LIVER:BODY MASS[1] RATIOS OF LAB RATS DRINKING WATER WITH CALORIC AND HYPO-CALORIC SWEETENERS ADDED

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KEYWORDS: Sucrose, Fructose, Aspartame, Sucralose, Liver Tissue.

Abstract

CARBOHYDRATES are metabolised in the livers of mammals. In order to determine the effect of consumption of sweeteners on the liver, mass ratios of the mass of the liver to total body mass were assessed in experiments carried out in laboratory animals. Weaned male Wistar rats were fed daily with a solid diet *ad libitum* (Global Teklad), and given water with: 1) Fructose, 15%; 2) Sucrose, 10%; 3) Aspartame, 0.3%; 4) Sucralose, 0.19%, and 5) Control, unsweetened water. After 73 days, the animals were sacrificed, and hepatic tissues were removed. Daily water volume consumption, amount of ingested food, and body mass gain were assessed. Results were statistically compared ($p<0.05$), showing that fructose in water promoted the highest mass gain in the rats. Rats that consumed sucrose had the lowest food consumption and the lowest final mass gain, statistically similar to those of the control lot. Study animals that had water with hypo-caloric artificial sweeteners, aspartame and sucralose, ingested the same amount of food as the control counterparts but were fatter than control and sucrose lots, but lower than the fructose-drinking specimens. The fructose-consuming lot showed a tendency to greater liver mass:body mass ratio even though it did not have statistically-significant values with respect to the other lots evaluated. A contrary tendency of liver:body mass ratios, smaller than the other three lots, was produced in the lots ingesting the two hypo-caloric sweeteners, although they did not reach statistically-significant values. These results should be further studied in a longer term experiment (250–300 days).

Introduction

The prevalence of obesity is increasing around the world, and is a significant public health problem in many countries. Consumers are increasingly concerned about the quality and safety of

[1] 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. For example, a person with 60 kg **mass**, weighs 60 **kg-force** in Earth's surface; but, the same person, in the Moon surface would only weigh around 10 kg-force; however, his/her mass will be 60 kg. In common language, the term 'weight' is wrongly used as a synonym of 'mass'. The mass unit in the IS is the kilogram, kg

many products present in the diets of industrialised countries, in particular, the use of artificial sweeteners and dietary supplements.

General apprehension also exists regarding the possible long-term health effects. Of particular concern are the potential carcinogenic effects of these products (Vermunt *et al*., 2003; Soffritti *et al*., 2006). Fructose is a monosaccharide found in fruits and honey that in recent decades has been enzymatically obtained from starch hydrolysis, mainly from maize (Bray *et al*., 2004). Some authors have suggested that the ingestion of fructose syrups may be responsible for much of the obesity problem seen in today's world (Aeberli *et al*., 2007; Gaby, 2005; Johnson *et al*., 2007).

Fructose is directly transported to the liver without increasing insulin in blood plasma, mobilising fats, and at a higher rate than glucose, which is controlled by phosphofructokinase (Voet and Voet, 1992). The liver, kidneys, and small intestine are the main metabolic utilisation sites for sugar (Murray *et al*., 1994). Aspartame and sucralose, some of the most commonly-added artificial sweeteners to foods and drinks, have also been studied, but still no conclusive data are available (Jürgens *et al*., 2005, Teff *et al*., 2004).

To corroborate the effects that these sweeteners have in liver when chronically consumed, especially in infancy and adolescence, these sweeteners were added daily to the drinking water of male Wistar rats after they were weaned, using them as models, until they reached 'adulthood'. Both food and water were given *ad libitum*.

Materials and methods

The investigation protocol was approved by 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). Male Wistar rats were used with an average initial body mass of 39.2± 0.4 g.

Rats were placed in individual boxes and acclimated for seven days in a controlled environment at a temperature of 23±1°C and a 12 hour cycle of light/dark and fed with the same solid diet *ad libitum* (Global Teklad) and tap drinking water. At the end of the inuring week, they were randomly distributed in five groups of 9 rats each one. Four groups had different sweeteners in their drinking water.

Two treatments were with natural sugars comparable to those found in soft drinks:

1) Fructose solution, 15% (Jürgens *et al.,* 2005), and
2) Sucrose solution, 10% (González, 2006).

Two other treatments utilised hypocaloric sweeteners:

3) Aspartame solution, 0.3% (González, 2006), and
4) Sucralose solution, 0.19% (González, 2006).

Finally, the fifth group received

5) Unsweetened drinking water (control).

Sweeteners were dissolved in drinking water. After daily intake was measured, residual water was discarded and freshly-prepared solutions were placed after water troughs were perfectly washed in order to avoid bacterial contamination. Body mass gain was individually measured for each of the nine rats of the five groups by means of an analytical weighing scale (OHAUS model Scout II), three times per week; the average was calculated by group/day. In the same way, food and liquid were measured daily for each rat.

After 73 days of feeding, the animals were sacrificed and their livers removed for histological analysis (Genneser, 1989; Ross *et al*., 1992). Experimental data obtained were statistically processed with analysis of variance (ANOVA, $p \leq 0.05$), between: a) Body mass gained by specimens and lots for the duration of the experiment, and b) Differences in the mass ratios of the amounts of food ingested by specimens and lots for the duration of the experiment. Once it was corroborated that statistically-significant differences existed, a comparison between groups, using a student's *t*-distribution ($p \leq 0.05$), was performed (Pedrero and Pangborn, 1996).

Results and discussion

Figure 1 presents the average values of liver mass with respect to total mass gain from the nine rats of each group. A tendency towards higher liver:body mass ratios was observed in the animals that ingested fructose. Even though these data were not statistically different ($p<0.05$), this finding will have to be studied in a longer term experiment (250–300 days) to establish its possible meaning.

Results obtained in this research show that drinking fructose water solutions promoted the highest mass gain in Wistar rats in comparison to other caloric and hypo-caloric sweeteners (Figure 2). Statistical differences were found among the rat group that ingested water containing caloric sweeteners (fructose, with respect to sugar and control), with the highest mass gain for the fructose-consuming group and the lowest for the sugar-consuming group.

There were no statistically-significant differences in mass gain between the sugar-consuming and control lots ($p<0.05$). When a comparison was made between both groups consuming hypo-caloric sweeteners, there were no significant differences. However, they were statistically different with respect to the other three groups (sucrose, fructose, and control).

The highest body mass gain from these two groups consuming hypo-caloric sweeteners was observed in the aspartame-consuming group. Once the rats reached adulthood, daily water consumption was up to 110 mL for the sugar group whereas for the control and aspartame-consuming groups, it was only 30 mL, with the fructose-consuming group ca. 90 mL, and the sucralose-consuming group around 55 mL (Figure 3).

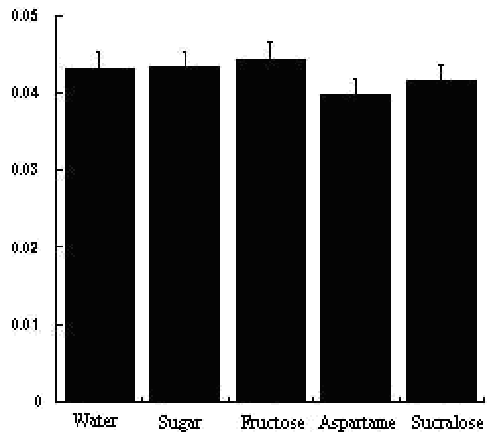

Fig. 1—Average ratio, (g) liver mass /(g) body mass, of model animals after 73 days of drinking water with different sweeteners added. Values are mean±S.D. ($p<0.05$) (n=9).

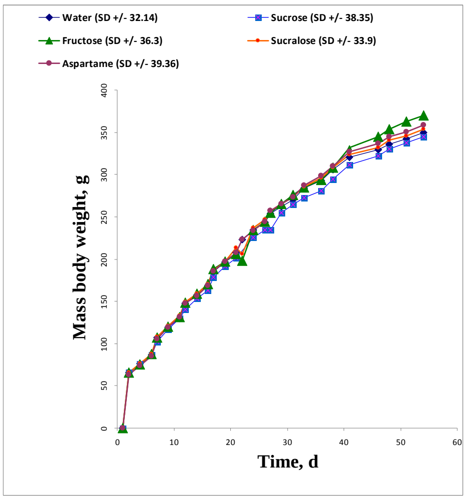

Fig. 2—Average body mass of animals, in (g), from day 0 to day 73 drinking water with different sweeteners added: (◊) Control (△Unsweetened water); (□) Sugar; ( ) Fructose; (+) Sucralose; (*) Aspartame. Values are mean±S.D. ($p<0.05$) (n=9).

Daily food intake was on average 19 g for the groups consuming hypo-caloric sweeteners and controls, and 12 g for the groups consuming caloric sweeteners (Figure 4). As had been expected, a preference was observed on the part of the animals for the liquid containing caloric sweeteners, along with a reduction of the ingestion of solids, where the daily caloric ingestion was concerned. Histological analysis showed that the hepatic tissue of the rats that drank fructose solution showed a greater accumulation and concentration of extracellular lipids.

It is interesting to observe that in spite of the similar daily consumption of water, and food with similar caloric intake (Martínez-Tinajero *et al.*, 2009), the animals that consumed hypo-caloric sweeteners are statistically fatter than those of the control group. A recent paper suggests that metabolic alterations might indeed be occurring after ingestion of these artificial sweeteners (Dyer *et al.*, 2007).

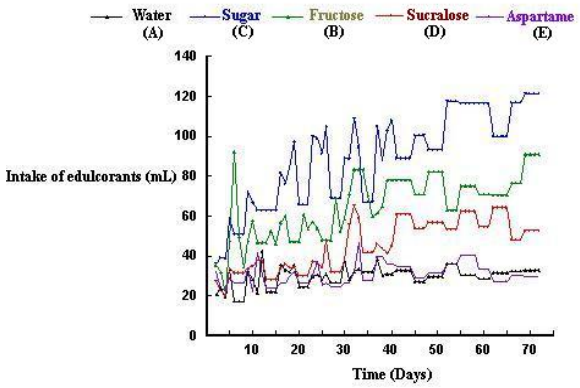

Fig. 3—Volume of drinking water ingested, in (mL/d), by laboratory animals drinking water with different sweeteners added: (◊) Control (Unsweetened water); (☐) Sugar; ( ) Fructose; (+) Sucralose; (*) Aspartame. Values are mean±S.D. (p<0.05) (n=9)

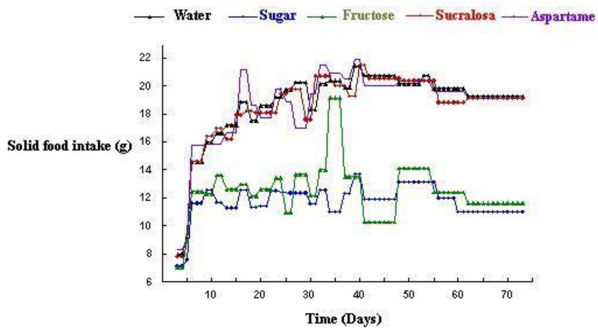

Fig. 4—Daily food intake of animals, in (g), after 73 days of drinking water with different sweeteners added: (◊) Control (Unsweetened water); (☐) Sugar; ( ) Fructose; (+) Sucralose; (*) Aspartame. Values are mean±S.D. (p<0.05) (n=9)

On the other hand, the groups ingesting hypo-caloric sweeteners showed a tendency to lower liver:body mass ratio, even though this did not reach statistically-significant values. (Figure 1). Rats that consumed hypo-caloric sweeteners had similar solid food intakes to that of the control group.

Nevertheless, when the liquid intake was analysed, it was observed that the animals receiving the hypo-caloric sweeteners showed a statistical difference where the sucralose-consuming lot was concerned (p<0.05). This behaviour should also be evaluated in a longer term experiment (250–300 days) to establish its possible meaning.

Conclusions

Caloric sweeteners promote water intake. However, fructose promotes a higher mass gain than sugar. Moreover, ingestion of fructose brings about an accumulation of extracellular lipids in liver tissue, as well as a slightly higher liver:body mass ratio when it is compared to sugar-ingesting and control groups.

Although hypo-caloric sweeteners did not contribute to caloric intake, they promoted greater mass gain than sugar-ingesting and control groups. Additionally, a slight tendency to a lower liver:body mass ratio was found among the sucralose- and aspartame-ingesting group when compared to the sugar-ingesting and control groups. These effects on liver:body mass ratios for both natural and hypo-caloric sweeteners should be studied further.

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RAPPORT FOIE : POIDS CORPOREL DE RATS DE LABORATOIRE CONSOMMANT DE L'EAU CONTENANT DES EDULCORANTS CALORIQUES ET HYPO-CALORIQUES

Par

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Mots Clés : Saccharose, Fructose, Aspartame, Sucralose, Tissus Hépatiques.

Résumé

LES CARBOHYDRATES sont métabolisés dans le foie des mammifères. Afin de déterminer l'effet de la consommation d'édulcorants sur le foie, les rapports de la masse du foie et la masse du corps total ont été évalués lors des expériences menées sur des animaux de laboratoire. Des rats adultes mâles Wistar ont été nourris quotidiennement avec une alimentation solide ad libitum et de l'eau contenant (1) 15% de fructose, (2) 10% de saccharose, (3)0.3% d'aspartame, (4) 0.19% de sucralose, et (5) de l'eau non sucré, comme contrôle. Après 73 jours, les animaux ont été sacrifiés et les tissus hépatiques retirés. La consommation journalière du volume d'eau, la quantité des aliments ingérés et le gain de poids corporel ont été évalués. Les résultats ont été comparés statistiquement ($p<0.05$), montrant que le fructose dans l'eau favorise le gain de poids le plus élevé chez les rats. Les rats consommant du saccharose avaient la plus faible consommation alimentaire et le gain de poids le plus bas, statistiquement similaire au contrôle. L'étude des animaux qui avaient bu de l'eau avec les édulcorants hypo caloriques artificiels, l'aspartame et le sucralose, ont consommé la même quantité de nourriture que ceux du contrôle mais étaient plus gros que les lots de contrôle et de saccharose, et inférieur à ceux qui avaient consommé du fructose. Ces derniers ont montré une tendance à un plus grand rapport entre la masse du foie et du corps, même si les différences n'étaient pas statistiquement significatives. Une tendance contraire au ratio foie:poids corporel, plus faible que les trois autres lots, a été noté dans les lots avec les deux édulcorants hypo-caloriques, bien qu'ils n'ont pas atteint des valeurs statistiquement significatives. Ces résultats devraient être étudiés plus profondément dans une expérience à long terme (250–300 jours).

INDICES TEJIDO HEPÁTICO:MASA CORPORAL EN RATAS DE LABORATORIO QUE BEBIERON AGUA ENDULZADA CON EDULCORANTES CALÓRICOS E HIPO CALORICOS

Por

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Palabras Clave: Sacarosa, Fructosa, Aspartame, Sucralosa, Tejido Hepático.

Resumen

LOS CARBOHIDRATOS son metabolizados en el hígado de los mamíferos. Con el propósito de determinar el efecto que tiene en el hígado el consumo de edulcorantes, se llevó un a cabo un estudio de las razones de la masa del hígado y la masa corporal total de un grupo de animales de laboratorio. Ratas macho Wistar destetadas fueron alimentadas diariamente con una dieta sólida *ad libitum* (Global Teklad) y se les dio a beber agua que contenía uno de los siguientes edulcorantes: 1) Fructosa, 15%; 2) Sacarosa, 10%; 3) Aspartame, 0.3%; 4) Sucralosa, 0.19%, y 5) Control, agua sin edulcorante. Después de 73 días, los animales fueron sacrificados y el tejido hepático fue removido. Se tenía información del volumen de agua consumido por día, cantidad de alimento y ganancia en masa corporal. Los resultados fueron comparados estadísticamente ($p<0.05$), mostrando que la fructosa en agua provocó la mayor ganancia de masa en las ratas. Las ratas que consumieron sacarosa tuvieron la menor ingesta de alimento y la ganancia de masa más baja, estadísticamente similar a los animales del grupo control. Los animales bajo estudio que tomaron agua con edulcorantes artificiales hipo-calóricos, aspartame y sucralosa, consumieron la misma cantidad de comida que los animales en el grupo control pero estaban más gordos que el control y que el grupo que consumió sacarosa, aunque menos pesados que los animales que tomaron agua con fructosa. El grupo que consumió fructosa mostró una tendencia a tener un radio masa hepática:masa corporal más alto aunque los resultados no fueron estadísticamente significativos respecto a los otros grupos. Una tendencia contraria en cuanto al radio masa hepatica:masa corporal se notó en los grupos de ratas consumiendo los dos edulcorantes hipo-calóricos, aunque los valores tampoco fueron estadísticamente significativos. Estos resultados deben estudiarse más a fondo en experimentos de más largo plazo (250–300 días).