International Journal of Environmental Health Research

ISSN: 0960-3123 (Print) 1369-1619 (Online) Journal homepage: https://www.tandfonline.com/loi/cije20

Evaluation of sucrose-enriched diet consumption in the development of risk factors associated to type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in a murine model

Carolina Gabriela Plazas Guerrero, Selene De Jesús Acosta Cota, Francisco Humberto Castro Sánchez, Marcela De Jesús Vergara Jiménez, Efrén Rafael Ríos Burgueño, Juan Ignacio Sarmiento Sánchez, Lorenzo Antonio Picos Corrales & Ulises Osuna Martínez

To cite this article: Carolina Gabriela Plazas Guerrero, Selene De Jesús Acosta Cota, Francisco Humberto Castro Sánchez, Marcela De Jesús Vergara Jiménez, Efrén Rafael Ríos Burgueño, Juan Ignacio Sarmiento Sánchez, Lorenzo Antonio Picos Corrales & Ulises Osuna Martínez (2019): Evaluation of sucrose-enriched diet consumption in the development of risk factors associated to type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in a murine model, International Journal of Environmental Health Research, DOI: 10.1080/09603123.2019.1680817

To link to this article: https://doi.org/10.1080/09603123.2019.1680817

Published online: 31 Oct 2019.

INTERNATIONAL JOURNAL OF ENVIRONMENTAL HEALTH RESEARCH
https://doi.org/10.1080/09603123.2019.1680817

Taylor & Francis
Taylor & Francis Group

ARTICLE

Evaluation of sucrose-enriched diet consumption in the development of risk factors associated to type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in a murine model

Carolina Gabriela Plazas Guerrero[a], Selene De Jesús Acosta Cota[a], Francisco Humberto Castro Sánchez[b], Marcela De Jesús Vergara Jiménez[b], Efrén Rafael Ríos Burgueño[c], Juan Ignacio Sarmiento Sánchez[d], Lorenzo Antonio Picos Corrales[a] and Ulises Osuna Martínez[a]

[a]Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Culiacán de Rosales, Mexico; [b]Facultad de Ciencias de la Nutrición y Gastronomía, Universidad Autónoma de Sinaloa, Culiacán de Rosales, Mexico; [c]Centro de Investigación y Docencia en Ciencias de la Salud, Universidad Autónoma de Sinaloa, Culiacán de Rosales, Mexico; [d]Facultad de Ingeniería Civil, Universidad Autónoma de Sinaloa, Culiacán de Rosales, Mexico

ABSTRACT

Overconsumption of sucrose, the main contributor of the total added sugar intake in the world, has been associated with negative metabolic effects related to non-communicable diseases. However, this relationship continues to be a controversial topic and further studies are needed. The aim of this study was to evaluate the sucrose-enriched diet consumption in the development of risk factors associated with type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in a murine model. Sucrose-enriched diet-fed rats showed a decrease in food, lipids and protein intake as well as in serum total cholesterol levels, an increase in carbohydrates intake, glucose, insulin, triglycerides, VLDL-c and HDL-c levels and a greater degree of insulin resistance, steatosis and non-alcoholic steatohepatitis. Our results show that sucrose-enriched diet consumption during 25 weeks contribute to the development of risk factors associated with type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease in male Wistar rats.

ARTICLE HISTORY
Received 9 July 2019
Accepted 9 October 2019

KEYWORDS
Sucrose; non-communicable diseases; risk factors

Introduction

Non-communicable diseases (NCDs), especially cardiovascular diseases (CVD), cancer, chronic respiratory diseases and diabetes represent a global important pandemic and the major worldwide health systems challenge (OMS 2017). Currently, NCDs are the main cause of morbidity and mortality in the world, do not discriminate between age or nationality (OMS 2017) and also are an important cause of premature disability (Córdova-Villalobos et al. 2008) causing significant socioeconomic problems (IMCO 2015; OMS 2017).

Overweight/obesity, hypertension, hyperglycemia and hyperlipidemia consider the major metabolic risk factors related to NCDs, have dramatically increased in recent years and it seems like this is mainly associated with sedentary lifestyle and unhealthy diet (OMS 2017). Therefore, many research projects have focused on studying the possible association between the intake of specific dietary components and

CONTACT Lorenzo Ulises Osuna Martínez ulises.osuna@uas.edu.mx Facultad de Ciencias Químico Biológicas. Universidad Autónoma de Sinaloa., Av. las Américas y Blvd. Universitarios S/N, CP 80010, Culiacán, Sinaloa, Mexico

NCDs (OMS 2003). It has been suggested that elevated consumption of simple carbohydrates, like added sugars, may contribute to the increased of NCDs prevalence (Barclay et al. 2008; Augustin et al. 2015).

Sucrose, commonly known as table sugar, is a disaccharide composed of glucose and fructose and is widely used as added sugar by the food industry in a large variety of food products and beverages (Cummings and Stephen 2007; Gómez Candela and Palma Milla 2013) being the main contributor of the total added sugar intake in the world and representing a high percentage of total daily energy intake in many countries (Pinto et al. 2016) as United States, Britain (Gibson et al. 2013), Chile and México (Singh et al. 2015).

Scientific evidence in both human and animal models support the suggestion that excess sugar consumption has negative metabolic effects such dyslipidemia, a rise in inflammatory markers, weight gain, increased risk for type 2 diabetes and other alterations associated with NCDs (Hochuli et al. 2014; Schultz et al. 2015; Gallagher et al. 2016). However, in other research studies or meta-analysis, no clear and determinant relationship between the consumption of this disaccharide and some of the NCDs related-risk factors has been found (Bravo et al. 2013; Rippe and Angelopoulos 2013; Tsilas et al. 2017)

For this reason, the impact of sugar consumption on health continues to be a controversial topic and further studies are needed to clarify the existing inaccuracies, to fill these evidence gaps and to know more of the potential metabolic and molecular mechanisms involved in this process.

The aim of this study, therefore, was to evaluate the sucrose-enriched diet consumption in the development of risk factors associated with type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease (NAFLD) in a murine model.

Materials and methods

Animals and experimental design

Twelve 4 weeks old-healthy male Wistar rats with an average weight of 80 ± 10 g were donated by Cinvestav Sede Sur (México City, México). The rats were housed in acrylic cages and maintained on a 12 h light-dark cycle in a temperature-controlled room (22 ± 2°C) with food and water available *ad libitum* throughout the study. All the experimental procedures were approved by the Comité Científico y Ético de Investigación de la Unidad Académica de Ciencias de la Nutrición y Gastronomía and were performed in accordance with the Mexican Official Norm Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999).

Experimental design is shown in Figure 1, briefly, before the intervention period, the rats received 7 days of acclimatization. After that, rats were randomly divided into two experimental groups ($n = 6$ each) which received free access to one of the two different diets for 25 weeks. Control group (CG) was fed with a standard laboratory diet (Rat Diet 5012, LabDiet, St. Louis, MO) containing 27.02% protein, 13.10% fat, 59.87% carbohydrates and 6.4% ash according to manufacturer´s information provided, and tap water. Sucrose group (SG) received the same laboratory diet and water enriched with 50% w/v sucrose. Body weight, food, macronutrient and total caloric intake were determined. At the end of the intervention period, animals were fasted for 12 h before being sacrificed. Blood samples, hepatic and aortic tissues were collected. Glucose (GLU), insulin, total cholesterol (TC), triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-c), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) levels were determined in serum. Liver and aortic tissues were stained with hematoxylin and eosin (H&E) and a histopathological analysis was made for both.

Food, macronutrients and total caloric intake

The food, macronutrients and total caloric intake was determined weekly for each group over the last 5 weeks of intervention (weeks 21 to 25). Food and water were weighed and replenished every 3 days and the consumption of both was calculated. The macronutrients and total caloric intake of the

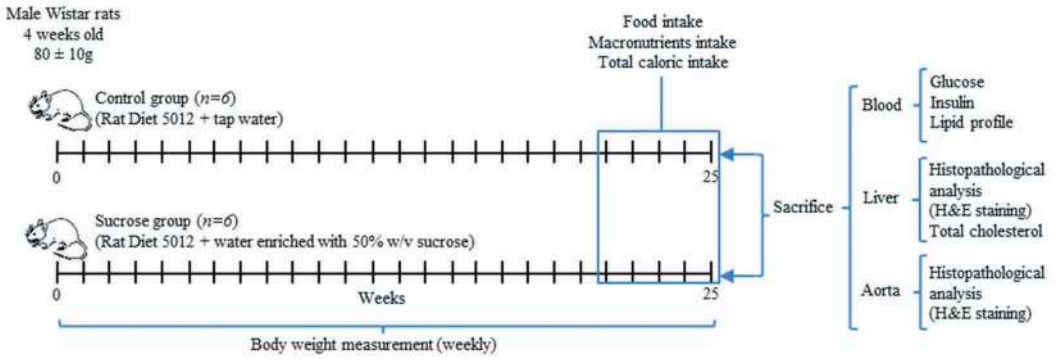

Figure 1. Experimental design. Animals were randomly divided into two experimental groups which received two different diets for 25 weeks. Control group (CG) was fed with a standard laboratory diet and tap water. Sucrose group (SG) received the same laboratory diet and water enriched with 50% w/v sucrose. Body weight was measured weekly. Food, macronutrient and total caloric intake was calculated weekly over the last 5 weeks of intervention. At the end of the intervention period, animals were sacrificed. Blood samples and hepatic and aortic tissues were collected. Glucose, insulin and lipid profile were determined. Liver and aortic tissues were stained with hematoxylin and eosin (H&E) to evaluate different parameters.

experimental groups was determined based on the nutritional composition of the standard laboratory diet (Rat Diet 5012, LabDiet, St. Louis, MO) and the sucrose content in the water (50% w/v).

Body weight

The animals were weighed weekly with a precision electronic scale throughout the 25 weeks of intervention.

Glucose, insulin and insulin resistance

Fasting blood GLU and insulin levels were measured in serum samples. GLU and insulin levels were measured using an enzymatic colorimetric method with a commercially available kit (Randox Laboratories, Crumlin, UK) and an Enzyme-Linked Immunoabsorbent Assay kit (ALPCO, New Hampshire, USA), respectively. In both cases following the manufacturer´s instructions.

The insulin resistance was determined according to the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) using the following formula: HOMA-IR = [fasting glucose (mg/dL) x fasting insulin (μIU/mL)/405] (Olguin et al. 2015; Schultz et al. 2015).

Lipid profile

The serum concentrations of TC, TG and LDL-c were assessed with enzymatic colorimetric kits (TC and TG: Randox Laboratories, Crumlin, UK; LDL-c: Wiener Laboratories Group, Rosario, Argentina). HDL-c levels were measured in serum using a combination of precipitation and enzymatic colorimetric methods with the respective commercial kits (Randox Laboratories, Crumlin, UK). VLDL-c was calculated according to the following formula: VLDL-c = [triglycerides (mg/dL)/5] (Aminlari et al. 2018; Uriarte et al. 2013).

Liver histopathological analysis

Liver steatosis

After sacrifice, livers were dissected, fixed in formalin (formaldehyde 10% 100 mL/L (J.T. Baker), NaH_2PO_4 4 g/L (Vetec), Na_2HPO_4 6.5 g/L (Fermont), distilled water 900 mL, pH 7.4) (Acosta-Cota et al. 2019), dehydrated, clarified, embedded in paraffin (Leica Paraplast) and cut into 5 μm tissue

sections using a microtome (Leica RM 2145 RTS). Following deparaffination and hydration, tissue sections were stained with H&E. The samples were examined by light microscopy (ZEISS Primo Star LED, Carl Zeiss, Gottingen, Germany) and the most representative images were taken using Zen Zeiss imaging blue edition software (Carl Zeiss, Gottingen, Germany). A total of 50 fields of each stained sample were analyzed at 40x.

The percentage of steatosis was calculated by counting the amount of hepatocytes with steatosis and without it in the tissue sample. Then, we determined the non-alcoholic steatohepatitis (NASH) degree according to the histological score system of the clinical research network of NASH (LaBrecque et al. 2012). This score determines eight grades of activity or development of NASH (grades 0 to 8) according to the percentage of steatosis and the presence of ballooned cells and inflammatory infiltrates in each hepatic tissue sample.

Hepatic total cholesterol quantification

Quantitative measurement of hepatic TC was performed according to the methodology used by Zhou et al. (Zhou et al. 2017). Briefly, 0.15 g of sample were collected, minced and lipids were extracted by addition of 3 mL of chloroform/methanol (2:1, v/v). The mixture was homogenized for 2 min, sonicated by 30 s and shaken for 2 h. Subsequently, 1 mL of bidistilled water was added and samples were centrifuged for 20 min at 3500 rpm. The lipid phase was collected (the bottom phase) and incubated overnight (12 h). The next day, the lipids were dissolved in absolute ethanol, sonicated and filtered through a 0.45 μm filter. Finally, TC was measured using an enzymatic colorimetric method with a commercial kit (Randox Laboratories, Crumlin, UK). The results are expressed as mg/g of liver. Therefore, the average liver weight of the experimental groups is also included. For this purpose, once the liver was obtained from each rat after sacrifice, it was weighed using a precision electronic scale.

Aorta histopathological analysis

Aortic tissue samples were fixed in 10% formalin followed by paraffin embedding. Each sample was cut into 5 μm sections and stained with H&E using standard laboratory protocols. Histopathological analysis of the intima, media and adventitia tunics of each aorta sample was performed using light microscopy and Zen Zeiss imaging blue edition software (Carl Zeiss, Gottingen, Germany) at 40X. Histopathological changes were expressed as percentage of the total tissue samples of each group.

Statistical analysis

Data obtained were analyzed using the statistical software SigmaPlot 12.0. Parametric variables were food and macronutrient intake, body weight, insulin, TG, TC, HDL-c, VLDL-c, liver weight and total hepatic cholesterol levels. In this case, results are expressed as mean values ± standard errors of the mean (SEM). Statistical tests performed for each variable are presented below. Differences among groups in body weight, a two-way repeated measures ANOVA was performed, followed by Bonferroni *post hoc* test. Food intake, macronutrients intake, insulin, TG, TC, HDL-c, VLDL-c, liver weight and total hepatic cholesterol levels were compared between the two groups using a t-test. Non-parametric variables were caloric intake, GLU, insulin resistance and LDL-c. In this case, results are expressed as medians and ranks. Statistical tests run for each variable are presented below. Results in total caloric intake were compared using a Friedman test. Mann–Whitney U-test was employed for statistical analysis of GLU levels, insulin resistance (HOMA-IR) and LDL-c. A p value of <0.05 was considered significant for parametric and non-parametric variables.

Results

Food, macronutrients and total caloric intake

A sucrose-enriched diet consumption significantly ($p < 0.05$) decreased food intake in SG (438.60 ± 20.59 g) *vs* CG (1136.96 ± 44.51 g), consuming 61.43% less food than CG over the last 5 weeks of intervention (Figure 2(a)).

Regarding the macronutrients intake, SG consumed significantly ($p < 0.05$) 1.31-fold higher carbohydrates (896.58 ± 28.60 g) intake over CG (680.69 ± .26.65 g). Otherwise, the consumption of protein (118.51 ± 5.56 g) and lipids (57.45 ± 2.69 g) was decreased ($p < 0.05$) 61.43% on both cases compared to CG (307.20 ± 12.02 g and 148.94 ± 5.83 g, respectively) (Figure 2(b)).

Although no significant differences ($p > 0.05$) were found in total caloric intake among the experimental groups, SG showed a slight decrease in its total caloric intake compared to CG (4580.43 cal; 5217.81 cal, respectively) (Figure 2(c)). Furthermore, the number of calories consumed through food was significantly different in the two groups. SG (2010.42 ± 94.38 cal) ingested 61.43% fewer calories from solid food than CG (5211.59 ± 204.04 cal). Otherwise, sucrose provided the 56.07% of the total caloric intake to SG (2567.05 ± 95.73 cal) while this disaccharide represented only the 1.52% of total caloric intake to CG (80.49 ± 3.15 cal, $p < 0.05$) (Figure 3(d)).

Body weight

There was no significant difference in body weight from weeks 0 to 2 and from weeks 15 to 24 of intervention among experimental groups. Although final body weight was slightly increased in SG

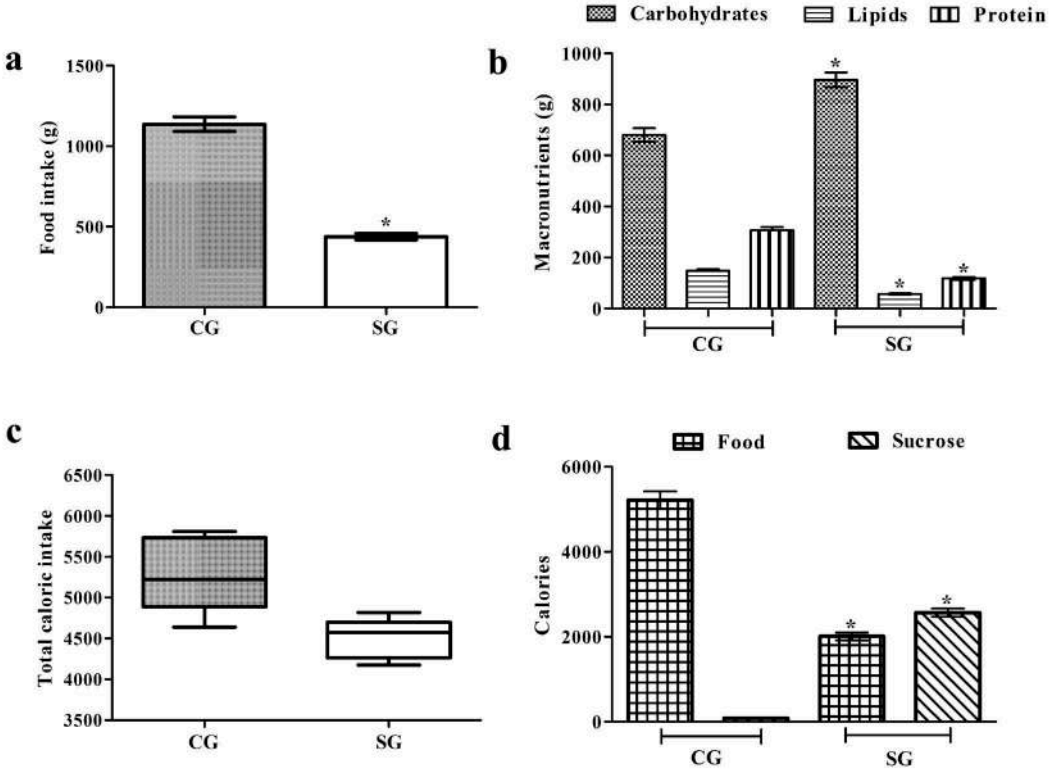

Figure 2. Effect of sucrose consumption on food (a), macronutrients (b), total caloric intake (c) and main food energy sources (d) of experimental groups over the last 5 weeks of intervention. CG: control group, SG: sucrose group. For food and macronutrients intake and for main food energy sources mean values ± SEM are plotted. For total caloric intake, median values and ranks are plotted. *$p < 0.05$.

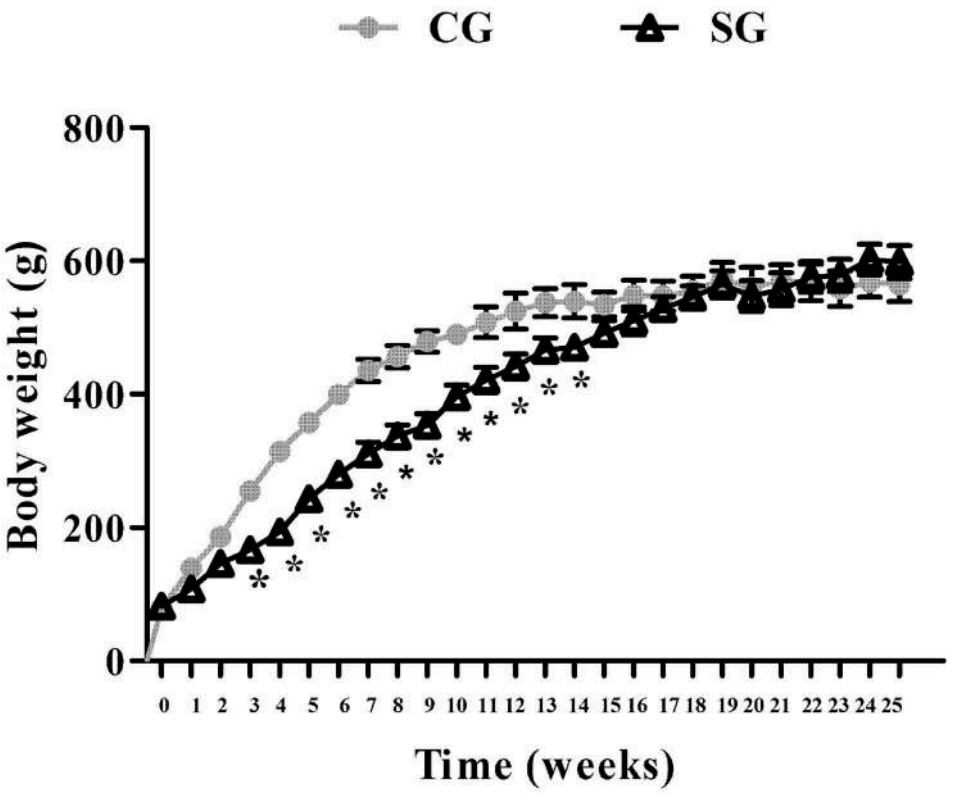

Figure 3. Effect of sucrose consumption on body weight. CG: control group, SG: sucrose group. Mean values ± SEM are plotted. *$p < 0.05$.

(598.03 g) compared to CG (564.26 g) on week 25, this did not reach statistical significance ($p > 0.05$). Nevertheless, SG had significantly lower body weight from week 3 to week 14 of intervention *vs* CG ($p < 0.05$) (Figure 3).

Glucose, insulin and insulin resistance

GLU levels were 1.42-fold higher ($p < 0.05$) in SG (172.25 mg/dL) compared to CG (120.69 mg/dL) (Figure 4) at the end of the intervention period (Figure 4(a)).

Additionally, a sucrose-enriched diet consumption induced an increased insulin levels which were 2.40-fold higher in SG (27.64 ± 5.44 µIU/mL) *vs* CG (11.51 ± 2.14 µIU/mL) (Figure 4(b)), and 3.37-fold greater insulin resistance according to the HOMA index in SG (11.53) *vs* CG (3.42) ($p < 0.05$) (Figure 4(c)).

Lipid profile

TG (111.75 ± 5.55 mg/dL), VLDL-c (22.35 ± 1.11 mg/dL) and HDL-c (31.23 ± 1.13 mg/dL) levels were significantly higher ($p < 0.05$) in sucrose-enriched diet-fed rats compared to CG (61.11 ± 10.14 mg/dL, 12.22 ± 2.02 mg/dL, and 26.33 ± 1.16 mg/dL, respectively), after 25 weeks of intervention (Figures 5(a,b) and 6(b)). Otherwise, TC levels in SG (57.44 ± 1.14 mg/dL) were 34.17% decreased ($p < 0.05$) compared to CG (87.25 ± 5.42 mg/dL) after 25 weeks of intervention (Figure 6(a)). On the other hand, even when LDL-c levels were slightly increased in SG (45.80 ng/

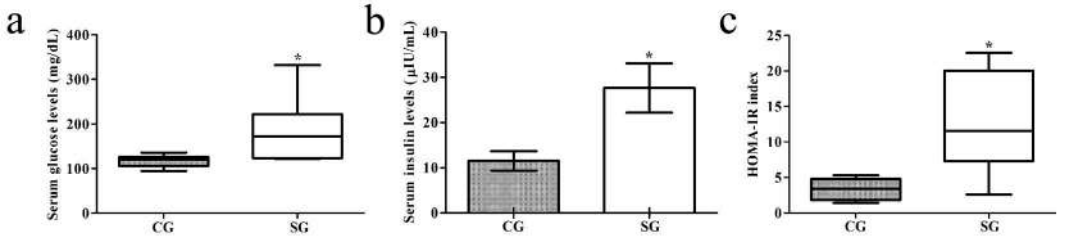

Figure 4. Effect of sucrose consumption on glucose (a), insulin (b) and insulin resistance (c). CG: control group, SG: sucrose group. Serum glucose levels and insulin resistance (HOMA-IR index) are plotted as median values and ranks while serum insulin levels are plotted as mean ± SEM. *$p < 0.05$.

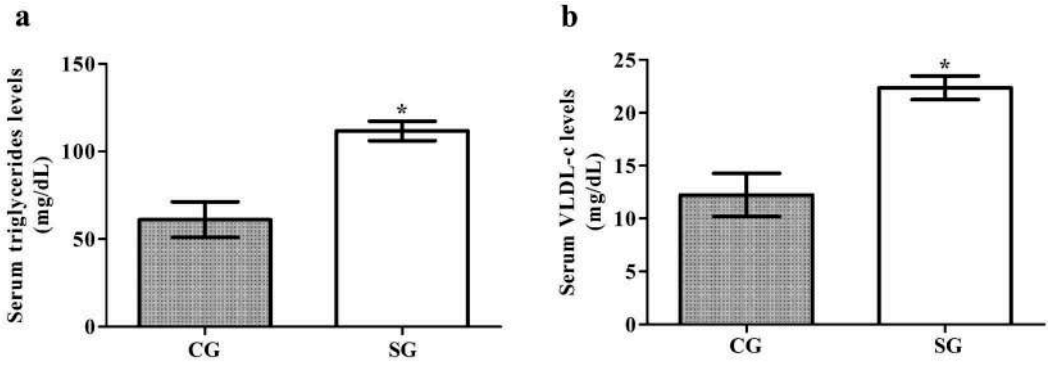

Figure 5. Effect of sucrose consumption on lipid profile. Effect of sucrose consumption on serum triglycerides (a) and VLDL-c (b) levels. CG: control group, SG: sucrose group. Mean values and ± SEM are plotted. *$p < 0.05$.

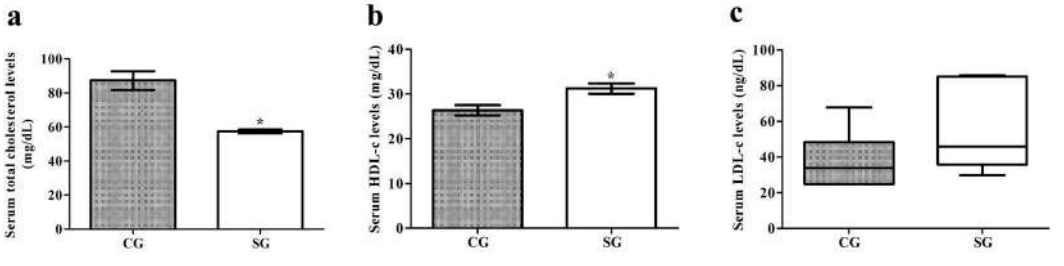

Figure 6. Effect of sucrose consumption on lipid profile. Effect of sucrose consumption on serum total cholesterol (a), HDL-c (b) and LDL-c (c) levels. CG: control group, SG: sucrose group. Serum total cholesterol and HDL-c levels are plotted as mean ± SEM while serum LDL-c levels are potted as median values and ranks. *$p < 0.05$.

dL) compared to CG (33.80 ng/dL), no statistical significance was observed ($p < 0.05$) between the experimental groups (Figure 6(c)).

Liver histopathological changes

In hepatic histological samples of the CG, a normal parenchyma formed by hepatocytes with a conserved architecture was observed, in which the cytoplasm and the nucleus can be appreciated (Figure 7(a)). Nevertheless, according to the histological score system of the clinical research network of NASH (LaBrecque et al. 2012), this group developed grade 1 of NASH showing 5.48% of microvesicular steatosis (Figure 6(a,c and e)).

On the other hand, histopathological analysis of SG showed a hepatic parenchyma in which the structure of a large number of hepatocytes was altered, showing a pronounced displacement of the nucleus towards the periphery of the cell due to the accumulation of a huge amount of lipids in their cytoplasm (Figure 7(b)). This group presented 65.49% of macrovesicular steatosis and 8.74% of microvesicular steatosis (zones I and II) with a hepatic steatosis mean of 74.22%. Furthermore, this group showed two ballooned cells per field (Figure 7(b,d)) and one inflammatory infiltrate (not shown), positioning it in grade 7 of NASH (LaBrecque et al. 2012). (Figure 7(b,d,f)).

Hepatic total cholesterol accumulation

Regarding to liver weight, no significant difference was found between the experimental groups (SG: 18.04 ± 1.21 g; CG: 14.96 ± 0.69 g, $p > 0.05$) (Figure 8(a)). On the other hand, although the higher hepatic TC levels found in SG (SG: 151.18 ± 12.66 mg/g; CG: 130.21 ± 4.53 mg/g), no significant difference ($p > 0.05$) was observed in this parameter between both experimental groups (Figure 8(b)).

Aortic histopathological changes

After aorta histopathological analysis, no significant differences were found between experimental groups. No significant lesions were observed in the intima, media and adventitia tunics of the aorta in the samples analyzed and no atheroma plaque formation or atherosclerosis development was observed (Figure 9). Aorta tissue samples of CG presented a slight wall thickening (16.6%), lymphocytes on the wall (50%), hemorrhage (50%) and vascular congestion (33.3%). On the other hand, histopathological analysis of SG samples, showed a slight wall thickening (16.6%), presence of few lymphocytes in the wall (100%) as well as vascular congestion (50%) and haemorrhage (50%). Besides, aorta tissue samples of SG showed higher amount of perivascular adipose tissue (PVAT) than those of the CG.

Discussion

Food, macronutrients and total caloric intake

Regarding to food intake, our results showed that sucrose-enriched diet consumption significantly decreased this parameter compared to CG over the last 5 weeks of intervention, which has been similarly reported in previous studies (Olguin et al. 2015; Pinto et al. 2016; Acosta-Cota et al. 2019). This finding may be attributed to the sweet taste of the sucrose which is mostly preferred by rats (Petykó et al. 2009) because indicates them that food has essential nutrients for their survival (Matsuo et al. 2011) and it is also able to triggers an hedonic response through the stimulation of the dopaminergic centers in the brain (Kampov-Polevoy et al. 2006; Kilpatrick et al. 2014).

Otherwise, high-sucrose consumption promotes higher insulinemic responses which triggers signals that are important in the control of food intake (Petykó et al. 2009). Insulin has an anorexigenic effect by decreasing the expression of orexigenic peptide Neuropeptide Y (NPY) and stimulating other satiety signals such as CCK and corticotropin-releasing hormone (CRH) (Hita et al. 2006). Insulin also stimulates leptin synthesis by adipocytes which increases satiety and decreases food intake since leptin acts on the hypothalamus by inhibiting the synthesis of NPY and increases the expression of anorexigenic peptides such as CRH (Sánchez 2005; Hita et al. 2006). This possible increase in blood leptin levels has already been observed in previous studies with mice fed with a high-sucrose diet (Oliveira et al. 2014; Castellanos Jankiewicz et al. 2015; Harris 2018).

Regarding the macronutrients intake, results showed that sucrose-enriched diet consumption significantly increased carbohydrates intake and decreased protein and lipids intake over the last 5 weeks of intervention compared to CG. Although no scientific evidence from similar studies that reports specific macronutrients intake was found, our findings may be related to those of food

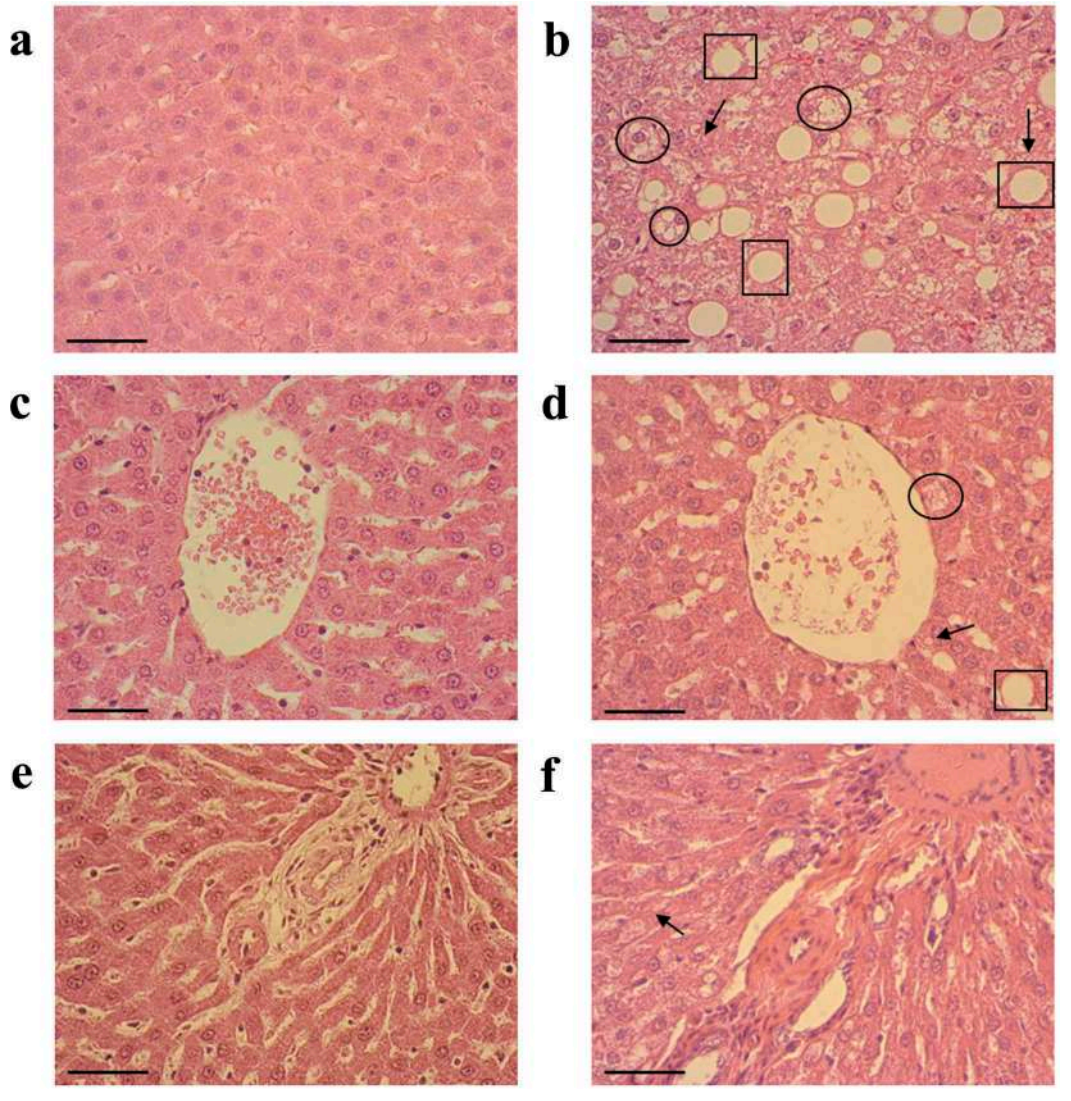

Figure 7. Effect of sucrose consumption on morphology and histopathology of liver. CG: control group, SG: sucrose group. Left column images (a, c and e) belong to CG, right column (b, d and f) images belong to SG. Parenchyma (a and b), central veins (c and d) and portal triads (e and f) of both experimental groups are shown. Microvesicular steatosis is indicated by arrows. Macrovesicular steatosis is indicated by circles and ballooned cells are into squares. Hematoxylin and eosin, 40X magnification. Scale bars = 50 µm.

intake observed in the experimental groups. SG consumed significantly more sucrose which caused a significant increase in carbohydrates intake and made of these macronutrients their main energy source. In addition, this group consumed significantly less solid food leading to a decrease in the consumption of other nutritional compounds found in the laboratory diet such as protein and lipids, as was observed in this group.

In the present study, results showed that sucrose-enriched diet consumption did not produce a significant difference in the total caloric intake over the last 5 weeks of intervention between the experimental groups, which has been similarly reported in a previous study (Schultz et al. 2015). However, SG showed a slight decrease in total caloric intake compared to CG. This may due to the significantly less food, protein and lipids intake observed by SG, which are important energy sources in the diet.

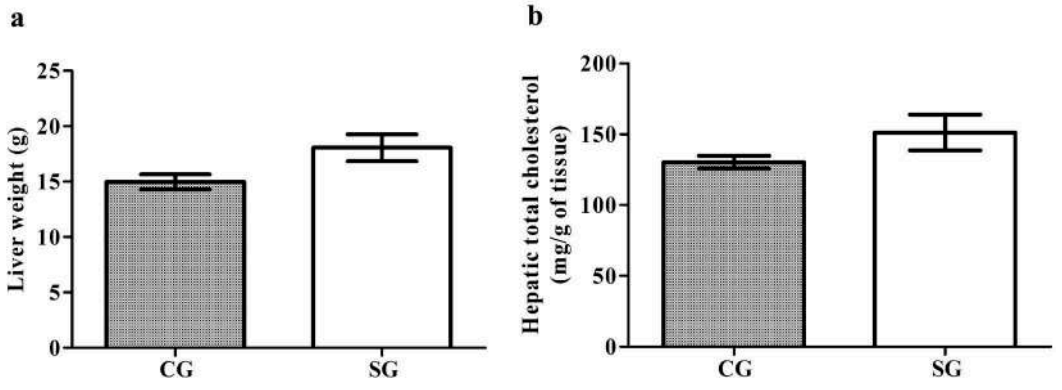

Figure 8. Effect of sucrose enriched-diet consumption on hepatic total cholesterol accumulation. CG: Control group, SG: Sucrose group. Hepatic total cholesterol levels (a) and liver weight (b) are shown. Mean values and ± SEM are plotted. $p > 0.05$.

Respecting the distribution of energy intake, our findings showed that calories provided by solid food (no sucrose) represented significantly less proportion of the total caloric intake in SG compared to CG, which was similarly observed by Sheludiakova et al. in Hooded Wistar rats fed with sugary drinks, one of them with sucrose (Sheludiakova et al. 2012).

Body weight

At the end of intervention period sucrose-enriched diet consumption during 25 weeks did not produce significant differences on rats body weight between the experimental groups, similar to other reports (Oliveira et al. 2014; Packard et al. 2014; Olguin et al. 2015; Schultz et al. 2015; Acosta-Cota et al. 2019). This may be due to the significantly decreased in food, protein and lipids intake, as well as the slight decreased in total caloric intake by SG. Additionally, the lowest food intake by SG could cause deficiency of micronutrients such as vitamin A or zinc, which has been related to weight loss in rats (Esteban-Pretel et al. 2010; Kumari et al. 2011).

Glucose and insulin levels and insulin resistance

Regarding to GLU levels, results showed that sucrose-enriched diet consumption during 25 weeks induced significantly higher fasting GLU levels in SG, as previously reported studies (Sheludiakova et al. 2012; Oliveira et al. 2014; Pinto et al. 2016). This elevation in fasting blood GLU levels may be due to an alteration in GLU metabolism such as glucose intolerance which has been observed in previous studies (Sheludiakova et al. 2012; Oliveira et al. 2014; Packard et al. 2014; Pinto et al. 2016; Acosta-Cota et al. 2019) and could be indicative of peripheral insulin resistance (Sheludiakova et al. 2012).

The hyperinsulinemia and the presence of insulin resistance observed to a greater extent in SG in the present study and as previously reported (Oliveira et al. 2014; Schultz et al. 2015), are findings that allow us to suggest the presence of this alteration. These results in insulin levels and insulin resistance are related to the potential of sucrose to influence glucose tolerance and insulin sensitivity through the insulinemic response to glucose (Pinto et al. 2016).

High GLU consumption, a component of sucrose, leads to hyperglycemia and hyperinsulinemia due to its ability to stimulate higher insulin secretion by pancreatic β-cells, which has serious implications in carbohydrates and lipids metabolism. It is well reported that hyperinsulinemia stimulates lipolysis in adipose tissue causing raised free fatty acids in the bloodstream and elevating their tissues uptake, especially at hepatic level (Basciano et al. 2005; Pérez Cruz et al. 2007).

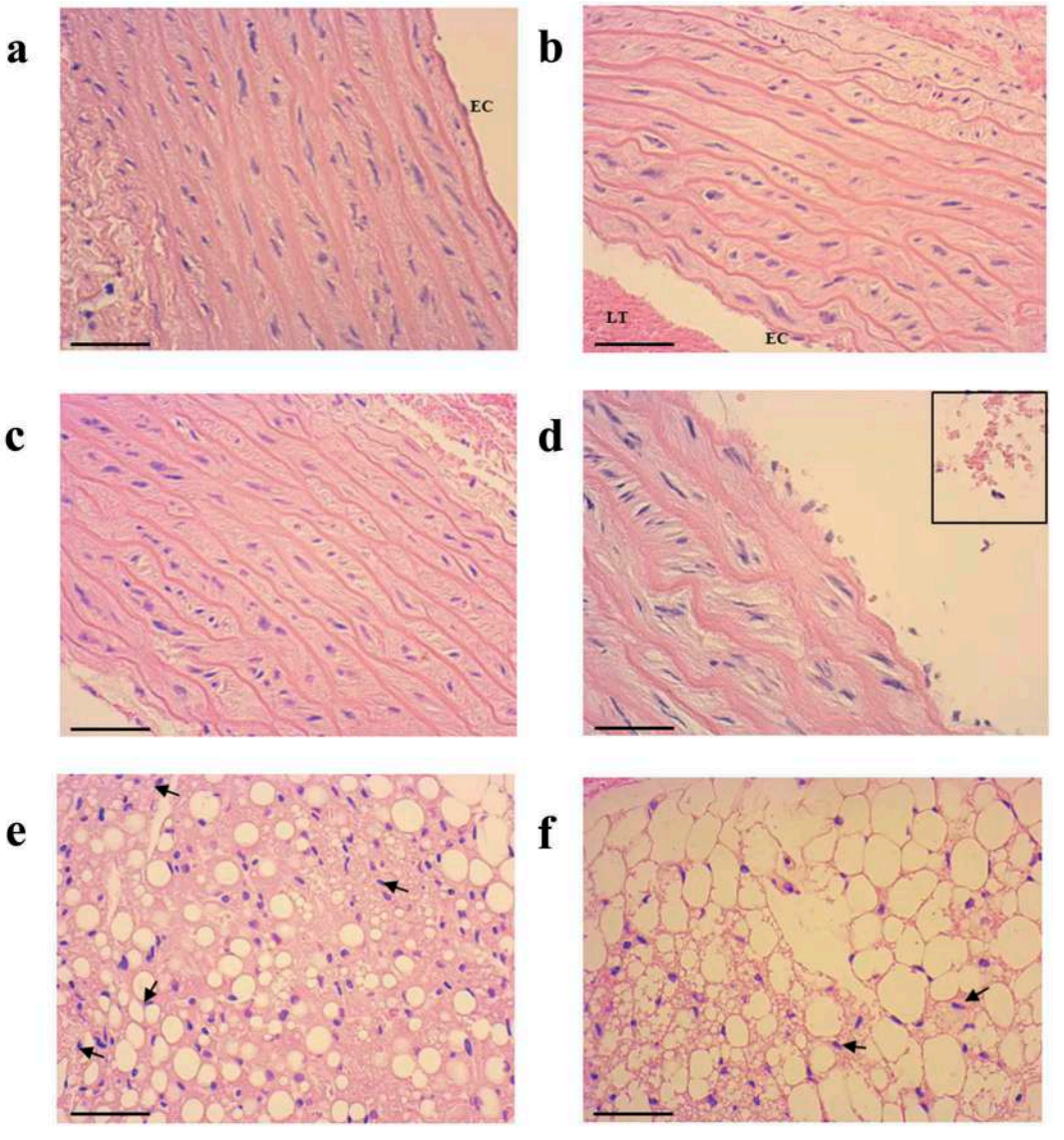

Figure 9. Effect of sucrose-enriched diet consumption on morphology and histopathology of aorta. CG: control group, SG: sucrose group. Left column images (a, c and e) belong to CG, right column (b, d and f) images belong to SG. Healthy intima, media and adventitia tunics (a and b), slight wall thickening (c and d) as well as PVAT (e and f) of both experimental groups are shown. Some lymphocytes are indicated by arrows, vascular congestion is into a square, LT letters indicate a luminal thrombus and EC letters indicate endothelial cells. Hematoxylin and eosin, 40X magnification. Scale bars = 50 µm.

In chronic hyperglycemic and hyperinsulinemic states, the levels and uptake of free fatty acids are higher, stimulating hepatic lipid synthesis and impairing β-oxidation (Ragab et al. 2015). This lipid synthesis, and as consequence its accumulation, is mainly due the stimulation of lipogenic pathways by insulin action that promotes the expression of Sterol regulatory element-binding protein-1 (SREBP-1) and carbohydrate responsive element-binding protein (ChREBP) in the liver, transcription factors involved in the expression of lipogenic genes, such as those involve in the synthesis of enzymes related with these pathways (Basciano et al. 2005; Pérez Cruz et al. 2007; Castro et al. 2014; Schultz et al. 2015).

Thereby, it has been reported that long-term exposure to excessive lipid deposition and free fatty acids directly influence insulin signaling by activation of several serine/threonine kinases reducing the tyrosine phosphorylation of insulin receptor substrate and affecting the phosphatidylinositol-3-kinase pathway activation (Zhao et al. 2015) decreasing the translocation of GLU transporters in insulin-dependent tissues (Acosta-Cota et al. 2019) leading to the development of hepatic and peripheral insulin resistance which is associated with a greater risk of type 2 diabetes (Sheludiakova et al. 2012) mostly due to lipotoxicity and the subsequently pancreatic β-cells failure (Zhao et al. 2015).

On the other hand, fructose is also a component of sucrose (Schultz et al. 2015) and it has been suggested that chronic exposure to it may cause hyperinsulinemia and insulin resistance due the increase in hepatic *de novo* lipogenesis in an insulin-independent manner especially because fructose is mainly metabolized in the liver and to a much greater extent than GLU (Basaranoglu et al. 2013, 2015).

Lipid profile

Regarding to TG levels, sucrose-enriched diet consumption significantly raised TG levels, similar to other reports (Sheludiakova et al. 2012; Olguin et al. 2015; Acosta-Cota et al. 2019), which is one of the most frequently reported findings in the scientific literature after high-sucrose consumption. This hypertriglyceridemia is closely related to high GLU and insulin levels of SG observed in our study which could cause the increased TG synthesis and their accumulation at hepatic level derived from the uncontrolled lipogenesis mentioned above.

After a certain period of time, the excessive TG synthesis is greater than liver capacity to store them and, due hepatic insulin resistance, the ability of insulin to suppress production of GLU and VLDL-c is impaired, so TG are release to bloodstream through VLDL-c lipoproteins (Koek et al. 2011; Rolo et al. 2012), leading to dyslipidemias. This is in accordance with our results observed on serum VLDL-c levels after sucrose-enriched diet consumption and it is important to mention that increased levels of VLDL-c and TG are important atherogenic risk factors (Basciano et al. 2005; Pérez Cruz et al. 2007).

In addition, high GLU and fructose levels are able to continuously stimulate glycolysis, producing high amounts of GLU, glycogen, lactate and pyruvate, which stimulates the release of insulin and provides a greater amount of glycerol and acyl groups used in the synthesis of TG and cholesterol, promoting the maintenance of metabolic alterations mentioned before (Basciano et al. 2005; Pérez Cruz et al. 2007).

Our results showed that sucrose-enriched diet consumption during 25 weeks significantly decreased serum TC levels. One possible explanation for this result is that SG consumed a significantly lower amount of solid food (as seen in our results), lipids and consequently less cholesterol from the laboratory diet compare to CG.

In addition, it has been reported that rats are an HDL-c pattern animal model, meaning that most of their serum cholesterol is transported by these lipoproteins (Osorio 2013; Osorio et al. 2013). In our study, sucrose-enriched diet consumption trigger increased serum HDL-c levels which could explain the lower TC levels found in SG (Schaefer et al. 2009). However, reports that correlate increased HDL-c levels with sucrose-enriched diet consumption were not found.

On the other hand, high HDL-c levels are not always considered as a protection factor from atherosclerosis. Composition and size of these lipoproteins play a fundamental role in this regard. If HDL particles are large and rich and cholesterol and TG esters, as it is possible same thing could have happened in our study given the HDL pattern of the model used, these lipoproteins represent and atherogenic risk factor due to the change in their composition and consequently in their function (Camont et al. 2011; Kapourchali et al. 2016). Thus, it is important to take into account other parameters that represent risk factors to atherosclerosis or CVD (Jacobson et al. 2015).

Regarding to serum LDL-c levels, our study showed that sucrose-enriched diet consumption during 25 weeks did not produce a significant difference in this parameter. This may be also related to the HDL pattern of the animal model used since this would mean that LDL would not be the main lipoproteins through which the serum cholesterol of rats would be transported. On the other hand, this finding may be related with high fructose ingestion as previously reported in patients with hyperinsulinemia (Schaefer et al. 2009) and with the high levels of TG and VLDL-c observed in SG in the present study. A significant percentage of VLDL particles it is converted to LDL as part of their metabolism (Basciano et al. 2005; Schaefer et al. 2009; Carvajal 2014).

Liver histopathological changes

Liver steatosis

Regarding to the histopathological changes induced by sucrose-enriched diet consumption during 25 weeks, results showed that both experimental groups developed non-alcoholic fatty liver disease (NAFLD). However, SG presented a higher steatosis percentage and a greater degree of NASH according to the scoring system (LaBrecque et al. 2012) used in our study which coincides with previous reports (Nojima et al. 2012; Schultz et al. 2015; Acosta-Cota et al. 2019).

It has been reported that high GLU and fructose consumption is related to the appearance of steatosis due to the ability of both monosaccharides to stimulate, in an insulin-dependent and independent manner, metabolic pathways and molecular mechanisms related to greater hepatic fatty acids uptake, increase hepatic *de novo* lipogenesis, imbalance between lipid synthesis and lipid degradation, lipid accumulation, mainly as TG, and the constant stimulation of the glycolytic pathway (Basciano et al. 2005; Pérez Cruz et al. 2007; Ragab et al. 2015) some of them previously mentioned.

The excessive fatty acids content in the liver could lead to mitochondrial dysfunction, increase in lipid peroxidation and increase reactive oxygen species production which have been related to the progression from steatosis to NASH (Angulo 2002; Koek et al. 2011; Rolo et al. 2012) due to the activation of inflammation pathways leading, among other things, to the proinflammatory cytokines, i.e. interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β) production and increase of chemotaxis (Castro et al. 2014; Acosta-Cota et al. 2019) which could explain the presence of the inflammatory infiltrate found in parenchyma of the liver histological samples of SG in our study.

Hepatic total cholesterol accumulation

To know the factors involve in the development of NASH is fundamental in the understanding of this condition and for creating effective therapies to prevent and cope with this disease (Arguello et al. 2015; Walenbergh and Shiri-Sverdlov 2015). However, the mechanisms contributing to this transition are not completely known. In this context, the 'multiple-hit' model has been proposed to explain this phenomenon (Buzzetti et al. 2016; Fang et al. 2018). According to this model, insulin resistance causes an increase in hepatic *de novo* lipogenesis and in lipolysis in adipose tissue with increased flux of fatty acids to the liver and altered secretion of adipokines and inflammatory cytokines (Buzzetti et al. 2016). TG accumulate excessively and lipotoxicity increases derived from high levels of fatty acids, cholesterol among other lipid metabolites leading to mitochondrial dysfunction and oxidative stress (Fang et al. 2018). Overall with the increased absorption of fatty acids and other pathogenic molecules from a dysfunctional gut to the liver leads to a chronic hepatic inflammatory state (Kirpich et al. 2015) which could progress to cell death and fibrosis (Caballero et al. 2009; Walenbergh and Shiri-Sverdlov 2015; Vega-Badillo 2016).

Regarding this, recent evidence in human and animal models suggest that hepatic cholesterol could be a key factor in the development and progression of NASH (Ioannou 2016; Ioannou et al. 2019). In the present study, sucrose-enriched diet consumption during 25 weeks did not produce

significant differences in hepatic cholesterol levels, which was similarly observed in other study executed with the same animal model (Torres-Villalobos et al. 2015). Nevertheless, scientific evidence that show association between these parameters is limited.

On the other hand, in the present study SG showed a slight increase of hepatic cholesterol levels, which could be correlated with the liver histopathological findings since scientific reports suggest that cholesterol metabolism is deregulated in animal models and patients with NAFLD as a result of its hepatic increased uptake and endogenous synthesis as well as its decreased removal through the bile which leads to greater liver damage (Arguello et al. 2015; Vega-Badillo 2016).

Otherwise, the mechanism by which cholesterol promotes the development of NASH is not completely elucidated; however, there are several possible suggested mechanisms. When hepatic cholesterol accumulates in the first stages of NAFLD, abundant cholesterol tends to crystallize within the lipid droplets leading to the aggregation of Kupffer cells in crown-like structures around the droplets which induce the activation of inflammation pathways and trigger the production of pro-inflammatory cytokines as TNF-α (Liangpunsakul and Chalasani 2018); Besides, stellate cells are also stimulated which generates fibrosis. Subsequently, different mechanisms that yield mitochondrial dysfunction, oxidative stress among others are activated and lead to hepatocyte apoptosis (Mota et al. 2016; Liangpunsakul and Chalasani 2018).

Aortic histopathological changes

CVD are recognized as the main cause of morbidity and mortality worldwide and atherosclerosis is the most contributing pathophysiological factor to these diseases (OMS 2017).

There are many atherosclerotic risk factors and unhealthy diet is closely related to them (OMS 2017). As well as high-fat diets (Wang et al. 2014), sugar-sweetened beverages consumption has been associated with several cardiometabolic effects and scientific evidence has indicated that may increase the risk of coronary heart disease (Huang et al. 2014; Shah 2017).

In the present study, sucrose-enriched diet consumption did not induce atheroma plaque formation. However, in both experimental groups, we observed a slight wall thickening which is considered the earliest microscopic vascular change (American Heart Association Type I lesion) in atherosclerosis classification (Sakakura et al. 2013). Couple with this, scientific evidence has proposed that intima-media thickening is a marker of atherosclerosis burden, a predictive factor of cardiovascular and stroke events in adults and has been associated with type 1 diabetes in children (Woo et al. 2004).

This finding may be firstly related to rats ages, which could explain that both experimental groups developed this characteristic. On the other hand, the higher lipids intake and hypercholesterolemia levels observed in CG are important atherogenic risk factors that could be related to this finding in this group (Lahoz and Mostaza 2007; Getz and Reardon 2012).

In respect to SG, greater levels of hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia and high VLDL-c levels could help to understand this finding in the group. Previous studies have shown that hyperglycemia can induce endothelial dysfunction which is considered to be a potential mediator to adverse cardiovascular effects of sugar-sweetened beverages (Shah 2017), early abnormality during atherogenesis and marker of arterial damage which precedes plaque formation (Woo et al. 2004). Persistent hyperglycemia is also associated with upregulation of cytokines and growth factors as interleukin- 1β and TNF-α in intimal cells of the arterial walls (Kishida et al. 2012). Besides, hyperglycemia may induce an increased intracellular reactive oxygen species (ROS) production and a decreased nitric oxide bioavailability (Hulsmans et al. 2012) increasing oxidative stress, inflammation and vascular dysfunction (Shah 2017). Insulin resistance also increased mitochondrial ROS from free fatty acids and by inactivation of antioxidant enzymes by ROS (Hulsmans et al. 2012).

High-fructose consumption is also related to atherosclerosis due to an increased *de novo* lipogenesis with its consequent elevation on TG and VLDL-c levels, consistent with our study. Also, increased small dense LDL-c that are in particular linked to cardiovascular risk (Kolderup and Svihus 2015).

Even when SG showed the most severe degree of NAFLD, both groups developed this condition which is tightly related to endothelial dysfunction and increased risk of atherosclerosis and CVD (Gaggini et al. 2013; Kim et al. 2014).

We observed that SG aorta tissue samples showed a higher amount of PVAT *vs* CG which is a very important finding. Normally, is recognized that atherosclerotic process initiates in intimal tunic and progress from the inside to the outside of the artery. Nevertheless, recently it has been suggested that inflammatory process also progresses from the outside toward the inside of the artery due to the action of PVAT (Tanaka and Sata 2018).

Finally, the fact that we did not observe the atheroma plaque formation despite the rats ages and the other metabolic alterations presented by them may be due to the intervention period. It is probable that the slight wall thickening observed in the aorta of the SG could progress to atheroma plaque, as normally occurs in humans, if the intervention period was longer and the high-sucrose consumption and metabolic alterations continue. However, it will be interesting to prove this dietary intervention in an animal model with a lipoprotein profile similar to humans with LDL present as the major form of circulating cholesterol, as guinea pigs (Ye et al. 2013).

Conclusion

The sucrose enriched-diet consumption during 25 weeks contributed to the development of risk factors associated with type 2 diabetes, atherosclerosis and non-alcoholic fatty liver disease such as hyperglycemia, hyperinsulinemia, insulin resistance, hypertriglyceridemia, high VLDL-c levels and NAFLD (steatosis and NASH) development in male Wistar rats. Our results, with those of previous studies, contributed to generate scientific evidence to help clarify the existing inaccuracies about the health negative effects associated with high-sucrose consumption with the purpose of implement effective combat and preventive strategies against NCDs and their risk factors.

Disclosure of interest

The authors report no conflict of interest.

Funding

This work was supported by the Universidad Autónoma de Sinaloa [PROFAPI2015/201].

ORCID

Marcela De Jesús Vergara Jiménez http://orcid.org/0000-0002-2679-8706
Juan Ignacio Sarmiento Sánchez http://orcid.org/0000-0001-8961-4436
Lorenzo Antonio Picos Corrales http://orcid.org/0000-0001-9029-2614
Ulises Osuna Martínez http://orcid.org/0000-0002-7824-6639

References

Acosta-Cota SJ, Aguilar-Medina EM, Ramos-Payan R, Ruiz-Quinonez AK, Romero-Quintana JG, Montes-Avila J, Rendon-Maldonado JG, Sanchez-Lopez A, Centurion D, Osuna-Martinez U. 2019. Histopathological and biochemical changes in the development of nonalcoholic fatty liver disease induced by high-sucrose diet at different times. Can J Physiol Pharmacol. 97(1):23–36.

Aminlari L, Shekarforoush SS, Hosseinzadeh S, Nazifi S, Sajedianfard J, Eskandari MH. 2018. Effect of Probiotics Bacillus coagulans and Lactobacillus plantarum on Lipid Profile and Feces Bacteria of Rats Fed Cholesterol-Enriched Diet. Probiotics Antimicrob Proteins. 1–9. doi:10.1007/s12602-018-9480-1

Angulo P. 2002. Nonalcoholic fatty liver disease. N Eng J Med. 346(16):1221–1231. doi:10.1056/NEJMra011775.

Arguello G, Balboa E, Arrese M, Zanlungo S. 2015. Recent insights on the role of cholesterol in non-alcoholic fatty liver disease. Biochim Biophys Acta. 1852(9):1765–1778. doi:10.1016/j.bbadis.2015.05.015.

Augustin LS, Kendall CW, Jenkins DJ, Willett WC, Astrup A, Barclay AW, Björck I, Brand-Miller JC, Brighenti F, Buyken AE. 2015. Glycemic index, glycemic load and glycemic response: an international scientific consensus summit from the international carbohydrate quality consortium (ICQC). Nutr Metab Cardiovas Dis. 25 (9):795–815. doi:10.1016/j.numecd.2015.05.005.

Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, Brand-Miller JC. 2008. Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. Am J Clin Nutr. 87(3):627–637. doi:10.1093/ajcn/87.3.627.

Basaranoglu M, Basaranoglu G, Bugianesi E. 2015. Carbohydrate intake and nonalcoholic fatty liver disease: fructose as a weapon of mass destruction. Hepatobiliary Surg Nutr. 4(2):109. doi:10.3978/j.issn.2304-3881.2014.11.05

Basaranoglu M, Basaranoglu G, Sabuncu T, Sentürk H. 2013. Fructose as a key player in the development of fatty liver disease. World J Gastroenterol. 19(8):1166. doi:10.3748/wjg.v19.i8.1166.

Basciano H, Federico L, Adeli K. 2005. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab (Lond). 2 (1):5. doi:10.1186/1743-7075-2-5.

Bravo S, Lowndes J, Sinnett S, Yu Z, Rippe J. 2013. Consumption of sucrose and high-fructose corn syrup does not increase liver fat or ectopic fat deposition in muscles. App Physiol Nutr Metab. 38(6):681–688. doi:10.1139/apnm-2012-0322.

Buzzetti E, Pinzani M, Tsochatzis EA. 2016. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 65(8):1038–1048. doi:10.1016/j.metabol.2015.12.012.

Caballero F, Fernandez A, De Lacy AM, Fernandez-Checa JC, Caballeria J, Garcia-Ruiz C. 2009. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. J Hepatol. 50(4):789–796. doi:10.1016/j.jhep.2008.12.016.

Camont L, Chapman MJ, Kontush A. 2011. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med. 17(10):594–603. doi:10.1016/j.molmed.2011.05.013.

Carvajal C. 2014. Lipoproteínas: metabolismo y lipoproteínas aterogénicas. Medicina Legal De Costa Rica. 31 (2):88–94.

Castellanos Jankiewicz AK, Rodriguez Peredo SM, Cardoso Saldana G, Diaz Diaz E, Tejero Barrera ME, Del Bosque Plata L, Carbo Zabala R. 2015. Adipose tissue redistribution caused by an early consumption of a high sucrose diet in a rat model. Nutr Hosp. 31(6):2546–2553. doi:10.3305/nh.2015.31.6.8935

Castro AVB, Kolka CM, Kim SP, Bergman RN. 2014. Obesity, insulin resistance and comorbidities? Mechanisms of association. Arquivos Brasileiros De Endocrinologia Metabologia. 58(6):600–609. doi:10.1590/0004-2730000003223.

Córdova-Villalobos JÁ, Barriguete-Meléndez JA, Lara-Esqueda A, Barquera S, Rosas-Peralta M, Hernández-Ávila M, de León-May ME, Admon L, Aguilar-Salinas CA. 2008. Las enfermedades crónicas no transmisibles en México: sinopsis epidemiológica y prevención integral. Salud Pública De México. 50:419–427. doi:10.1590/S0036-36342008000500015.

Cummings J, Stephen A. 2007. Carbohydrate terminology and classification. Eur J Clin Nutr. 61(S1):S5. doi:10.1038/sj.ejcn.1602936.

Esteban-Pretel G, Marín MP, Cabezuelo F, Moreno V, Renau-Piqueras J, Timoneda J, Barber T. 2010. Vitamin A deficiency increases protein catabolism and induces urea cycle enzymes in rats. J Nutr. 140(4):792–798. doi:10.3945/jn.109.119388.

Fang Y-L, Chen H, Wang C-L, Liang L. 2018. Pathogenesis of non-alcoholic fatty liver disease in children and adolescence: from "two hit theory" to "multiple hit model". World J Gastroenterol. 24(27):2974. doi:10.3748/wjg.v24.i27.2974.

Gaggini M, Morelli M, Buzzigoli E, DeFronzo R, Bugianesi E, Gastaldelli A. 2013. Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients. 5(5):1544–1560. doi:10.3390/nu5051544.

Gallagher C, Keogh JB, Pedersen E, Clifton PM. 2016. Fructose acute effects on glucose, insulin, and triglyceride after a solid meal compared with sucralose and sucrose in a randomized crossover study, 2. Am J Clin Nutr. 103 (6):1453–1457. doi:10.3945/ajcn.115.129866.

Getz GS, Reardon CA. 2012. Animal models of atherosclerosis. Arterioscler Thromb Vasc Biol. 32(5):1104–1115. doi:10.1161/ATVBAHA.111.237693.

Gibson S, Gunn P, Wittekind A, Cottrell R. 2013. The effects of sucrose on metabolic health: a systematic review of human intervention studies in healthy adults. Crit Rev Food Sci Nutr. 53(6):591–614. doi:10.1080/10408398.2012.691574.

Gómez Candela C, Palma Milla S. 2013. Una visión global, actualizada y crítica del papel del azúcar en nuestra alimentación. Nutrición Hospitalaria. 28:1–4. doi:10.3305/nh.2013.28.sup4.6791.

Harris RBS. 2018. Source of dietary sucrose influences development of leptin resistance in male and female rats. Am J Physiol Regul Integrcomp Physiol. 314(4):R598–R610. doi:10.1152/ajpregu.00384.2017.
Hita MEG, Macías KGA, Enríquez SS. 2006. Regulación neuroendócrina del hambre, la saciedad y mantenimiento del balance energético. Investigación En Salud. 8(3):191–200.
Hochuli M, Aeberli I, Weiss A, Hersberger M, Troxler H, Gerber PA, Spinas GA, Berneis K. 2014. Sugar-sweetened beverages with moderate amounts of fructose, but not sucrose, induce fatty acid synthesis in healthy young men: a randomized crossover study. J Clin Endocrinol Metab. 99(6):2164–2172. doi:10.1210/jc.2013-3856.
Huang C, Huang J, Tian Y, Yang X, Gu D. 2014. Sugar sweetened beverages consumption and risk of coronary heart disease: a meta-analysis of prospective studies. Atherosclerosis. 234(1):11–16. doi:10.1016/j.atherosclerosis.2014.01.037.
Hulsmans M, Van Dooren E, Holvoet P. 2012. Mitochondrial reactive oxygen species and risk of atherosclerosis. Curr Atheroscler Rep. 14(3):264–276. doi:10.1007/s11883-012-0237-0.
IMCO. 2015. Kilos de más, pesos de menos: Los costos de la obesidad en México.
Ioannou GN. 2016. The role of cholesterol in the pathogenesis of NASH. Trends Endocrinol Metab. 27(2):84–95. doi:10.1016/j.tem.2015.11.008.
Ioannou GN, Landis CS, Jin GY, Haigh WG, Farrell GC, Kuver R, Lee SP, Savard C. 2019. Cholesterol crystals in hepatocyte lipid droplets are strongly associated with human nonalcoholic steatohepatitis. Hepatol Commun. 3 (6):776–791. doi:10.1002/hep4.1348
Jacobson TA, Ito MK, Maki KC, Orringer CE, Bays HE, Jones PH, McKenney JM, Grundy SM, Gill EA, Wild RA. 2015. National lipid association recommendations for patient-centered management of dyslipidemia: part 1—full report. J Clin Lipidol. 9(2):129–169. doi:10.1016/j.jacl.2015.02.003.
Kampov-Polevoy AB, Alterman A, Khalitov E, Garbutt JC. 2006. Sweet preference predicts mood altering effect of and impaired control over eating sweet foods. Eat Behav. 7(3):181–187. doi:10.1016/j.eatbeh.2005.09.005.
Kapourchali FR, Surendiran G, Goulet A, Moghadasian MH. 2016. The role of dietary cholesterol in lipoprotein metabolism and related metabolic abnormalities: a mini-review. Crit Rev Food Sci Nutr. 56(14):2408–2415. doi:10.1080/10408398.2013.842887.
Kilpatrick LA, Coveleskie K, Connolly L, Labus JS, Ebrat B, Stains J, Jiang Z, Suyenobu BY, Raybould HE, Tillisch K. 2014. Influence of sucrose ingestion on brainstem and hypothalamic intrinsic oscillations in lean and obese women. Gastroenterology. 146(5):1212–1221. doi:10.1053/j.gastro.2014.01.023.
Kim EJ, Kim B-H, Seo HS, Lee YJ, Kim HH, Son -H-H, Choi MH. 2014. Cholesterol-induced non-alcoholic fatty liver disease and atherosclerosis aggravated by systemic inflammation. PLoS One. 9(6):e97841. doi:10.1371/journal.pone.0097841.
Kirpich IA, Marsano LS, McClain CJ. 2015. Gut–liver axis, nutrition, and non-alcoholic fatty liver disease. Clin Biochem. 48(13–14):923–930. doi:10.1016/j.clinbiochem.2015.06.023.
Kishida K, Funahashi T, Shimomura I. 2012. Molecular mechanisms of diabetes and atherosclerosis: role of adiponectin. Endocr Metab Immune Disord Drug Targets. 12(2):118–131. doi:10.2174/187153012800493468.
Koek G, Liedorp P, Bast A. 2011. The role of oxidative stress in non-alcoholic steatohepatitis. Clin Chim Acta. 412 (15–16):1297–1305. doi:10.1016/j.cca.2011.04.013.
Kolderup A, Svihus B. 2015. Fructose metabolism and relation to atherosclerosis, type 2 diabetes, and obesity. J Nutr Metab. 2015:1–12. doi:10.1155/2015/823081.
Kumari D, Nair N, Bedwal RS. 2011. Effect of dietary zinc deficiency on testes of Wistar rats: morphometric and cell quantification studies. J Trace Elem Med Biol. 25(1):47–53. doi:10.1016/j.jtemb.2010.11.002.
LaBrecque D, Abbas Z, Anania F, Ferenci P, Ghafor-Kjan A, Goh KL. 2012. Enfermedad del hígado graso no alcohólico y esteatohepatitis no alcohólica. Guías De La Organización Mundial De Gastroenterología. 1(1):1–31.
Lahoz C, Mostaza JM. 2007. La aterosclerosis como enfermedad sistémica. Revista Española De Cardiología. 60 (2):184–195. doi:10.1157/13099465.
Liangpunsakul S, Chalasani N. 2018. Lipid mediators of liver injury in nonalcoholic fatty liver disease. Am J Physiol Gastrointestinal Liver Physiol. 316(1):G75–G81. doi:10.1152/ajpgi.00170.2018.
Matsuo E, Mochizuki A, Nakayama K, Nakamura S, Yamamoto T, Shioda S, Sakurai T, Yanagisawa M, Shiuchi T, Minokoshi Y. 2011. Decreased intake of sucrose solutions in orexin knockout mice. J Mol Neurosci. 43 (2):217–224. doi:10.1007/s12031-010-9475-1.
Mota M, Banini BA, Cazanave SC, Sanyal AJ. 2016. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. Metabolism. 65(8):1049–1061. doi:10.1016/j.metabol.2016.02.014.
Nojima K, Sugimoto K, Ueda H, Babaya N, Ikegami H, Rakugi H. 2012. Analysis of hepatic gene expression profile in a spontaneous mouse model of type 2 diabetes under a high sucrose diet. Endocr J. 60(3):261–274. doi:10.1507/endocrj.ej12-0258.
Olguin MC, Posadas MD, Revelant GC, Labourdette V, Marinozzi DO, Venezia MR, Zingale MI. 2015. Efectos del consumo elevado de fructosa y sacarosa sobre parámetros metabólicos en ratas obesas y diabéticas. Revista Chilena De Nutrición. 42(2):151–156. doi:10.4067/S0717-75182015000200006.

Oliveira LSC, Santos DA, Barbosa-da-Silva S, Mandarim-de-Lacerda CA, Aguila MB. 2014. The inflammatory profile and liver damage of a sucrose-rich diet in mice. J Nutr Biochem. 25(2):193–200. doi:10.1016/j.jnutbio.2013.10.006.
OMS. 2017. Enfermedades no transmisibles. [accessed 2019 Jun 20]. http://www.who.int/mediacentre/factsheets/fs355/es/.
OMS. SDIT. 2003. Dieta, nutrición y prevención de enfermedades crónicas. Geneva, Switzerland: OMS (Organizacion Mundial de la Salud).
Osorio JH. 2013. Determinación de los niveles de colesterol LDL en una especie con patrón HDL. Revista De Investigaciones Veterinarias Del Perú. 24(3):277–282. doi:10.15381/rivep.v24i3.2575.
Osorio JH, Suárez YJ, Pérez JE. 2013. Comparación de dos métodos para la determinación de los niveles de colesterol HDL en caninos. Biosalud. 12(2):60–65.
Packard AE, Ghosal S, Herman JP, Woods SC, Ulrich-Lai YM. 2014. Chronic variable stress improves glucose tolerance in rats with sucrose-induced prediabetes. Psychoneuroendocrinology. 47:178–188. doi:10.1016/j.psyneuen.2014.05.016.
Pérez Cruz E, Zúñiga AES, Mier GM. 2007. Efectos benéficos y deletéreos del consumo de fructosa. Revista de Endocrinología y Nutrición. 15(2):67–74.
Petykó Z, Tóth A, Szabó I, Gálosi R, Lénárd L. 2009. Neuronal activity in rat medial prefrontal cortex during sucrose solution intake. Neuroreport. 20(14):1235–1239. doi:10.1097/WNR.0b013e32832fbf30.
Pinto BAS, Melo TM, Flister KFT, França LM, Kajihara D, Tanaka LY, Laurindo FRM, de Andrade Paes AM. 2016. Early and sustained exposure to high-sucrose diet triggers hippocampal ER stress in young rats. Metab Brain Dis. 31(4):917–927. doi:10.1007/s11011-016-9830-1.
Ragab SM, Elghaffar SKA, El-Metwally TH, Badr G, Mahmoud MH, Omar HM. 2015. Effect of a high fat, high sucrose diet on the promotion of non-alcoholic fatty liver disease in male rats: the ameliorative role of three natural compounds. Lipids Health Dis. 14(1):83. doi:10.1186/s12944-015-0087-1.
Rippe JM, Angelopoulos TJ. 2013. Sucrose, high-fructose corn syrup, and fructose, their metabolism and potential health effects: what do we really know?. Adv Nutr 4(2):236–245. doi:10.3945/an.112.002824.
Rolo AP, Teodoro JS, Palmeira CM. 2012. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. Free Radical Biol Med. 52(1):59–69. doi:10.1016/j.freeradbiomed.2011.10.003.
Sakakura K, Nakano M, Otsuka F, Ladich E, Kolodgie FD, Virmani R. 2013. Pathophysiology of atherosclerosis plaque progression. Heart Lung Circ. 22(6):399–411. doi:10.1016/j.hlc.2013.03.001.
Sánchez JC. 2005. Perfil fisiológico de la leptina. Colomb Med. 36(1):50–59.
Schaefer EJ, Gleason JA, Dansinger ML. 2009. Dietary fructose and glucose differentially affect lipid and glucose homeostasis. J Nutr. 139(6):1257S–1262S. doi:10.3945/jn.108.098186.
Schultz A, Barbosa-da-Silva S, Aguila MB, Mandarim-de-Lacerda CA. 2015. Differences and similarities in hepatic lipogenesis, gluconeogenesis and oxidative imbalance in mice fed diets rich in fructose or sucrose. Food Funct. 6(5):1684–1691. doi:10.1039/C5FO00251F.
Shah PK. 2017. Sugar-sweetened beverage and vascular function: not so sweet after all. Arterioscler Thromb Vasc Biol. 37(6):1020–1021. doi:10.1161/ATVBAHA.117.309450.
Sheludiakova A, Rooney K, Boakes RA. 2012. Metabolic and behavioural effects of sucrose and fructose/glucose drinks in the rat. Eur J Nutr. 51(4):445–454. doi:10.1007/s00394-011-0228-x.
Singh GM, Micha R, Khatibzadeh S, Lim S, Ezzati M, Mozaffarian D. 2015. Estimated global, regional, and national disease burdens related to sugar-sweetened beverage consumption in 2010. Circulation. 132(8):639–666. doi:10.1161/CIRCULATIONAHA.114.010636.
Tanaka K, Sata M. 2018. Roles of perivascular adipose tissue in the pathogenesis of atherosclerosis. Front Physiol. 9:3. doi:10.3389/fphys.2018.00003.
Torres-Villalobos G, Hamdan-Pérez N, Tovar AR, Ordaz-Nava G, Martínez-Benítez B, Torre-Villalvazo I, Morán-Ramos S, Díaz-Villaseñor A, Noriega LG, Hiriart M. 2015. Combined high-fat diet and sustained high sucrose consumption promotes NAFLD in a murine model. Ann Hepatol. 14(4):540–546. doi:10.1016/S1665-2681(19)31176-7.
Tsilas CS, de Souza RJ, Mejia SB, Mirrahimi A, Cozma AI, Jayalath VH, Ha V, Tawfik R, Di Buono M, Jenkins AL. 2017. Relation of total sugars, fructose and sucrose with incident type 2 diabetes: a systematic review and meta-analysis of prospective cohort studies. Can Med Assoc J. 189(20):E711–E720. doi:10.1503/cmaj.160706.
Uriarte G, Paternain L, Milagro F, Martínez J, Campion J. 2013. Shifting to a control diet after a high-fat, high-sucrose diet intake induces epigenetic changes in retroperitoneal adipocytes of Wistar rats. J Physiol Biochem. 69(3):601–611. doi:10.1007/s13105-012-0231-6.
Vega-Badillo J. 2016. Alteraciones en la homeostasis del colesterol hepático y sus implicaciones en la esteatohepatitis no alcohólica. TIP Revista Especializada En Ciencias Químico-Biológicas. 20(1):50–65. doi:10.1016/j.recqb.2016.11.005.

Walenbergh SM, Shiri-Sverdlov R. 2015. Cholesterol is a significant risk factor for non-alcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 9(11):1343–1346. doi:10.1586/17474124.2015.1092382

Wang S, Xiaoling G, Pingting L, Shuqiang L, Yuaner Z. 2014. Chronic unpredictable mild stress combined with a high-fat diets aggravates atherosclerosis in rats. Lipids Health Dis. 13(1):77. doi:10.1186/1476-511X-13-77.

Woo K, Chook P, Yu C, Sung R, Qiao M, Leung S, Lam C, Metreweli C, Celermajer D. 2004. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. Int J Obes. 28(7):852. doi:10.1038/sj.ijo.0802539.

Ye P, Cheah IK, Halliwell B. 2013. High fat diets and pathology in the guinea pig. Atherosclerosis or liver damage? Biochim Biophys Acta Mol Basis Dis. 1832(2):355–364. doi:10.1016/j.bbadis.2012.11.008.

Zhao Z-Z, Xin L-L, Xia J-H, Yang S-L, Chen Y-X, Li K. 2015. Long-term high-fat high-sucrose diet promotes enlarged islets and β-cell damage by oxidative stress in bama minipigs. Pancreas. 44(6):888–895. doi:10.1097/MPA.0000000000000349.

Zhou S, Wang Y, Jiang Y, Zhang Z, Sun X, Yu L. 2017. Dietary intake of structured lipids with different contents of medium-chain fatty acids on obesity prevention in C57BL/6J mice. J Food Sci. 82(8):1968–1977. doi:10.1111/jfds.2017.82.issue-8.