Effects on Body Mass of Laboratory Rats after Ingestion of Drinking Water with Sucrose, Fructose, Aspartame, and Sucralose Additives

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Abstract: The excessive consumption of natural sweeteners is considered to be a major cause of increase in body mass. The authors wished to establish whether hypocaloric artificial sweeteners also promoted mass gain in laboratory rats (Harlan Wistar male rats). *Ad libitum* sweeteners were added to the drinking water of five groups of nine male rats each weighing *circa* 40g: Group 1, 15% fructose; group 2, 10% sucrose; group 3, 0.3% aspartame; group 4, 0.19% sucralose; and group 5 (control), ordinary drinking water. The daily volume of water consumption, the amount of ingested food, and gain of body mass were assessed during 73 days. Histological sections of the liver tissue of these rats were analyzed using Sudan and Hematoxylin-Eosin red staining. Results indicated that the fructose solution promoted the highest final gain in body mass, statistically different from the control and sucrose groups ($p<0.05$). The caloric consumption was similar to that of the sucrose group, but different from that of the control and one of the groups consuming hypocaloric sweeteners, aspartame ($p<0.05$). Rats that ingested sucrose solutions had the lowest final body mass in spite of the fact that their total caloric intake was one of the highest, and as mentioned, similar to fructose. Rats that drank water with hypo-caloric artificial sweeteners, aspartame and sucralose ingested the same amount of food, and the caloric intake was similar to the control group ($p<0.05$). They were fatter than the control and sucrose groups, although their caloric consumption was lower than that of the fructose-drinking specimens, apparently confirming recent findings about glucose absorption with ingestion of artificial sweeteners. The behavior of the sucralose group, with a body mass higher than those of the control and sucrose groups, should be further studied, since this group showed a tendency to drink more water over time when compared to the control and aspartame groups. Liver-to-body mass ratios were not statistically different ($p<0.05$) among the five groups, but both groups consuming hypocaloric sweeteners had slightly lower ratios than the sucrose, fructose, and control groups. As has been mentioned in previous research, ingestion of fructose solutions led to an increase of lipids in the liver tissue, in comparison with the other groups studied. Groups consuming hypocaloric sweeteners also showed a slight increase in lipid accumulation in liver tissue but not as much as the fructose-consuming group. The results of these experiments indicate the advisability of a long term experiment focusing on the ingestion of these sweeteners and their role in the increase in body mass.

Keywords: Mass gain, sucrose, fructose, aspartame, sucralose, drinking water, liver tissue.

INTRODUCTION

Health problems attributable to the excessive intake of carbohydrates have become frequent among populations of developed countries, and recently in the emerging countries [1]. Some sugars, like sucrose, found in sugar cane and fruits, represent the well-known type of sweetener widely distributed in nature. Common sources of carbohydrates include sugars in soft drinks such as sucrose and fructose-glucose from hydrolyzed corn [2,3]. Several studies using animal models for experimental purposes have indicated that fructose is responsible for the increased incidence of such conditions as diabetes and cardio-kidney diseases [4-7]. The addition of small amounts of fructose to food and drink increases the hepatic synthesis of glycogen in humans and reduces the glucemia response in subjects with Type 2 diabetes mellitus, suggesting the importance of fructose in liver metabolism [8-11]. Large amounts of fructose provide an irregular source of precursors of carbon to affect hepatic lipogenesis [12-14].

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Hypo-caloric sweeteners, such as aspartame and sucralose, are also commonly used. However, the effects of long term consumption of these products has not yet been established [15-17].

The purpose of this research was to observe the effects on body mass and liver-to-body mass ratio on rats ingesting caloric and hypo-caloric sweeteners dissolved in water versus a control drinking only tap water. This research studied male rats (Wistar), over 73 days, from weaning through adulthood. Rats were individually provided with drinking water *ad libitum* and a standardized commercial diet also *ad libitum*. Food and water intake, as well as body mass, were recorded daily.

MATERIALS AND METHODS

The research protocol was approved by the Ethics Committee of the Institutional Program for the Care and Use of Animals from the Animal Experimentation Unit, Complex E, Faculty of Chemistry, UNAM (*Universidad Nacional Autónoma de México*, National Autonomous University of Mexico in Spanish). For the study, 45 male Wistar rats were used (Production Center of Laboratory Animals for UNAM-Harlan, Mexico City, Mexico), with an average initial body mass of 31±0.4 g. The rats were placed in individual cages and acclimated for seven days in a controlled environment at a temperature of 23±1°C and a 12 hour cycle of light/dark. At the end of the inuring week, the rats were randomly distributed into five groups of nine rats each. The groups were formed according to the following treatments: Four different sweeteners were used in the drinking water. Two treatments were with natural sugars: 1) Fructose solution, 15% (56 kcal/100 mL) [18], and 2) sucrose solution, 10% (40 kcal/100 mL) [18], Two other treatments utilized hypo-caloric sweeteners: 3) Aspartame solution, 0.3% [18] (kcal/100 mL, negligible), and 4) sucralose solution, 0.19% [18] (kcal/100 mL, negligible). Finally, a control group was given 5) unsweetened water. All the groups received the same solid diet *ad libitum* (Global Teklad 2018s, 3.4 kcal/g feedstuff). After 73 days, the animals were euthanized, their livers removed and, using a precision scale (OHAUS model Scout II), their mass determined. The hepatic tissue was prepared for histological analysis.

Daily Preparation of Drinking Water

According to procedures mentioned above, sweeteners were dissolved in drinking water. After daily intake was measured, residual water was discarded and fresh solutions were placed after water troughs were carefully washed to avoid bacterial contamination. In the case of hypocaloric sweeteners, the small amount of glucose added to enhance the sweet flavor was considered negligible for the total caloric intake [5].

Gain in Body Mass and Measurements of Food Intake

Body mass gain was individually measured three times a week for each of the nine rats in the five groups by means of an analytical balance (OHAUS model Scout II); the average was calculated by group/day. Food intake was daily measured for each rat in the same way.

Histological Analysis

Once the hepatic organs were obtained, they were set up into a formaldehyde solution buffered to pH 7.4. Later on, histological cuts of approximately six micrometers thick were made using a cryostat. The cuts were stained with Sudan and Hematoxylin-Eosin (HE) techniques in order to highlight acylglycerols in the tissues [19,20].

Statistical Analyses

Experimental data obtained were statistically processed with the analysis of variance (ANOVA) to observe significant differences ($p<0.05$) between: a) Body mass gained by specimens and groups during the time of the experiment, b) Differences in the volumes of water solutions ingested by specimens and groups during the experiment, c) Differences in the amounts of food ingested, and d) Differences in total caloric intake per group at the end of the experiment. Once it was established that statistically significant differences existed, a comparison among groups, by means of a paired Student's *t*-distribution ($p\leq0.05$), was performed [21]. Also, a comparison between pairs of groups was carried out: caloric sweeteners (fructose-sucrose), hypocaloric sweeteners (aspartame-sucralose), using paired Student's *t*-distribution ($p\leq0.05$) [21].

RESULTS AND DISCUSSION

The effects of sweeteners on body mass, and solid food and liquid intake for the five groups of rats are presented in the following paragraphs. It should be mentioned that no abnormalities (no vomitus, low activity or diarrhea) were observed during the 73 days of the experiments in any of the animals of the groups studied. The only anomaly was the death of one specimen during the inuring week (adaptation

Table 1. Final Data for the Laboratory Rats (Five Groups after 73 Days)

	*Final mean body mass after 73 days, g	*Mean total caloric intake along 73 days, kcal	*Mean ratio, liver mass/body mass (g/g)
Control	353.88±26.01[a]	4460±315.7[a]	0.043±0.005[a]
Sucrose	348.68±36.38[b]	5334±323.9[b]	0.044±0.009[a]
Fructose	373.60±26.27[c]	5371±598.5[b]	0.043±0.004[a]
Aspartame	363.71±33.04[c,d]	4466±459.7[a]	0.040±0.006[a]
Sucralose	358.11±28.88[d]	4524±502.2[a]	0.041±0.007[a]

*SD data are for the values at day 73 ($p<0.05$) (n=9)

[a,b,c,d]Paired Student's t test

after weaning), leaving one group with eight animals instead of nine.

Increase in Body Mass

After 73 days, the final gain in body mass of the groups, in decreasing order, was: fructose>aspartame>sucralose>control>sucrose (Table **1**). There were statistical differences among them ($p<0.05$). Contrary to expectations, the group that consumed sucrose gained the least mass (Fig. **1a**). Data for the inuring week and final data for body mass are shown in Fig. (**1b**).

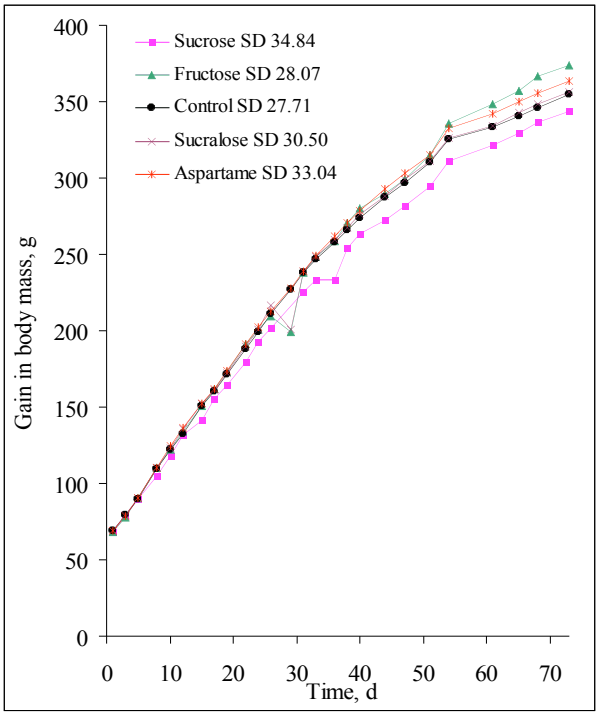

Fig. (1a). Gain in body mass from day 0 to day 73 for the laboratory rats during the experiment with drinking water with different sweeteners: control group; fructose group; sucrose group; sucralose group; aspartame group. Values are mean±S.D. ($p<0.05$) (n=9).

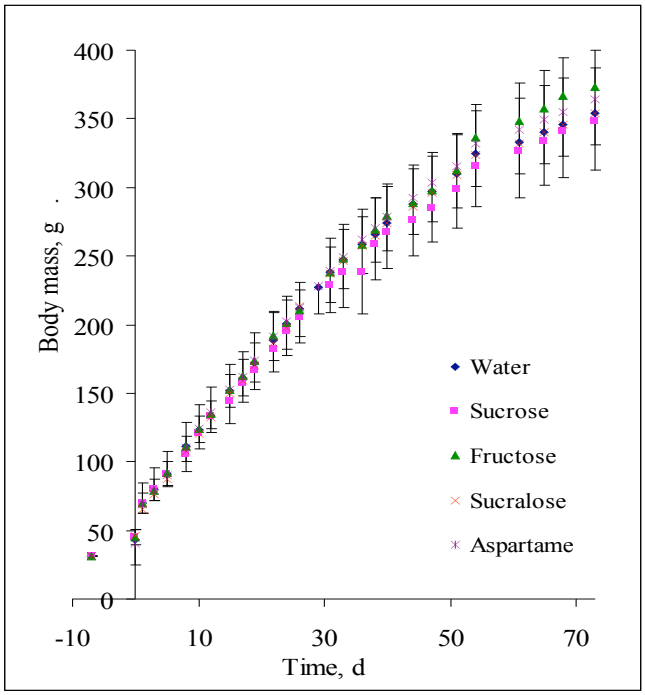

Fig. (1b). Body mass from day -7 to day 0 (inuring week) and from day 0 to day 73 for the laboratory rats during the experiment with drinking water with different sweeteners: control group; fructose group; sucrose group; sucralose group; aspartame group. Values are mean±S.D. ($p<0.05$) (n=9).

Volume of Food and Water Intake, and Type of Sweetener in Solution

Volume of water ingested daily by specimens appears in Fig. (**2a**), and its equivalent in kilocalories in Fig. (**2b**). No statistical differences were found between the caloric intake of those specimens drinking sucrose and fructose solutions, as previously indicated in the literature on these fluid concentrations [5]. However, gain in body mass is quite different, and this fact provides an interesting subject for

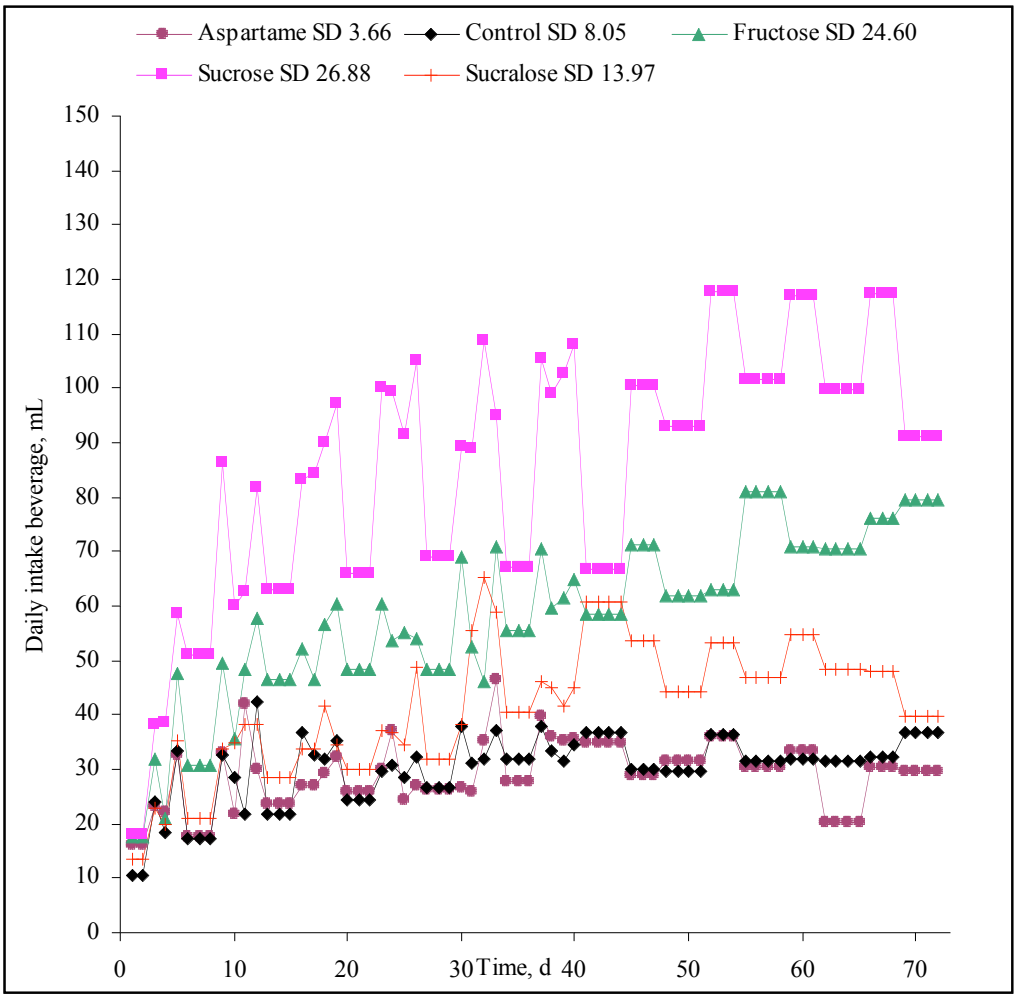

Fig. (2a). Water ingested, in (mL/d), by laboratory rats during the experiment with different sweeteners: control group; fructose group; sucrose group; sucralose group; aspartame group. Values are mean. SD is for day 72 ($p<0.05$) (n=9).

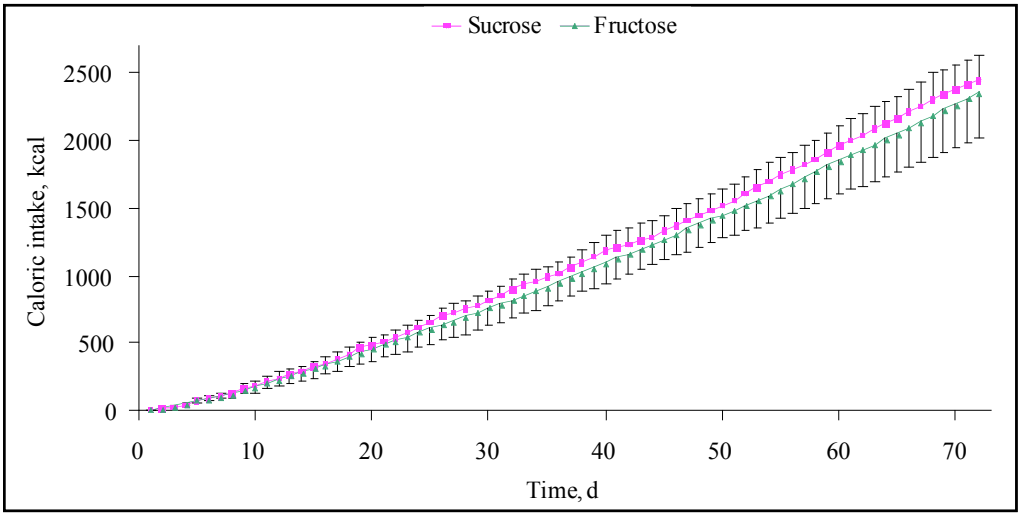

Fig. (2b). Caloric intake, in kcal/d, by laboratory rats drinking water with fructose and sucrose added. Values are mean±SD ($p<0.05$) (n=9).

further study, especially because some researchers have found different results concerning fructose versus glucose, the two monosaccharides in sucrose [8,22,23].

The sucrose- and fructose-consuming groups ingested the lowest amount of solid food, a fact that is to be expected, since their combined caloric intake is the highest for the five groups studied (Figs. **3a,b**). However, gain in mass was higher for the fructose-consuming group than the sucrose-consuming one, the difference becoming greater over time. Upon review of the literature, this outcome would be unexpected [15,22], suggesting longer-term experiments need to be carried out to determine whether these differences continue.

Groups consuming hypocaloric sweeteners in drinking water ingested similar amounts of solid food to that of control specimens (Table **1**). An interesting fact was observed concerning the daily volume consumed by the aspartame group and the sucralose group. The first group showed a similar pattern to the control. However, the sucralose-consuming group showed a growing tendency towards greater ingestion of water at the end of the experiment. Statistical differences were found for the sucralose-consuming group with respect to the aspartame and control groups with respect to daily liquid ingestion, but not final body mass. Total caloric ingestion was no different from that of the control group ($p<0.05$).

Recent papers mention that "Artificial sweeteners increase glucose absorption in the order acesulfame potassium > sucralose > saccharin, in parallel with their ability to increase intracellular calcium concentration" [24,25]. Therefore, further studies on these two hypo-caloric sweeteners, aspartame and sucralose, as well as those

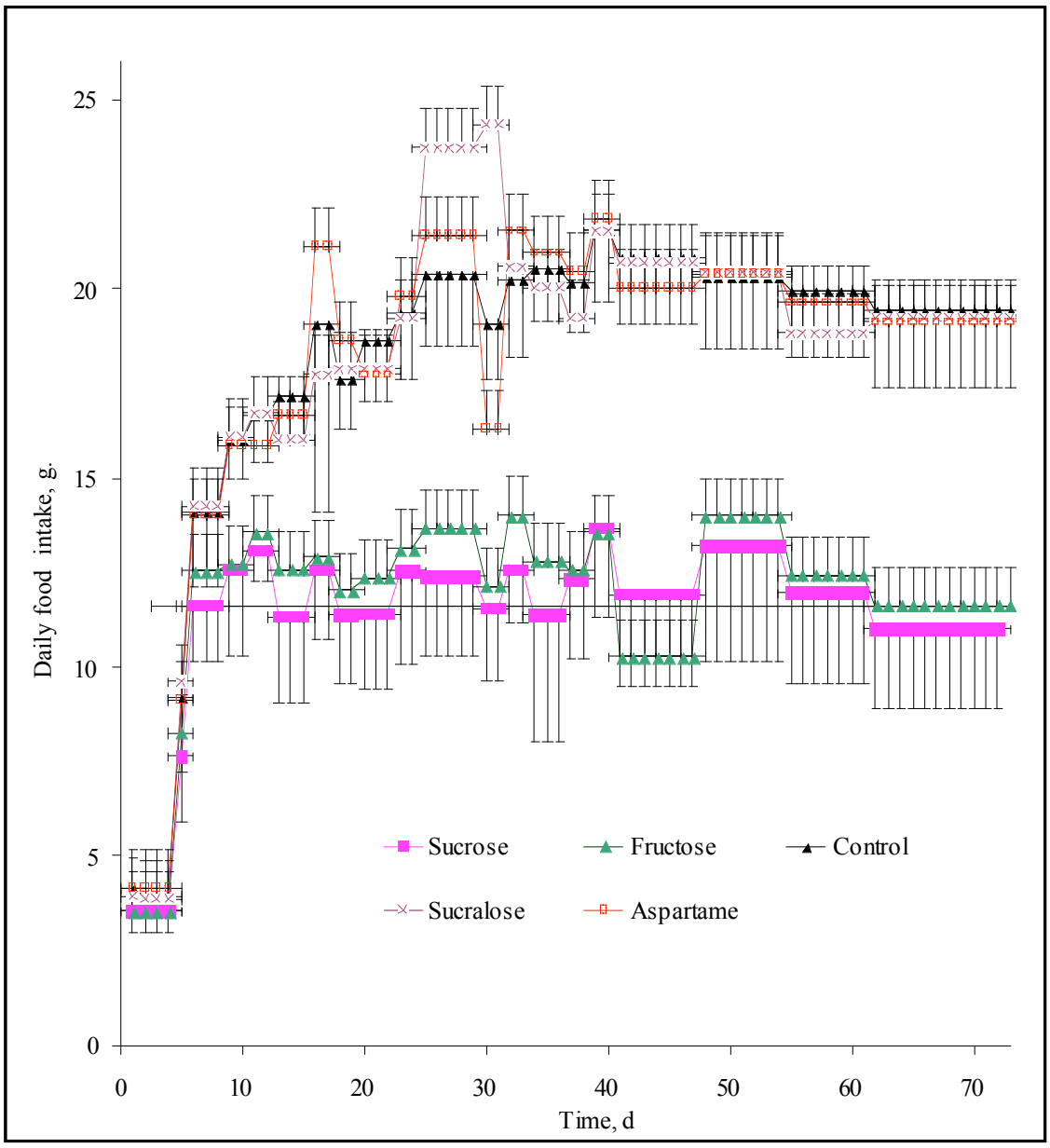

Fig. (3a). Solid food intake, in (g/d), by laboratory rats during the experiment drinking water with different sweeteners added: control group; fructose group; sucrose group; sucralose group; aspartame group. Values are mean±SD ($p<0.05$) (n=9).

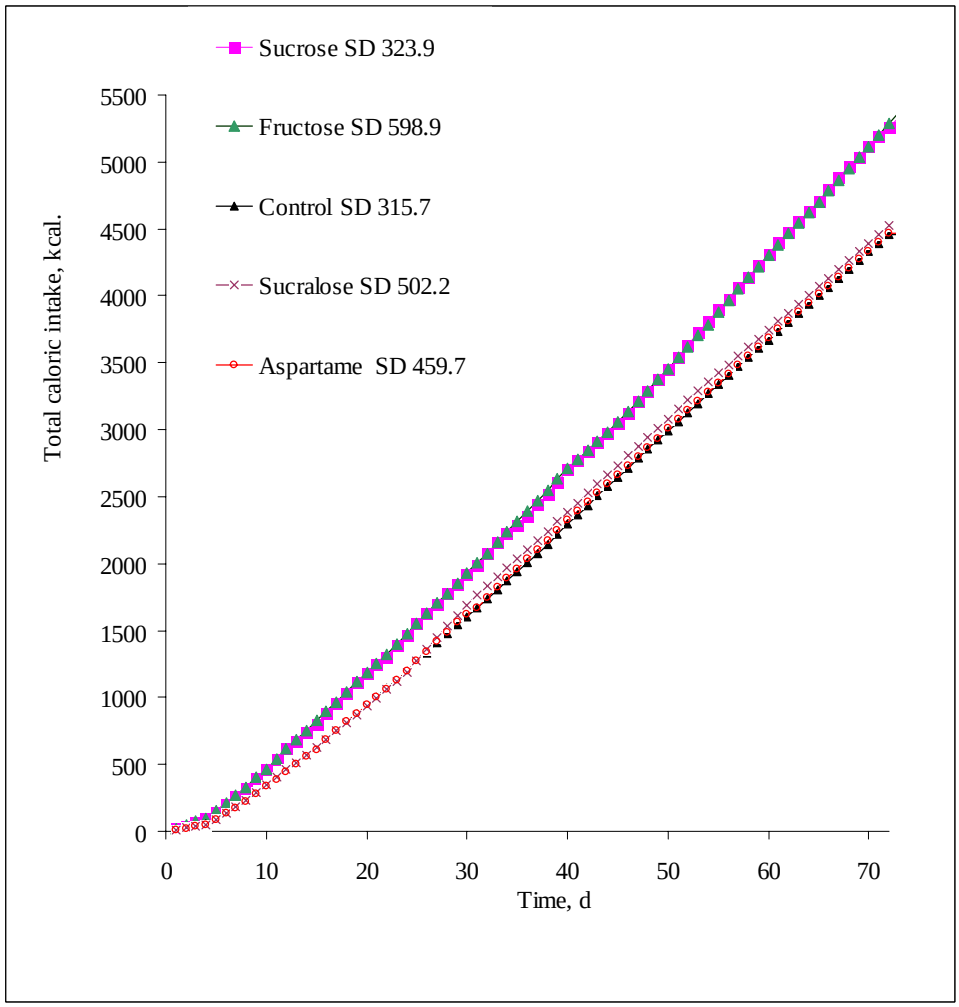

Fig. (3b). Total caloric intake (food and drink), in (g/d), by laboratory rats drinking water with different sweeteners added: control group; fructose group; sucrose group; sucralose group; aspartame group. Values are mean±SD ($p<0.05$) (n=9).

mentioned in these contributions, should be addressed in order to explain these differences and their effects on metabolism.

Effects of the Addition of Sweeteners on the Histology of Hepatic Tissue

Because the sweeteners are metabolized in the liver, a histological study was performed in order to observe the

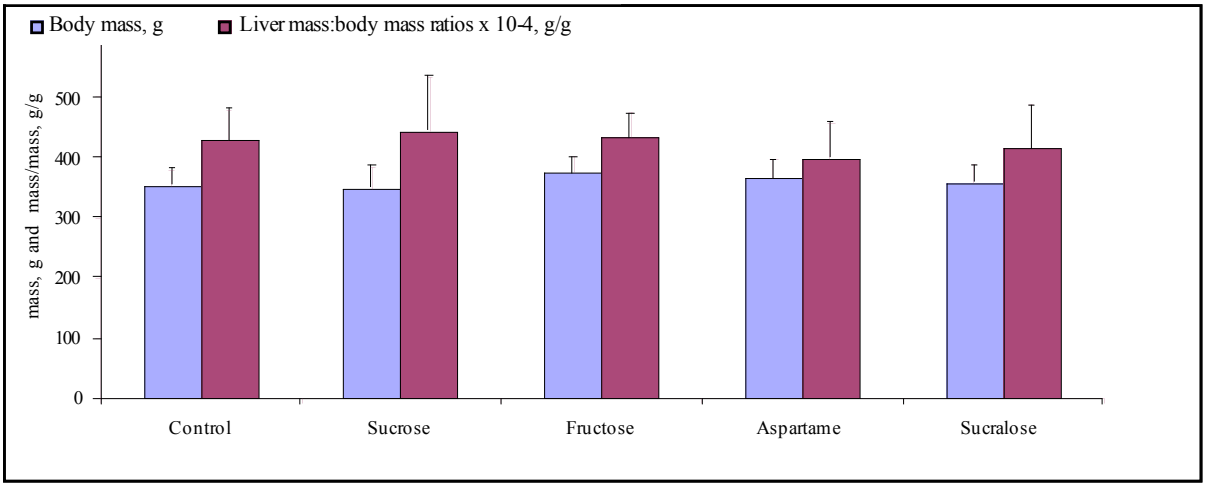

Fig. (4). Body mass of laboratory rats (blue columns) for the five groups (control; fructose group; sucrose group; sucralose group; aspartame group), in g, and liver mass/body mass ratios (magenta columns), in g/g, at 73 days. Values are mean±SD ($p<0.05$) (n=9).

presence of lipids *in situ*. Animals' liver mass/body mass ratio was quantified (Fig. **4**). It was observed that the sucrose- and fructose-ingesting groups tended to have greater liver mass/body mass ratios. On the other hand, groups ingesting hypo-caloric sweeteners showed a lower ratio (Table **1**). Although these data were not statistically different ($p<0.05$), this could be another topic for study in a longer-term experiment to establish possible metabolic implications. This is particularly relevant, considering recent papers on the subject [8,22,24,25].

Histology assessment utilized Hematoxylin-Eosin and Sudan staining. Results indicated the presence of a greater amount of lipid clusters in the livers of the rats drinking the fructose solution compared to the livers of specimens from the other four groups. As an example, Fig. (**5**) shows Sudan staining results.

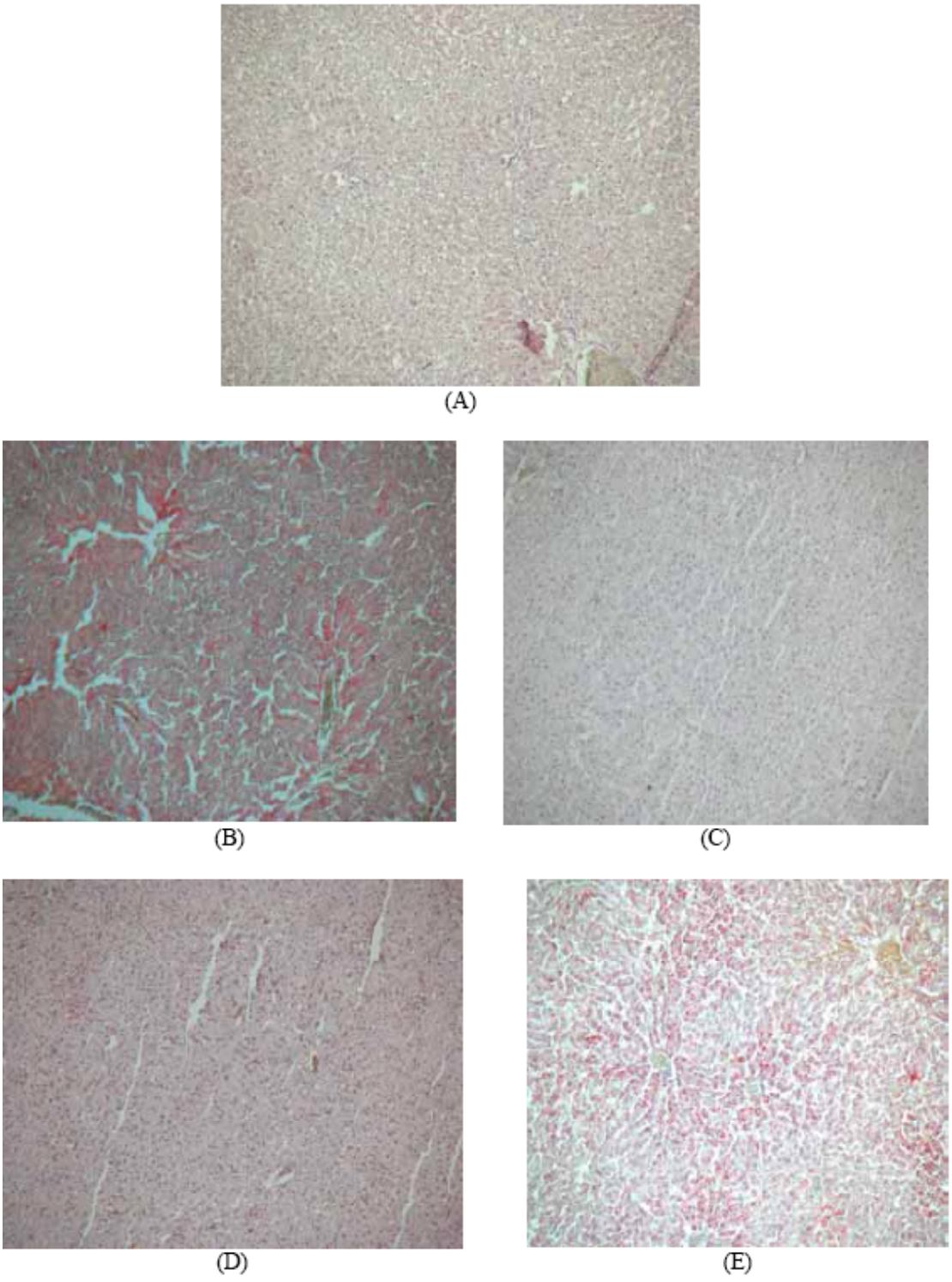

Fig. (5). Samples of histological studies using Sudan staining of lipid clusters in liver tissue of laboratory rats drinking water with different sweeteners added. **A:** control group; **B:** fructose group; **C:** sucrose group; **D:** sucralose group; **E:** aspartame group.

CONCLUSIONS

Results obtained in this research show that groups of laboratory rats consuming water with fructose, aspartame, and sucralose from weaning age to adulthood had a gain in body mass higher than that of the sucrose-consuming group and the control, in spite of the fact that sucrose- and fructose-, and sucralose-, aspartame-, and control-consuming groups had similar caloric intakes ($p<0.05$).

Additionally, histological analysis demonstrated that the hepatic tissue of the rats drinking the fructose solution showed a greater accumulation of extracellular lipids, which has been mentioned by other researchers.

On the other hand, the groups of rats consuming hypo-caloric sweeteners had similar solid food intakes to those of the control. Nevertheless, when liquid intake was analyzed, it was observed that the animals receiving the hypo-caloric sweeteners showed a difference with respect to the sucralose group. A certain preference for sucralose by the specimens was assessed. This preference should be studied further. Although a tendency towards a lower liver mass/body mass ratio was observed, it did not reach statistically significant values. These results should be also further studied in a longer-term experiment.

The purpose of this research was to observe the effects on body mass and liver-to-body mass ratio on rats consuming caloric and hypo-caloric sweeteners dissolved in water as compared to a control drinking only tap water. This research was carried out in Wistar laboratory rats from weaning to adulthood. The findings point to the importance of continuing this research to study effects of caloric and hypocaloric sweeteners on gain in body mass and/or increased visceral adiposity, as well as modifications in other organs, such as the kidneys and heart.

NOTE

"In the sciences, mass and weight are different properties. Mass is a measure of the amount of matter in the body while weight is a measure of the force on the object caused by a gravitational field. In routine laboratory use, the reading on a precision scale when a stainless steel standard is placed upon it is actually its conventional mass; that is, its true mass minus buoyancy. Also, any object compared to a stainless steel mass standard has *its* conventional mass measured; that is, its true mass minus an unknown degree of buoyancy. For certain high-precision disciplines, the density of a sample is sometimes known or can be closely estimated (such as when weighing aqueous solutions) and the effect of buoyancy is compensated for mathematically" (http://en.wikipedia.org/wiki/Mass_versus_ weight).

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