Maternal exercise during pregnancy modulates genetic and biochemical damage caused by high consumption of fructose in blood and liver of offspring

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Abstract

Pregnancy is a period that is characterized by several metabolic and physiological changes and requires special attention, especially with regard to the relationship between feeding and fetal development. Therefore, the objective of this study is to evaluate whether the practice of voluntary physical exercise in combination with chronic consumption of fructose from the beginning of life and/or until the gestational period causes biochemical and genotoxic changes in pregnant females and in their offspring. 70 Swiss female mice received fructose in the hydration bottle and/or practiced voluntary physical exercise (VPE) for 8 weeks (pre-pregnancy/pregnancy). After the lactation period, the offspring groups were separated by sex. It was observed that the consumption of fructose affected the food consumption, serum concentration of fructose and glycemic profile in the mothers and that the VPE decreases these parameters. In addition, fructose was genotoxic in the mothers' peripheral tissues and VPE had a preventive effect on these parameters. The offspring showed changes in food consumption, serum fructose concentration and body weight, in addition to an increase in the adiposity index in male offspring in the FRU group and a decrease in the FRU+VPE group. Fructose lead to hepatic steatosis in the offspring and VPE was able to decrease the area of steatosis. In addition, fructose led to genotoxicity in the offspring and VPE was able to modulate this effect, reducing damages. In conclusion, we observed that all interventions with voluntary physical exercise had nutritional, genetic and biochemical benefits of the mother and her offspring.

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ABSTRACT

Pregnancy is a period that is characterized by several metabolic and physiological changes and requires special attention, especially with regard to the relationship between feeding and fetal development. Therefore, the objective of this study is to evaluate whether the practice of voluntary physical exercise in combination with chronic consumption of fructose from the beginning of life and/or until the gestational period causes biochemical and genotoxic changes in pregnant females and in their offspring. 70 Swiss female mice received fructose in the hydration bottle and/or practiced voluntary physical exercise (VPE) for 8 weeks (pre-pregnancy/pregnancy). After the lactation period, the offspring groups were separated by sex. It was observed that the consumption of fructose affected the food consumption, serum concentration of fructose and glycemic profile in the mothers and that the VPE decreases these parameters. In addition, fructose was genotoxic in the mothers' peripheral tissues and VPE had a preventive effect on these parameters. The offspring showed changes in food consumption, serum fructose concentration and body weight, in addition to an increase in the adiposity index in male offspring in the FRU group and a decrease in the FRU+VPE group. Fructose lead to hepatic steatosis in the offspring and VPE was able to decrease the area of steatosis. In addition, fructose led to genotoxicity in the offspring and VPE was able to modulate this effect, reducing damages. In conclusion, we observed that all interventions with voluntary physical exercise had nutritional, genetic and biochemical benefits of the mother and her offspring.

Keywords: Fetal programming; Fructose; Physical exercise; Genotoxicity; Mutagenicity

Introduction

It is believed that an inadequate intrauterine environment can affect fetal growth and development, leading to aberrant, permanent programming of the metabolic and endocrine function (Deodati et al., 2020). Fetal programming is an adaptive response of the organism to the environment. This ability is called "developmental plasticity" which allows the development of a spectrum of phenotypes from a single genotype (Lucas et al., 1991; Gluckman and Hanson, 2004). It allows the organism to respond to the surrounding environment, especially during early development, when cells are differentiating and tissues are developing. This plasticity is based on modifications of cellular pathways leading altered gene expression and the induction of specific phenotypes, but without modification of DNA sequence (Hanson and Gluckman, 2008; Barouki et al., 2012). Thus, early metabolic events during the pre-natal period may be associated with susceptibility to chronic diseases later in the adult life (Gluckman et al., 2005).

Maternal obesity and gestational diabetes, for example, have been shown to provoke long-term detrimental changes in the pathways that regulate energy balance in offspring. Notably, the increase in obesity and diabetes, as well as metabolic syndrome and fatty liver disease coincided with the increase in fructose in recent years (Muriel et al., 2021). In recent decades the daily intake of free fructose or as high fructose corn syrup has increased markedly. These forms of fructose are used in the food industry for their sweetness, palatability, solubility, low cost and high production efficiency compared to sugar (Cioffi et al., 2017). Ancestral diets showed that the average fructose intake was 2 kg per year, while the current global average consumption is 25 kg per year (Kmietowicz, 2012). More recently, fructose consumption has been estimated at 117 g/day in women and 162 g/day in men in world, and that, to consume this excess fructose via industrialized foods it is necessary to consume a 350 mL can of soda a day, while when eating fruits one would have to consume 1.3 kg of banana, 4.3 kg of strawberry, 800 g of apple and 900 mL of orange juice a day. In the latest data from

Vigitel Brazil for 2018, it was observed that, in the set of 27 cities evaluated, the frequency of consumption of five or more groups of ultra-processed foods (rich in fructose and glucose) was 18.2%, being higher among men (21.8%) than among women (15.1%) (Ministry of Health, 2019).

Research with animals, showed that after 8 weeks of high fructose consumption, rats developed features of the metolinic syndrome along with mitochondrial structural changes (Mamikutty et al., 2014). In this context, Magenis et al. (2020) evaluated the effects of fructose treatment during pregnancy and lactation in mice, and observed an increased in DNA damage, hyperglycemia and hyperlipidemia in females that received doses of 10 and 20%/L fructose/day. Corroborating with this, Magenis et al. (2022) observed that the offspring (males and females) of these females, in both dosage groups, demonstrated genotoxicity (assessed by the comet assay) and oxidative stress (assessed by nitrite concentration, sulfhydryl content, and superoxide dismutase activity) in peripheral tissues. In addition, they showed nutritional and metabolic changes due to increased food consumption, hyperglycemia, hyperlipidemia and metabolic syndrome. Therefore, it was suggested that high fructose consumption by pregnant females was harmful to their offspring. However, it is believed that early dietary changes, both in mothers and children, significantly contribute to alleviate or assist in the treatment of these pathologies (Frias and Grove, 2012).

In this context, seeking alternatives to protect the damage caused to fetal programming are necessary in order to search new treatments. Although, the practice of physical exercise increased among Brazilians according to Vigitel data (2019), the proportion of women performing some type of physical activity dropped from 15.7% in 2013 to 14.2% in 2018 (Ministry of Health, 2019). More worrying is the sedentary lifestyle during pregnancy, since only 4.7% of pregnant Brazilian women are active throughout this period and 12.9% of women reported some physical activity during pregnancy as recommended by the American College of Obstetricians and Gynecologists (ACOG) (Domingues and Barros, 2007). In addition, it is observed that 50% of women at reproductive age and 20–25% of pregnant women in Europe are overweight or obese at the first antenatal visit (Stevens et al., 2012).

Guidance for physical exercise during pregnancy has been encouraged and recommended by the ACOG since the 1990s, but only in 2002 was this practice recognized as safe and indicated for healthy pregnant women. Through a systematic review and meta-analysis, Davenport et al. (2018) observed that pregnant women who exercised have a 39% reduced chance of having a baby weighing more than four kilos compared to those who did not exercise. It was also shown that the practice of exercise during pregnancy is not related to intrauterine growth restriction, premature birth and low birth weight, thus being safe and beneficial for the fetus. The ACOG advises pregnant women, even if they are sedentary, to perform at least 30 minutes of physical exercise on as many days of the week as possible (ACOG, 2002). In São Paulo, it was observed in the pregnant population that only 13% developed some type of physical activity, and only half of them performed exercises with frequency guided by the ACOG (Surita et al., 2014).

So far research in animals observed that maternal exercise before and during pregnancy does not seem to affect the body composition of offspring in healthy mice (Kelly et al., 2015). In an animal model of maternal obesity induced by a high-fat diet and treated with treadmill exercise, it was observed that the intervention had beneficial effects in relation to insulin resistance, excess placental lipid deposition and its hypoxia in offspring (Fernandez-Twinn et al. al., 2017). Moreover, Brito et al. (2016) observed that maternal physical exercise decreases DNA damage and oxidative stress caused by doxorubicin in offspring cardiomyocytes. In addition, Netto et al. (2018) observed that the practice of swimming in pregnant females with streptozotocin-induced diabetes by contributed to the reduction of DNA damage and lipid peroxidation in the mother and in her offspring.

To understand the effect of lifestyle and levels of DNA damage on pregnancy, further studies are needed, with sample collection from preconception, pregnancy and its offspring. Thus, the objective of the study is to evaluate whether the practice of voluntary physical exercise associated with chronic consumption of fructose from the pre-pregnancy period and/or until the pregnancy period causes biochemical and genotoxic alterations in breeding females, as well as genotoxic and biochemical alterations in their offspring.

2. Materials and Methods

2.1 Animals, ethical principles and experimental design

The animals were obtained from the Animal Center of the University of Southern Santa Catarina (Unesc, Brazil) and housed in polypropylene boxes and maintained under a 12 h light/dark cycle with access to plain water or water containing fructose (20%/L) in a temperature-controlled (23 ± 1°C) room. This study was approved by the Ethics Committee of the University of Southern Santa Catarina (approval No. 023/2019-1) and was performed in accordance with the ethical principles of the Brazilian College of Animal Experimentation (COBEA). The experiments followed the guidelines of ARRIVE (Animal Research: Reporting of In Vivo Experiments). Moreover, every effort was made to minimize animal suffering and to reduce the number of animals used in the experiments. The standard feed of the animals was supplied from the Puro Lab 22PB laboratory, which corresponds to 2.93kcal/g, 20%, 10% and 70% of the calories supplied with protein, fat and carbohydrates, respectively

Seventy Swiss female mice with 21 days of life were used, which received fructose (20%/L) in the hydration bottle and/or practiced voluntary physical exercise (VPE) for 8 weeks of treatment (pre-pregnancy). Twenty-one male 60-day-old Swiss mice were used for copulation, 1 male placed every 5 females (the males were kept with the females for only 7 days, the period intended for copulation) in order to obtain offspring (males and females).

Females were initially divided into 4 treatment groups (G) starting pre-pregnancy: G1- Water: females that received water and did not practice voluntary physical exercise (n=10); G2 – Water + VPE: females that received water and practiced voluntary physical exercise (n=20); G3 –FRU: females that received 20% fructose/L (n=10); G4 –FRU + VPE: females that received 20% fructose/L and practiced voluntary physical exercise (n=30) (Figure 1). Blood samples from the mothers were collected pre-pregnancy (week 8), during pregnancy (week 12) and post-pregnancy (week 15) via tail venipuncture in heparin tubes.

Subsequently they were subdivided into 7 experimental groups for the period of copulation, pregnancy and lactation: G1 - Water: females that received water and did not practice voluntary physical exercise until the end of pregnancy (n=10); G2a – Water + VPE: females that received water and practiced voluntary physical exercise until pregnancy (n=10); G2b – Water + VPE/Water: females that received water and practiced voluntary physical exercise until copulation and then stopped the VPE and continued only with water until the pregnancy period (n=10); G3 – FRU: females that received 20% fructose/L (n=10); G4a – FRU + VPW: females that received 20% fructose/L and practiced voluntary physical exercise until pregnancy (n=10); G4b –FRU + VPE/Water + VPE: females that received 20% fructose/L and practiced voluntary physical exercise until copulation, after this period, they stopped fructose consumption and continued receiving only water and practicing voluntary physical exercise during pregnancy (n=10); G4c –FRU + VPE/Water: females that received 20% fructose/L and practiced voluntary physical exercise until copulation, after stopped fructose consumption and voluntary physical exercise and received only water during pregnancy (n=10) (Figure 1).

After the lactation period, the offspring (males and females, separated by sex) were separated into the same 7 offspring groups (OG) in correspondence to the treatments performed on the mother females. The mothers were euthanized and tissues were collected for further analysis. The offspring receive standard show and water until 60 days of age, at which point they were sacrificed and tissues were collected for genotoxic and biochemical tests (Figure 1).

2.2 Treatments

2.2.1 Fructose

Mothers received drinking water or fructose at a concentration of 20%/L ad libitum in hydration bottles during pre-pregnancy (8 weeks of treatment), copulation (7 days), pregnancy (21 days) and lactation (21 days) periods. These dosages and treatment time were chosen according to data previously described in the literature (Mamikutty et al., 2014; Magenis et al., 2020). The route of administration and the period of treatment were chosen in order to maintain the relevance of the applicability of human studies. Fructose

(Sigma Aldrich) was dissolved in drinking water and prepared three times a week at a concentration of 20%/L (Magenis et al., 2020).

2.2.2 Voluntary Physical Exercise (VPE)

Voluntary physical exercise was performed using a voluntary running wheel placed inside the cages during the treatment time (varying according to each group), thus allowing free access by the animals. The exercise mileage of the animals was controlled through an infrared equipment placed next to the wheel and was divided by the animals present in the cage (5 animals) (Muller et al., 2011). This analysis was performed in the pre-pregnancy period (8th week) and during pregnancy (12th week). The females during the pre-pregnancy period ran an average of 3143 meters/day and in the gestation period 5649m/day, and this amount traveled was an estimate per animal.

2.3 Body composition and food consumption of animals

The mothers were weighed throughout the experiment according to their periods (pre-pregnancy at week 8, at the end of pregnancy at week 12 and post-pregnancy at week 15) and the offspring (males and females) at ages (7, 14, 21, 30 and 60 days of age). Food consumption (food and fructose solution) was calculated weekly by weighing the total amount of food (g) and liquids (mL) given to the animals and subtracting the food (g) and liquids (mL) from the remainder in the cage and hydration bottle throughout the experiment (Diniz et al., 2004; Diniz et al., 2005). From this, the daily calories ingested on the day were calculated, since the chow provides 3.36 kcal/g and the fructose solution 4 kcal/mL (Kending et al., 2014).

To assess the adiposity index of the animals, epididymal, mesenteric, perirenal and retroperitoneal adipose tissue were extracted to calculate the adiposity index (gram of fat/gram body weight x 100). This evaluation was performed after euthanasia of the offspring (males and females) at 60 days of age.

2.4 Biochemical analyses

2.4.1 High Performance Liquid Chromatography (HPLC)

The quantification of serum fructose levels was determined by High Performance Liquid Chromatography (HPLC) (Rahman et al., 2008) and was carried out in serum samples from pre-pregnancy (week 8) and pregnant mothers (week 12) and their offspring (males and females) at 7 and 60 days of life. Serum samples were obtained by centrifugation. The chromatographic system was a Shimadzu Prominence HPLC system, equipped with an LC-20AT pump coupled to a SIL-20AHT autosampler, a CTO-20A thermostat and a SPD-20A UV-Vis detector, in addition to the LC Solution software (Shimadzu, Kyoto, Japan). Chromatographic separations were performed on an Ascentis® C18 chromatographic column, reference code 581305 (250 mm × 2.1 mm, 5 µm; Supelco®, Bellefonte, PA, USA) and the column temperature was 30°C. The injection volume of the samples was 20 µL. The mobile phase was eluted in isocratic profile and consisted of HPLC grade acetonitrile and ultra-pure water (Milli-Q) (55:45, v/v). The mobile phase flow rate was 0.4 mL/min and detection was performed at a wavelength of 195 nm. The identification of fructose in the samples was by comparing the retention time in the standard solution. The results were quantified by means of a calibration curve (r^2 [?]0.99) prepared with 1.25, 2.5, 5, 10 and 20 mg/mL of fructose standards and the respective peak areas.

2.4.2 Fasting glucose and insulin tolerance test (ITT)

To assess fasting blood glucose, blood was collected through a small incision at the tip of the tail of the animals, after fasting period of 6 hours (Ayala et al., 2010), and blood glucose concentration was measured using a glucometer for determination of fasting blood glucose (mother females and their offspring).

The ITTs were only performed for the mothers pre- and post-pregnancy (weeks 8 and 12), since this test cannot be performed during pregnancy due to insulin administration. In the offspring (males and females), this test was performed when they completed 60 days of life. After that, all animals received a dose of 2U/kg of intraperitoneal insulin and subsequent blood glucose measurements at times 5, 10, 15, 20, 25 and 30min post-insulin injection were performed (adapted from Wang and Liao, 2012). The animals that presented

glucose values below 30mg/dL during the test were kept warm and received intraperitonially glucose. For demonstration and data analysis, they were presented as absolute glucose data (mg/dL), as area under the ITT curve calculated by dividing glucose data (mg/dL) by the time post-insulin injection (0 to 30min).

2.5 Assessing genome damage

2.5.1. Comet assay to assess DNA strand breaks

The comet assay was performed under alkaline conditions as described by Collins et al. (2023). Blood was collected using a tip and a micropipette and placed in heparinized and refrigerated microtubes, while liver samples were dissected and immersed in refrigerated phosphate buffer (PBS). Liver samples of about 3x3mm were individually homogenized with the aid of a syringe, through the back and forth movement, in order to obtain a cell suspension.

Blood cells (5 µL whole blood aliquots) and dissociated liver cell suspensions (10 µL aliquots) were embedded in low melting point agarose (0.75%, w/v, 115 µL or 110 µL, respectively). The mixture was added to a microscope slide pre-coated with 1.5% normal melting point agarose, covered with a coverslip and then placed in the refrigerator for approximately 5 minutes at 4°C for solidification. Soon after, the coverslips were carefully removed and the slides immersed in lysis buffer (2.5M NaCl, 100mM EDTA and 10mM Tris, pH 10.0-10.5, with the addition of 1% Triton X - 100 and 10% DMSO) at 4ºC for a minimum of 12 hours and a maximum of 48 hours.

The slides were incubated in alkaline solution (300mM NaOH and 1mM EDTA, pH>13) for 20 minutes for DNA unfolding, followed by electrophoresis at ˜ 1V/cm for approximately 20 minutes. All these steps were performed under weak yellow indirect light. Afterwards, the slides were neutralized with 0.4M Tris (pH 7.5) and, at the end, the DNA was stained by Syber Gold (1:10.000) (Invitrogen, USA) for further analysis.

Evaluation of 100 cells per individual and per tissue (50 cells in each duplicated slide) was performed. Resulting comet shapes were visually evaluated, being classified into five classes, according to the shape and size of the comet tail, with the classification for absence of tail considered 0, up to 4 when almost all DNA has moved to the tail, leaving a tiny comet head (Collins et al., 1997). In this way, we have a Damage Index for each animal ranging from zero (100 X 0 = 0; 100 cells observed completely without damage) to 400 (100 X 4 = 400; 100 cells observed with maximum damage).

International guidelines and recommendations for the comet assay consider the visual scoring of 100 comets to be a well-validated assessment method. It has a high correlation with computer image analysis (Collins et al., 1997). Negative and positive controls in in whole peripheral blood cells from healthy individuals were used for each assay to ensure the reliability of the procedure. Damage index of negative control = 13.00 ± 4.6 and Damage index of positive control: 105.30 ± 22.7). All slides were coded for blind analysis. The aspects of the comet assay procedures were reported following the guidelines of MIRCA (Minimum Information for Reporting on the Comet Assay) (Moller et al., 2020).

2.5.2 The enzyme modified comet assay to detect DNA oxidation

An enzyme-modified comet assay was performed as described by Collins et al. (2023), only with peripheral blood of the mothers. For the measurement of oxidized purines, there is an additional step included after the lysis: the slides were washed three times with ice-cold buffer (0.1 M KCl, 0.5 mm Na2 EDTA, 40 mm HEPES, and 0.2 mg/ml bovine serum albumin; pH 8.0) and incubated for 40 min at 37°C with formamidopyrimidine DNA glycosylase (FPG) (Unisciencer, 1:1000) or buffer. Further steps were performed as mentioned for the comet assay under alkaline conditions (Collins et al., 2023). Net FPG sensitive sites were calculate as the difference between the samples incubated with FPG and the samples incubated with buffer; Damage Index of $sample_x$/FPG – Damage Index of $sample_x$/buffer.

2.5.3 Micronucleus (MN) test to assess chromosome damage

This test was performed according to the guidelines of the GeneTox program of the US Environmental Protection Agency (Mavournin et al., 1990; Krishna e Hayashi, 2000) and following the new OECD test

guideline (TG) 474 for the in vivo mammalian erythrocyte micronucleus (MN) test (29 July 2016).

Bone marrow was extracted from animal femurs, and a smear was deposited directly onto a microscope slide using a drop of fetal calf serum. The slides were subjected to Giemsa staining (5%) then air-dried and coded for blinded analysis. To avoid false negative results and as a toxicity measure, the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) was determined for 500 erythrocytes per animal. The presence of the MN was determined in 4000 erythrocytes per animal (2000 from each of the two slides) by bright field microscopy (1000× magnification with immersion oil). For individual animals, mean values of MnPCE were used as the experimental units, and variation was based on differences among animals within the same group.

2.6 Histological analysis

The liver of the offspring (males and females) were sectioned, immediately immersed in 4% paraformaldehyde (PFA) fixative solution and buffered for 48 hours for subsequent histological processing. The material was embedded in paraffin and cut with a microtome, obtaining 4 μm thick sections. Slides were stained with hematoxylin & eosin (H&E) staining for image acquisition and histopathological analysis of liver histoarchitecture.

To analyze protein expression in an automated way, the technique of color morphometry was used, which was adapted to obtain the intensity and destruction of nuclear expression of the protein. Therefore, the H&E stained slides were submitted to the Axio Scan.Z1®(ZEISS, Jena, Germany) to capture images of all sample areas. After pre-processing these images with Adobe Photoshop CS6 v 13.0 ® (Adobe, San Jose, CA), in order to improve quality and separate images, they were analyzed with the Image Pro Plus v.4.5.0.29 (Media Cybernetics, Rockville, MD) software using the color morphometry tool. Initially, the software analyzed the total distribution of the white steatosis area in the tissue. With the help of the software, the steatosis area was marked in red. This method of marking a particular structure based on its color was called "mask".

The software automatically provided the marked areas, quantified in square micrometers, through the following formula: (value of the white steatosis area/value of the image field area). Subsequently, the results were transported to an excel spreadsheet and quantified.

2.7 Statistical analysis

Data are expressed as mean and standard deviation of the mean (mean ± SD). For statistical analysis, the normality of the variables was analyzed using the Bartlett test. For non-parametric variables, Kruskal-Wallis was used followed by Dunn's post hoc. For parametric variables, one-way variance (ANOVA) by Tukey's post hoc was used, and for specific comparisons between groups, the student t-test was used.

For the evaluation of the mothers, food consumption and body weight, Kruskal-Wallis followed by Dunn's post hoc were used. Fasting blood glucose, insulin tolerance test, and the comet assay were analyzed by one-way ANOVA with Tukey's post hoc. Differences in the fructose serum levels and micronucleus test were tested with the student t-test.

For the evaluation of the offspring (males and females), food consumption, fructose serum levels, fasting glucose, insulin tolerance test, adiposity index and comet assay, ANOVA with Tukey's post hoc was used. Micronucleus test were analyzed with the student t-test. Body weight and histological parameters were tested by Kruskal-Wallis followed by Dunn's post hoc. Differences in micronuclei was tested by student t-test.

Differences between groups were considered significant when P values were less than 0.05 ($p<0.05$), using the GraphPad Prism 5.0 program.

3 Results

3.1 Characteristics of the mothers

3.1.1 Food consumption, body weight and fructose uptake

During the treatment period, the amounts of liquids, animal feed and daily calories ingested by the mothers were measured, in addition to the monitoring of body weight.

Table 1 shows the average of these parameters by group during the three periods.

In the pre-pregnancy period, an increase in liquid consumption was observed in the water + VPE group and in the FRU group in relation to the water group ($p<0.05$). Concerning food consumption, only the water + VPE group demonstrated an increase in relation to the water group ($p<0.05$). However, daily caloric consumption was increased in the FRU group compared to the water group (Table 1 and Figure 2). Regarding body weight, no statistically significant difference was observed among the groups (Table 1).

During pregnancy, liquid consumption increased in the FRU group compared to the water group, and the groups FRU + VPE/Water + VPE and FRU+VPE/Water consumed less water compared to the FRU group ($p<0.05$). Food consumption was only decreased in the FRU + VPE/Water group in comparison to the FRU + VPE/Water + VPE group ($p<0.05$). Regarding caloric consumption, an increase was observed in the FRU group compared to the water group ($p<0.05$) (Table 1 and Figure 2). Regarding body weight, there was no statistically significant difference among the groups (Table 1).

In the post-pregnancy period, there was no statistically significant difference between the groups in the consumption of liquids, feed and calories. Apart from the caloric consumption of the FRU group, which was increased compared to the water group ($p<0.05$) (Table 1 and Figure 2). In terms of body weight, it was observed that the groups FRU + VPE/Water + VPE and FRU+VPE/Water had a decreased weight compared to the FRU group ($p<0.05$) (Table 1).

The average consumption of daily calories consumed via the liquids and feed in the pre-pregnancy, pregnancy and post-pregnancy period, is presented in figure 2.

In addition to the consumed liquid and food containing fructose (Table 1), the concentration of fructose in the serum of the mothers was quantified in the pre-pregnancy and pregnancy period through HPLC. In the pre-pregnancy period, it was observed that the FRU group had higher serum fructose concentrations compared to the water group ($p<0.05$) and the FRU+VPE group showed decreased levels compared to the FRU group ($p<0.05$) (figure 3). The same happened in the pregnancy period, where the FRU group showed increased the serum concentrations of fructose in comparison to the water group, while the interventions FRU+VPE and FRU+VPE/Water + VPE reduced this concentration compared to the FRU group ($p<0$, 05) (figure 3). These evaluations were not performed in the post-pregnancy period due to sampling problems.

3.1.2 Effect on blood glucose and insulin sensitivity

During the treatment period, the glycemic profile was evaluated through fasting glucose (Table 2) and the insulin tolerance test (Figure 4).

Fasting blood glucose levels pre-pregnancy were increased in the FRU group compared to the water group, whereas the FRU+VPE group showed a decrease in glucose levels compared to the FRU group ($p<0$ .05). During pregnancy, blood glucose increased in the FRU group in comparison to the water group, while FRU+VPE/Water + VPE and FRU+VPE/Water groups showed decreased glucose levels compared to the FRU group ($p<0.05$). Regarding the post-pregnancy period, the water + VPE group showed higher glucose levels in comparison to the water group, whereas the interventions FRU+VPE/Water+VPE and FRU+VPE/Water increased these levels in relation to the FRU+VPE group ($p<0.05$) (Table 2).

In the pre-pregnancy period, an increase in AUC in the FRU group was observed compared to the water group and a decrease in the FRU+VPE group compared to the FRU group ($p<0.05$), indicating that at the time of mating the females were resistant the insulin. However, in the post-pregnancy period, there was no statistically significant difference between the groups, since the glycemic curve of the animals was similar (Figure 4).

3.1.3 Effect on DNA strand breaks and DNA oxidation

In the pre-pregnancy period, in both strand breaks and oxidized purine levels increased in the FRU group compared to the water group (Figure 5A; $p<0.05$). A decrease in oxidized DNA damage was observed in the FRU+VPE treated group compared to the FRU group ($p<0.05$).

The same happened in the pregnancy period, an increase in strand breaks and oxidized DNA damage was observed in the FRU group compared to the water group (Figure 5B; $p<0.05$). Regarding the interventions, it was observed that there was a decrease in strand breaks and oxidized DNA in the FRU+VPE, FRU+VPE/Water + VPE and FRU+VPE/Water group in comparison to the FRU group ($p <0.05$).

In the post-pregnancy period, we observed an increase in strand breaks and oxidized DNA damage in the FRU group compared to the water group (Figure 5C; $p<0.05$). In addition, a decrease in strand breaks was observed in the FRU+VPE, FRU+VPE/Water + VPE and FRU+VPE/Water groups compared to the FRU group ($p<0.05$).

In the liver (post-pregnancy period), an increase in DNA damage (detected as strand breaks) was observed in the FRU group in comparison to the water group and a reduction in DNA damage was observed in the FRU+VPE/Water group compared to the FRU group (Figure 6 ; $p<0.05$).

3.1.4 Levels of chromosome damage

To evaluate whether the consumption of fructose and the practice of voluntary physical exercise causes additional genotoxic effects in the mothers in the post-pregnancy period, the micronucleus test was performed on bone marrow cells (Table 3).

Significantly higher levels of MnPCE were observed in mothers treated with FRU in comparison to the water group ($p<0.05$). In addition, a decrease in the number of MnPCE was observed in the FRU+VPE, FRU+VPE/Water + VPE and FRU+VPE/Water group compared to the FRU group ($p<0.05$). Regarding the PCE/NCE ratio, no statistically significant differences were observed between the groups, demonstrating that the production of erythrocytes was occurring normally in the bone marrow, with no evidences of cytotoxicity.

3.2 The effect of maternal fructose consumption and exercise on the offspring

3.2.1 Fructose uptake by the offspring and food consumption, body weight

Knowing that there was a consumption of fructose by the matrices females (Table 1), we sought to quantify its concentration in the serum of the offspring at 7 days of life (lactation period) and at 60 days (adult life) through HPLC (Figure 7).

Regarding the offspring with 7 days of life, we observed in both (males and females) that there was an increase in fructose concentration in the FRU group in relation to the water group and a decrease in fructose concentration in the FRU+VPE, FRU+VPE /Water+VPE and FRU+VPE/Water groups in relation to the FRU group ($p<0.05$). In the 60-day-old offspring (males and females), we did not find statistically significant differences. However, we observed that there is an increase in the concentration of serum fructose in the offspring during the period of exposure via mother.

During the life follow-up period, the amount of animal food and daily calories ingested by the offspring were measured (Table 4). In addition, body weight was monitored during 7, 14, 21, 30 and 60 days of life, these results are shown in table 5.

Regarding food consumption, it is observed that both offspring (males and females) had higher consumption of chow in the water + VPE group compared to the water group ($p<0.05$). Furthermore, it was observed that the FRU+VPE/Water+VPE group had a decrease in consumption compared to the FRU+VPE group ($p<0.05$). Regarding daily calories, statistical differences were observed only in the male offspring, where the FRU+VPE/Water + VPE group showed a reduction in calories compared to the FRU+VPE group ($p<0.05$).

In addition to the consumption of liquids, the weight gain was also analyzed in the animals throughout their lives (Table 5).

In the female offspring, we observed that at 7 and 14 days of age the FRU group had an increase in body weight in relation to the water group. However, at 7 days a decrease in weight in the FRU+VPE group compared to the FRU group ($p<0.05$) was observed. At 21 days of age, was observed only an increase in weight in the FRU+VPE/Water group in relation to the FRU+VPE group ($p<0.05$). At 30 days of age, there was a decrease in body weight in the FRU+VPE group in relation to the FRU group ($p<0.05$). Finally, at 60 days of life, an increase in body weight in the FRU group in relation to the water group ($p<0.05$) was also observed, whereas the FRU + VPE and FRU+VPE/Water+VPE groups showed weight reduction compared to the FRU group ($p<0.05$). In addition, an increase in body weight was observed in the FRU+VPE/Water group in relation to the FRU+VPE group ($p<0.05$). Thus, we observed in the female offspring that the offspring from the FRU group has an increase in body weight, especially in the first days of life and in adulthood, since no differences were observed throughout life. Furthermore, we observed that interventions can decrease this body weight.

In the male offspring, at 7 days of age, we observed an increase in body weight in the FRU group in relation to the water group ($p<0.05$) and a decrease in body weight in the FRU+VPE and FRU+VPE/Water in relation to the FRU group ($p<0.05$). Furthermore, an increase in body weight was found in the FRU+VPE/Water+VPE group in relation to the FRU+VPE group ($p<0.05$). At 14 and 21 days of age, an increase in body weight in the FRU group in relation to the water group ($p<0.05$) was observed. At 30 and 60 days of life, no statistically significant differences were observed. Thus, we observed in male offspring that the weight is changed until 21 days of age and then it is brought to eutrophy in relation to the other groups.

3.2.2 The effect on the offspring's body adiposity

To assess whether the FRU increases the adipose tissue of the animals and whether the VPE could reverse this situation, the body adiposity index was calculated (figure 8) by weighing the epididymal, mesenteric, retroperitoneal and perirenal fats.

According to the results obtained in the figure 8, it was possible to verify that in the female offspring there was no statistically significant difference between the groups. However, in males, an increase in body adiposity index was observed in the FRU in relation to the water group ($p<0.05$) and a decrease in the FRU+VPE in relation to the FRU group ($p<0.05$).

3.2.3 Fasting blood glucose and insulin sensitivity of the offspring

The glycemic profile was evaluated in the offspring (females and males) through fasting glucose (Table 6) and the insulin tolerance test (Figure 9).

A decrease in fasting glucose was observed in offspring females in the FRU+VPE/Water+VPE group compared to the FRU group and FRU+VPE ($p<0.05$). In males, no significant differences were observed.

In the oral insulin tolerance test (figure 9), no significant differences were found in females, showing that the offspring were not insulin resistant. In offspring males, we observed a decrease in the area under the curve in the FRU+VPE/Water+VPE group compared to the fructose group and the FRU+VPE group ($p<0.05$). However, the fructose group did not demonstrated insulin resistance.

3.2.4 Levels of hepatic steatosis in the offspring

To evaluate hepatic steatosis, histology of the liver of the offspring (female and male) was performed and the quantification was through the percentage of the steatosis area as observed in the graph and in the morphology of the liver cells, evidenced by the black arrows (figure 10).

In female offspring, was observed an increase in the area of steatosis in the FRU group in relation to the water group ($p<0.05$). The treated groups, FRU+VPE, FRU+VPE/Water+VPE and FRU+VPE/Water reduced the area of steatosis in relation to the FRU group ($p<0.05$). In addition, it was observed

that the FRU+VPE/Water+VPE group decreased in relation to the FRU+VPE group, already and the FRU+VPE/Water group increased in relation to the FRU+VPE/Water+VPE ($p<0.05$).

In male offspring, there was a decrease in the area of steatosis in the FRU group compared to the control group ($p<0.05$). The treated groups, FRU+VPE, FRU+VPE/Water+VPE and FRU+VPE/Water reduced the area of steatosis in relation to the FRU group ($p<0.05$). In the FRU+VPE/Water group there was also a reduction in the area of steatosis in relation to the FRU+VPE and FRU+VPE/Water+VPE group ($p<0.05$).

Thus, our results demonstrate that the VPE made by the mothers exposed to fructose before and during pregnancy was able to decrease the area of hepatic steatosis in the offspring (females and males).

3.2.5 Levels of DNA damage in the offspring

The alkaline comet assay was performed on peripheral tissues (blood and liver) in the offspring (females and males) using the damage index parameter (0-400) (figure 11).

In the evaluation of the female offspring, in the blood (figure 11), our results demonstrated an increase in DNA damage index in the FRU group in relation to the water group ($p<0.05$). In addition, was observed a decrease in DNA damage in the FRU+ VPE, FRU+VPE/Water+VPE and FRU+VPE/Water, in relation to the FRU group ($p<0.05$). No significant differences were found in the liver.

In the male offspring was found an increase in DNA damage in the water+VPE and FRU group in relation to the water group ($p<0.05$) in the blood. A decrease in DNA damage in the FRU+VPE, FRU+VPE/Water+VPE and FRU+VPE/Water groups compared to the FRU group ($p<0.05$) was observed. Regarding the liver, an increase in DNA damage was observed in the FRU group when compared to the water group and a decrease in the damage index in FRU+VPE, FRU+VPE/Water+VPE and FRU+VPE/Water in relation to the FRU group ($p<0.05$).

3.2.6 Levels of chromosome damage in the offspring

Table 7 presents the results of the micronucleus test in the female and male offspring, evaluating whether the consumption of fructose and the practice of voluntary physical exercise in the mothers causes mutagenic effects in the bone marrow cells of the offspring.

Significantly higher levels of MnPCE were observed in the female and male offspring from mothers treated with FRU in comparison to the water group ($p<0.05$). Regarding the PCE/NCE ratio, no statistically significant differences were observed between the groups, demonstrating that the production of erythrocytes was occurring normally in the bone marrow, with no evidence of cytotoxicity.

4. Discussion

Fetal programming during pregnancy can generate a healthy or poor environment for the offspring (Deodati et al., 2020). Maternal obesity and gestational diabetes, for example, have been shown to provoke long-term changes in the mechanisms that regulate energy balance in offspring. The increase in childhood obesity and diabetes in children is often attributed to an increase in calorie-dense diets, reduced levels of physical activity and exercise frequency by the mother. However, it is believed that early dietary changes, both in mothers and children, significantly contribute to alleviate or assist in the treatment of these pathologies (Frias and Grove, 2012). In this context, the current study evaluated the effects of maternal chronic consumption of fructose (20%) during pregnancy and early-life on the genome and metabolism of the mothers and their offspring, and assessed the modulating effect of interventions with voluntary physical exercise.

4.1 Maternal fructose consumption increases their own and their offsprings' caloric intake, while exercise reduces it

In our study, the fructose serum concentration of the mothers in the groups treated with fructose was higher compared to those consuming water, confirming proper uptake of the fructose by the body, and a decrease was observed in the groups doing voluntary physical exercise. The higher exposure to fructose

before and during pregnancy increased the liquid consumption of the mothers, and that intervention with voluntary physical exercise was able to reduce this 20%/L fructose liquid consumption, especially in the groups FRU+VPE/Water+VPE and FRU+VPE/Water. Food consumption seemed to be more associated with doing exercise, as only slight non-significant increases were observed for the fructose only group (G3) compared to the water only group (G1). However, caloric intake did increase significantly in the fructose consuming groups during all the study periods.

Corroborating with our results, Magenis et al. (2020) observed that fructose consumption at 10 and 20%/L in mice can lead to an increase in food and caloric intake in female mice during pregnancy, as well as, increase in food consumption in offspring of mothers that were exposed to fructose. Clayton et al. (2015) suggest that maternal fructose (10%) consumption may alter maternal responsiveness to leptin and lead to impaired signaling in the fetus, thus altering food consumption. The imbalance of this hormone can affect appetite regulation and lead to insufficient satiety, increasing food intake (Kao et al., 2021). It is further suggested that fructose consumption may be a significant factor in the obesity epidemic since human studies using fructose concentrations ranging from 7.5% to 25% of caloric intake have demonstrated that its long-term consumption may be associated with the development of hyperlipidemia and insulin resistance (Vos et al., 2008; Stanhope et al., 2015).

Physical exercise can bring benefits in food control as observed in this study. Platt et al. (2015) observed that the physical exercise of the voluntary wheel did not change the caloric consumption of pregnant females, as in our study. Hsu et al. (2021) evaluated the effects of aquatic exercise in mice fed with a high-fructose diet and observed that fructose increases food consumption and that physical exercise decreases this consumption when associated with fructose.

In the offspring (male and female), an increase in serum fructose concentration was observed during the lactation, showing that maternal fructose exposure passes via the placental barrier and that exposure continues via breast milk to offspring. However, upon reaching adulthood and without maternal exposure, levels returned to normal. These results prove that fructose was taken up by the mothers, and that the offspring were exposed to fructose via the mothers during pregnancy and lactation. When fructose was consumed in combination with voluntary physical exercise, these levels decreased. As suggested by Egli et al. (2016) exercise performed immediately after fructose ingestion increases fructose oxidation and suppresses fructose and glycogen storage.

Indeed, we observed that food consumption, and caloric intake (especially for the males), was lower in the FRU+VPE/Water+VPE intervention compared to the FRU group, showing that stopping fructose and continuing to practice physical exercise during pregnancy has better regulation of food consumption in the offspring. Interestingly, the uterine environment may influence taste preferences and healthy eating in offspring. The flavors of the maternal diet are found in the amniotic fluid and consequently ingested by the fetus, thus flavors experienced in the intrauterine period determine a preference that persists into childhood or even into adulthood, along with possible complications such as type 2 diabetes mellitus and obesity (Trout and Effinger, 2012). Koo et al. (2021) have observed the negative effects of maternal fructose intake during pregnancy and lactation on their offspring, leading to the appearance of metabolic syndrome, suggesting that nutritional status and food intake during pregnancy and lactation may affect fetal programming (Koo et al., 2021).

4.2 The effects on body composition in the mothers and their offspring

As changes were found in food consumption, as well as in levels of exposure with physical exercise, we sought to understand body weight and body composition in mothers and their offspring. Regardless the increase in maternal caloric intake, no significant increases in maternal weight was observed. In the post-pregnancy period a decrease was observed, which may be due to the postpartum period. Indeed in our previous study by Magenis et al. (2020), this weight drop was observed in the lactation period, as expected due to the physiological demand postpartum with the birth of offspring. However, the weight of the fructose group remained high post-pregnancy. Interestingly, the exercise interventions (G4a, G4b and G4c) reduced

maternal weight (especially the "FRU+VPE/Water+VPE" and "FRU+VPE/Water" groups compared to the FRU group) and brought it down to similar weights as in the water treatment groups.

The weight of the offspring was followed up from 7 days after birth until adulthood at 60 days. In female offspring, the maternal fructose exposure lead to increased body weight in early life (significant at 7 and 14 days of age) and in adulthood (60 days of age). While maternal exercise was able to decrease and modulate the offsprings' body weight, especially in adulthood. Although, the adiposity index of the female offspring showed an increase for the fructose group, this difference was not statistically significant compared to the water group. In the male offspring an increase in body weight was mainly observed early in life (7-14 days of age) and the maternal exercise only showed a significant reduction in body weight at 7 days of age. Regardless, maternal fructose consumption led to an increase in the male offspring's adiposity index (OG3 = FRU group) and maternal exercise significantly decreased adiposity in the FRU+VPE group (OG4a) at 60 days of life.

It is known that fructose can increase body weight in offspring, as observed in our previous study by Magenis et al. (2022), where the offspring were followed up to 30 days of age and an increase in body weight was observed. Furthermore, it is suggested, as observed in our current study, that physical exercise can reduce intrahepatic lipids and abdominal fat with or without weight loss (Thyfault and Rector, 2020). It is believed that physical exercise is able to increase adipogenesis in brown adipose tissue, favoring mitochondrial biogenesis with an increase in PGC-1alpha and UCP1 in male and female offspring of female mice that performed exercise during pregnancy (Son et al. 2020). Overall, our current results indicate that the maternal fructose consumption can change the weight and adiposity of the offspring throughout life, and that maternal voluntary physical exercise is able to reduce these adverse effects.

4.3 The effect on disease risk markers

The alarming increase in metabolic problems, such as non-alcoholic fatty liver disease (NASH), metabolic syndrome and type 2 diabetes mellitus are worrying and studying the effects of life-style interventions, such as physical exercise, is of great importance for their prevention and treatment (Muriel et al., 2021). In view of this, the glycemic profile of mothers and offspring and liver steatosis in the offspring were evaluated in the current study.

4.3.1 The effects on insulin resistance in mothers and their offspring

Factors associated with insulin resistance include obesity, particularly abdominal obesity, increased waist circumference, familial history of type 2 diabetes, sedentary lifestyle, hypertension, and fatty liver. Furthermore, is insulin resistance also characterized by hypertriglyceridemia, lower concentrations of high-density lipoprotein (HDL) cholesterol in blood, and increased inflammation. As a result, glucose metabolism deteriorates, resulting in hyperglycemia, impaired glucose tolerance or overt type 2 diabetes mellitus. Weight loss and regular moderate intensity physical activity/exercise are significant factors for insulin resistance prevention and/or treatment (Papakonstantinou et al., 2022). In our work, we used voluntary physical exercise as an intervention and it was observed to improve the glycemic profile in the mothers and their offspring.

The glycemic profile in mothers was altered with FRU and the VPE had the potential to modulate this effect, in different periods. Regarding fasting glycemia and insulin tolerance test, in pre-pregnancy mothers we observed an increase in fasting glycemia and insulin resistance in the FRU group, while in combination with modulation the exercise intervention (FRU+VPE group) there was a decrease in fasting glycemia and modulation of insulin resistance. During pregnancy, we observed a similar change with a decrease in fasting glucose in response to the physical activity interventions (FRU+VPE/Water+VPE and FRU+VPE/Water groups). In contrast, in the post-pregnancy period, an increase in fasting glucose was observed in the Water+VPE group, however we did not observe insulin resistance in any group during this period.

In the female offspring, we did not observe changes in fasting blood glucose in the fructose group, but a possible modulation in the FRU+VPE/Water+VPE group, due to the decrease in blood glucose. However, in males no differences were found, only a decrease in the area under the curve in the insulin tolerance test.

With these results, we observed that fructose exposure can cause a glycemic imbalance in the mothers and that physical exercise may have the potential to modulate, but not enough to be a modulator of insulin resistance in offspring.

Similar to our result, Mamikutty et al. (2014) observed that fructose consumption at doses of 20% and 25% for a period of 8 weeks was able to alter food consumption and lead to metabolic disorders such as obesity, dyslipidemia, hypertension and hyperglycemia. This change in the glycemic profile and insulin resistance is due to the fact that, unlike glucose, fructose does not stimulate insulin secretion from pancreatic β cells. Furthermore, reduced insulin sensitivity in the state of hypertriglyceridemia can lead to the formation of hyperglycemia (Bray et al., 2004). Furthermore, excess fructose can increase the production of its triosis phosphate metabolites and enter the Krebs cycle for intrahepatic oxidation or synthesis of glucose, lactate and/or lipids (Horst and Serlie, 2017). The change in the gestational period, on the other hand, corroborates the results found in the study by Magenis et al. 2020 and 2022, where the consumption of fructose during the gestational period in mice was able to increase the fasting glucose of the females and offspring. However, we did not observe insulin resistance in the post-pregnancy period and in the offspring, and the same was found in previous studies (Magenis et al., 2020; Magenis et al., 2022).

Pregnancy is characterized by myriad metabolic adaptations that affect glucose levels and may also alter the effects of exercise on glycemic control. Glucose is the primary source of fetal energy and is required for optimal fetal growth and development. Accordingly, hormonal changes during pregnancy and in the placenta drive preferential feto-placental glucose delivery, since maternal glucose production increases and maternal insulin sensitivity is reduced as pregnancy progresses, particularly at the level of skeletal muscle, in order to shift glucose to the developing fetus (Motola and Artal, 2016; Barbour et al. 2007). We believe that the mechanisms of fructose metabolic disorders are more associated with an increase in free fatty acid, so there is an increase in lipotoxicity and, consequently, oxidative stress and inflammation, often not causing type 2 diabetes mellitus initially, only a glycemic imbalance (Lipke et al. al., 2022).

Based on this assumption, physical exercise is necessary as an adjuvant for the prevention and treatment of obesity and associated comorbidities. To date, studies show that resistance and aerobic exercises are recommended as effective treatments for people with obesity and type 2 diabetes mellitus (Yang et al., 2014; Xiao and Fu, 2015; Villareal et al., 2017; García-Hermoso et al., 2018). Furthermore, the ACOG recommends the practice of physical exercise during the pre- and gestational period in order to prevent gestational diabetes (ACOG, 2018). Among healthy pregnant women, regular exercise during pregnancy reduces insulin resistance and upregulates skeletal muscle glucose transporter GLUT4, (Davenport et al., 2018). Although our glycemic results are not conclusive regarding insulin resistance, we suggest that physical exercise has the potential to control the mother's fasting glucose and can bring benefits to her offspring, as described in the literature.

4.3.2 The risk of fatty liver disease in the offspring

It has been previously shown that excessive fructose consumption leads to dysfunctions in various tissues and organs, including liver, adipose tissue, pancreatic, skeletal muscle, kidney, heart, brain, and intestine (Muriel et al.,2021). The primary metabolites of fructose metabolism are produced in the liver and secreted into the circulation, directly affecting tissue and organ functions (Zhang et al., 2019). In humans, 70% of fructose is metabolized in the liver, so a high fructose diet induces hepatic - fatty acid synthesis and triglyceride accumulation (Muriel et al., 2021). Since uncontrolled lipid metabolism is associate with the development of non-alcoholic fatty liver disease (NASH) hepatic steatosis was studied in the offspring. It is a term widely used to describe excessive fatty infiltration in the liver in the absence of alcohol, autoimmune disorders, or viral hepatitis; it is attributed to obesity, high sugar and fat consumption, and sedentarism (Horst and Serlie, 2017). The current study observed that maternal fructose consumption during pregnancy leads to an increased area of steatosis in the livers of male and female offspring, compared to the water groups.

Furthermore, it is suggested that physical exercise is capable of decreasing intrahepatic lipids and the formation of lipid droplets, decreasing free fatty acids and the effects of lipotoxicity, since increased levels of free fatty acids can be toxic to the body cells, and their sequestration by lipid droplets provides a buffering

capacity that prevents lipotoxicity (Olzmann & Carvalho, 2019). Indeed in our current study, the maternal physical exercise interventions were able to reduce hepatic steatosis, demonstrating that the practice of voluntary physical exercise by the mothers has a hepatic protective effect in the offspring.

4.5 Fructose consumption enhances genome damage and VPE can prevent this

The metabolization of fructose results in liver glycogen and de novo lipogenesis of free fatty acids (FFA) that can cause lipotoxicity (Zhang et al., 2019), which is defined as the deleterious effect caused by high concentrations of lipids and lipid derivatives that manifest as a set of metabolic disorders in non-adipose tissue cells. The mechanisms involved in lipotoxicity (also known as lipotoxic effects) include oxidative stress, endoplasmic reticulum (ER) stress, and induction of inflammation (Lipke et al., 2022). Since lipid peroxidation and oxidative stress can cause damage to the genome, the genotoxic and mutagenic effects of the fructose consumption was evaluated in the blood and liver of the mothers and their offspring through the comet assay and micronucleus test.

In our study, the exposure to fructose led to increased DNA damage in mothers and offspring and the intervention with physical exercise was able to modulate and reduce the detected genome damage. In the mothers increased DNA oxidation and DNA strand-breaks (considered as markers of short-term exposures) were observed in both the blood and liver in response to fructose consumption (during all the (pre/post)pregnancy periods), as well as an increased number of MN (marker of long-term exposures). Maternal fructose consumption led to increased DNA damage (markers of short-term damage) in the blood of the offspring, while DNA damage levels in the liver were only significantly increased in male offspring due to fructose. The number of MN (marker of long-term exposures) was increased in both female and male offspring from mothers who consumed fructose. The VPE intervention led to the reduced genotoxicity of fructose in mothers and offspring. Overall these results indicate that the consumption of high concentrations of fructose in a fetal programming model was genotoxic in the mother and their offspring and that physical exercise was capable to protect genomic stability. The genotoxic results found in liver in the offspring corroborate the results found in histology, where we observed an increase in the area of steatosis in the FRU group and a decrease in FRU+VPE.

Similar to these results, our previous studies also showed that fructose leads to genomic instability in the mothers and their offspring (Magenis et al., 2020; Magenis et al., 2022). The fructose can lead to decreased expression of hepatic PGC-1alpha, a regulator of mitochondrial biogenesis in fructose-fed rats, due to increased ROS and DNA damage (Cioffi et al., 2017).

Diet rich in fructose promotes ROS imbalance through the simultaneous increase in ROS production and the negative regulation of antioxidant defense mechanisms, having as a result, widespread damage to macromolecules such as lipids, proteins and DNA (Cioffi et al., 2017). Furthermore, we suggest that maternal voluntary exercise has the ability to positively modulate the antioxidant defense system, thus decreasing the action of ROS and other reactive species that lead to DNA damage (Saiyin et al., 2019). Tryfidou et al. (2020) also showed that physical exercise can decrease inflammation, ROS and increase mitochondrial biogenesis, thus improving genomic instability. Corroborating, Kasper et al. (2021) showed that maternal physical exercise mediates hepatic metabolic programming via activation of the AMPK-PGC1α axis in the offspring of obese mothers, which lead to reduced DNA damage in liver.

Conclusion

In this study, we sought to evaluate the different combinations and durations of interventions with voluntary physical exercise during the gestational period. Although, we did not find similar combinations of interventions in the literature as in our metabolic programming model, it has been reported that combining a change in eating habits and lifestyle make the benefits more effective in healthy adults (Martinez-Avila et al., 2020; Lelis et al., 2020).

We showed that the practice of physical exercise in the control group did not lead to maternal and offspring changes, ie, it does not cause harm. Overall, we observed that maternal voluntary physical exercise has a

protective effect on genomic stability in mothers and their offspring, reversing the adverse effects observed in blood and liver in response to the chronic high consumption of maternal fructose. In addition, we provide evidence for metabolic programming caused by the various maternal treatments that have a lasting effect in the offspring until adulthood. However, the intervention with VPE was not able to reach the point of returning to the similar levels as the controls. It is possible that the effects on pregnant mice, as well as the transplacental effect of the direct passage of fructose, confer indirect and direct metabolic risk to the offspring later in life. However, more studies are needed in order to understand its long-term effects on the adult life of the offspring and also to study other metabolic pathways.

To conclude, our data indicate that, regardless the period during which the exercise was performed (pre-, post- or during pregnancy), the practice of exercise during pregnancy becomes essential to obtain the observed metabolic and genomic benefits for the mother and offspring, and that when combined with changing to water, the benefits are even better.

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Histological analyzes were performed using the Axio Scan.Z1® (ZEISS, Jena, Germany) to capture images of all sample areas. After pre-processing these images with Adobe Photoshop CS6 v 13.0 ® (Adobe, San Jose, CA) in order to improve quality and separate the images, they were analyzed with Image Pro Plus v.4.5.0.29.

Benefit-Sharing Statement: A research collaboration was developed with scientists from the countries providing genetic samples, all collaborators are included as co-authors, the results of research have been shared with the provider communities and the broader scientific community (see above), and the research addresses a priority concern, in this case the conservation of organisms being studied. More broadly, our group is committed to international scientific partnerships, as well as institutional capacity building.

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Tables

Table 1. Food consumption and body weight of the mothers that received water or fructose and/or practiced voluntary physical exercise pre-pregnancy, during pregnancy and post-pregnancy.

	Treatment	**Liquid (mL/day)**	**Food (g/day)**	**Calories (kcal/day)**	**Body**
Pre-pregnancy	Week 0-8				
G1	Water	5.8 ± 1.09	4.5 ± 0.72	16.7 ± 2.55	30.8 ±
G2	Water + VPE	7.2 ± 1.26 [a]	5.6 ± 0.40[a]	19.3 ± 2.52	30.7 ±
G3	FRU	9.0 ± 3.88[a]	4.9 ± 0.42	39.6 ± 21.7[a]	39.3 ±
G4	FRU + VPE	7.3 ± 1.89	5.0 ± 0.49	36.8 ± 15.37	30.8 ±
Pregnancy	Week 9-12				
G1	Water	10.9 ± 2.22	7.4 ± 2.13	28.5 ± 7.77	40.8 ±
G2a	Water + VPE	9.28 ± 2.37	7.5 ± 0.72	25.9 ± 3.05	40.6 ±
G2b	Water + VPE/Water	10.7 ± 3.14	7.3 ± 1.04	26.7 ± 4.78	40.8 ±
G3	FRU	14.6 ± 5.8[a]	7.8 ± 0.70	57.9 ± 12.4[a]	45.4 ±
G4a	FRU + VPE	13.0 ± 5.17	7.0 ± 0.72	59.7 ± 15.4	40.6 ±
G4b	FRU + VPE/ Water + VPE	10.0 ± 2.86[b]	9.0 ± 1.11	54.4 ± 11.4	41.5 ±
G4c	FRU + VPE/Water	9.9 ± 2.96[b]	6.6 ± 0.25[c]	47.7 ± 10.0	41.3 ±
Post-pregnancy	Week 12-15				
G1	Water	16.4 ± 4.06	9.6 ± 2.80	30.1 ± 8.14	35.3 ±
G2a	Water + VPE	14.3 ± 4.11	15.8 ± 2.42	55.2 ± 8.74	34.4 ±
G2b	Water + VPE/Water	14.0 ± 3.71	12.0 ± 3.47	31.7 ± 12.0	37.8 ±
G3	FRU	18.5 ± 5.19	13.8 ± 2.06	97.0 ± 6.46[a]	48.2 ±
G4a	FRU + VPE	15.5 ± 5.50	13.1 ± 3.77	75.2 ± 28.5	35.5 ±
G4b	FRU + VPE/ Water + VPE	14.4 ± 4.25	9.7 ± 3.11	86.3 ± 16.08	33.6 ±
G4c	FRU + VPE/Water	17.2 ± 6.94	11.8 ± 3.67	86.11 ± 18.7	32.4 ±

Data are expressed as mean ± standard deviation of the mean (n=10 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a] Significant difference in relation to the water group ($p < 0.05$, Kruskal-Wallis, *post hoc*Dunn's);[b]Significant difference in relation to the fructose group ($p < 0.05$, Kruskal-Wallis, *posthoc* Dunn's); [c]Significant difference in relation to the group FRU + VPE/ Water + VPE ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);

Table 2. Fasting blood glucose (mg/dL) of mothers during the various stages, while or after having received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy.

	Treatment	**Glucose (mg/dL)**
Pre-pregnancy	Week 0-8	
G1	Water	109.0 ± 5.91
G2	Water + VPE	115.4 ± 15.7
G3	FRU	142.5 ± 18.2[a]
G4	FRU + VPE	116.7 ± 13.7[b]
Pregnancy	Week 9-12	
G1	Water	104.3 ± 9.29
G2a	Water + VPE	115.3 ± 7.89
G2b	Water + VPE/Water	106.8 ± 14.2
G3	FRU	131.0 ± 5.24[a]
G4a	FRU + VPE	110.6 ± 5.59
G4b	FRU + VPE/ Water + VPE	101.4 ± 15.4[b]
G4c	FRU + VPE/Water	92.83 ± 12.1[b]
Post-pregnancy	Week 12-15	
G1	Water	119.6 ± 1.47
G2a	Water + VPE	151.8 ± 7.29[a]
G2b	Water + VPE/Water	139.6 ± 10.0
G3	FRU	130.0 ± 9.59
G4a	FRU + VPE	107.2 ± 24.6
G4b	FRU + VPE/ Water + VPE	141.0 ± 13.9[c]
G4c	FRU + VPE/Water	140.3 ± 15.6[c]

Data are expressed as mean ± standard deviation of mean fasting blood glucose (n=6 animals per group). FRU- Fructose; VPE- voluntary physical exercise. [a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the fructose group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[c]Significant difference in relation to the FRU + VPE group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

Table 3. Number of micronucleated polychromatic erythrocytes (MnPCE) observed in bone marrow samples from mothers post-pregnancy, after they had received water or fructose and/or practiced voluntary physical exercise pre-pregnancy and/or during pregnancy.

Treatment	**MnPCE**	**PCE/NCE**
Water	0.33 ± 0.51	0.53 ± 0.02
Water + VPE	0.75 ± 0.95	0.53 ± 0.03
Water + VPE/Water	0.83 ± 0.75	0.54 ± 0.01
FRU	6.0 ± 1.15[a]	0.56 ± 0.02
FRU + VPE	3.25 ± 1.70[b]	0.52 ± 0.03
FRU + VPE/ Water + VPE	3.60 ± 1.67[b]	0.53 ± 0.02
FRU + VPE/Water	3.40 ± 1.51[b]	0.53 ± 0.02

Data are expressed as mean of 4000 cells analyzed per sample ± standard deviation of the mean (n=8 animals per group).[a]Significant difference in relation to the water group ($p< 0.05$, Test t -student);[b]Significant difference in relation to the fructose group ($p < 0.05$, Test t -student). MnPCE: micronucleated polychromatic erythrocytes; PCE/NCE: the ratio of polychromatic to normochromatic erythrocytes; FRU- Fructose; VPE-

voluntary physical exercise.

Table 4. Food consumption of offspring (females and males) born to mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy.

	Treatment	**Food (g/day)**	**Calorie (kcal/day)**
Female			
OG1	Water	3.6 ± 0.92	14.6 ± 2.55
OG2a	Water + VPE	5.8 ± 1.90[a]	17.6 ± 4.64
OG2b	Water + VPE/Water	4.1 ± 0.95	13.2 ± 2.3
OG3	FRU	5.1 ± 1.29	18.8 ± 8.46
OG4a	FRU + VPE	5.4 ± 1.71	19.3 ± 6.62
OG4b	FRU + VPE/ Water + VPE	3.7 ± 1.09[b]	12.2 ± 3.0
OG4c	FRU + VPE/Water	4.4 ± 1.42	16.9 ± 5.27
Male			
OG1	Water	3.6 ± 0.92	12.4 ± 3.63
OG2a	Water + VPE	5.8 ± 2.42[a]	17.7 ± 3.87
OG2b	Water + VPE/Water	4.1 ± 0.95	16.5 ± 5.06
OG3	FRU	5.1 ± 1.29	17.1 ± 4.36
OG4a	FRU + VPE	5.4 ± 1.71	22.1 ± 7.75
OG4b	FRU + VPE/ Water + VPE	3.7 ± 1.09[b]	14.9 ± 6.36[b]
OG4c	FRU + VPE/Water	4.4 ± 1.4	15.8 ± 4.11

Data are expressed as mean ± standard deviation of mean (n=10 animals per group). [a]Significant difference in relation to the water group ($p < 0.05$, ANOVA , *post hoc* Tukey's);[b]Significant difference in relation to the FRU + VPE group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

Table 5. Body weight (g) at different ages of offspring (females and males) born to mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy.

	Treatment	**7 days (g)**	**14 days (g)**	**21 days (g)**	**30 days (g)**	**60 days (g)**
Female						
OG1	Water	5.2 ± 0.49	6.5 ± 0.54	19.6 ± 0.52	26.6 ±1.91	30.1 ± 2.59
OG2a	Water + VPE	5.0 ± 0.47	7.6 ± 0.57	17.8 ± 0.72	24.0 ± 1.27	31.3 ± 2.16
OG2b	Water + VPE/Water	5.8 ± 0.33	7.0 ± 0.73	18.8 ± 2.29	27.5 ± 1.48	33.0 ± 1.62
OG3	FRU	7.8 ± 1.05[a]	10.7 ± 1.16[a]	24.4 ± 0.90	28.2 ± 0.95	37.3 ± 2.47[a]
OG4a	FRU + VPE	5.7 ± 1.46[b]	7.2 ± 1.70	18.4 ± 2.28	21.97 ± 1.3[b]	31.3 ± 2.45[b]
OG4b	FRU + VPE/ Water + VPE	6.6 ± 0.84	8.3 ± 1.63	19.9 ± 2.12	24.6 ± 0.49	30.6 ± 1.25[b]
OG4c	FRU + VPE/Water	6.4 ± 0.54	8.1 ± 0.98	24.5 ± 1.3[c]	26.1 ± 0.68	34.4 ± 2.47[c]
Male						
OG1	Water	5.6 ± 0.64	6.2 ± 0.66	20.6 ± 1.35	28.6 ± 4.02	32.5 ± 4.11
OG2a	Water + VPE	5.9 ± 0.91	7.5 ± 1.29	16.8 ± 1.05	27.5 ± 1.79	35.9 ± 1.82
OG2b	Water + VPE/Water	5.4 ± 0.92	6.8 ± 1.09	21.4 ± 0.89	27.5 ± 2.43	36.3 ± 2.26
OG3	FRU	7.8 ± 1.17[a]	10.2 ± 1.19[a]	29.1 ± 2.55[a]	29.7 ± 6.0	34.1 ± 3.01

	Treatment	7 days (g)	14 days (g)	21 days (g)	30 days (g)	60 days (g)
OG4a	FRU + VPE	5.7 ± 1.32[b]	9.3 ± 5.77	24.2 ± 1.06	26.5 ± 2.35	36.0 ± 3.35
OG4b	FRU + VPE/ Water + VPE	7.2 ± 1.03[c]	10.0 ± 2.44	24.3 ± 2.0	27.6 ± 5.42	36.0 ± 1.37
OG4c	FRU + VPE/Water	6.1 ± 0.40[b]	7.0 ± 1.09	24.7 ± 1.45	28.5 ± 2.11	37.5 ± 2.73

Data are expressed as mean ± standard deviation of mean (n=10 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the water group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);[b]Significant difference in relation to the FRU group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);[c] [c]Significant difference in relation to the FRU + VPE group ($p < 0.05$, Kruskal-Wallis, *post hoc*Dunn's).

Table 6. Fasting blood glucose (mg/dL) of offspring (females and males) of mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy.

	Treatment	Glucose (mg/dL)
Female		
	Water	139.8 ± 17.1
	Water + VPE	139.6 ± 17.6
	Water + VPE/Water	145.5 ± 26.4
	FRU	158.8 ± 4.92
	FRU + VPE	159.4 ± 12.5
	FRU + VPE/ Water + VPE	123.6 ± 9.4[a.b]
	FRU + VPE/Water	149.8 ± 23.6
Male		
	Water	131.2 ± 12.6
	Water + VPE	121.4 ± 12.3
	Water + VPE/Water	135.6 ± 11.7
	FRU	130.6 ± 15.9
	FRU + VPE	133.5 ± 9.0
	FRU + VPE/ Water + VPE	131.6 ± 21.2
	FRU + VPE/Water	125.0 ± 5.0

Data are expressed as mean ± standard deviation of mean (n=6 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the fructose group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the FRU + VPE group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

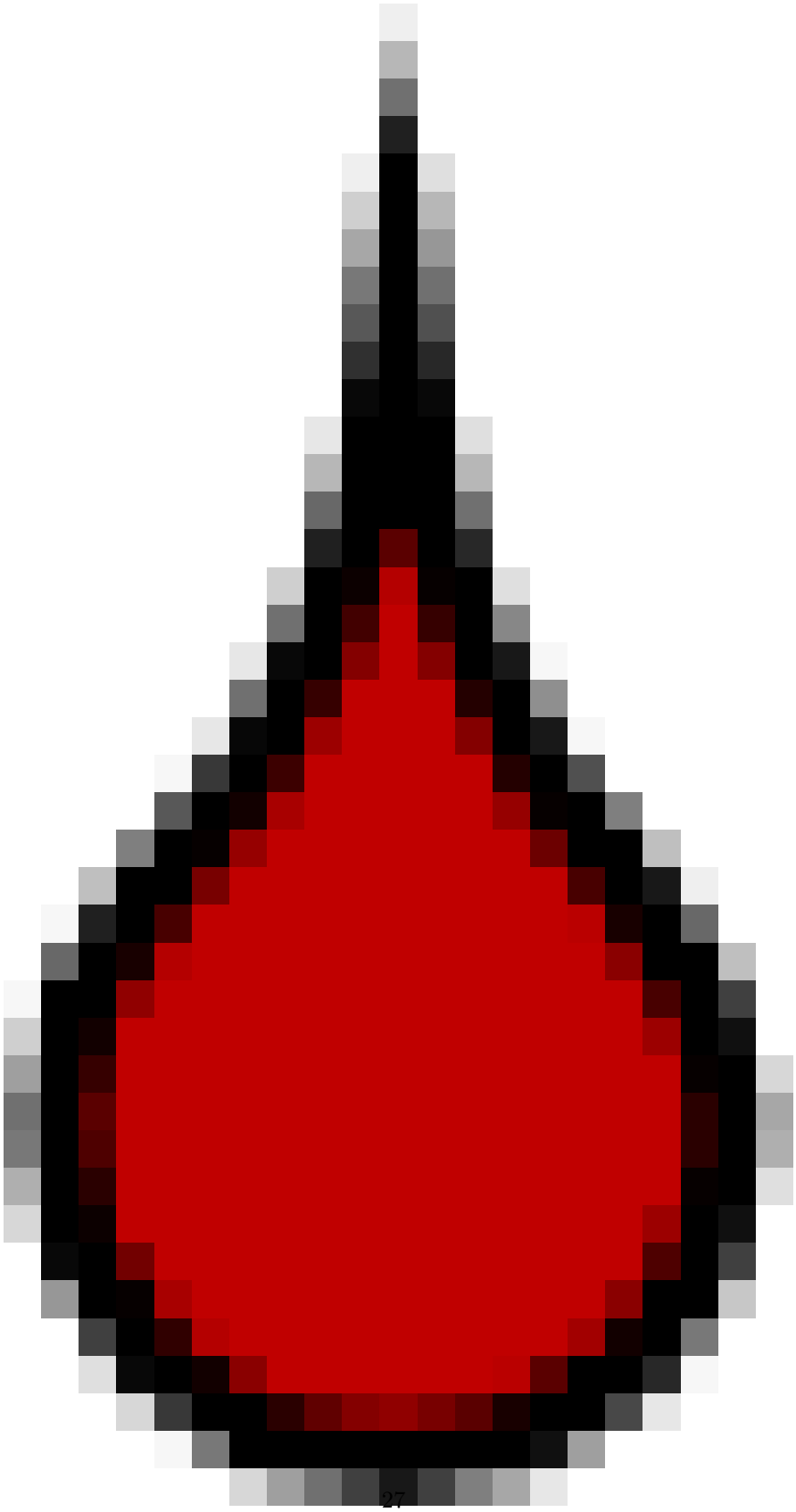
Table 7. Number of micronucleated polychromatic erythrocytes (MnPCE) observed in bone marrow samples from offspring (males and females) of mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy

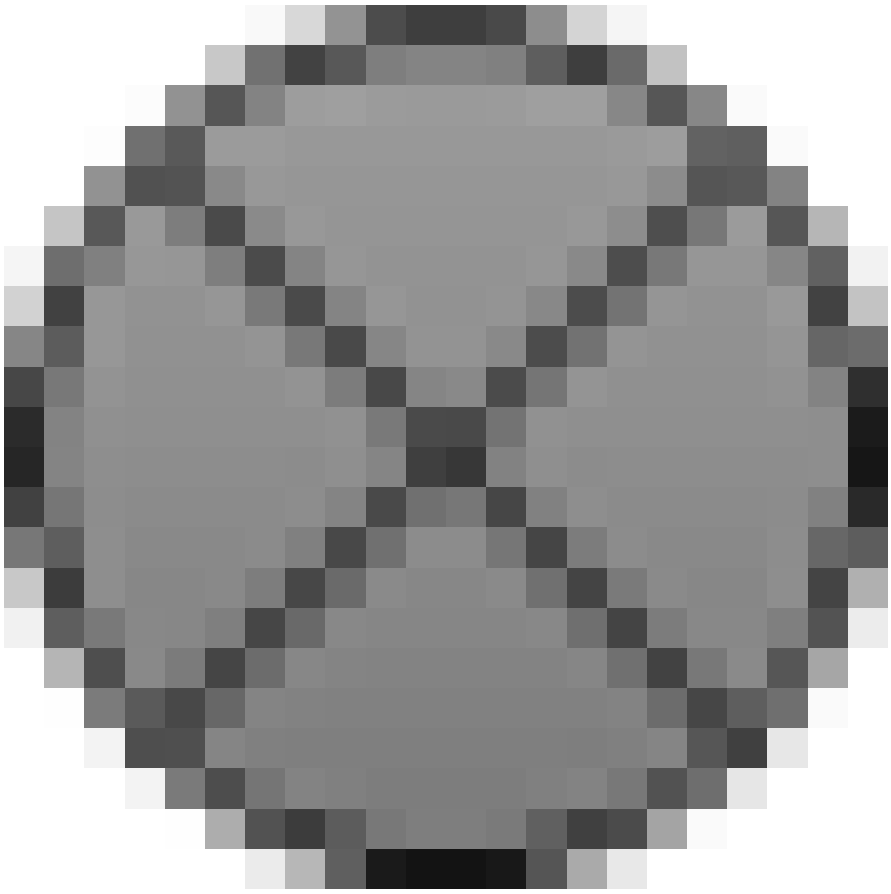
	Treatment	MnPCE	PCE/NCE
Female			
OG1	Water	0.16 ± 0.40	0.52 ± 0.02
OG2a	Water + VPE	0.17 ± 0.41	0.54 ± 0.03
OG2b	Water + VPE/Water	0.40 ± 0.54	0.54 ± 0.02

	Treatment	**MnPCE**	**PCE/NCE**
OG3	FRU	2.5 ± 1.87[a]	0.54 ± 0.03
OG4a	FRU + VPE	1.83 ± 0.75	0.53 ± 0.02
OG4b	FRU + VPE/ Water + VPE	1.66 ± 0.81	0.51 ± 0.02
OG4c	FRU + VPE/Water	1.80 ± 0.83	0.54 ± 0.02
Male			
OG1	Water	1.28 ± 0.95	0.52 ± 0.02
OG2a	Water + VPE	1.16 ± 0.75	0.53 ± 0.02
OG2b	Water + VPE/Water	1.75 ± 1.25	0.53 ± 0.02
OG3	FRU	3.66 ± 1.21[a]	0.51 ± 0.01
OG4a	FRU + VPE	2.14 ± 2.54	0.52 ± 0.02
OG4b	FRU + VPE/ Water + VPE	2.16 ± 1.72	0.49 ± 0.09
OG4c	FRU + VPE/Water	2.14 ± 2.11	0.53 ± 0.02

Data are expressed as mean of 4000 cells analyzed per sample ± standard deviation of the mean (n=8 animals per group).[a]Significant difference in relation to the water group ($p < 0.05$, Test t -student). MnPCE: micronucleated polychromatic erythrocytes; PCE/NCE: the ratio of polychromatic to normochromatic erythrocytes; FRU- Fructose; VPE- voluntary physical exercise.

Figures





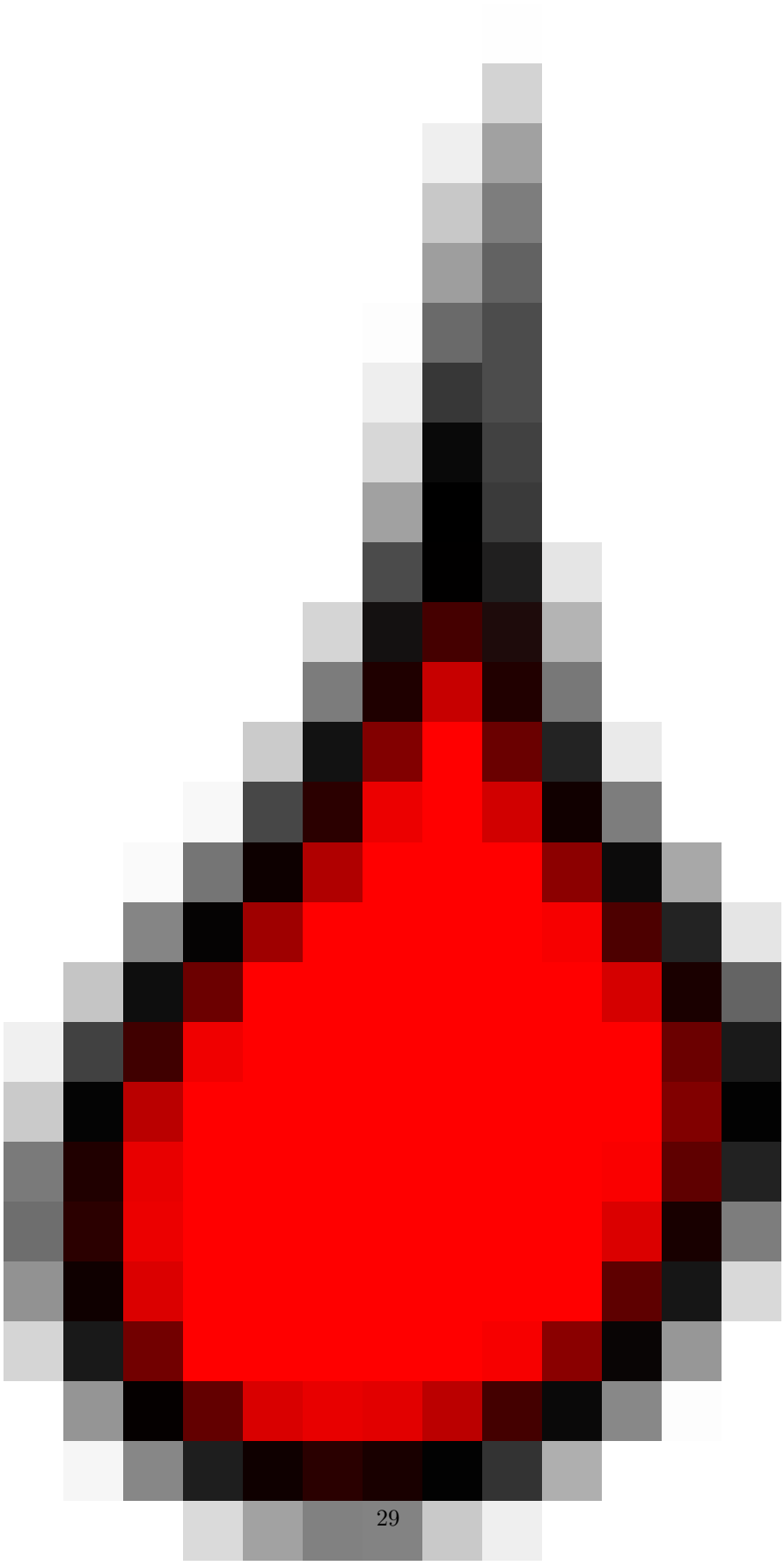


Figure 1 . Experimental Design. The mothers were divided into four experimental groups during the pre-pregnancy period, until week 9: G1- Water: females that received water and did not practice voluntary physical exercise (n=10); G2 – Water + VPE: females that received water and practiced voluntary physical exercise (n=20); G3 –FRU: females that received 20% fructose/L (n=10); G4 –FRU + VPE: females that received 20% fructose/L and practiced voluntary physical exercise (n=30). After this period, interventions were carried out for which the females were subdivided into 7 groups: G1 - Water: females continued to receive water and did not practice voluntary physical exercise until the end of pregnancy (n=10); G2a – Water + VPE: females that received water and practiced voluntary physical exercise until the end of pregnancy (n=10); G2b – Water + VPE/Water: females that received water and practiced voluntary physical exercise until copulation and then stopped the VPE and continued only with water until the end of the pregnancy period (n=10); G3 – FRU: females that continued to receive 20% fructose/L (n=10); G4a – FRU + VPW: females that received 20% fructose/L and practiced voluntary physical exercise until the end of pregnancy (n=10); G4b –FRU + VPE/Water + VPE: females that received 20% fructose/L and practiced voluntary physical exercise until copulation, after this period stopped fructose consumption and continued receiving only water and practiced voluntary physical exercise during pregnancy (n=10); G4c – FRU + VPE/Water: females that received 20% fructose/L and practiced voluntary physical exercise until copulation, after which they stopped fructose consumption and voluntary physical exercise and received only water during pregnancy (n=10). *During lactation only fructose supplementation was continued, while VPE was stopped due to limited space in the cages of the mothers and the pups. After weaning (week 15), the offspring continued to be monitored according to the seven experimental groups, referred to as offspring groups (OG). FRU: fructose; VPE: Voluntary Physical Exercise;

blood collection from mother;

assessment of VPE;

blood collection from offspring.

Figure 2. Caloric intake (kcal) of mothers during the various stages, while or after receiving water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean consumption of calories (n=10 animals per group).[a]Significant difference in relation to the water group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's).

Figure 3 . Fructose concentration (mg/mL) determined by high performance liquid chromatography in the serum of mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean (n=6 animals per group).[a]Significant difference in relation to the water group ($p < 0.05$, Test t -student); [b]Significant difference in relation to the fructose group ($p < 0.05$, Test t -student).

Figure 4. Insulin tolerance test of mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean (n=6 animals per group).[a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's); [b]Significant difference in relation to the fructose group ($p < 0.05$, ANOVA, *post hoc* Tukey's). AUC: Area under the curve. Based on the graphs on the left, the areas under the curves were computed and presented in the graphs on the right in the insulin tolerance test.

Figure 5. DNA damage index in peripheral blood cells of mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of the mean (n=8 animals per group). 5A: peripheral blood in pre-pregnancy; 5B: peripheral blood in pregnancy; 5C: peripheral blood in post-pregnancy.[a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the fructose group ($p < 0.05$, ANOVA, *post hoc* Tukey's). FPG: formamidopyrimidine DNA glycosylase.

Figure 6. DNA damage index (assessed as strand breaks) in liver cells of mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy.

Data are expressed as mean ± standard deviation of the mean (n=8 animals per group). [a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the fructose group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

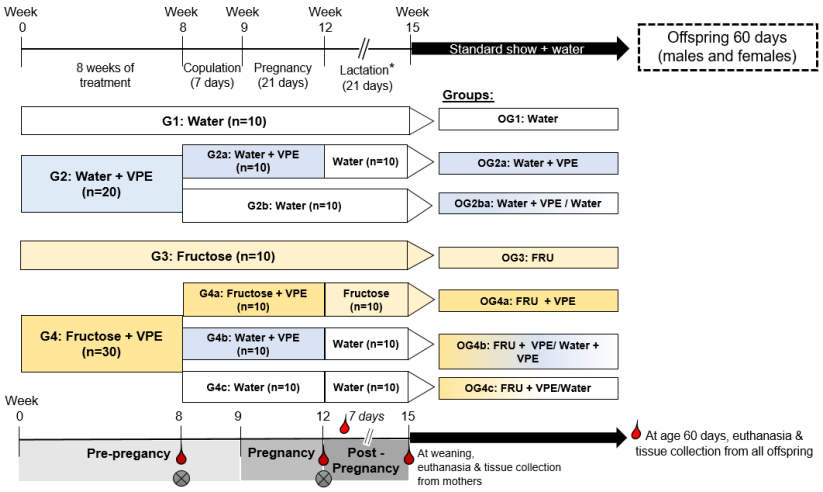
Figure 7 . Fructose concentration (mg/mL) determined by high performance liquid chromatography of the offspring (females and males) of mothers that received water or fructose and/or practiced voluntary physical exercise during the pre-pregnancy period /or pregnancy. Data are expressed as mean ± standard deviation of mean (n=6 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the FRU group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

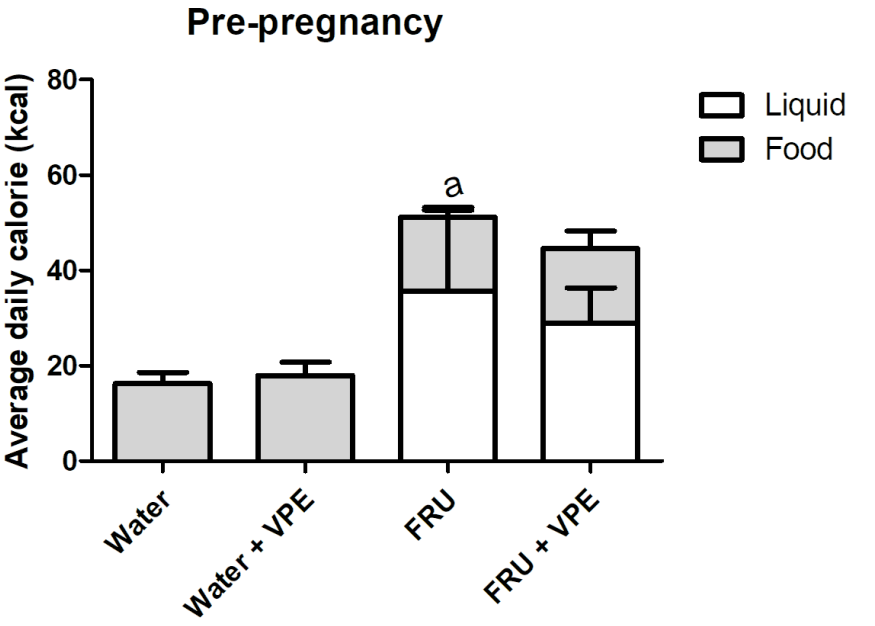
Figure 8. Body adiposity index of offspring (females and males) of mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean (n=6 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* de Tukey's);[b]Significant difference in relation to the FRU group ($p < 0.05$, ANOVA, *post hoc* de Tukey's).

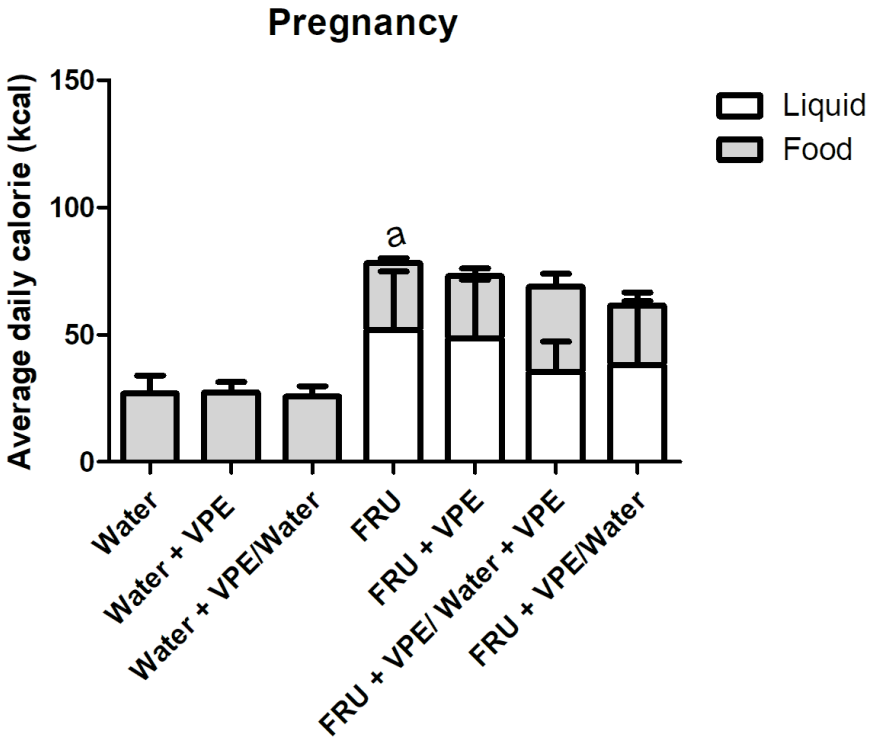
Figure 9. Insulin tolerance test of offspring (females and males) of mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean (n=6 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the FRU group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference compared to the FRU + VPE group ($p < 0.05$, ANOVA, *post hoc* Tukey's). AUC: area under the curve.

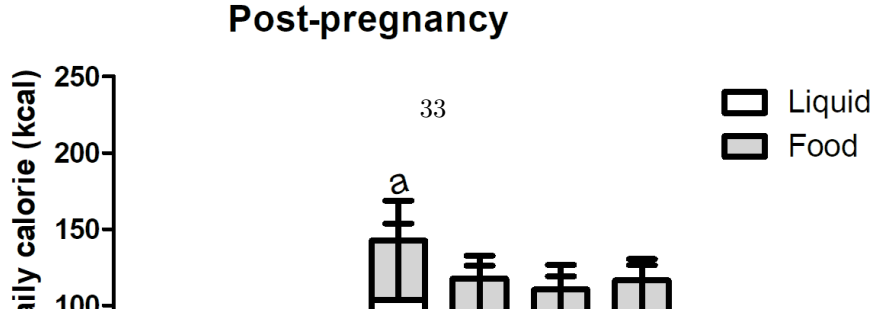
Figure 10. Histological profile of the hepatic tissue of the offspring (females and males) of mothers that received water or fructose and/or practiced voluntary physical exercise during pre-pregnancy and/or pregnancy. The upper pannel shows the representative images of female and male offspring. The lower pannel shows the quantified percentage of steatosis of female and male offspring. Representative images of histological photomicrographs of cross-sections of the liver of the animals. A: Water; B: Water + VPE; C: Water + VPE/Water; D: FRU; E: FRU + VPE; F: FRU + VPE/Water + VPE; G: FRU + VPE/Water. Data are expressed as mean ± standard deviation of mean (n=8 animals per group). FRU- Fructose; VPE- voluntary physical exercise.[a]Significant difference in relation to the water group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);[b]Significant difference in relation to the FRU group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);[c]Significant difference in relation to the FRU + VPE group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's);[d]Significant difference compared to the FRU + VPE/Water + VPE group ($p < 0.05$, Kruskal-Wallis, *post hoc* Dunn's).

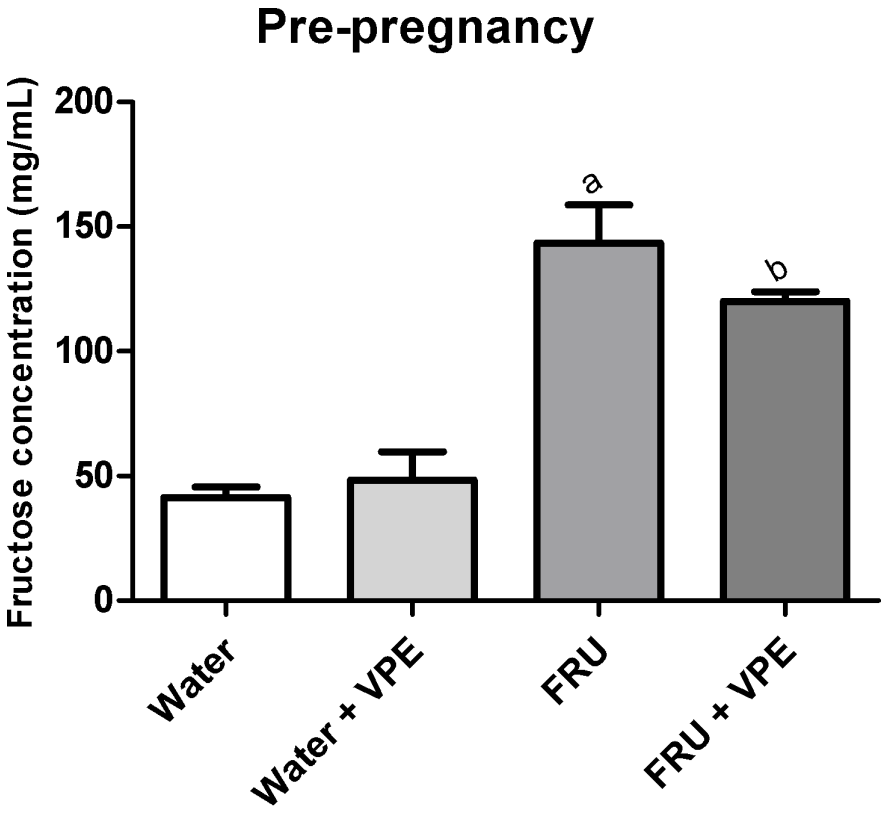
Figure 11. DNA damage index in peripheral tissue cells of the offspring (female and male) of mothers that received water or fructose (FRU) and/or practiced voluntary physical exercise (VPE) during pre-pregnancy and/or pregnancy. Data are expressed as mean ± standard deviation of mean (n=8 animals per group).[a]Significant difference in relation to the water group ($p < 0.05$, ANOVA, *post hoc* Tukey's);[b]Significant difference in relation to the FRU group ($p < 0.05$, ANOVA, *post hoc* Tukey's).

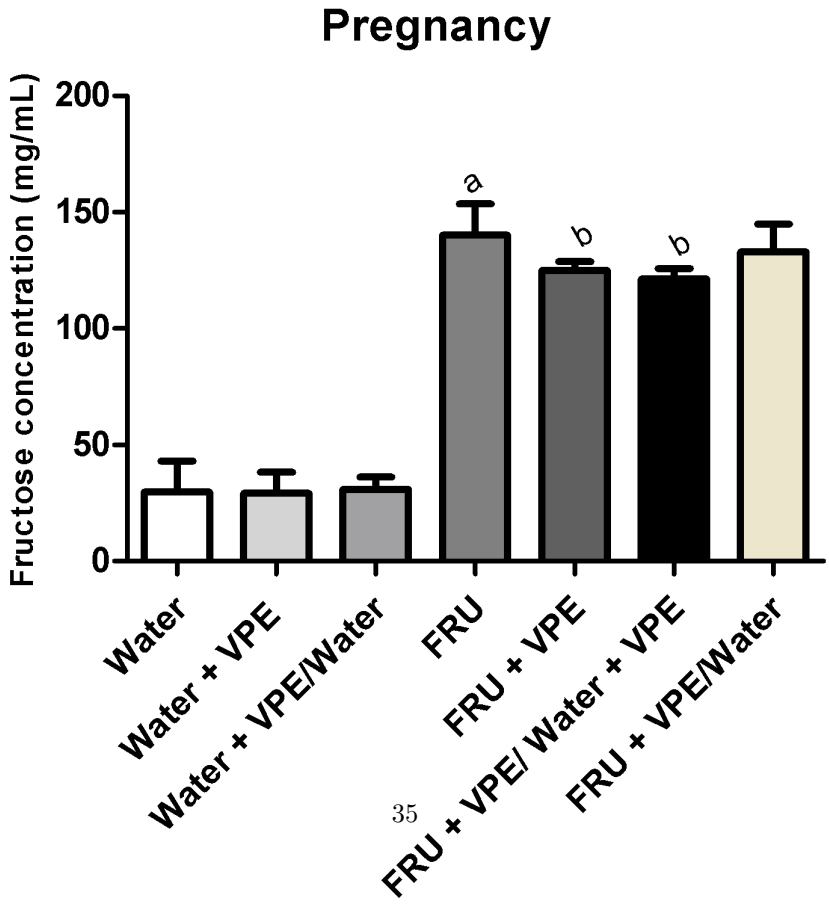


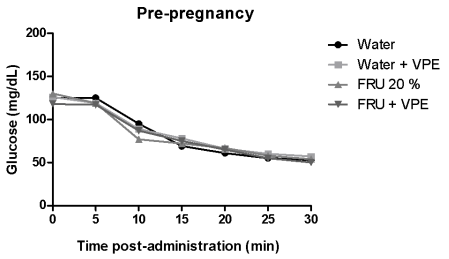


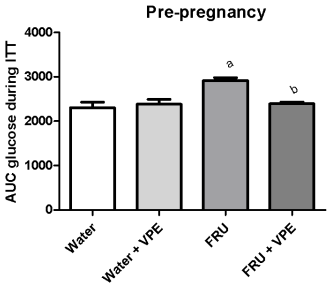


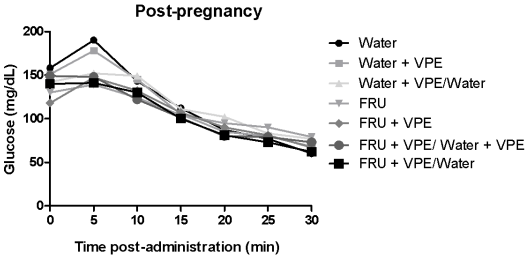


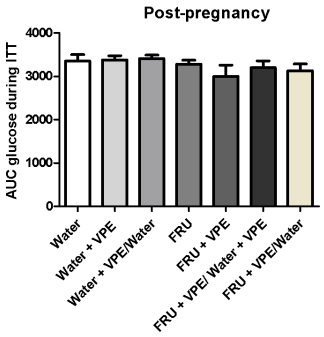


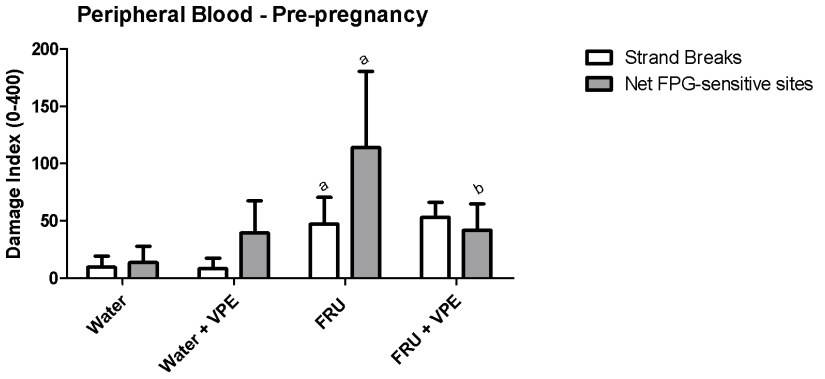


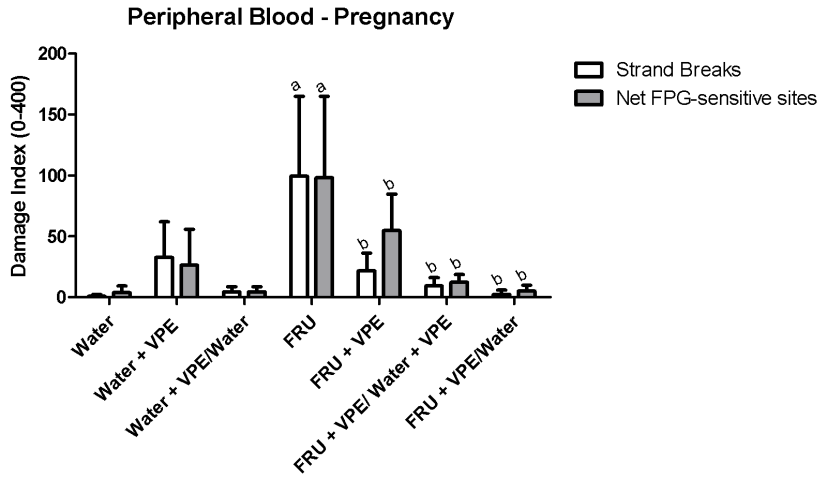


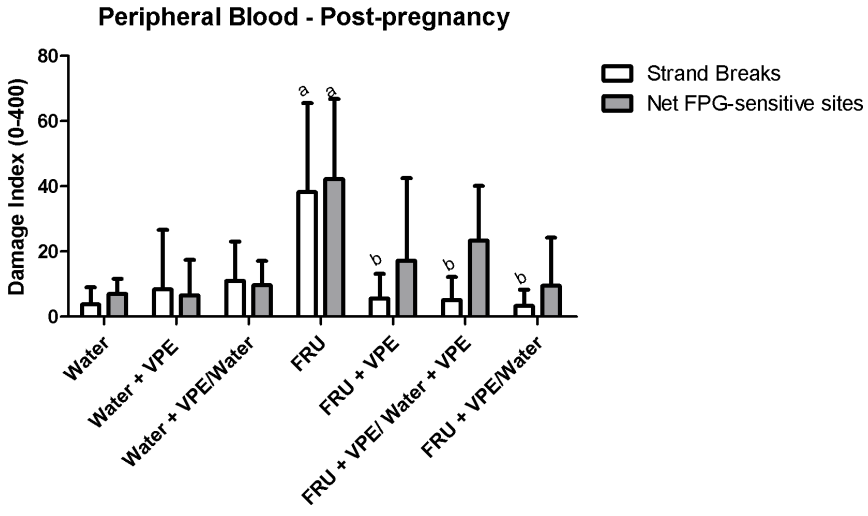


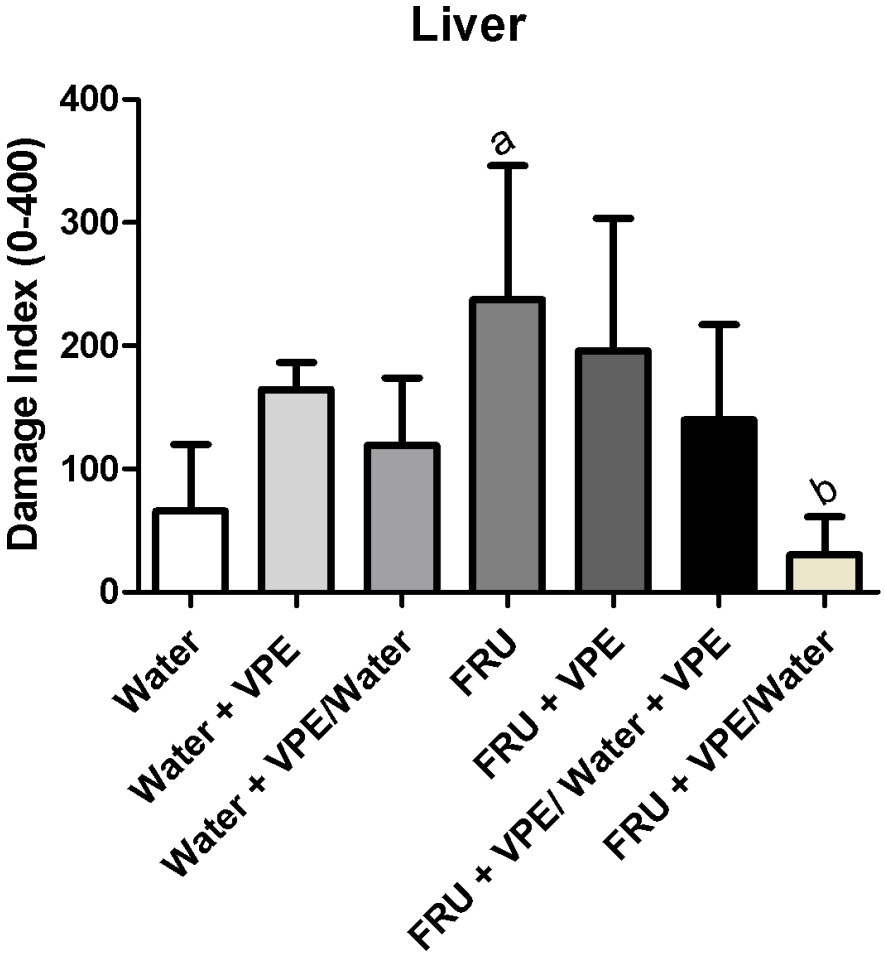


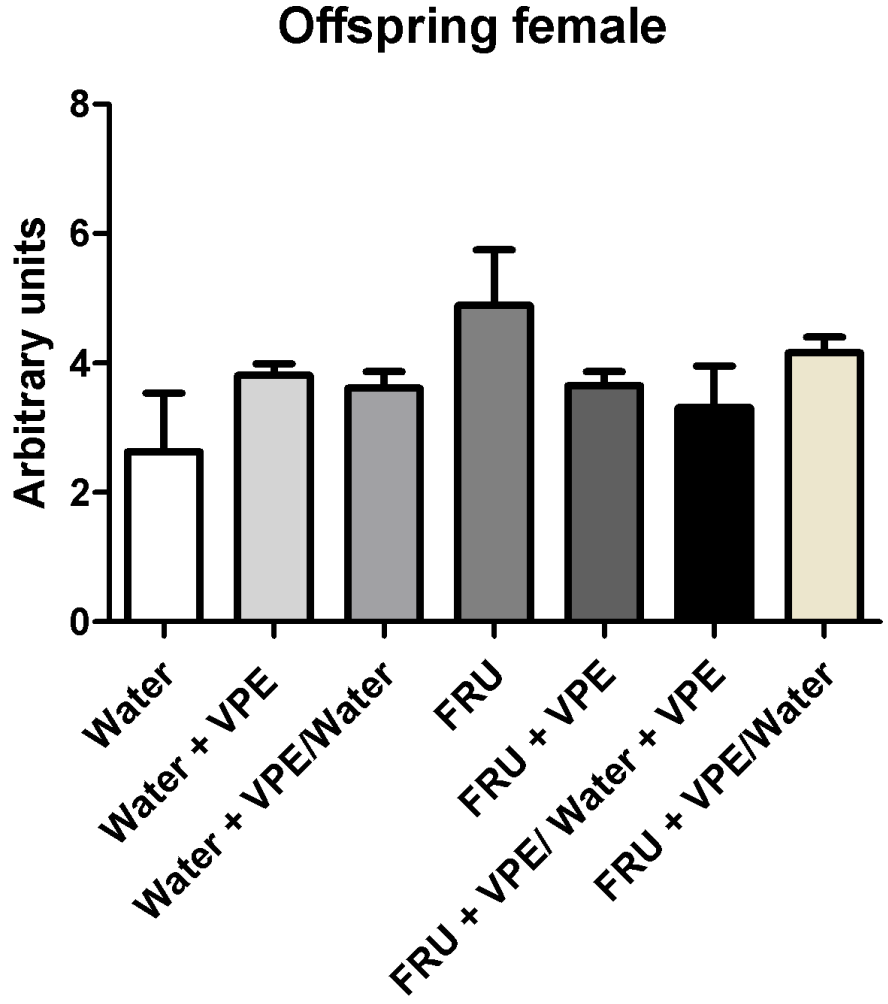


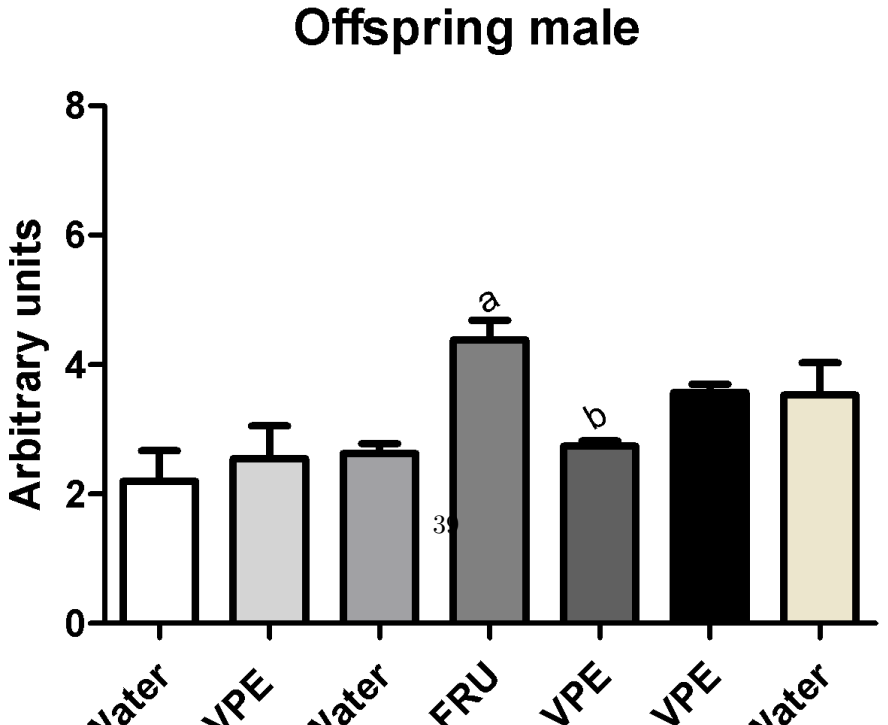


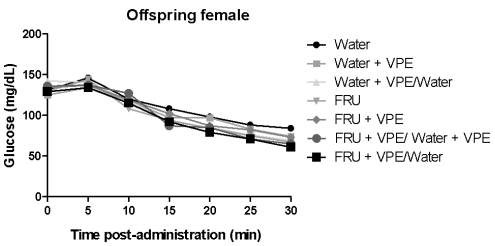


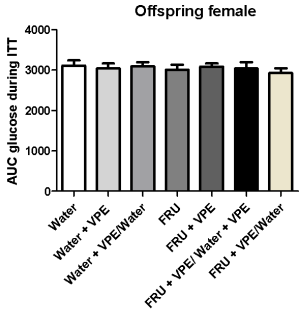


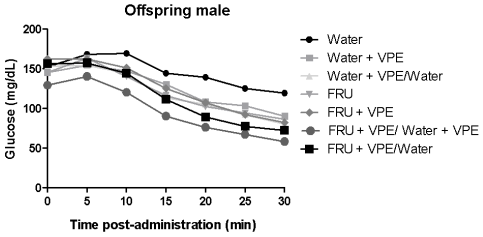


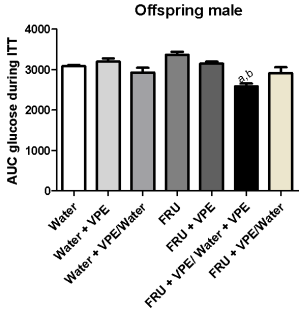


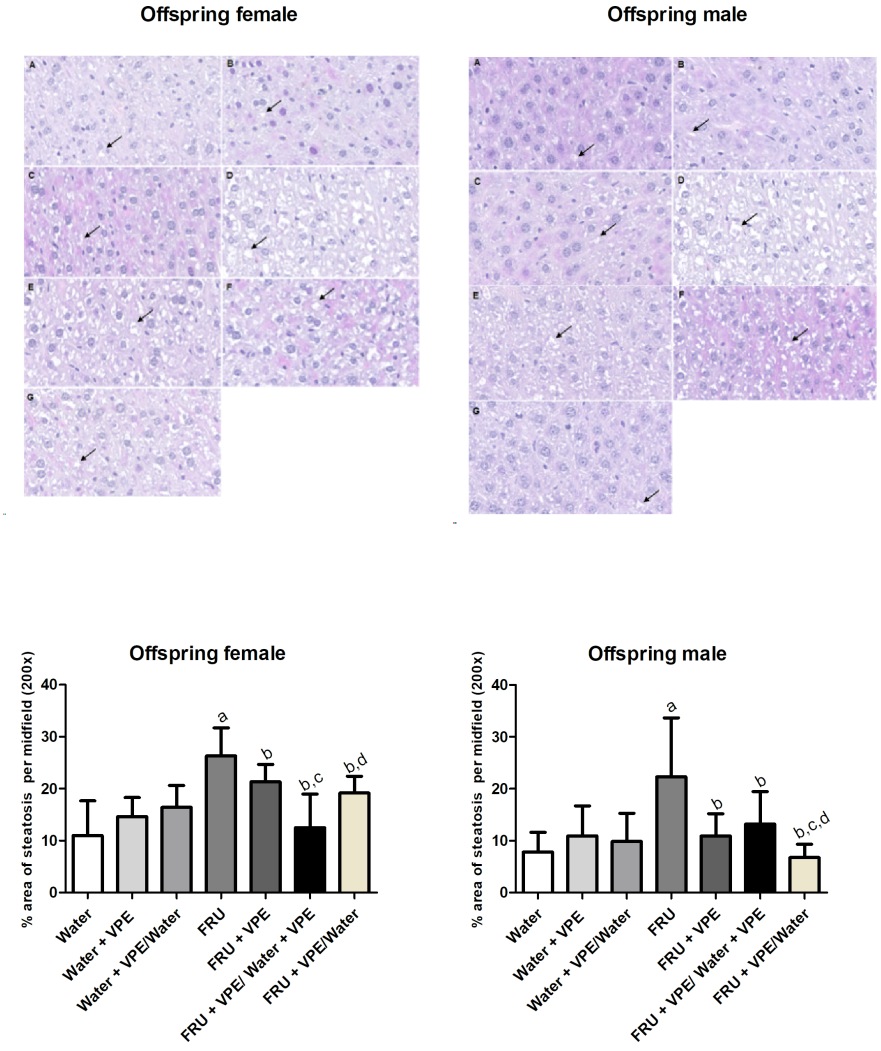


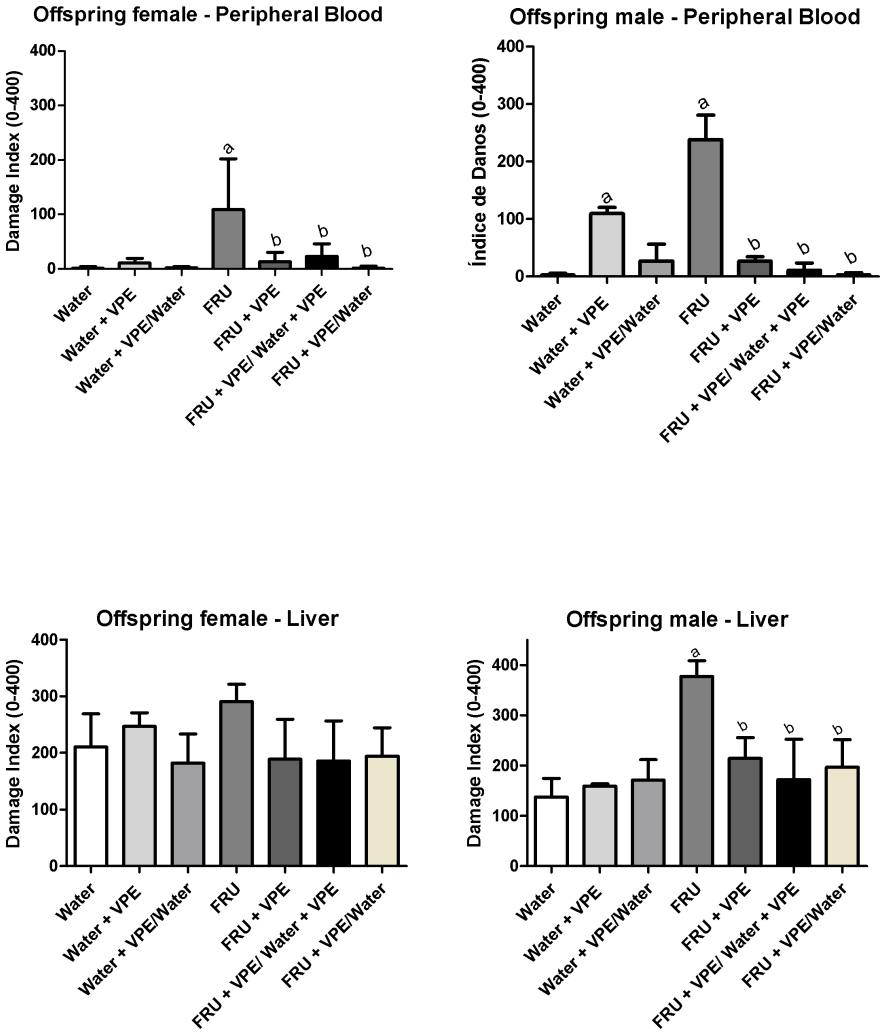












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