

INFLUENCE OF SUGAR AND SWEETENERS ON MOUSE BODY WEIGHT, STRESS-INDUCED BODY WEIGHT CHANGES AND LIFE EXPECTANCY

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Abstract

Artificial sweeteners are commonly used for sugar substitution in drinks and sweets for better body weight control. Many animal studies have shown that these substances can induce hunger and increased food consumption leading to obesity instead of the expected weight loss. This effect is attributed to an increased insulin secretion due to the sweet taste. Our goal was to administer sweet solutions (sugar, artificial sweeteners, or sugar + artificial sweeteners) to mice and to evaluate body weight and life expectancy changes. Solutions of artificial sweeteners administered instead of drinking water increased with about 10 % the body weight of male mice; female mice's body weight remaining uninfluenced. This effect is abolished by replacing a part of the sweeteners with sugar, suggesting that after meal sweeteners don't increase the sensation of hunger, since calories are provided together with the sweet taste. The administration of sugar solutions had no significant effect on mice's body weight. Body weight loss was induced by moving animals in different types of cages; no effect of treatment compared to control was recorded. Even if some effects of treatment on survival curves were observed in the male group, statistical significance was not achieved.

Rezumat

Îndulcitorii artificiali sunt frecvent utilizați pentru înlocuirea zahărului în băuturi și dulciuri de tip „light” (dietetice) pentru un mai bun control al greutatei corporale. Studii efectuate pe animale demonstrează că aceste substanțe cresc secreția de insulină, ceea ce poate avea ca rezultat un aport crescut de alimente. Scopul lucrării de față a fost urmărirea greutății corporale și a speranței de viață a șoarecilor cărora li s-au administrat în loc de apă soluții dulci (conținând zahăr, îndulcitori artificial sau ambele produse). Greutatea corporală a șoarecilor masculi care au consumat soluții de îndulcitori artificiali a crescut cu 10 %, în cazul femelelor greutatea corporală nu a fost influențată. Acest efect de creștere a greutății corporale este anulat prin înlocuirea unui procent de îndulcitori cu zahăr, sugerând că după masă îndulcitorii artificiali nu mai cresc senzația de foame datorită aportului caloric. Administrarea soluției cu zahăr nu influențează semnificativ greutatea corporală a șoarecilor. S-a testat și influența îndulcitorilor artificiali asupra pierderii de masă corporală provocată de stres; nu s-a observat o diferență între lotul tratat și cel martor. În cazul

loturilor tratate se observă o influență a substanțelor testate asupra speranței de viață; diferențele însă nu sunt semnificative din punct de vedere statistic.

Keywords: artificial sweeteners, weight gain, weight loss, life expectancy, maximum life span.

Introduction

Artificial sweeteners were introduced as sugar substitutes for diabetic patients. Nowadays, these substances are widely used for sugar substitution in “diet” drinks and sweets with the purpose of weight control. Even some successes in body weight control were recorded when artificial sweeteners substituted sugar in diet [10]. Most commonly used products to date are saccharin, cyclamate, aspartame, and acesulfam K; maximum accepted daily intakes are stated for each one. So far most concerns regarding these sweeteners have been their possible carcinogenic effects. Saccharin and cyclamate were classified by IARC (International Agency for Research on Cancer) in group 3: agents that are not classifiable as to their carcinogenicity to humans [5]. European Food Safety Authority concluded, after reviewing all scientific papers published about aspartame's toxicity, that there was no evidence of aspartame's carcinogenicity to humans [4]. Other health effects found during animal experiments were found to be irrelevant to humans [4, 5].

In the past few years, a new and interesting side effect was correlated with the use of artificial sweeteners: increased body weight directly related to consumption of these substances.

Many experiments were carried out on laboratory animals. Rats given artificially sweetened diets gained more weight compared to those receiving glucose-sweetened ones. The increased body weight appeared regardless of the used sweetener and persisted after discontinuation of the artificially sweetened diet [9, 18, 19].

Body weight increase is due to increased food consumption when artificially sweetened water is used. The sweet taste was considered to contribute to this effect [21, 22, 23]. It was presumed that, based on the Pavlovian conditioning principles, consumption of non-nutritive sweeteners could result in sweet taste no longer serving as consistent predictor of nutritive postingestive consequences. The dissociation between the sweet taste and the caloric consequences could lead to a decrease in the ability of sweet taste to evoke physiological responses that serve to regulate energy balance [18]. Increased insulin secretion was observed in rats fed with artificial sweeteners. Interestingly, increased food consumption is maintained even after insulin blood levels return to normal. Hepatic

vagotomy ceased the effects of saccharin suggesting that a hepatic mechanism is involved in the observed effects. Temporary alteration of the disposition of metabolic fuels towards storage and away from oxidation was assumed as a mechanism of action in the artificial sweetener-induced obesity [20, 23].

Increased food consumption was found in females with eating restraint when they used aspartame-sweetened lemonade [7].

Increased body weight was correlated with the use of artificial sweeteners in many human studies, too [27]. The effect is probably due to the fact that sweetness decoupled from caloric effect offers partial, but not complete, activation of the food reward pathways. Lack of food satisfaction, likely because of the failure to activate the postingestive component, further stimulates the food seeking behavior, thus contributing to obesity [14, 18, 27].

There are, however, clinical studies of which results do not agree with the findings stating that artificial sweeteners can increase hunger and food consumption. These studies show that there are no changes or that even weight lost can be obtained by using artificial sweeteners [1, 2, 10, 11, 15].

Using artificial sweetener instead of sugar for body weight control seems reasonable because a lower caloric intake could be attained. Some animal studies showed an increased body weight gain when different carbohydrates were added to their diet [13]. Interestingly, some human studies are not confirming this finding: in a study after a month of using sweetened beverages there were no changes in food consumption in 24 human subjects of both sexes [25]. Furthermore, large retrospective studies made on large numbers of individuals failed to correlate increased body mass index with the consumption of sugar sweetened beverages [6, 16].

The purpose of this paper is to complete the available studies with the following aspects:

- studying the body weight gain of mice receiving solutions of artificial sweeteners with low amounts of sugar
- studying the influence of artificial sweeteners, sugar or mixture of both upon bodyweight loss
- studying the life expectancy of mice receiving sugar solutions, artificial sweetener solutions or mixtures of sugar and artificial sweetener solutions. It was considered that life expectancy better describes life style induced changes in health than any other parameter, since many health problems can go undetected for long periods of times.

Materials and Methods

Materials

- Sugar, SC Zahăr Oradea SA, Romania.
- Commercially available artificial sweetener: Diamant, Kruger GmbH & Co, Germany; contains: 40 mg sodium cyclamate and 4 mg sodium saccharine/tablet.
- Animal feed: granulated feed obtained from mixed grains produced by Kabai Tap Zrt., Hungary. Nutritional qualities: humidity 14.00 %, protein 14.99 %, fat 2.38 %, ash 6.54 %, fibers 6.73 %, lysine 0.56 %, vitamin A 8000 NE/kg, vitamin D 1340 NE/kg, vitamin E 66 mg/kg.
- Drinking fluids:
 - I. water
 - II. sugar-sweetened water (70 g/L sugar)
 - III. artificially sweetened water (80 mg/L sodium saccharine + 800 mg/L sodium cyclamate)
 - IV. 1:1 mix of II and III.

All three sweet solutions had the same intensity of sweetness. The concentrations were chosen by tasting, taking account of the sweetening power of the sweeteners. Concentrations of sweeteners were of the same order of magnitude as the commercial products used for human consumption [3].

- Animal housing: 1.20x0.60 m glass aquariums for the first 21 weeks of experiment, and then identical sized steel cages. The housing floor was covered with wood shavings (layer thickness: 1-2 cm).
- Temperature: 20 °C, relative humidity 40-60 %.
- Laboratory animals: white mice AKR2 line.

Methods

320 mice (160 females and 160 males) were randomly divided in four test groups. All groups received feed and one of the drinking fluids *ad libitum*. Females and males in each test group were caged separately and every animal received a unique code so weight gain could be individually recorded.

The experiment started by administrating the solutions to the mice when they reached the age of 30 days and ended with the natural death of the last animal. Body weight recording started one week after weaning and moving to the new location, and that weight is considered the starting value in all figures throughout this work (8.09 ± 1.74 g in the case of female mice

and 7.75 ± 1.11 g in the case of male mice). This value was also used for computing body weight gains of the animals.

Animal weights were recorded for 52 weeks (weekly in the first month, twice in the next month and then bimonthly until the end of the 52 weeks). After this period, only the animals death was recorded, while keeping the same treatment. The animal weighing was realized using a balance (Digital Scale, China) with 0.1 g accuracy, putting the animals in a glass cup to avoid the weighing errors caused by the animals' motion.

Animals were housed according to international regulations and with our University's Committee on Ethics approval. Principles of laboratory animal care (NIH publication No. 86-23, revised 1985) were followed. No animal was harmed in any way during this study, weight measurements being the only tests carried out. There was no need to euthanize any animal during the study.

Statistical analysis

Relationship between body weight and sweetened solutions ANOVA and Bonferroni's Multiple Comparison statistical tests were used in order to evaluate the differences between the tested groups. When a difference was found, unpaired, two tailed "t" test was used to confirm the result and to compute the p value. Parametric statistical tests were used since, as expected, D'Agostino and Pearson normality test showed that during the study, the body weight of animals followed a Gaussian distribution in all groups. Statistical significance was considered for a p value less than 0.05. Males and females were evaluated separately.

Survival of animals

Survival of animals was recorded until the last animal died from natural causes. Log-rank (Mantel-Cox) test and Gehan-Breslow-Wilcoxon tests were used in order to compare the differences between survival curves.

Life expectancy was calculated by averaging the age of all animals, belonging to a group, at the time of their death. Maximum life span of animals (the mean life span of the most long-lived 10 % of a given cohort) was also calculated. Maximum life span is considered determined by the rate of aging, while life expectancy varies with susceptibility to disease, accident or other factors [26].

Results and Discussion

Study rationale

Sugar and sugar-sweetened beverages were considered a cause of increased body mass index and obesity. Artificial sweeteners were introduced for general use in order to reduce sugar consumption and give

better body weight control. Even if this should work in theory, scientific studies showed that sugar-sweetened beverages are not as significant to obesity as thought before and that artificial sweeteners have the ability to increase body mass index [2, 9]. Many studies, especially animal experiments, proved this unwanted effect. The conclusion of these studies is that the sweet taste increases insulin production leading to a decreased blood glucose concentration and hunger feeling [12, 17]. One can presume that consuming beverages with low glucose content that contain artificial sweeteners is advantageous. Such products provide carbohydrates so no hypoglycemia occurs, but significant amounts of sugar are replaced with calorie free substances while the taste remains the same. This is the reason why we chose to have four study groups of animals: the control group (I) receiving water, group II receiving sugar solutions, group III receiving artificially sweetened solution, and group IV receiving a solution consisting of a mixture of artificial sweeteners and sugar.

Animal studies that correlate body weight loss and consumption of artificial sweeteners are not available. We decided to examine if artificial sweeteners can alter a body weight loss pattern in mice.

Many toxic substances, extremely stressful conditions and food restrictions are known to reduce the body weight of experimental animals. The first two conditions are not relevant to a human extrapolation, and the last would lead to fights between animals. Earlier findings in our animal care facility suggested that animals are voluntarily restraining their food consumption for a limited amount of time, when they are moved in unfamiliar places, even if temperature, food quality, general housing conditions are kept the same.

It was decided to move the animals after 21 weeks of experiment from glass aquariums to steel cages and into a different room, but all other conditions to be kept unchanged, in order to assess how body weight would be affected by such minor environmental changes and what influence on the consumption of artificial sweeteners would have.

Another goal of this experiment was to observe changes in animals' life expectancy as a measure of general health conditions, even if this parameter is not commonly used in toxicological experiments. It was considered that life expectancy better describes life style induced changes in health more than any other parameter, since many health problems can go undetected for long periods of times. For example many smokers die from natural causes but their life expectancy is significantly less than that of nonsmokers [8]. Even United Nations Development Program uses human

life expectancy in the estimation process of International Human Development Indicators [24].

All three sweet solutions had the same intensity of the sweet taste; concentrations were chosen by tasting. Concentrations of sweeteners were of the same order of magnitude as the commercial products used for human consumption [3]. Because in such products usually more than two sweeteners are used we had to increase cyclamate concentration to match the sweet taste. The amounts of artificial sweeteners consumed by mice, adjusted to the body surface, were less than the maximum accepted daily intakes stated for humans. It means that any effect on the survival rate would be related to changes in body weight, insulin concentration, etc. rather than a direct toxic effect of these substances.

Relations between body weight and sweetened solutions

The results of the statistical analysis can be found in Table I.

Table I

Differences recorded between the body weights of the tested groups

Week	Males			Females		
	ANOVA	t test	% DBM	ANOVA	t test	% DBM
1	NS	NS		NS	NS	
2	GI>GIV	p=0.0031**	11.4	NS	NS	
3	GI>GIV	p=0.0140*	8.8	NS	NS	
4	GI>GIV GIII>GIV	p=0.0094** p=0.0039**	9.3 8.9	GI>GIV	p=0.0328*	5.5
6	NS	NS		GI>GIV	p=0.0132*	8.5
8	NS	NS		NS	NS	
14	NS	NS		NS	NS	
21	GIII>GI	p<0.0001***	12.0	NS	NS	
27	GIII>GI	p=0.0018**	8.8	NS	NS	
33	NS	NS		NS	NS	
41	NS	NS		NS	NS	
52	GIV>GII	p=0.0040**	13.5	NS	NS	

G – group (I, II, III, IV), % DBM - percent of difference between means, NS – non significant

Male mice

Figure 1 shows the changes in the body weight of male mice recorded for 52 weeks.

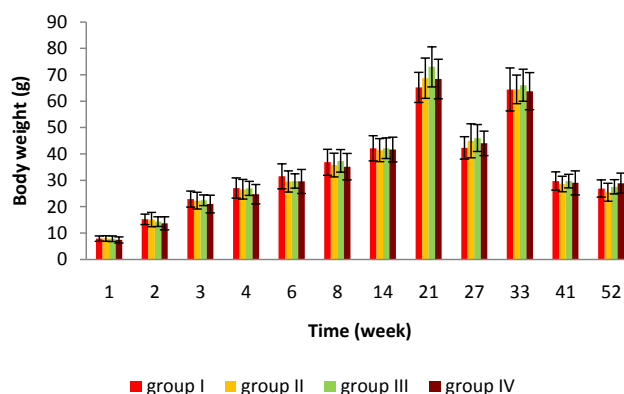


Figure 1.

Body weight dynamics in male mice during 52 weeks of testing.

Results show that substitution of water with artificially sweetened solutions was able to increase the body weights of male mice. The effect is visible only after daily administration of the artificially sweetened solution for about 20 weeks. Interestingly, in the first 6 weeks of administration, body weights of Group I (water) were higher than in the other groups, but reached statistical significance only when compared with Group IV (sugar + artificial sweeteners).

Group II (sugar) had a trend similar to that of Group IV: lower body weight in the first period of administration and higher after 20 weeks; however statistical significance was not attained in any period. It can be seen that reducing sugar content of the drinking solution but maintaining unaltered the sweet taste doesn't have such high impact on the body weight of male mice as in the case of administering artificially sweetened solutions.

It was also confirmed the hypothesis that it is reasonable to replace sugar with artificial sweeteners, but only when a minimal caloric intake is maintained, this way hypoglycemia induced by sweet taste without caloric content will be abolished. The similarity between body weight patterns seen in Group II and IV is a confirmation of the fact that effects seen in Group III are generated only by the caloric free sweet taste of the artificial sweeteners and other "pharmacological" or toxicological effects can be excluded.

When animals were moved to a different location and cages were changed, even if all other conditions (temperature, light, humidity) were kept identical, they lost about one third of their weight. This shows that inducing weight loss in mice is extremely easy and methods that induce suffering are to be avoided because of ethical considerations. The weight

loss was followed by a recovery phase after animals adapted to the new situation and resumed their usual food consumption. Even during the weight loss phase animals in Group III had larger body weights than those in the control Group I.

After the body weight recovery phase, due to aging, animals started to lose weight. However, at this stage a large number of animals were already dead and further comparison between groups could bring some errors since it is reasonable to presume that the most affected animals died first.

Female mice

In the case of female mice, those receiving artificial sweeteners had lower body weight than those receiving water, but the difference was not statistically significant during the entire period of testing. In the first period of life, replacing water with sugar + artificial sweeteners led to a reduced body weight. Statistical significance for such finding was recorded only in week four of administration ($p=0.0328^*$). Adding sugar to artificial sweeteners was also a source of reduced body weight compared to water administration in week four ($p=0.0132^*$). This was consistent with results obtained in the case of males. In the remaining measuring intervals artificial sweeteners, sugar and combination of these tended to reduce body weights compared to the water group but no statistically significant difference was found (Figure 2).

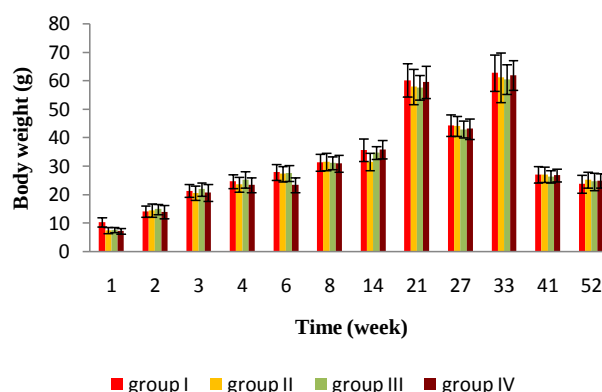


Figure 2.

Body weight variation in female mice during 52 weeks of testing

Results show that in the first period of life female mice tend to have lower body weights when using sugar + solutions sweetened with artificial sweetener instead of water. Contrary to males, at maturity, absolutely no increase in body weight is brought by the use of artificial sweeteners. On the

contrary, a statistically non-significant reduction in body weight was observed through their maturity.

As in the case of males, a weight loss of the animals can be induced by a simple change in the aspect of the environment. The body weight loss and regain pattern was not changed in any way by the administration of sweetened solutions instead of water.

Interestingly, these findings show that male mice respond better to changing conditions, being able to adjust their body weight, better than females do.

Survival rate

Male mice

Figure 3 shows the cumulative deaths of animals recorded over 101 weeks of experiment (1.9 years).

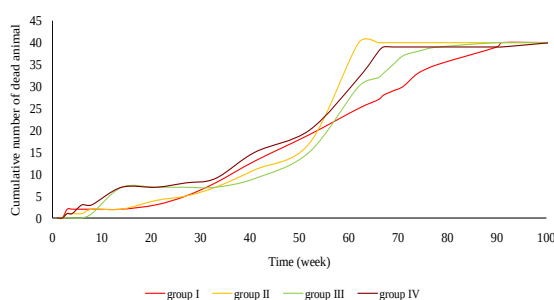


Figure 3.

Cumulative number of dead male mice for the four tested groups

The males in all tested groups had higher death rates and lower maximum life spans than the control group. However, difference between survival curves reached statistical significance only for the difference between group II and I and only in one statistical test ($p=0.0426^*$; Log-rank (Mantel-Cox) test).

Female mice

Figure 4 shows the cumulative deaths of female mice recorded over 116 weeks of experiment (2.2 years).

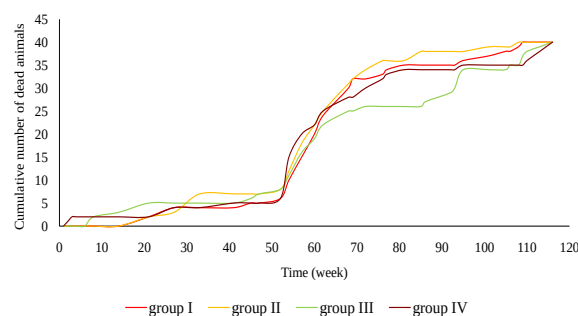


Figure 4.

Cumulative number of dead female mice in the four tested groups

In the case of females the lowest death rate was observed in Group III. Statistical significance was recorded only in the Log-Rank (Mantel-Cox) test for differences between group III and II ($p=0.0327^*$). In this case too, lower maximum life span was recorded in group II. The only consistent finding in this survival test is the fact that sugar consumption negatively affects death rates of animals and their maximum life expectancy.

Comparative analysis of survival rate differences recorded in male and female mice

Table II shows that even if differences were recorded in the survival curves of animals, life expectancy values were not considerably different between groups.

Table II

Life expectancy and maximum life span recorded for the four tested groups

	Males		Females	
	Life expectancy (week) $\pm$ SD	Maximum life span (week)	Life expectancy (week) $\pm$ SD	Maximum life span (week)
Group I	55.9 $\pm$ 22.6	94.2	67.5 $\pm$ 20.5	109.7
Group II	52.5 $\pm$ 14.6	64.5	63.7 $\pm$ 18.8	98.7
Group III	57.4 $\pm$ 20.7	82.2	71.6 $\pm$ 29.0	116.0
Group IV	52.6 $\pm$ 21.8	79.0	67.7 $\pm$ 26.0	118.5

It seems that very little negative effect, if any, affects older animals that lived longer than the average for their species.

Maximum life span however was lower with 31.5 % in the case of male mice that drank sugar-sweetened solutions compared with the control group. Unfortunately, there is no statistical test for the maximum life span of animals to evaluate differences between groups. Because of this, a negative effect of sugar administration on the general health of animals cannot be stated until further tests are made to confirm our results.

Because of the fact that all the recorded differences failed to reach statistical significance simultaneously in both statistical tests used, we can conclude that administering sugar or artificially sweetened solutions to mice does not seem to affect their general health when life expectancy is used for such evaluation.

In both cases, males and females, effects of sugar administration on death rate were visible only when animals reached their average life expectancy. These findings are interesting and further experiments need to be done to be confirmed.

Conclusions

In this work the effect of cyclamate and saccharine on body weight gain was studied in an animal study. Groups of mice received drinking solutions sweetened by artificial sweeteners, sugar or both. The relations between body weight and sweetened solutions, survival of animals and life expectancy were evaluated.

Contrary to expectations and according to literature findings for humans, sugar sweetened solutions do not lead to increased body weight in animals, probably due to the fact that caloric intake brought by sugar is compensated by a reduced caloric intake from solid foods.

Our study is also a confirmation of human studies that show a lack of increased food intake when artificially sweetened solutions are consumed after meal. In such circumstances replacing sugar with artificial sweeteners could be of advantage due to the fact that caloric intake is assured by the previously consumed meal and the individual would be pleased by additionally consuming a sweet solution.

Using commercially available juices that contain small amounts of sugar together with artificial sweeteners would probably be useful for body weight control in the case of humans too, especially when such products are consumed outside of regular meals.

Even if weight increase due to the use of artificial sweeteners seems moderate (about 10%) if extrapolated to humans can be very important since in many cases a 10% weight loss is desired by people who undergo a weight loss program.

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