Research report

Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels

Fernanda de Matos Feijó, Cíntia Reis Ballard, Kelly Carraro Foletto, Bruna Aparecida Melo Batista, Alice Magagnin Neves, Maria Flávia Marques Ribeiro, Marcello Casaccia Bertoluci

Programa de Pós-Graduação em Medicina: Ciências Médicas, Faculdade de Medicina da Universidade Federal do Rio Grande do Sul, Ramiro Barcelos 2400, CEP 90035-003, Porto Alegre, Brazil
Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Sarmento Lette 500, CEP 90050-170, Porto Alegre, Brazil
Serviço de Medicina Interna, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, CEP 90035-903, Sala 700, 7º andar, Porto Alegre, Brazil

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A B S T R A C T

It has been suggested that the use of nonnutritive sweeteners (NNSs) can lead to weight gain, but evidence regarding their real effect on body weight and satiety is still inconclusive. Using a rat model, the present study compares the effect of saccharin and aspartame to sucrose in body weight gain and in caloric intake. Twenty-nine male Wistar rats received plain yogurt sweetened with 20% sucrose, 0.3% sodium saccharin or 0.4% aspartame, in addition to chow and water ad libitum, while physical activity was restrained. Measurements of cumulative body weight gain, total caloric intake, caloric intake of chow and caloric intake of sweetened yogurt were performed weekly for 12 weeks. Results showed that addition of either saccharin or aspartame to yogurt resulted in increased weight gain compared to addition of sucrose, however total caloric intake was similar among groups. In conclusion, greater weight gain was promoted by the use of saccharin or aspartame, compared with sucrose, and this weight gain was unrelated to caloric intake. We speculate that a decrease in energy expenditure or increase in fluid retention might be involved.

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Introduction

Increased NNS consumption has been historically associated with the increasing prevalence of obesity (Fowler et al., 2008; Popkin & Nielsen, 2003; Yang, 2010). It has also been suggested that either the use of saccharin or aspartame is associated with an increased feeling of hunger (Appleton & Blundell, 2007; Blundell & Green, 1996; Lavin, French, & Read, 1997; Rogers, Carlyle, Hill, & Blundell, 1988).

Rats whose diet was sweetened with saccharin for over 5 weeks, presented greater weight gain and adiposity as well as a decrease in the central body temperature when compared to glucose supplementation (Switers & Davidson, 2008). On the other hand, some studies show that exposure to NNS may help control body weight (Blackburn, Kanders, Lavin, Keller, & Whatley, 1997; Phelan, Lang, Jordan, & Wing, 2009; Raben, Vasilaras, Møller, & Astrup, 2002) and have no effect on satiety (Anton et al., 2010; Canty & Chan, 1991; Drewnowski et al., 1994; Van Wymelbeke, Béridot-Thérond, de La Guéronnière, & Fantino, 2004). However, the effects of NNS on body weight and satiety are still inconclusive.

The aim of the present study was to examine the long-term effects in weight gain and caloric intake of supplementing the diet of Wistar rats with NNS (either saccharin or aspartame), compared with sucrose, in the context of ad libitum ingestion of chow.

Methods

Animals

Adult male Wistar rats (n = 30), weighing 200–300 g at the start of testing, were randomly divided into three groups (n = 10 per group): saccharin (SAC), aspartame (ASP) and sucrose (SUC), according to the type of sweetener added to yogurt supplement. Standard chow and water were offered daily ad libitum for all groups. The animals were housed in individual transparent acrylic cages, with controlled humidity (65–70%) and temperature (22 ± 1 °C), maintained through a 12 h light and dark cycle. In order to minimize the impact of physical activity in weight changes we restrained the rats’ movements by confining them in...
Dietary manipulation

Based on the Swithers & Davidson protocol (2008), the preparation of diets included 20 ml of plain yogurt (Piá™) to which 20% sucrose (União™), 0.3% sodium saccharin (Finn™) or 0.4% aspartame (Gold™) were added. During preparation, 10 ml pure water was added in all yogurt diets to adjust the viscosity. Yogurt diets were offered 5 days a week, in special bottles with beaks adapted for possible leakage. Yogurt bottles were offered at 6 pm and remained available for approximately 22 h. The exclusion criterion was ingesting less than 70% of sweetened yogurt offered.

The caloric density (CD) of watered down yogurts was ~0.9 kcal/g for yogurt sweetened with sucrose and ~0.5 kcal/g for yogurt sweetened with saccharin or aspartame. Throughout each week of testing, the SAC group received a total of ~139 kcal per week from the yogurt diet, while the SAC and ASP groups received ~75 kcal per week.

In addition to yogurt diet, standard chow pellet (CD: 2.93 kcal/g, Nuvital CR-1, Nuvilab™) and water were offered ad libitum 7 days a week, at 9 am and removed after 24 h. The large solid pellets were deposited in grid feeders, with a bottom crumb collector, on the outside of the cage where the rats were kept.

Measurements of food intake and weight gain

The control of food intake (chow and yogurt diets) was conducted daily by subtraction of the quantity remaining (g) from the quantity supplied (g). Cages were carefully monitored for any evidence of chow spillage and crumbs were considered for the control of chow intake. The yogurt bottles were also checked for any sign of leaking or clogging. The rats were weighed weekly at the same time in the morning. An electronic precision balance (AS 5500, Marte™) was used for all these measures and data collectors were blinded to group assignments.

Determination of variables

Variables over time

Cumulative weight gain was calculated by the subtraction of the basal weight from the weight obtained every week, and expressed in grams.

Cumulative total caloric intake (including yogurt and chow), cumulative caloric intake of yogurt and cumulative caloric intake of chow were calculated by the sum of calories ingested along each week, and corrected by the corresponding rat weight at the end of each week. These data were calculated in the cumulative mode for the 12-week period and were expressed as kcal/g of rat.

Single variables

The total weight gain was defined by the difference between the basal weight and the final weight, in the 12th week. Mean total caloric intake per week, mean caloric intake from yogurt per week, and mean caloric intake from chow per week were calculated as the mean of 12 periods of week caloric ingestion corrected by rat weight (g) at the end of each week and expressed as kcal/g/wk.

Statistical analysis

To evaluate the effect over time, Linear Mixed Model (Cleophas, Zwinderman, & van Oeverkerk, 2010; Shek & Ma, 2011; West, 2009) with random slopes and weekly measurement as a repeated effect were applied to these variables. In these analyses, basal weight was used as a covariate, the fixed effects in the model were the groups and the interaction group-weeks, while rats and weeks were treated as a random effect. First-order autoregressive covariance structure [AR(1)] were used in all these analysis.

For single variables, one-way analysis of variance (ANOVA) with the Dunnett’s test was used to identify differences between groups, and was also used to compare the basal weight, and percentage of intake of yogurt diets. Normality assumptions were verified by Kolmogorov–Smirnov test, with Lilliefors significance correction, and Shapiro–Wilk statistic.

Reported values were mean ± SE, and p < 0.05 was set for all analyses. We used the SPSS™ software version 19 (IBM Corporation, Somers, NY) for statistical analyses.

Results

Cumulative weight gain

Body weight was similar among groups in the beginning of the experiment, F(2, 26) = 0.09, p = 0.92 (263.49 ± 10.60) (Table 1). Weight increased considerably in all groups. In the linear mixed model analysis, SAC and ASP presented greater weight gain than the SAC group (Fig. 1A): SAC (β = 3.82, SE = 1.31, p = 0.005) and ASP (β = 2.70, SE = 1.34, p = 0.048), F(2, 73.47) = 4.51, p = 0.01. In the ANOVA analysis, total weight gain was 28% greater in SAC (p < 0.003) and 20% in ASP (p < 0.04) in relation to SUC, F(2, 26) = 6.73, p = 0.004 (Table 1).

Total caloric intake

Cumulative total caloric intake (yogurt plus chow) was similar among all groups over the 12 weeks of the experiment, F(2, 27.17) = 0.12, p = 0.89 (Fig. 1B). Mean total week caloric intake corrected by rat weight was also similar in all groups, F(2, 26) = 0.04, p = 0.96 (Table 1).

Calories from yogurt

In the mixed model analysis, the cumulative caloric intake of sweetened yogurt during the study was lower in both SAC and ASP compared to SUC (β = 0.16, SE = 0.01, p < 0.001) but did not differ between SAC and ASP (Fig. 1D).

Likewise, the weekly average intake of yogurt corrected by weekly weight was confirmed to be lower in ASP and SAC groups, F(2, 26) = 60.64, p < 0.001 after the ANOVA analysis (Table 1). One rat from the ASP group was excluded from the study due to an ingestion of less than 70% of yogurt, the remaining rats presented an average intake of 88.52% (±1.68), F(2, 26) = 2.23, p = 0.13 (Table 1).

Calories from chow

The cumulative caloric intake from chow during the study was significantly increased in SAC vs. SUC (β = 0.17, SE = 0.03, p < 0.001) and in ASP vs. SUC (β = 0.18, SE = 0.03, p < 0.001), F(2, 35.07) = 27.07, p < 0.001 (Fig. 1C). The mean weekly intake of chow corrected by weekly weight was 16% higher in both the SAC and ASP groups in relation to the SUC group, F(2, 26) = 40.85, p < 0.001 (Table 1). This decrease in calories from chow in SAC
Table 1
Weight and caloric intake parameters.

<table>
<thead>
<tr>
<th></th>
<th>SAC (n = 10)</th>
<th>ASP (n = 9)</th>
<th>SUC (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yogurt intake (% of total offered)</td>
<td>88.21 ± 2.21</td>
<td>84.28 ± 4.39</td>
<td>92.66 ± 1.15</td>
</tr>
<tr>
<td>Basal weight (g)</td>
<td>260.83 ± 21.97</td>
<td>259.41 ± 18.81</td>
<td>269.82 ± 15.54</td>
</tr>
<tr>
<td>Total weight gain (g)</td>
<td>175.31 ± 6.47***</td>
<td>164.28 ± 10.65*</td>
<td>137.37 ± 5.51</td>
</tr>
<tr>
<td>Mean total caloric intake per week (kcal/g/wk)*</td>
<td>1.45 ± 0.02</td>
<td>1.45 ± 0.03</td>
<td>1.44 ± 0.02</td>
</tr>
<tr>
<td>Mean calories from yogurt per week (kcal/g/wk)**</td>
<td>0.18 ± 0.01***</td>
<td>0.18 ± 0.01***</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>Mean calories from chow per week (kcal/g/wk)**</td>
<td>1.27 ± 0.01***</td>
<td>1.27 ± 0.02**</td>
<td>1.10 ± 0.02</td>
</tr>
</tbody>
</table>

Group labels: saccharin (SAC), aspartame (ASP) and sucrose (SUC).
Data are Mean ± SE. Analysis by ANOVA with Dunnett test. Asterisks indicate comparison with SUC.
* p < 0.05.
** p = 0.005.
*** p < 0.001.
* Mean weekly intake throughout the 12 week study period.

Fig. 1. Cumulative effect of the sweetened supplements [saccharin (SAC), aspartame (ASP) or sucrose (SUC)] on feeding pattern and body weight gain during 12 weeks of consumption. The groups that received nonnutritive sweeteners (NNSs) consumed more chow than the group offered sucrose (D), compensating the caloric deficit of these sweeteners (B). Thus, the total calorie consumption (yogurt plus chow) did not differ among the experimental groups (C). However, the NNS groups gained more weight than the group supplemented with sucrose (A). Analysis by linear mixed model, n = 10 (9 for ASP). Asterisks indicate comparison with SUC: ***p < 0.001; **p = 0.005 and *p < 0.05. Error bars indicate SE.
Discussion

In the present study, Wistar rats receiving yogurt sweetened with either saccharin or aspartame and free chow diet during 12 weeks presented increased weight gain compared to animals receiving the same free chow diet plus yogurt sweetened with sucrose. Although saccharin and aspartame promoted relatively fewer calories from yogurt intake when compared to sucrose, increases in calories from chow intake effectively compensated for decreases in calories from yogurt, in such a way that there was a similar total caloric intake among all groups after the 12-week period of the experiment. These data are consistent with the hypothesis that animals adjust for calories consumed on one occasion by reducing their caloric intake on subsequent opportunities to eat (Booth, 1972; Foltin, Fischman, Moran, Rolls, & Kelly, 1990; Mazlan, Horgan, & Stubbs, 2006; Rowland, Nasrallah, & Robertson, 2006).

However, it was surprising to find that the NNS were able to induce weight gain without an increase in total caloric intake, suggesting that other mechanisms such as decreased caloric expenditure may occur after NNS use.

Our data are in accordance with the study of Swithers and Davidson (2008) who, using a similar protocol in Sprague–Dawley rats, observed that rats did gain more weight when using saccharin compared to glucose, despite a similar total energy intake after 5 weeks of experimentation. Our results are also in accordance with the Polyák et al. study (2010), in which CBA/Ca inbred mice with free chow were given saccharin or aspartame added to the water and compared to controls using pure water, during 25 weeks. They observed that animals exposed to NNS in water gained more weight than those drinking non-sweetened water, despite a similar total caloric intake. These studies taken together are consistent in demonstrating that positive caloric compensation in chow intake occurs among NNS-exposed rats, but the increased weight gain observed in these animals was not explained by caloric intake, which was similar in all three groups.

Possible explanations for weight-gain in saccharin and aspartame groups without increasing energy intake are still widely speculative. We could not attribute it to technical bias because we had strict daily control of chow and yogurt intake. A possible cause could be the reduction of energy expenditure induced by NNS. Swithers and Davidson (2008) demonstrated that body core temperature can be increased acutely minutes after the ingestion of glucose compared to non-sweetened fluid, an effect that was not observed when saccharin is used. The authors consider that food intake elicits a reflexive cephalic phase thermogenic response, and the heat production may be mediated by orosensory stimuli that signal the absorption of nutrients in the gut. However, there has not yet been any conclusion regarding a more prolonged effect induced by saccharin.

A second possibility would be that hyperinsulinemia could be induced by saccharin, with consequent weight gain (Corkey, 2012). In vitro studies, using islet cells from Wistar rats were able to demonstrate an insulinotropic effect due to saccharin and other NNS, although not with aspartame. Insulin secretion increased three times from basal levels when cells were exposed to increased saccharin concentrations. The authors speculated that the bitter taste of saccharin, due to hexose pentaacetate esters could act through cellular mechanisms similar to that involved in bitter-taste by taste buds. This effect is supported by the finding that islet cells are equipped with GUST27 receptors also expressed in taste buds which are mediated by G proteins activated with calcium release. These mechanisms are linked to the blockage of potassium channels and activation of CAMP breakdown which regulates insulin secretion (Malaisse, Vanonderbergen, Louchami, Jijakli, & Malaisse-Lagae, 1998). Increased insulin secretion induced by NNS however still remains to be established in animal and human studies.

We also considered the possibility of increased fluid intake and retention due to sodium content in saccharin and also to the sweet taste per se. However, in our study, yogurt caloric intake was decreased both in saccharin and in aspartame groups compared to sucrose, making this hypothesis less likely. Likewise, since weight gain was similar in saccharin and in aspartame groups, we believe that the impact of sodium (from saccharin) may have been of minor or significance.

Conclusion

We observed significantly greater weight gain among Wistar rats fed diets supplemented with NNS—whether saccharin or aspartame—compared with sucrose. This increase was not the result of increased total caloric intake. This discrepancy raises the possibility that weight gain in NNS-fed rats might result from decreases in energy expenditure, which have been observed in other studies. Further studies are necessary to address energy expenditure after NNS exposure in rats as well as long-term clinical trials to evaluate weight gain increases in humans.

References


