for weight according to previous studies22,23 and separated into six experimental groups, with ten animals each group, as represented in the Figure 1.
Figure 1. Experimental Groups: 1. Control group (CON); 2. Caloric soft drink group (CS); 3. Non-caloric soft drink group (NCS); 4. Cafeteria diet group (CD); 5. Cafeteria diet with caloric soft drink group (CD+CS); 6. Cafeteria diet with a non-caloric soft drink (CD+NCS).

Experimental Diet

The foods included in the cafeteria diet were sandwich cookies, wafers, sausages, chips, and condensed milk. The cafeteria diet was based on previous studies\textsuperscript{17,18,23}. The standard chow and all foods in the cafeteria diet were offered fresh daily, the same food items daily. The nutritional composition of the diets is shown in Table 1. The animals receiving the cafeteria diet also had access to standard chow and water. The control of the animals’ food and drink intake were performed simultaneously by the staff daily. Daily food and drink consumptions were calculated by subtracting the amount offered from leftovers after 24 h. This value was summed up and divided by the number of animals in the box (two animals/box), resulting in individual intake estimates, and divided by the average weight of the animals.

Weight parameters and liposomatic index

Body weight was assessed weekly, from the beginning to the end of the dietary treatment. After 12 weeks, the animals were euthanized by decapitation after a 12-hour fasting period. The weight at the start of treatment was subtracted from the last recorded weight before euthanasia, to obtain the total weight gain of the animals. The intra-abdominal (retroperitoneal and mesenteric) and perigonadal adipose tissue were obtained by dissecting the animals’ fat after euthanasia and weighed jointly in a semi-analytical balance (Shimadzu BL3200), as described by Cinti (2005)\textsuperscript{24}. The visceral fat was obtained from the combination of intra-abdominal and perigonadal adipose tissues and used to calculate the liposomatic index (LI). The LI was calculated by dividing the weight of visceral fat by the body weight of the animals at the end of 12 weeks.
Table 1. Nutritional composition of standard chow, cafeteria diet foods, and soft drink

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard chow</th>
<th>Snack Yokitos</th>
<th>Sandwich cookies strawberry</th>
<th>Wafer chocolate</th>
<th>Condensed milk</th>
<th>Frankfurter</th>
<th>Regular soft drink cola</th>
<th>Zero soft drink cola</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>1,234.0</td>
<td>1,916.0</td>
<td>2,016.0</td>
<td>1,920.0</td>
<td>1,345.0</td>
<td>820.0</td>
<td>178.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>40.5</td>
<td>64.0</td>
<td>70.0</td>
<td>53.3</td>
<td>55.0</td>
<td>4.8</td>
<td>11.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Protein</td>
<td>55.0</td>
<td>48.4</td>
<td>54.4</td>
<td>48.4</td>
<td>68.7</td>
<td>8.7</td>
<td>98.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Lipids</td>
<td>16.2</td>
<td>6.8</td>
<td>5.0</td>
<td>4.7</td>
<td>7.5</td>
<td>14.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sodium</td>
<td>270.0</td>
<td>640.0</td>
<td>223.3</td>
<td>163.3</td>
<td>90.0</td>
<td>1,082.0</td>
<td>5.0</td>
<td>28.0</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Data obtained from the manufacturer’s website. *Nuvilab CR-1, Brazil; b Yoki, Brazil; c Kraft Foods Brasil; d Bauducco, Brazil; e Elegê, Brazil; f Excelsior, gCoca-Cola, Brazil®.

*Values are expressed in grams of nutrients (carbohydrates, protein, and lipids) in 100g of food.

Biological material collection and biochemical assays

After 12 weeks, the animals were euthanized by decapitation after a 12-hour fasting period. Blood was collected, after euthanasia of the animals, and placed in test tubes and allowed to rest for 30 minutes before centrifugation. It was subsequently separated into aliquots and stored at -80 °C until the completion of serum analysis. Commercial kits were used to determine serum glucose concentration (Glucose PAP Liquiform, Labtest, Brazil) and lipid profile (Cholesterol Liquiform, HDL-cholesterol, and Triglycerides Liquiform, Labtest, Brazil). Serum insulin and leptin levels were determined by enzyme-linked immunosorbent assay (ELISA) using reagents specific for rats (Millipore), with a detection sensitivity of 0.2 ng/mL. The homeostasis model assessment of insulin resistance (HOMA-IR) was used to calculate approximate insulin resistance (fasting glucose [mg/dL] × fasting insulin [mIU/mL] /2430)\(^{25}\).

Statistical Analysis

The distribution of variables was analyzed using the Kolmogorov-Smirnov test, and values were expressed as the mean and standard error. The effects of each treatment on the intake of chow, nutrients, energy, water, total liquid, body weight, adipose tissue weight, glucose, insulin, leptin, total cholesterol, HDL-cholesterol, triglycerides, and HOMA-IR were analyzed at the end of the treatment by one-way ANOVA with Tukey’s post hoc test. Student’s t-test was used to analyze the energy and carbohydrate intake of the two groups receiving caloric soft drinks at the end of the experiment, while correlation analyses were performed using Pearson correlation coefficient. All analyses were performed using SPSS®, version 18, with \(p<0.05\) being considered statistically significant.

RESULTS

Food, Beverages, and Energy Intake

There was a significant difference between the groups in chow intake (\(p>0.0001\)) (Figure 2A). In
the non-cafeteria groups, only the CS group showed a significant reduction in chow intake ($p<0.001$) when compared to the CON group (Figure 2A). The groups that received cafeteria diet (CD, CD+CS, and CD+NCS) showed decreased chow intake when compared to their respective non-cafeteria groups ($p<0.001$ for all) (Figure 2A). The animal groups that received the cafeteria diet consumed similar amounts of ultra-processed foods ($p>0.08$) (Figure 2B). Total solid food intake was similar among groups, except for the CS group, which presented a significant reduction in solid intake when compared to all other groups ($p<0.001$) (Figure 2C). There was a difference in water intake between the groups ($p<0.0001$) (Figure 2D). All groups showed decreased water intake when compared to the CON group ($p<0.001$ for all). The CS and CD+CS groups presented similar water intake; however, NCS drank significantly more water than CD+NCS ($p=0.003$). The consumption of caloric soft drinks was almost twice as high in the presence of standard chow when compared with CD+CS, as well as other groups that received caloric soft drinks (Figure 2E). The CS and CD+CS groups’ animals consumed more total liquids compared to the other groups (Figure 2F). Comparing only solid energy intake, the CS group consumed less energy compared to all other groups ($p<0.001$ for all) (Figure 2G). CON and NCS consumed similar solid energy intake and cafeteria groups consumed more than the standard chow groups ($p<0.001$ for all).The total energy intake was similar among the standard chow groups (Figure 2I), as well as among the cafeteria diet groups (Figure 2I). There was a significant difference in total energy intake, represented by the sum of solid and liquid energy between groups ($p<0.0001$) (Figure 2I). However, all cafeteria diet groups consumed more energy than the standard chow groups ($p<0.001$), except for the CS group that consumed total energy like the cafeteria group.

**Standard Chow and Experimental Diets Content**

There was a significant difference between the groups in chow intake ($p<0.0001$). Likewise, there was a significant difference between the groups in total carbohydrate intake ($p<0.0001$). In addition, the CS group had reduced carbohydrate intake in solid foods (standard chow), and increased carbohydrate intake in caloric soft drinks as compared to CON ($p<0.001$) and CD+CS ($p<0.001$) groups. The NCS group consumed fewer carbohydrates in total than the CS group ($p<0.001$), but similar to the other groups. There was a significant difference in protein intake between the groups ($p<0.0001$). The CS group consumed less protein than the CON ($p<0.001$) and NCS groups ($p<0.001$). Cafeteria diet intake led to a reduction in protein consumption in all groups when compared to the controls ($p<0.0001$). There was a significant difference in lipid intake between the groups ($p<0.0001$). Cafeteria diet groups (CD, CD+CS, and CD+NCS) consumed more lipids than their controls ($p<0.0001$ for all). No differences were observed among the cafeteria groups ($p>0.05$). There was a significant difference in total sodium intake between the groups ($p<0.001$). The total sodium intake in the cafeteria diet group was significantly higher than that in the CON ($p<0.0001$), CS ($p<0.001$), and NCS ($p<0.001$) groups. There was a significant difference in caffeine intake among the groups that received a soft drink ($p<0.0001$). The CS group showed an increase in caffeine intake compared to the CD + CS ($p<0.001$), NCS ($p<0.001$), and CD+NCS ($p<0.001$) groups. In contrast, the NCS group did not differ from the cafeteria group CD+NCS ($p>0.05$) (Table 2).
Figure 2. Average daily intake of: (A) standard chow (g/d), (B) Cafeteria diet (g/d), (C) Total solid food (g/d), (D) water (mL/day), (E) Soft drink (mL/day), (F) Total liquid (mL/day), (G) Solid food energy (kJ/day), (H) Soft drink energy (kJ/day), and (I) Total energy (kJ/day) during 12 weeks treatment. Data expressed as mean ± Standard Error (SE) of ten animals/group. CON (control); CS (caloric soft drink); NCS (non-caloric soft drink); CD (cafeteria diet); CD+CS (CD + caloric soft drink); NCS+CD (CD + non-caloric soft drink). a, b, c Different letters represent a significant difference (p<0.05; one way ANOVA with Tukey post hoc test).

Table 2. Nutrient daily average consumption during 12 weeks treatment

<table>
<thead>
<tr>
<th>Food/Beverage</th>
<th>CON ±SE</th>
<th>CS ±SE</th>
<th>NCS ±SE</th>
<th>CD ±SE</th>
<th>CD+CS ±SE</th>
<th>CD+NCS ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid carbohydrate (g)</td>
<td>14.6±0.3a</td>
<td>10.2±0.4b</td>
<td>14.5±0.4a</td>
<td>12.5±0.5c</td>
<td>10.6±0.4b</td>
<td>13.3±0.9ab,c</td>
</tr>
<tr>
<td>*Soft drink carbohydrate (g)</td>
<td>-</td>
<td>9.3±0.7a</td>
<td>-</td>
<td>-</td>
<td>5.5±0.3b</td>
<td>-</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>14.6±0.3ab,c</td>
<td>19.5±0.5b</td>
<td>14.5±0.4ab,c</td>
<td>12.5±0.5c</td>
<td>16.1±0.6b</td>
<td>13.3±0.9a</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.8±0.1a</td>
<td>4.1±0.1b</td>
<td>5.8±0.2a</td>
<td>2.9±0.2b</td>
<td>2.7±0.2a</td>
<td>2.9±0.1c</td>
</tr>
<tr>
<td>Lipid (g)</td>
<td>1.06±0.0a</td>
<td>0.7±0.0a</td>
<td>1.1±0.0a</td>
<td>4.8±0.2b</td>
<td>4.4±0.1b</td>
<td>4.5±0.2b</td>
</tr>
<tr>
<td>Solids Sodium (mg)</td>
<td>71.6±1.5a</td>
<td>49.9±1.8a</td>
<td>71.4±2.1a</td>
<td>180.7±10.9b</td>
<td>189.6±13.7b</td>
<td>161.0±8.7b</td>
</tr>
<tr>
<td>Liquids Sodium (mg)</td>
<td>-</td>
<td>4.3±0.3a</td>
<td>3.8±0.3a</td>
<td>-</td>
<td>2.8±0.3a</td>
<td>4.5±0.3a</td>
</tr>
<tr>
<td>Total Sodium (mg)</td>
<td>71.6±1.5a</td>
<td>54.3±1.6a</td>
<td>75.2±2.3a</td>
<td>180.7±10.9b</td>
<td>192.0±13.7b</td>
<td>165.6±8.9b</td>
</tr>
<tr>
<td>Caffeine (mg)</td>
<td>-</td>
<td>8.54±0.9a</td>
<td>2.7±0.6b</td>
<td>-</td>
<td>5.2±0.8c</td>
<td>3.24±1.5ab,c</td>
</tr>
</tbody>
</table>

Data expressed as mean ± Standard Error (SE) of ten animals/group. CON (control); CS (caloric soft drink); NCS (non-caloric soft drink); CD (cafeteria diet); CD+CS (CD+caloric soft drink); CD+NCS (CD+non-caloric soft drink). a, b, c Different letters represent a significant difference (p<0.05; one way ANOVA with Tukey post hoc test. We used the student's t-test for carbohydrate analysis in soft drinks. *Regular soft drink cola.
**Body Weight Gain and Liposomatic index**

The groups that received the cafeteria diet showed a significant increase in weight in relation to their controls ($p<0.0001$), as shown in percentage of weight gain in Table 3. The CD group showed increased the percentage of weight gain compared to the CON group ($p<0.001$), CD+CS compared to the CS group ($p<0.004$), and CD+NCS compared to the NCS group ($p<0.03$) (Table 3). Similar differences were observed in LI [CD versus CON ($p<0.0001$); CD+CS versus CS ($p<0.0001$); however, CD+NCS did not differ from the control NCS ($p<0.07$)]. Moreover, the CD+NCS group showed a decrease in LI compared to CD ($p<0.007$) and CD+CS groups ($p<0.008$); LI from NCS was not statistically different from the CD+NCS group (Table 3).

**Table 3.** Body weight before and after the treatment and Liposomatic index

<table>
<thead>
<tr>
<th>Variables</th>
<th>CON ±SE</th>
<th>CS ±SE</th>
<th>NCS ±SE</th>
<th>CD ±SE</th>
<th>CD+CS ±SE</th>
<th>CD+NCS ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>300.9</td>
<td>310.6</td>
<td>293.6</td>
<td>308.5</td>
<td>317.5</td>
<td>286.1</td>
</tr>
<tr>
<td>(g)</td>
<td>±12.7$^a$</td>
<td>±14.4$^a$</td>
<td>±10.1$^a$</td>
<td>±12.4$^a$</td>
<td>±15.7$^a$</td>
<td>±12.2$^a$</td>
</tr>
<tr>
<td>Final body weight</td>
<td>439.6</td>
<td>464.6</td>
<td>433.5</td>
<td>547.0</td>
<td>541.6</td>
<td>482.9</td>
</tr>
<tr>
<td>(g)</td>
<td>±10.1$^a$</td>
<td>±14.3$^b$</td>
<td>±11.6$^a$</td>
<td>±14.0$^c$</td>
<td>±12.7$^c$</td>
<td>±12.7$^b,c$</td>
</tr>
<tr>
<td>Weight gain percentage</td>
<td>46.0$^a$</td>
<td>49.6$^a$</td>
<td>47.6$^a$</td>
<td>77.3$^b$</td>
<td>70.6$^b$</td>
<td>68.8$^b$</td>
</tr>
<tr>
<td>Liposomatic index</td>
<td>0.025</td>
<td>0.024</td>
<td>0.027</td>
<td>0.063</td>
<td>0.071</td>
<td>0.049</td>
</tr>
<tr>
<td>(g)</td>
<td>±0.002$^a$</td>
<td>±0.003$^a$</td>
<td>±0.003$^b,c$</td>
<td>±0.005$^b$</td>
<td>±0.004$^b$</td>
<td>±0.007$^c$</td>
</tr>
</tbody>
</table>

*Liposomatic index (LI= weight of intra-abdominal and perigonadal adipose tissue divided by body weight) at the end of the experiment (12th week). Data expressed as mean ± Standard Error (SE) of ten animals/group. CON (control); CS (caloric soft drink); NCS (non-caloric soft drink); CD (cafeteria diet); CD+CS (CD+caloric soft drink); CD+NCS (CD+non-caloric soft drink). $^a,b,c$Different letters represent a significant difference ($p<0.05$; one way ANOVA with Tukey post hoc test).

**Biochemical Markers**

Serum biochemical levels at the end of treatment are shown in Table 4. Fasting glucose, total cholesterol, HDL cholesterol, and non-HDL cholesterol were similar for all groups at the end of treatment ($p>0.05$). There was a significant difference in HOMA-IR between the groups ($p<0.0001$). The cafeteria groups (CD and CD + CS) showed an increase in HOMA-IR as compared to CON groups [CD ($p<0.05$) and CD+CS ($p<0.0001$), respectively], but not in the CD+NCS group. A significant difference in triglyceride levels was identified between the groups ($p<0.0001$). The CD and CD+CS groups presented an increase in triglyceride levels as compared to the CON group ($p<0.001$ and $p<0.008$), CS group ($p<0.001$ and $p<0.01$), and NCS group ($p<0.002$ and $p<0.01$), without differences between the CD + NCS group and the control and cafeteria groups. Similar results were observed in insulin levels between the groups ($p<0.0001$). The CD and CD + CS groups showed a significant increase in insulin levels in relation to their controls [CON ($p<0.008$) and CD+CS ($p<0.0001$), respectively], except in the CD + NCS group. There was a significant difference in leptin levels between the groups ($p<0.0001$). The CD group showed an increase in leptin levels as compared to the CON, CS, and NCS groups ($p<0.004$, $p<0.002$, and $p<0.005$, respectively). The CD+CS group also showed an increase in leptin levels as compared to the CON ($p<0.001$), CS ($p<0.0001$), and NCS ($p<0.001$) groups. No difference was observed in the CD+NCS group as compared to the control and the other cafeteria groups ($p>0.05$).
Table 4. Serum biochemical levels at the end of the experiment (12th week)

<table>
<thead>
<tr>
<th>Variables</th>
<th>CON ± SE</th>
<th>CS ± SE</th>
<th>NCS ± SE</th>
<th>CD ± SE</th>
<th>CD+CS ± SE</th>
<th>CD+NCS ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>0.1±0.0a</td>
<td>0.05±0.0a</td>
<td>0.05±0.0a</td>
<td>0.12±0.0b</td>
<td>0.14±0.0b</td>
<td>0.1±0.0abc</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>65.7±9.9</td>
<td>67.5±9.9</td>
<td>69.7±9.9</td>
<td>127.9±10.9</td>
<td>118.3±10.5</td>
<td>89.9±9.9abc</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>1.3±0.2a</td>
<td>1.1±0.2a</td>
<td>1.2±0.3a</td>
<td>2.6±0.5b</td>
<td>3.0±0.5b</td>
<td>1.9±0.4abc</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>2.9±1.8a</td>
<td>2.3±1.8a</td>
<td>3.2±1.8a</td>
<td>13.3±1.9b</td>
<td>14.6±1.9b</td>
<td>8.5±1.7abc</td>
</tr>
</tbody>
</table>

Data expressed as mean ± Standard Error (SE) of ten animals/group. CON (control); CS (caloric soft drink); NCS (non-caloric soft drink); CD (cafeteria diet); CD+CS (CD+caloric soft drink); CD+NCS (CD+non-caloric soft drink). a, b, c Different letters represent a significant difference (p<0.05; one way ANOVA with Tukey post hoc test). HOMA-IR (homeostasis model assessment of insulin resistance).

Finally, correlation analyses were performed to assess the possible association between biochemical markers, morphological, and consumption parameters. There were statistically significant correlations between caffeine and carbohydrate consumption in CS (r = 0.731; p< 0.02), CD+CS (r = 0.947; p< 0.0001), and CD+NCS (r = 0.750; p< 0.001) groups. Also, relevant associations were found between Leptin and LI in CD (r = 0.873; p< 0.005), CD+CS (r = 0.897; p< 0.006), and CD+NCS (r = 0.871; p< 0.005) groups.

DISCUSSION

The present study demonstrates that experimental animals exposed to caloric soft drinks and/or cafeteria diet developed hyperphagia, significant changes in normal feeding patterns, and relevant correlations. These affirmations are made based on the results showing that exposure to caloric soft drinks decreases chow intake by 30%. This decrease in the consumption of standard chow was even more drastic when a caloric soft drink was offered in association with the cafeteria diet, leading to a drastic reduction (90%) in the consumption of standard feed. The caloric soft drink consumption was greater than the non-caloric soft drink intake regardless of its exposure alone (68% increase) or in conjunction with a cafeteria diet (38% increase). The chow intake of animals exposed to non-caloric soft drinks did not decrease compared to the control group, reflecting lower total energy consumption in non-caloric soft drinks compared to caloric soft drinks. However, the cafeteria diet was responsible for an increase in energy intake from solid foods. It was 32% more in CD than in CON, 24% more in CD+CS than in CS, and 34% more in CD+NCS than in NCS. These results demonstrated that the animals replaced the standard chow with caloric soft drinks and/or ultra-processed foods. Over the past few years, this concern has grown and motivated other studies that have shown similar results.

The higher caloric soft drink intake of the CS group resulted in an increase of 33% in carbohydrate consumption in the CON group, while the lipid and protein consumption was lower than that in the CON and NCS groups. However, the intake of non-caloric soda did not significantly alter the total solid foods and energy consumption, body weight gain, and LI when compared to the CON group. Owing to the greater consumption of soft drinks, the animals in the CS and NCS groups, which received only standard feed as solid food, consumed less water than CON. Water was replaced by caloric soda as it has a high content of sucrose (a simple carbohydrate) and is highly palatable. This is considered one of the main contributors to the increase in the number of overweight and obese people and abdominal adiposity. Similar results were found in another study by our group, which analyzed the effects of caloric and non-caloric soft drink consumption during 17 weeks of treatment in rats. A study on the food consumption in the Brazilian population, through a telephone survey, identified an increase in caloric soft drink consumption from 60% in 2007 to 67% in 2009.

Interestingly, although the CS group presented higher consumption of caffeine and carbohydrates, there was also a significant correlation between these diet components in the groups that received the
The dopaminergic system (reward system) may be involved in the increase in soda consumption, which in turn induces gastric distension and decreases the standard feed intake. When palatable foods such as those rich in sucrose are consumed, there is an increase in dopamine release in the nucleus accumbens, which stimulates food preference and increases sucrose intake, playing these roles in the preparatory and food consumption phases. The reward mechanism are also strongly involved with dorsal striatum and the orbitofrontal cortex. The feeling of satisfaction when eating palatable food, instead of generating acute pleasure, lasts for some time, which most likely contributes to the desire to eat again. Another factor that seems to contribute to reward generation during and after a meal is the action of nutrient sensors in the gastrointestinal tract.

The caloric soda intake and the consequent decrease in solid food consumption can be explained by the high palatability and satiety provided by caloric soda. Postprandial hyperglycemia activates satiety nuclei in the ventromedial hypothalamus, which inhibits the nucleus related to the feeding of the lateral hypothalamus and induces food intake suppression, which is known as the hunger glycostatic theory. The caloric soda consumption amount in the CS group was higher than that in CD + CS group because the latter, in addition to receiving caloric soda, received solid palatable food in the cafeteria diet.

The total solid food intake was similar for the groups that received the cafeteria diet, but higher than the CON, CS, and NCS groups in detriment of the standard feed consumption, demonstrating the high intake of the cafeteria diet. This resulted in high lipid and low protein consumption, leading to a 450% increase in the lipid consumption and a 50% reduction in the protein intake. The high palatability of the cafeteria diet contributes to increased energy consumption, triggering an increase in body fat, and consequent obesity in animal models and in humans. Our study showed a significant increase in the weight of animals receiving a cafeteria diet. These animals showed a 60% increase in body weight compared to animals in the control group, increased body fat, triglyceride, and leptin levels. These data reinforce the association between LI and leptin since leptin is produced by adipose tissue. Likewise, it was observed by another study carried out with Wistar rats fed a high-fat diet.

Thus, this diet increased the metabolic risk, as was observed, demonstrating HOMA changes, similar results were found in the study by Tunapong et al. with rats fed by high-fat diet. It should be noted that soft drink consumption can decrease adherence to healthy diets by decreasing the intake of nutrients such as fibers and micronutrients (e.g., folic acid and calcium) among children and young people. Another important result of our study is related to the increase in sodium levels among animals consuming the cafeteria diet. This result was expected because processed foods are rich in sodium content.

The use of drinks sweetened with ingredients that do not provide calories is controversial according to some authors. This study found that despite having solid food consumption and total energy like those in the CD and CD + CS groups, the CD + NCS group had a significantly lower LI, demonstrating less visceral fat deposition. The results of this study were different from those obtained by some studies which identified that artificial sweeteners caused an increase in body weight in the group that received sucrose, but the total energy consumption was not significantly different. However, a study carried out in Denmark observed an increase in weight and body fat in individuals who received drinks and foods sweetened with sucrose, and there was a reduction in body weight and body fat in individuals who received drinks and foods sweetened with artificial sweeteners. Similarly, another research found less weight gain in rats that received cola drinks and a high-fat diet as compared to controls who received the same diet but drank water for 28 weeks. The lower weight gain was attributed to the possible increase in muscle thermogenesis induced by caffeine in cola soft drinks. Other studies have also shown beneficial effects of caffeine on the adiposity of animals fed high-fat diets, demonstrating a reduction in body fat mass and percentage of body fat, possibly due to increased lipolysis via catecholamines. The lower LI in the CD + NCS group may be related to the lower insulinogetic effect of the non-caloric soda due to the greater presence of caffeine in the drink and the 17% reduction in carbohydrates compared to the CD + CS group. Caffeine generates a lipolytic effect by stimulating the release of adrenaline by...
the adrenal glands, thereby reducing lipogenesis\textsuperscript{46,49}. Another study demonstrated beneficial effects of caffeine consumption on health and a negative association with the incidence of DM2, as well as in helping control body weight\textsuperscript{50}. In this sense, our results demonstrate that the consumption of caffeine present in non-caloric cola-type soda prevented weight gain, but this effect may be more strongly related to the absence of sucrose. However, more studies are needed on the effects of calorie and non-caloric soda consumption with cafeteria diet on metabolism. It is also necessary to compare the sugary beverage intake effect with that of artificially sweetened and caffeine-free beverages associated with a cafeteria diet. It should be noted that this result needs to be interpreted carefully to avoid encouraging the consumption of non-caloric soda to the detriment of the consumption of water or healthier foods.

CONCLUSION

During the study period, the supply of balanced food along with caloric soda intake reduced solid food and water consumption and increased energy consumption and total liquid intake. Thus, caloric soda intake had a negative influence on the quantity and quality of consumed solid foods, causing a nutritional imbalance. The animals that received a cafeteria diet consumed more processed foods offered and did not ingest much standard feed, making their food intake low in nutritional value and causing an increase in the consumption of energy from sugars and lipids, greater weight gain, and visceral fat. We emphasize that the results regarding the consumption of non-caloric soft drinks need further studies, considering the consumption of this soft drink should not be encouraged.

Acknowledgments

The funding for this project was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (BR) (Grant - 482296/2013-5; Grant - 305395/2018-1) and Research Incentive Fund (FIPE Grant nº 13-0136).

Author Contribution

MEKH: coordinated the study design, was responsible for the experiment execution, analysis, and interpretation of data, literature review, and preparation of the manuscript.

ICM: contributed to the experiment execution, analysis, and interpretation of data, literature review, and preparation of the manuscript.

RUT: contributed to the experiment execution, literature review, analysis, and interpretation of data, and preparation of the manuscript.

ROF: contributed to the experiment execution, statistical analysis, data analysis, and revision of the manuscript.

JFG: contributed to the experiment execution, statistical analysis, data analysis, and revision of the manuscript.

GLS: contributed to the study design, data analysis, and revision of the manuscript.

ILT: contributed to the data analysis and revision of the manuscript.

Conflict of interest

Authors declare having no conflict of interest.
REFERENCES


