Research report

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

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ABSTRACT

It has been suggested that the use of nonnutritive sweeteners (NNSs) can lead to weight gain, but evidence regarding their real effect in 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 (Swithers & 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 as 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

$44 \times 34 \times 16$ cm cages during the 12 weeks of the experiment. Procedures were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23, revised 1985) and guidelines of the National Research Council for the Care and Use of Laboratory Animals (Council, 2010) in accordance with the Brazilian Law for the Scientific Use of Animals. Study protocols were approved by the Ethics Committee for Experimental Procedures of Hospital de Clínicas de Porto Alegre, Brazil.

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 SUC 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 Ouwerkerk, 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, $F(2, 26) = 0.09$, $p = 0.92(263.49 \pm 10.60)$ (Table 1). Weight increased considerably in all groups. In the linear mixed model analysis, SAC and ASP presented greater weight gain than the SUC group (Fig. 1A): SAC ($\beta = 3.82$, $SE = 1.31$, $p = 0.005$) and ASP ($\beta = 2.70$, $SE = 1.34$, $p = 0.048$), $F(2, 73.47) = 4.51$, $p = 0.01$. 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 ($\beta = 0.16$, $SE = 0.01$, $p < 0.001$) 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 ($\beta = 0.17$, $SE = 0.03$, $p < 0.001$) and in ASP vs. SUC ($\beta = 0.18$, $SE = 0.03$, $p < 0.001$), $F(2, 35.07) = 27.07$, $p < 0.001$ (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