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Sweet taste of saccharin induces weight gain without increasing caloric intake, not related to insulin-resistance in Wistar rats

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ABSTRACT

In a previous study, we showed that saccharin can induce weight gain when compared with sucrose in Wistar rats despite similar total caloric intake. We now question whether it could be due to the sweet taste of saccharin *per se*. We also aimed to address if this weight gain is associated with insulin-resistance and to increases in gut peptides such as leptin and PYY in the fasting state. In a 14 week experiment, 16 male Wistar rats received either saccharin-sweetened yogurt or non-sweetened yogurt daily in addition to chow and water *ad lib*. We measured daily food intake and weight gain weekly. At the end of the experiment, we evaluated fasting leptin, glucose, insulin, PYY and determined insulin resistance through HOMA-IR. Cumulative weight gain and food intake were evaluated through linear mixed models. Results showed that saccharin induced greater weight gain when compared with non-sweetened control ($p = 0.027$) despite a similar total caloric intake. There were no differences in HOMA-IR, fasting leptin or PYY levels between groups. We conclude that saccharin sweet taste can induce mild weight gain in Wistar rats without increasing total caloric intake. This weight gain was not related with insulin-resistance nor changes in fasting leptin or PYY in Wistar rats.

1. Introduction

Epidemiological studies suggest that products containing non-nutritive sweeteners (NNS) are associated with increased adiposity (Colditz et al., 1990; Fowler et al., 2008), type 2 diabetes mellitus (T2DM), metabolic syndrome and cardiovascular disease (Dhingra et al., 2007; Lutsey, Steffen, & Stevens, 2008). NNS may also interfere in the regulation of compensatory appetite promoting weight gain in animal experimental models (Davidson, Martin, Clark, & Swithers, 2011; Polyák et al., 2010; Rogers, Carlyle, Hill, & Blundell, 1988).

It has been suggested that NNS could elicit compensatory food intake in response to the absence of a caloric consequence following a sweet taste stimulus. In theory, this response could be related to the perception of sweet-taste involving the action of oral and gut sweet-taste receptors which are connected through vagal afferent conductions to thalamic central connections and to the reward system. The partial activation of this system by sweet taste stimulus without a caloric consequence may sub-activate reward system, breaking the signaling to satiety in the hypothalamus (Bellisle & Drewnowski, 2007; Berthoud, 2002; Cummings & Overduin, 2007; Renwick & Molinary, 2010; Saris, 2003; Smeets, Erkner, & de Graaf, 2010; Yang, 2010). In a series of experiments (Swithers & Davidson, 2008) demonstrated that saccharin may interfere in the ability to compensate calories after exposition to sweet taste, thus promoting greater caloric intake and weight gain.

Abbreviations: AR(1), covariance type first-order autoregressive; β, beta; GLP-1, glucagon-like peptide 1; NNS, nonnutritive sweeteners; PYY, peptide YY; SE, standard error; SST, serum-separating tube.

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This finding suggests that artificial sweeteners may interfere in the expectation of caloric intake altering the response in food intake, at least in animals.

In a previous study (Feijó et al., 2012), using a similar protocol to that of Swithers (Swithers & Davidson, 2008), we showed that saccharin and aspartame increased rat weight gain compared to sucrose, but we were surprised to find no differences in total caloric intake between groups. We now question if the observed weight gain could be due to the sweet taste of saccharin *per se*, or to an artifactual finding due to a decrease in weight in the control group (sucrose). This question was raised as it was recently demonstrated that fructose enriched diets could induce to weight loss (Madero et al., 2011). As sucrose molecule breaks into fructose (Lê & Tappy, 2006), it could be possible that the fructose interference could reduce weight when using sucrose. By this way, the primary objective of the present study was to test if the sweet taste of saccharin could induce weight gain in comparison to a non-sweetened vehicle. Our hypothesis is that, sweet taste *per se*, without caloric consequences, can induce weight gain in rats. Moreover, we also tested if this increases in weight gain would impact in insulin sensitivity as well as in fasting levels of leptin, insulin and PYY of these animals.

2. Materials and methods

2.1. Animals

The experiment was conducted with 16 adult male Wistar rats, 72 days-old, weighing ~300 g at the start. Mean baseline weight was similar between groups. Rats were randomly divided into 2 groups according to the supplement that was offered with yogurt: saccharin-sweetened and non-sweetened control. Animals were housed in individual transparent acrylic cages in a colonization room with controlled humidity (65–70%) and ventilation, and maintained during 12 h light–dark cycles with lights on at $7{:}00_{AM}$ in stable temperature at 22 ± 1 °C. In order to minimize the impact of physical activity in weight, we restrained rat movements by confining them into 44 × 34 × 16 cm individual cages during the 14 weeks of the experiment.

2.2. Sweetened yogurt supplement

Supplements were prepared using 20 ml of plain yogurt (Piá™) with or without 0.3% sodium saccharin (Finn™), according to Swithers & Davidson, (2008) and to our former protocol (Feijó et al., 2012) . During preparation, 10 ml of pure water was added into yogurt to adjust viscosity. Yogurt diets were offered 5 days a week, in special bottles with beaks adapted for possible leakage, and were available 22 h daily. The caloric density (CD) of watered down yogurts was ~0.5 kcal/g, and was similar between sweetened and non-sweetened yogurt. Each week, both groups received ~75 kcal per week. We excluded rats with lower than 70% of yogurt intake.

In addition to yogurt diets, all rats received daily water and standard chow pellet *ad lib* (CD: 2.93 kcal/g, Nuvital CR-1, Nuvilab™), every 24 h during the 14 weeks of the experiment. The standard chow is basically composed of ground whole corn, soybean meal, wheat bran, calcium carbonate, di-calcium phosphate, sodium chloride, mineral and vitamin pre-mix amino acids (22.5% protein, 4.5% fat, 55% carbohydrate, 10% mineral mix, 0.8% fiber, 12.5% humidity).

2.3. Measurements of food and water intake

Chow intake and water intake were controlled daily by subtraction of the quantity remaining from the quantity supplied using an electronic precision balance (AS 5500, Marte™). The large solid pellets were deposited in grid feeders, with a bottom crumb collector outside of the cage. Cages were carefully monitored for any evidence of chow spillage and crumbs were considered for the control of chow intake. Yogurt bottles were also checked for any sign of leaking or clogging. The researchers were blinded in relation to groups.

2.4. Cumulative intake

Cumulative water intake, 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 during each week, and corrected by the corresponding rat weight at the end of each week. Water intake also was calculated by the subtraction of the basal water intake from the weekly ingestion.

These data were calculated in the cumulative mode for the 14-week period and were expressed as Kcal/g or ml/g of rat.

2.5. Cumulative weight gain

Rats were weighed weekly at the same time in the morning, using an electronic precision balance, suitable for weighing animals in motion (AS 5500, Marte™). Cumulative weight gain was calculated by the subtraction of the basal weight from the weight obtained every week, and expressed in grams.

2.6. Euthanasia

At the end of the experiment, after 12–14 h fasting, animals were placed for 1 min in a CO_2 chamber and were killed by decapitation. Experimental 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 HCPA, Porto Alegre, Brazil.

2.7. Serum measurement

At the end of the experiment, fasting (12 h) blood samples were collected in specific tubes with a separator gel (SST) with the aid of individual funnels, after a 30 min for allowing coagulation was centrifuged at 3000 rpm for 15 min. The serum collected was stored in Eppendorf tubes and immediately frozen at −80 °C for later analysis.

Serum total-PYY, leptin and specific rat insulin were determined by Luminex xMAP technology, commercially available multiplex immunoassays manufactured by Millipore™. Glucose was analyzed separately by ELISA in the pathology service of HCPA (Porto Alegre, RS). All samples were assayed in duplicate and in a single assay to eliminate inter-assay variation.

Homeostasis model assessment 2 β-cell function ($HOMA_2$% β), insulin sensitivity ($HOMA_2$% S), and the degree of homeostasis model assessment insulin resistance ($HOMA_2$ IR) were determined from fasting glucose and insulin concentrations by homeostasis model assessment ($HOMA_2$) modeling. $HOMA_2$ parameters were calculated using the HOMA Calculator ("The Oxford Centre for Diabetes, Endocrinology and Metabolism. Diabetes Trial Unit. HOMA Calculator," 2004).

2.8. Statistical analysis

All statistical analyses were done using SPSS™ 20 (IBM Coorporation, Armonk, NY, USA), and graphs were generated using GraphPad Prism™ 5 (GraphPad Software Inc., La Jolla, CA, USA). Initially, we performed an exploratory analysis to verify the behavior of subjects over the time. Linear mixed models (Cleophas, Zwinderman, & van Ouwerkerk, 2010; Shek & Ma, 2011; West, 2009) with random slopes and weekly measurement as a repeated effect were applied to cumulative weight gain, cumulative water intake, cumulative caloric intake of yogurt, of chow and total over time. All intake measures were corrected by the corresponding weekly weight. In these analyses, the basal weight was used as a covariate. The fixed effects in the model were the groups and the interaction group*weeks. Subjects and weeks were treated as a random effect. A diagonal type covariance structure was used for the cumulative weight gain and for the other variables, the first-order autoregressive type was used [AR(1)].

Serum biochemical measures, $HOMA_2$ parameters, mean food intakes, mean water intake, basal weight and intake percentage of yogurt diets were compared using t-tests.

Normality assumptions and homogeneity variances were tested using the Shapiro–Wilk test and Levene's test, respectively. Reported values are means and standard error (SE), and $p < 0.05$ was taken as significant for all analyses.

3. Results

3.1. Cumulative weight gain

Baseline body weight did not differ between groups $t(14) = -0.63$, $p = 0.54$, [302.46 (7.49)] (Table 1). Over the 14 weeks, there was a quadratic effect of cumulative weight gain, which varied considerably in a magnitude and timing among groups. Saccharin presented a more marked weight gain compared to Control $F(1, 95.40) = 5.02$, $p = 0.027$ (Fig. 1).

3.2. Caloric intake

Cumulative total energy intake (chow plus yogurt) corrected by the weight at the end of each week was similar between all groups, $F(1, 14.66) = 0.71$, $p = 0.41$ (Fig. 2A). Mean weekly total caloric intake corrected by rat weight was also similar in all groups, $t(14) = 0.75$, $p = 0.46$ (Table 1).

3.3. Calories from yogurt

All rats ate more than 70% of the yogurt diet, with an average intake of 89% (1.88) of the offered, $t(14) = 0.75$, $p = 0.47$ (Table 1). In the mixed model analysis, Saccharin presented similar energy yogurt intake in relation to control, $F(1, 14.43) = 1.64$, $p = 0.22$ (Fig. 2B). Similarly, the weekly average intake of yogurt adjusted by weekly weight was confirmed to be similar between groups, since these were isocaloric, $t(14) = 1.26$, $p = 0.23$ after the t-test analysis (Table 1).

Table 1
Basal weight, food and water intake parameters.

	Saccharin (n = 8)	Control (n = 8)	*p*-Value
Yogurt intake (% of total offered)	87.45 (2.99)	90.32 (2.39)	0.47
Basal weight (g)	307.26 (12.33)	297.67 (9.05)	0.54
Mean total caloric intake (kcal/g/wk)[a]	1.44 (0.04)	1.47 (0.03)	0.46
Mean calories from yogurt (kcal/g/wk)[a]	0.17 (0.01)	0.18 (0.01)	0.23
Mean calories from chow (kcal/g/wk)[a]	1.27 (0.04)	1.30 (0.03)	0.56
Mean water intake (ml/g/wk)[a]	0.61 (0.05)	0.65 (0.03)	0.60

Data are mean and standard error (SE). Analysis by t-test. There was no difference between groups.
[a] Mean weekly intake (kcal or ml) corrected by weight weekly rat (g) throughout the 14 week study period.

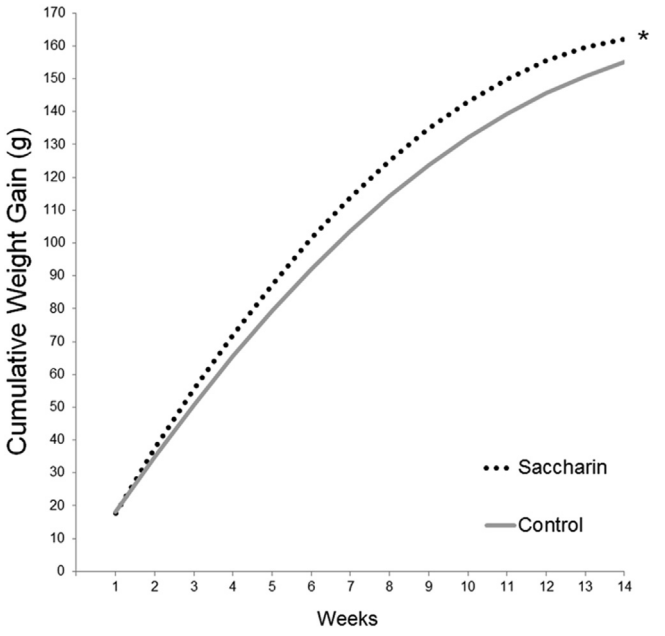

Fig. 1. Cumulative weight gain (g) over the 14 weeks, determined by linear mixed model with random effects (slope and intercept) for groups and controlling for time, using a quadratic model. Asterisk indicates significance: $^*p = 0.027$ (n = 8 per group).

3.4. Calories from chow

Saccharin ingested the same quantity of chow as the Control, $F(1, 14.82) = 0.40$, $p = 0.54$ (Fig. 2C). In this sense, the mean weekly intake of chow adjusted by weekly weight was also similar, $t(14) = 0.60$, $p = 0.56$ (Table 1).

3.5. Water intake

The groups did not show significant difference in relation to cumulative water intake correctly by the weight weekly, $F(1, 14.68) = 0.43$, $p = 0.52$ (Fig. 2D), as well as in the mean weekly intake of water, $t(14) = 0.54$, $p = 0.60$ (Table 1).

3.6. Serum measurements

Fasting serum concentrations determined at the end of 14-week period for PYY [$t(12) = 0.65$, $p = 0.53$], leptin [$t(10) = 0.68$, $p = 0.51$], blood glucose [$t(14) = 0.19$, $p = 0.86$] and insulin [$t(12) = 0.05$, $p = 0.96$] did not present significant differences between groups. There were also no differences between groups regarding $HOMA_2$ IR [$t(12) = 0.10$, $p = 0.92$], $HOMA_2\%$ β [$t(12) = -0.07$, $p = 0.94$] and $HOMA_2\%$ S [$t(12) = -0.31$, $p = 0.76$] (Fig. 3).

4. Discussion

The present results indicates that Wistar rats receiving saccharin-sweetened supplement along with a free chow diet for 14 weeks increase weight gain in comparison to controls receiving non-sweetened supplement. There were no differences in total

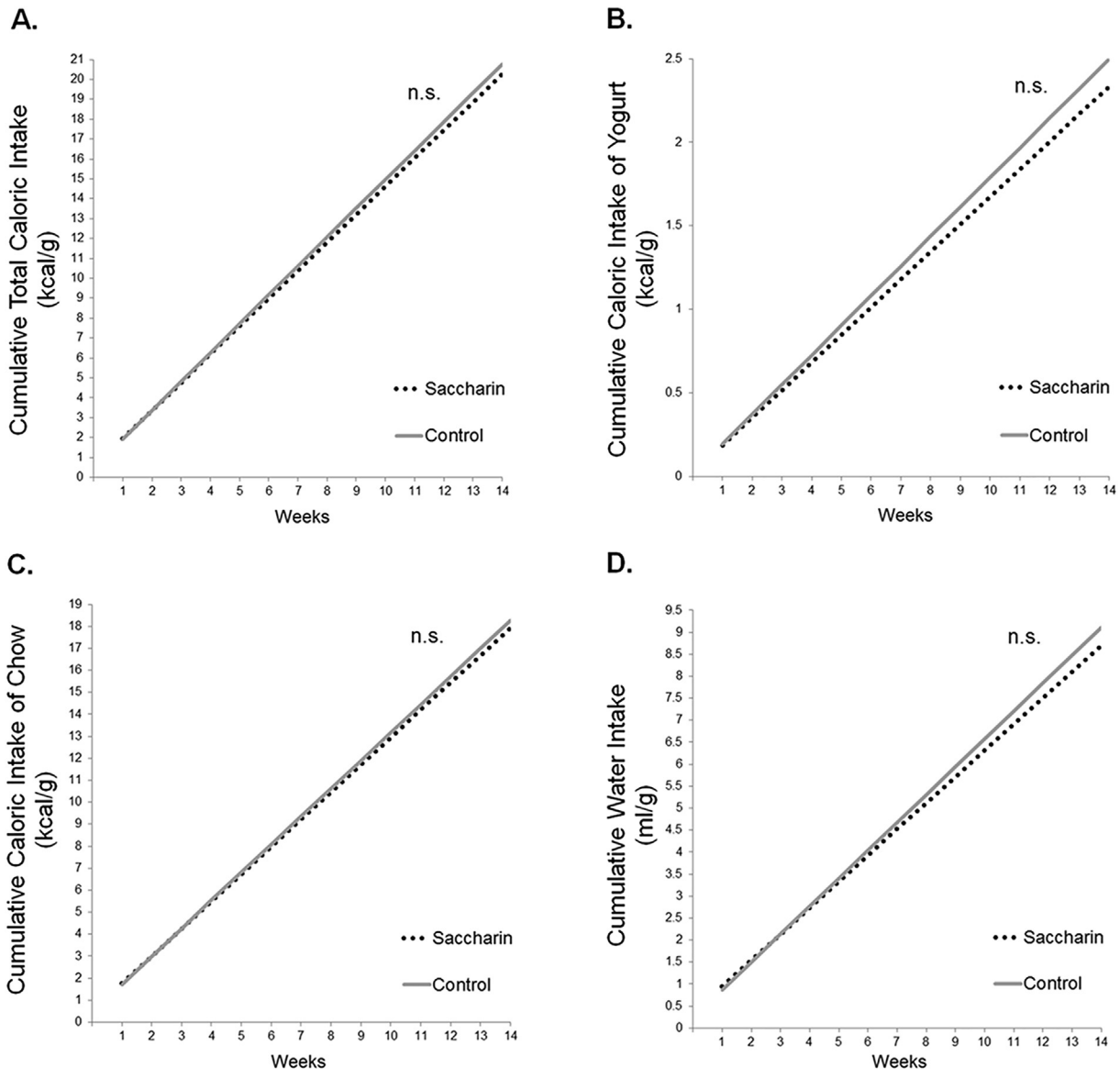

Fig. 2. Cumulative effect of the dietary treatment on feeding pattern (A, B and C) and water intake (D) along 14 weeks of consumption determined by linear mixed model, using a linear model, with random effects (slope and intercept) for groups and controlling for time, $n = 16$ (8 per group). There was no significance (n.s.) difference between the groups.

caloric or water intake between groups and these findings could be attributed solely to the sweet-taste of saccharin. The increase in weight gain, however, was not associated with hyperinsulinemia, increased insulin resistance, fasting leptin or PYY levels in this experimental model.

Our findings are in accordance with the study of Swithers and Davidson (2008) in which they observed that Sprague–Dawley rats gained more weight when using saccharin-sweetened yogurt than when using glucose-sweetened yogurt after 5 weeks of experiment. Similar results were also found in the study of Polyák (Polyák et al., 2010) in which CBA/CA inbred mice receiving saccharin on water for 26 weeks showed greater weight gain when compared with control rats receiving non-sweetened tap water.

In the present study, the greater increase in weight gain in the saccharin group began in week 5, becoming maximal between weeks 8 and 12, with a trend to atenuate the difference along weeks 12 and 14. This difference, however, was less prominent than that observed in our former study (Feijó et al., 2012), when sacharin was compared to sucrose. We believe the difference between both studies rely on differences in the comparator used. In that study, we used sucrose as a comparator, a molecule that can be broken into fructose. As it was recently observed in individuals with metabolic syndrome, fructose-enriched diet may induce more weight loss than individual using low-fructose diets, despite a similar caloric intake (Madero et al., 2011). By this way, fructose thus may have had an effect in reducing weight in the comparator group in that study (Lê & Tappy, 2006).

The rationale for the increased weight gain with saccharin without excess in total caloric intake is still speculative. Excessive sodium intake due to the sodium content in saccharin is plausible to cause water retention and weight gain, however, this mechanism is unlikely because sodium content was very small as we used diluted saccharin (0.3%). Moreover we observed that water intake was rigorously similar in both groups. A second hypothesis could be a possible down-regulation of total caloric expenditure due to saccharin effect (Swithers, Baker, & Davidson, 2009; Swithers & Davidson, 2008). Swithers's group have observed that the body core temperature, immediately after an acute overload of saccharin

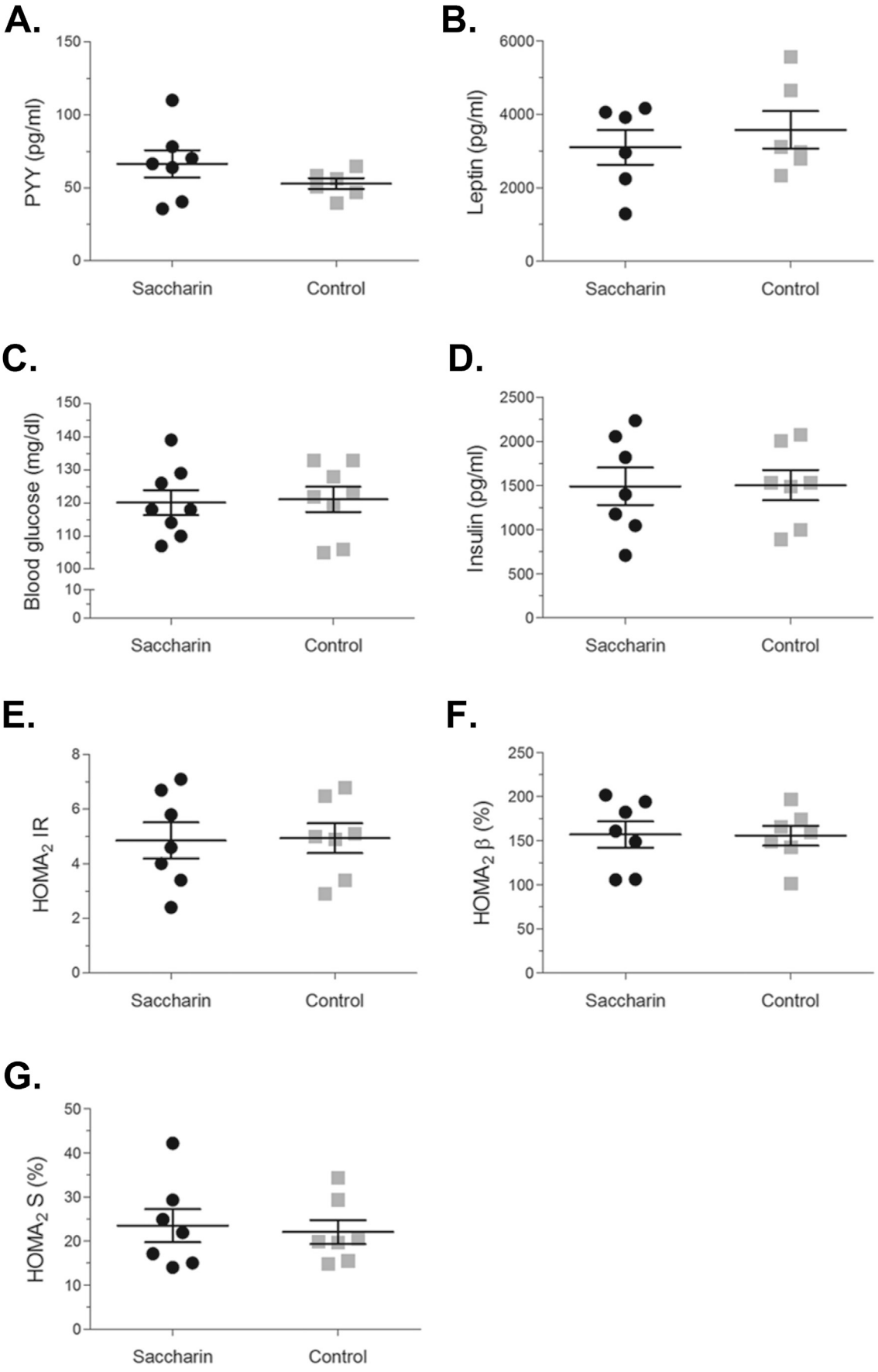

Fig. 3. Effect of the dietary treatment, at the end of 14-weeks period, on fasting PYY (A), leptin (B), blood glucose (C), insulin (D), insulin resistance (E), beta cell function (F) and insulin sensitivity (G) after 14 weeks of the experiment. There was no significant (n.s.) difference in these parameters. Determined by t-test, n = 6–8 per group.

was smaller than glucose in rats. It is still not clear, however, if this effect is due to a decrease in thermogenesis due to saccharin or to an increase in heat due to glucose metabolism.

Finally, it is possible that the sweet-taste of NNS could stimulate intestinal glucose transport through activation of gut SGLT1 and GLUT2 translocation after activating sweet-taste receptors T1R2 and T1R3, which are located in enterocyte brush cells, in rodents. T1R2 and T1R3 are G protein coupled taste receptors which couples to α-gustducin through which they can activate a phospholipase c (*PLC*)*β2*-dependent pathway to increase intra-cellular calcium concentration. By this way, the intra-cellular calcium influx could regulate apical GLUT-2 action (Mace, Affleck, Patel, & Kellett, 2007; Sclafani, 2007; McLaughlin, McKinnon, & Margolskee, 1992). The α-gustducin protein can also regulate the secretion of GLP-1 from entero-endocrine L cells (Jang et al., 2007). This increase in GLP-1, by this way, could promote the expression of SGLT1 through a paracrine effect in the enterocyte.

It also necessary to consider that changes in intestinal microbiota resulting from the use of NNS may have an impact in energy metabolism, with potential consequences in weight gain. (Bokulich & Blaser, 2014; Daly et al., 2015). Recently, Suez and cols. studied the impact of NNS in glucose tolerance by inducing changes in the microbiota in rodents. They demonstrated that transplanted microbiota from animals using NNS, which developed glucose intolerance, could induce glucose intolerance in previously normal germ-free recipients mice (Suez et al., 2014). These data, however still need to be reproduced.

The present results did not find association with weight gain and changes in insulin sensitivity or insulin secretion at fasting state. Weight gain was not associated to increases in fasting leptin, insulin, PYY or adiposity (data not shown). A possible explanation for this was that Wistar rats may not be a good model of insulin resistance when exposed to regular diet. This may have limited the relevance of these results to other rodents prone to insulin resistance.

5. Conclusion

We concluded that sweet-taste of saccharin can promote increases in weight gain without increasing total caloric intake or promoting insulin resistance in Wistar rats. The possibility that saccharin could decrease caloric expenditure or even stimulate glucose transport mediated by gut sweet-receptors should be evaluated in future studies.

Competing interests

The authors declare not having any personal or financial support or involvement with organizations with financial interest in the subject matter or any actual or potential conflict of interest.

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