

# Effect of artificial and natural sweeteners on glucose and insulin in plasma of rats

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## Abstract

**Introduction:** The role of artificial sweeteners in body-weight regulation is still unclear. Replacing sugar with low-calorie sweeteners is a common strategy for facilitating weight control. Whether using artificial sweeteners may augment positive energy balance through increased food intake was investigated. The effects of sweet taste and its sources (carbohydrate and non-caloric sweeteners) on diet growth efficiency, fasting and postprandial glucose and insulin plasma concentrations were also compared.

**Material and method:** 140 male Sprague-Dawley rats (initial body mass:  $325 \pm 19$ g) were randomly divided into 4 groups. Each group was provided with isoenergetic diets: 3 with the same sweet taste intensity (with sucralose – SU, sucrose – SC and maltodextrine – M) and one diet non-sweet (NS). Food intake was recorded daily and body weight measured twice a week/controlled every two days. After 3 weeks, animals were euthanized as follows: fasting and 30, 60, 120, 180 min. after an appropriate meal (n=7 from each group/time point).

**Results:** The group receiving sucralose consumed more feed and had significantly increased body weight compared to the other groups. The diet growth efficiency in groups SU was statistically higher than in NS and SC groups, but did not differ from group M. In SU group, postprandial increases in glucose and insulin levels were significantly lower compared to both SC and M groups, but no different from group NS. Fasting HOMA insulin resistance index was not significantly different among the groups.

**Conclusions:** The results indicate that the sweet taste of a diet derived from non-caloric sweeteners may increase food intake sufficiently to cause a significant increase in body weight gain. These results could support the hypothesis that artificial sweeteners weaken a predictive relationship between sweet taste and the caloric consequences of eating.

## Key words

non-caloric sweeteners, food intake, body mass gain, insulin, glucose

## INTRODUCTION

Excessive consumption of sucrose is one of the most serious causes of obesity and comorbidities, such as hypertension, diabetes, dyslipidaemia, and coronary heart diseases, which are associated with a significant increase in mortality and morbidity rates. Growing public awareness regarding the dangers imposed by refined sugars has prompted a reduction in their consumption by replacing them by artificial sweeteners. Artificial sweeteners are widely used in many products, such as desserts, gums, diet beverages and yogurts, as a means of combating body mass gain, metabolic syndrome, diabetes, and all risk factors for heart disease. According to the International Food Information Council Foundation (2012), 73% of respondents who were consuming noncaloric sweeteners reported doing so to reduce total calories consumed. However, in 2012, the American Heart Association (AHA) and American Diabetes Association (ADA) concluded that there are still insufficient data to determine the role played by artificial sweeteners in the regulation of energy balance, appetite, body weight, and influence on cardiometabolic risk factors [1].

There is evidence that non-caloric sweeteners increase appetite, promote overeating, and lead to body mass gain [2, 3, 4, 5, 6]. The mechanisms by which this occurs remains unknown. It is probable that a significant role in this mechanism is played by the sweet taste receptors T1R2 and T1R3, which recently have also been found in the duodenum, small intestine and pancreas [7, 8]. Stimulation of these receptors by artificial sweeteners changes the activity of clue metabolic hormones, including GIP, GLP-1 and insulin [7, 9, 10, 11, 12].

One of the hypothesis maintains that artificial sweeteners weaken a natural predictive relationship between sweet taste and the calorie intake during eating. As a consequence, the organism receiving non-caloric sweetener, which is very sweet and free from energy value, tends to offset energy deficit during the next meal [2, 6]. It is known that from the earliest years the body associates orosensory cues such as sweet taste with the postprandial caloric or nutritive consequences of eating [13, 14, 15].

In order to extend knowledge about the influence of dietary non-caloric sweeteners on energy balance, food intake and body mass was compared, as well as plasma levels of insulin and glucose in rats fed with non-sweet diet and diets differing in the source of sweet taste.

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## MATERIALS AND METHOD

Male Sprague-Dawley rats obtained from the Medical Research Centre of the Polish Academy of Sciences in Warsaw, Poland, were placed in individual cages and accommodated for seven days in standard environmental conditions at a temperature of  $23 \pm 1^\circ\text{C}$ , a 12-hour light/dark cycle, 50-65% humidity, with food (Labofeed) and water supplied *ad libitum*.

140 male Sprague-Dawley rats (initial body mass:  $325 \pm 19\text{g}$ ) were randomly divided into 4 groups. Each was provided with *ad libitum* isoenergetic diets (1g=3,766 kcal): 3 with the same sweet taste intensity which corresponded to 10 g of sucrose (with sucralose – SU, sucrose – SC and maltodextrine – M) and one diet non-sweet (NS). Diet composition is presented in Table 1.

**Table 1.** Diet composition (g/100g)

Ingredients	DIETS			
	NS	SC	M	SU
Wheat starch	62.9	52.9	0	62.9
Sucrose	0	10	0	0
Sucralose	0	0	0	0.02
Maltodextrine	0	0	62.9	0
Fiber	5	5	5	5
Casein	20	20	20	20
Soybean oil	7	7	7	7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
L-cystein	0.3	0.3	0.3	0.3
Choline bitartrate	0.25	0.25	0.25	0.25
Tert-butylhydroquinone	0.0014	0.0014	0.0014	0.0014

NS – non-sweet diet with, SC – diet with sucrose, M – diet with maltodextrine, SU – diet with sucralose

Food intake was recorded daily and body weight measured 3 times per week. After 3 weeks, the animals were anesthetized by isoflurane as follows: at fasting and 30, 60, 120, 180 min. after meal ( $n=7$  from each group/time point). Blood was taken by cardiac puncture and plasma stored at  $-80^\circ\text{C}$ . Plasma insulin concentration was measured by using rat insulin ELISA kit (Demeditec, Germany). Plasma glucose level was determined enzymatically using PTH Hydrex kit (Warsaw, Poland).

To assess body insulin sensitivity or resistance, the index HOMA-IR (Homeostatic Model Assessment-Insulin Resistance) was calculated. HOMA-IR is a valuable method that shows a strong relationship with an euglycemic-hyperinsulinemic clamp [16]. High HOMA-IR scores indicate low insulin sensitivity (i.e. insulin resistance). The HOMA-IR index was calculated for fasting groups using the formula: fasting glucose (mmol/L)  $\times$  fasting insulin (mU/mL)/22.5 [16, 17].

Statistical analysis (multifactor analysis of variance followed by *post-hoc* Tukey test) was performed using STATISTICA 10 (Statsoft).  $p$ -values  $<0.05$  were considered statistically significant.

## RESULTS

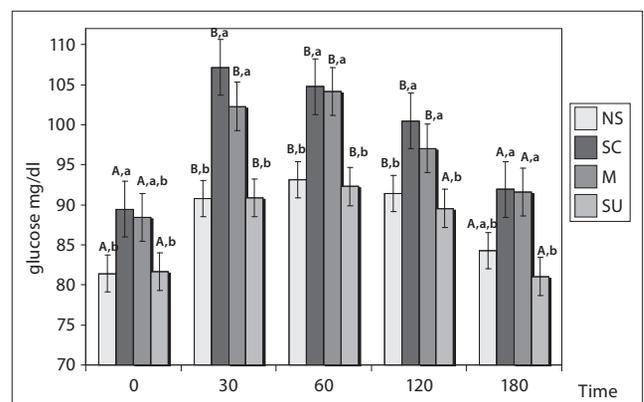
**Body weight and food intake.** After 3 weeks of the experiment, the highest daily body mass gain was observed in SU group compared to NS ( $p<0.001$ ), SC ( $p<0.001$ ), M ( $p<0.001$ ) groups. Daily body mass gain in SC group was significantly higher than in groups NS ( $p<0.001$ ) and M ( $p=0.009$ ).

Daily energy intake and daily food intake expressed in g of diet were higher in SU group than in groups: NS ( $p<0.001$ ), M ( $p<0.001$ ) and SC ( $p<0.001$ ). In SC group, food intake was higher than in both NS ( $p<0.001$ ) and M ( $p<0.001$ ) groups. Diet growth efficiency in group SU was statistically significantly higher than in NS ( $p<0.001$ ) and SC ( $p<0.001$ ) groups and not different from group M (Tab. 2).

**Table 2.** Daily body weight gain, intakes of food and total energy in rats fed experimental diets. Values statistically significantly different ( $p<0.05$ ) are indicated by different letters

Variables	Dietary groups	Mean	SE
Daily body mass gain (g)	NS	2.73 <sup>a</sup>	0.51
	SC	3.31 <sup>b</sup>	0.29
	M	3.09 <sup>c</sup>	0.26
	SU	3.68 <sup>d</sup>	0.32
Food intake (g /day)	NS	25.16 <sup>a</sup>	1.56
	SC	29.35 <sup>b</sup>	0.49
	M	27.03 <sup>a</sup>	1.64
	SU	32.19 <sup>c</sup>	1.15
Diet growth efficiency (g body weight gain /g of food intake)	NS	0.11 <sup>a</sup>	0.02
	SC	0.11 <sup>a</sup>	0.01
	M	0.12 <sup>b</sup>	0.01
	SU	0.12 <sup>b</sup>	0.01
Daily energy intake (kJ /day)	NS	94.76 <sup>a</sup>	5.87
	SC	115.25 <sup>b</sup>	1.96
	M	101.02 <sup>a</sup>	6.13
	SU	119.67 <sup>c</sup>	4.61

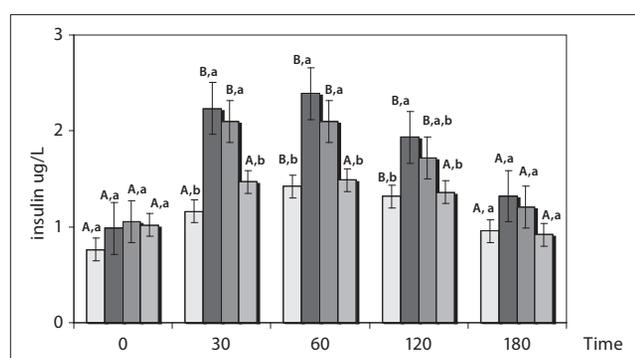
**Insulin and glucose concentrations in plasma.** Fasting plasma glucose was significantly higher in SC group compared to NS ( $p=0.04$ ) and SU ( $p=0.04$ ) groups, but did not differ from group M (Fig. 1). The highest postprandial



**Figure 1.** Plasma concentrations of glucose [mg/dl] in rats fed NS, SC, M and SU diets during 3 weeks of experiment. Values are expressed as mean with their standard errors for 7 animals. Different a, b letters indicate significant differences within fasting group and 30, 60, 120, 180 min. after meal ( $p<0.05$ ). Different A, B letters indicate significant differences between groups – NS, SC, M, SU ( $p<0.05$ )

glucose concentration in SC group was observed 30 min. after consumption of meal, whereas in groups NS, M and SU, after 60 min. These peaks were followed by a decrease in glucose concentration which, at 180 min. after consumption of a meal, had returned to fasting values in each group. At 30, 60, 120 and 180 min., plasma glucose levels in SU group were significantly lower compared to group SC ( $p < 0.0001$ ,  $p < 0.001$ ,  $p < 0.0007$ ,  $p < 0.005$ , respectively) and M ( $p < 0.004$ ,  $p < 0.002$ ,  $p < 0.01$ ,  $p < 0.007$ , respectively), and did not differ from that observed in NS group. At 30, 60 and 120 min., plasma glucose concentrations in SC and M groups were significantly higher than in NS group ( $p < 0.001$ ,  $p < 0.003$ ,  $p < 0.02$ , respectively) and did not differ at 180 min. At no point did the glucose levels in SC and M groups differ significantly.

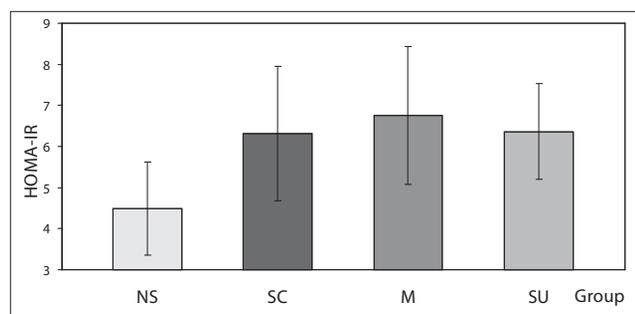
Both at fasting and 180 min., postprandial plasma insulin concentrations did not differ statistically significantly between groups (Fig. 2). The patterns of postprandial changes



**Figure 2.** Plasma concentrations of insulin [ $\mu\text{g/L}$ ] in rats fed NS, SC, M and SU diets during 3 weeks of experiment. Values are expressed as mean with their standard errors for 7 animals. Different a, b letters indicate significant differences within fasting group and 30, 60, 120, 180 min. after meal ( $p < 0.05$ ). Different A, B letters indicate significant differences between groups - NS, SC, M, SU ( $p < 0.05$ ).

in plasma insulin concentrations were similar in SC and M groups, and in NS and SU groups. A significant increase above fasting level in SC and M groups occurred 30 min. after a meal, and persisted to 120 min., then fell after 180 min. to values that did not differ from those observed during fasting. At 30, 60 and 120 min. after consumption of a meal, in SC group plasma insulin levels were significantly higher compared to groups NS ( $p < 0.00004$ ,  $p < 0.0002$ ,  $p < 0.02$ , respectively) and SU ( $p < 0.003$ ,  $p < 0.0005$ ,  $p < 0.03$ , respectively). In M group at 30 and 60 min., insulin concentrations were higher than in NS ( $p < 0.0003$ ,  $p < 0.008$ , respectively) and SU ( $p < 0.01$ ,  $p < 0.02$ , respectively) groups.

Fasting HOMA-IR did not differ significantly in the experimental groups (Fig. 3).



**Figure 3.** HOMA-IR index in rats fed NS, SC, M and SU diets during 3 weeks of experiment. Values are expressed as mean with their standard errors for 7 animals

## DISCUSSION

The results indicate that both the presence of sweet taste in diet and the type of its source influence feed intake and growth. It can be supposed that sweet taste increased food intake, since the lowest consumption was observed in rats receiving unsweetened feed. However, food intake in this group did not differ significantly from that observed in rats receiving maltodextrine. Among the groups given different sweet sources, the highest food intake was observed in the group fed a diet with sucralose. It is noteworthy that the food intake in this group exceeded that observed in the groups receiving natural sweeteners, saccharose or maltodextrine. Body weight gain reflected the amount of food consumed and was the highest in rats given sucralose, which indicate that non-caloric sweeteners may increase food intake sufficiently to cause a significant increase in weight gain. On the other hand, other results suggest that artificial sweeteners may promote body weight gain. Martinez et al. [3] showed that rats who drank water with aspartame and sucralose were fatter than both control and sucrose groups, in spite of the fact that total caloric intake in the sucrose group was higher than in both groups with artificial sweeteners.

The results of human studies also prompt the assumption that artificial sweeteners may promote body mass gain. The Multi-Ethnic Study of Atherosclerosis [MESA] conducted over an 8-year period on 5,011 participants aged 45-84 years, revealed that consumption of artificial sweeteners in beverages at least daily was associated with significantly greater risks of select incident metabolic syndrome components (36% greater) and type 2 diabetes (67% greater) [18]. Fowler et al. confirmed a positive correlation between artificial sweetener consumption and body mass gain in 3,682 participants [19]. It was reported that consuming more than 21 artificially-sweetened beverages per week was associated with an almost-doubled risk of overweight and obesity. Average BMI among artificial sweetener users were 47% greater than among nonusers. Similarly, Appleton et al. reported higher BMI in artificial-sweetened beverages users who consumed more than 825 ml of artificially sweetened beverages per day, compared to non-users [20].

Body mass gain and overeating after consumption of products containing artificial sweeteners can be explained by the hypothesis that artificial sweeteners disrupt the body's natural ability to predict the caloric contents of food on the basis of sweet taste, which leads to greater body mass gain through increased food intake during the next meal to compensate the energy deficit and/or diminished energy expenditure. Rats given saccharine solutions both ate significantly more and gained significantly more weight than the rats given the glucose solution [5, 6].

Mattes and Popkin in their review listed various mechanisms of the effects of artificial sweeteners on compensatory appetite, food intake and hunger through their influence on cephalic phase stimulation, gut peptide response, palatability, and natural ability to learn to like a repeated exposure to sweet taste which may preserve a preference for sweetness in the diet, including products sweetened with natural sweeteners [21].

Is possible that increased consumption of diets containing non-caloric sweeteners can also be associated with their effects on satiety. Drinking saccharin solution, but not water, elevated the mRNA levels of orexin and neuropeptide Y.

Activation of orexigenic neuropeptides, such as orexin and neuropeptide Y induced by the sweetness of saccharin, but not by neutral fluid, suggests that palatability is one of the factors that augments food intake during application of artificial sweeteners [19].

Consumption of sweeteners such as sucrose or saccharin elevate  $\beta$ -endorphin levels, both in the cerebrospinal fluid and plasma more effectively than the intake of other taste solutions or water. In turn, opioids are involved in the generation of palatability and facilitation of food consumption [22].

Rats from SC group ate more and elicited greater body mass gain than rats from M group. This could be due to the presence of fructose derived from hydrolysis of sucrose in SC diet. It is noteworthy that, based on the differences in the metabolic pathways for fructose and glucose, fructose consumption gives less satiety, leads to increased food intake and body mass gain compared to glucose [23, 24, 25].

The lowest daily food intake and weight gain were recorded in NS group. These results are consistent with the observation of Graaf et al. [26] who found significantly higher sweet diet intake than with a starch diet. Other authors claim that sweet taste promotes palatability of the diet, which in turn contributes to overconsumption [27, 28, 29, 30].

Postprandial increase in glucose and insulin levels in SU group was at the level of NS group, and significantly lower than in groups receiving natural sources of sweet taste, which indicates a lower glycemic index of SU diet. It is generally accepted that a lower glycaemic index of food is connected with lower lipogenic potential. Unfortunately, rat body composition was not estimated and therefore it could not be assessed whether higher body mass gain in SU groups was due to greater body fat. But if this was the fact, it could indicate that SU diet promoted body mass gain, despite a lower glycaemic index.

Lack of differences between fasting and postprandial plasma glucose in SU and NS groups is consistent with the research by Grotz et al. [31] which demonstrated that sucralose consumption for 3 months had no effect on glucose homeostasis in individuals with type 2 diabetes, and was as equally well tolerated as the placebo. However, some authors have observed an increase in plasma glucose and insulin levels in rats in response to acesulfame potassium, sucralose or saccharin [10, 32]. This was mediated via stimulation of sweet-taste receptors in L-cells by artificial sweetener, and subsequent increase in GLP-1 [9] and GIP secretion, as well as SGLT1 [33] and GLUT2 expression [32].

Insulin plays a key role in both short- and long-term increase of satiety and termination of eating [34, 35]. Postprandial increase in the level of plasma insulin inhibits the secretion of ghrelin [36]. It is possible that the considerably lower postprandial insulin plasma concentration in SU group reduced satiety, leading to increased food intake. The higher body mass weight in SC and M groups compared to NS group may be due to lipogenic effects of insulin on adipocytes [37].

## CONCLUSIONS

1. Non-caloric sweeteners can increase food intake sufficiently to cause a significant increase in weight gain.
2. Dietary sucralose intake induces lower levels of plasma glucose and insulin than both sucrose and maltodextrin.
3. The body weight increasing effect of sucralose occurs despite the low glycaemic index of a sucralose containing diet.

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