NUTRITIONAL AND BIOCHEMICAL EFFECTS OF ASPARTAME INTAKE IN RATS UNDER AN EXPERIMENTAL DIET

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KEYWORDS

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ABSTRACT

The present study evaluates the effect of aspartame intake in rats under a diet mimicking the trends of fat consumption in the society. The composition of the experimental diet was within the recommended human limits except the saturated fat amount supplying from coconut fat. Rats under the experimental diet showed an increase in the body weight, transitory increase in the blood pressure and in plasma values of glucose and triglycerides alongside a transitory reduction in plasma urea versus the standard group. Rats under the experimental diet plus aspartame intake (54.8 ± 7.3 mg/kg bw/day) did not show any increase of body weight and in plasma values of glucose and triglycerides while showed an improvement in the plasma value of urea with respect the group under only the experimental diet. However, the aspartame group showed a more maintained increase of blood pressure. In conclusion, experimental diet produces negative effects on cardiovascular risk factors of the rats while the aspartame intake under the experimental feeding had mixed effects on the cardiometabolic factors of the animals.

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1 Introduction

Aspartame a synthetic nonnutritive sweetener is a widespread component in the current human diet. This sweetener employed for diverse proposes such as the reduction in sugar consumption, decrease of caloric intake and to maintain or lose body weight keeping the palatability of the food. Aspartame approved as a sweetening agent and a flavor enhancer in more than 90 countries and has been added to over 6000 products worldwide (Butchko et al., 2002; Magnuson et al., 2007). The acceptable daily intake for aspartame in humans was established at 40 or 50 mg/kg of body weight (bw) according to the European Union's Scientific Committee on Food or the U.S. Food and Drug Administration, respectively (Magnuson et al., 2007).

Premarketing and postmarketing evaluations of aspartame demonstrated their safe use for human consumption (Butchko et al., 2002; Magnuson et al., 2007). However, other studies have been demonstrated that the consumption of aspartame may cause deleterious effects such as hepatocellular injury, carcinogenic effects and systemic hypertension (Roberts, 2004; Soffritti et al., 2006; Gombos et al., 2007; Abhilash et al., 2011).

In the same manner, ubiquitous components of the food supply such as fats, sugars and proteins have physiological functions in the body (Joint WHO/FAO/UNU Expert Consultation, 2002; Johnson et al., 2009; Nettleton et al., 2013). However, changes in the dietary and lifestyle patterns contribute to the causal factors underlying the rapidly increase of the worldwide burden of chronic diseases including obesity, diabetes, cardiovascular disease, hypertension, stroke and some types of cancer. For example, intake of dietary fats has a remarkable increase in the world over the past three decades where North America and Europe have the high consumption amounts (Joint WHO/FAO Expert Consultation, 2003). Consequently, the Joint World Health Organization / Food and Agricultural Organization (WHO/FAO) recommends a human consumption of saturated fat of <10%, added sugar of <10% and protein of 10-15% of energy alongside other recommendations to prevent the diet-related diseases (Joint WHO/FAO Expert Consultation, 2003; FAO Expert Consultation, 2010).

A study assaying the aspartame intake under a similar pattern of the society feeding relating to the WHO/FAO recommended limits has not been reported (Frey, 1976; Knopp et al., 1976; Ishii et al., 1981; Stegink et al., 1983; Shahangian et al., 1984; Stegink, 1987; Colagiuri et al., 1989; Gupta et al., 1989; Butchko et al., 2002; Gougeon et al., 2004; Roberts, 2004; de la Hunty et al., 2006; Soffritti et al., 2006; Gombos et al., 2007; Magnuson et al., 2007; Soffritti et al., 2007; Roberts, 2008; Anton et al., 2010; Abhilash et al., 2011; Aune, 2012; Schernhammer et al., 2012; Feijó et al., 2013; Palmnäs et al., 2014). Therefore, the aim of this study was to evaluate the nutritional and biochemical effects of aspartame in Wistar rats

consuming a fat plus sucrose enriched diet relating to the WHO/FAO values.

2 Materials and Methods

2.1 Animals and diets

After one week of acclimatization, thirteen-week-old male Wistar rats were randomly divided in the standard (S) group and experimental (E) group. The S group (n=6) had free access to standard pellets (Rodent Laboratory Chow 5001, PMI Nutrition Int'l, LLC, Brentwood, MO) while the E group (n=12) fed *ad libitum* coconut fat and sucrose supplemented standard pellets for 22 weeks. The caloric density for the S diet was 3.19 kcal/g with 28.9% of the energy (%E) from protein, 58.4%E of total carbohydrates, 12.7%E of total fat and 4.4%E of saturated fat while the E diet was 3.99 kcal/g with 15.4%E of protein, 57.0%E of total carbohydrates, 10.3%E of free sucrose, 27.7%E of total fat and 15.9%E of saturated fat. The nutritional information of diets was obtained from the supplier and food analysis in the Center of Investigation and Postgraduate Studies (CIEP) from the University of San Luis Potosí. The animals were housed in individual acrylic cages with free access to water under a controlled humidity (65-70%) and temperature (24 ± 2 °C), maintained on a 12 h light and dark cycle. The E group was divided into two groups in the week 11. The first group maintained the original condition while the second group drank a solution of aspartame instead water and consumed the experimental diet (EA group, n=6). The E pellets were prepared weekly and stored at 4°C. The aspartame solution was prepared daily and maintained at room temperature. The animals were handled according to the National Research Council Guide for the Care and Use of Laboratory Animals (2011) and the study was approved by the Research Ethics Committee of the host institution.

2.2 Nutritional measurements and blood pressure recording

The animals were weighed weekly at the morning time. Food intake was calculated daily by the subtraction of the remaining amount from the supplied amount of the diet taking account the body weight of the animal. In the same manner, the aspartame intake was calculated daily using the concentration of the aspartame solution, the supplied and remaining volume of the solution and the body weight of the animal. The daily caloric intake was obtained from the multiplication of the food intake with the caloric density of the diet. These measurements were taken during the weeks 1 to 22. After warming rats for 20 minutes on a 37 °C chamber, the systolic and diastolic blood pressures were recorded in completely conscious rats using the Non-Invasive Blood Pressure System for Rodents (Panlab s.l., Barcelona, Spain) during the weeks 10, 14, 16, 18, 20 and 22. At the end of the study, the mesenteric, epididymal and perirenal adipose tissues were weighed after the sacrifice of each animal.

2.3 Biochemical measurements

One milliliter of blood of each rat in fast was drawn from the tail vein using a Heparin-coated needle during the weeks 10, 14, 18 and 22. The blood sample was centrifuged at 12,100 rpm for 10 min at 10°C in a Sorvall RMC 14 Refrigerated Microcentrifuge (Sorvall Products, L.P., CT, USA). Then, the plasma was collected and it was stored at -80°C until use. In the following day of the blood sampling, each rat was placed in a metabolic cage for the collection of 1 mL of urine sample and recording of the 24-urinary volume. The urine sample was centrifuged using the same blood procedure and the supernatant was stored at -80°C until use. Glucose, triglycerides and urea were measured in each plasma sample while the creatinine was measured in urine and plasma samples using the automated analyzer Vitalab Selectra Junior® (Vital Scientific NV, Dieren, the Netherlands). All biochemical assays were done according to the Official Mexican Normativity for clinical laboratories (NOM-166-SSA1-1997). The creatinine clearance was calculated employing the formula used by Hazelhoff et al. (2012).

2.4 Statistical analysis

The data is presented as the mean only or mean $\pm$ standard deviation (SD) and was analyzed employing the two-way ANOVA with Bonferroni post test. Only the information from adipose tissue was analyzed using the one-way ANOVA with Tukey post test. A P value of <0.05 was considered statistically significant. GraphPad Prism 5 software (San Diego, CA, USA) was used for statistical analysis.

3 Results

The results of the study revealed that aspartame intake in the group EA was 54.8 ± 7.3 mg/kg of the bw/day. The nutritional parameters of animal groups are shown in Figure 1. The food consumption was lower in E and EA groups than the S intake starting in the week 4 (Figure. 1A). However, the caloric intake in the E group was superior to S data from the week 1 until the

week 8. After this week, the caloric intake was similar in all groups (Figure. 1B). The body weight information showed higher values in the E group than the S and EA data after the week 10 (Figure. 1C). However, the values from the adipose tissue did not have statistical significant difference between these groups at the week 22 (Figure. 1D). The Figure. 2 shows the recording of blood pressure of the animals. The E diet caused an increase of the systolic blood pressure in the rats only in the week 16 versus the S values. Meanwhile, the EA feeding increased the systolic blood pressure in the rats in the weeks 14, 16 and 18 with respect to S and E values (Figure. 2A). The diastolic blood pressure was only increased in the EA group versus S data in the week 14 (Figure. 2B). The E diet caused an increase of the glucose and triglycerides alongside a reduction of the urea in the plasma samples from rats between the weeks 10 and 18 versus the S data. The EA group had a reduction of the plasma urea versus the S data without other significant changes (Figure. 3A, B and C). Finally, the plasma creatinine was reduced in the EA group versus the S and E values (Figure. 4A). The remainder of values in all groups did not have significant changes (Figure. 4B and C).

4 Discussion and conclusions

All of data from the S group were in accordance with the literature normal values (Olmsted et al., 1951; Fisher, 1965; López-Varela et al., 1995; Zulet et al., 1999; Palm & Lundblad, 2005; Buettner et al., 2006; Romestaing et al., 2007; Jhonson-Delaney, 2008; Amin et al., 2011; Hazelhoff et al., 2012; Moura et al., 2012; Poudyal et al., 2012). However, the trend to reduced intakes of food and calories in the S group through the time of the study were contradictory to previous normal data (Buettner et al., 2006; Romestaing et al., 2007; Amin et al., 2011). This difference may be explained by the declined energy intake due to increasing age (Thomas et al., 2002) because the present study used older animals than prior studies. This study used adult animals because the highest consumption of aspartame in humans is by individuals aged 25 to 59 (FSANZ, 2004).

Figure 1 Nutritional status of animals. The differences (*) had P values of <0.01 versus S group (A), of <0.05 versus S group (B), of <0.001 versus E group (C) or not showed significant changes (D). Standard group (S, pink circle), experimental group (E, orange square) and experimental plus aspartame group (EA, brown triangle). bw; body weight.

Figure 2 Blood pressures of animals. The differences (*) and (***) had P values of <0.05 versus S group and <0.001 versus S group plus <0.01 versus E group, respectively (A). The difference (**) had a P value of <0.01 versus S group (B). Standard group (pink bar), experimental group (orange bar) and experimental plus aspartame group (brown bar).

Coconut fat and sucrose were incorporated into present experimental chow. The final composition of the E diet was within the WHO/FAO recommended limits for humans except the saturated fat amount, where the source of this excess was the coconut fat. In this manner, the E diet caused a reduction in the food ingestion, similar caloric intake, slow increase in the body weight, transient changes in plasma values (urea, triglycerides and glucose) and blood pressure, and unalterable parameters of creatinine in the rats versus the S data. In present study E information is in accordance with the unchanged values of creatinine and plasma modifications of triglycerides and urea in animals under a coconut fat diet (Cera et al., 1990; Buettner et al., 2006; Buettner et al., 2007).

In this case, the reduction of urea in plasma is also probably due to low amount of protein from the consumed diet due to the reduced food consumption versus the S data (Hosten, 1990).

On the other hand, the E data are contrary to the fast weight gain and on set of hyperphagia observed in prior coconut fat or sucrose studies (Buettner et al., 2006; Buettner et al., 2007; Amin et al., 2011; Cahova et al., 2012), increased values of creatinine and urea in serum due to a high sucrose diet (Amin et al., 2011) and unaltered sera values of glucose in previous coconut fat or sucrose evaluations ((Cera et al., 1990; Buettner et al., 2006; Buettner et al., 2007; Amin et al., 2011; Cahova et al., 2012). These differences are probably due to the lowest amounts of fat and sucrose included simultaneously in present diet than all the prior individual studies. In addition, food-calorie-body weight relation of present study is in accordance with other studies using rats under high calorie diets (Moura et al., 2012), with the significant increase of the sera glucose value in animal models under a diet with high saturated fat content (Cahova et al., 2012) and the registration of blood pressure was not done in the prior coconut or sucrose studies (Cera et al., 1990; Buettner et al., 2006; Buettner et al., 2007; Amin et al., 2011; Cahova et al., 2012).

Figure 3 Biochemical values of the rat groups. The differences (*), (**) and (***) had P values of <0.05 versus S group, <0.01 versus S group and <0.001 versus S group, respectively (A and C). The difference (+++) had a P value of <0.001 versus the groups S and E (B). Standard group (pink bar), experimental group (orange bar) and experimental plus aspartame group (brown bar).

Therefore, the increasing body weight of rats via high saturated fat in the E diet versus WHO/FAO values caused metabolic disorders such as impaired glycemia, high systolic blood pressure and hypertriglyceridemia in according with the information from overweight-obesity related diseases (Jia, 2015). However, all the metabolic changes in the E group returned to normal values probably due to the reduced amounts of fat and sucrose added in this experimental diet.

On the other hand, the rat group under the experimental diet plus aspartame intake had a consumption of this sweetener slightly greater than the accepted daily intake for humans (Magnuson et al., 2007).

The intake patterns of food and calories in the EA group are explained by the effects of the E diet as described above. However, the aspartame intake avoids the high body weight as

observed in the E group. The weight loss by aspartame has been reported extensively (Ishii et al., 1981; Butchko et al., 2002; de la Hunty et al., 2006; Palmnäs et al., 2014). In this case, the reduction of the body mass cannot be explained by a significant loss of adipose tissue. Hence, the possible additional mechanism for the weight loss is through the reduction of muscle mass because the high body weight observed in the E group was not due to a significant increase of the adipose tissue and the plasma creatinine was only diminished in the EA group. It is important to note that the serum creatinine is a reliable marker for the evaluation of the muscle mass if the kidney function and dietary protein intake is undertaken as was done for the E versus EA data (Perrone et al., 1992; Patel et al., 2013). Changes in muscle mass or serum creatinine during loss of body weight due to aspartame were not evaluated previously (Ishii et al., 1981; Butchko et al., 2002; de la Hunty et al., 2006; Palmnäs et al., 2014).

Figure 4 Creatinine results of the rat groups. The difference (**) had a P value of <0.01 versus the groups S and E (A). Plate B and C did not show significant changes. Standard group (pink bar), experimental group (orange bar) and experimental plus aspartame group (brown bar). bw; body weight.

The changes in the remainder of biochemical parameters (glucose, triglycerides and urea) due to the E diet were avoided or diminished by the aspartame ingestion in the EA group. Lipid outcome for the present study is in agreement with the failure of hypertriglyceridemia due to aspartame intake in humans following lipid loading (Magnuson et al., 2007).

Furthermore, glucose and urea changes by aspartame were not reported previously; however, all prior studies were conducted under different experimental designs (Butchko et al., 2002; Magnuson et al., 2007; Palmnäs et al., 2014). Finally, the consumption of aspartame by the rats under the E diet caused an early and prolonged increase of the blood pressure than the animals under water intake and the same E diet. This situation was probably caused by the presence of vasoactive substances came from the aspartame metabolism. The systemic

hypertension due to aspartame was associated in prior human data (Butchko et al., 2002; Roberts, 2004; Roberts, 2008). Hence, this is the first experimental evidence of transient hypertension via aspartame in animals under a high saturated fat diet. All of data from the EA group suggest that the aspartame intake by adult rats under an unhealthy diet with respect to WHO/FAO values has a beneficial effect on cardiovascular risk factors such as overweight/obesity, raised blood glucose and raised blood triglycerides. However, these changes were accompanied by raised blood pressure which is the leading risk factor for cardiovascular diseases alongside reduced creatinine in plasma which is a risk factor for type 2 diabetes (WHO, 2011; Moon et al., 2013). In this manner, further studies are needed to increase the evidence of the aspartame effects under high fat feedings instead the

commonly evaluations using normal diets, including diets with other sources and amounts of fat.

In summary, experimental diet used in present study have high saturated fat with respect to the WHO/FAO limit produces negative effects on cardiovascular risk factors of the rats while the aspartame intake under the experimental feeding has mixed effects on the cardiometabolic factors of the animals.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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