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Original Article

Quantification of the liver structure of zebrafish (*Danio rerio*) submitted to different diets and physical exercise

Quantificação da estrutura hepática de zebrafish (*Danio rerio*) submetidos à diferentes dietas e exercício físico

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Abstract

The zebrafish has been used in research for over 80 years. In the last three decades, discoveries about the fundamental properties of development, regeneration, cancer, and other diseases have established the zebrafish as an important model organism in biomedical research. This study aimed to evaluate liver alterations in zebrafish by quantitatively assessing the areas occupied by hepatocytes, as well as connective and adipose tissues. Forty-eight adult Danio rerio (38 males and 10 females) of approximately 13 months of age were used. They were divided into four groups, with 12 animals each. The fish were randomly distributed to form the groups, which received a maintenance and/or hypercaloric diet, with or without the addition of physical exercise. The animals underwent six hours of forced exercise (5 cm/s) for thirteen weeks. The animals that practiced physical exercise had a higher volumetric density of the area occupied by hepatocytes (65.92%±1.81 - GMex and 50.75%±2.24 GHex) among the groups. The GH group had a higher volumetric density of the area occupied by connective tissue (15.12%±0.72), followed by the GHex group (13.53%±1.43). Regarding the volumetric density of the area occupied by adipose tissue, the GH group had a higher density (27.21%±1.36), followed by the GHex group (21.66%±1.11) with statistically significant differences. The GMex had a volumetric density of the area occupied by adipose tissue of 3.5%±0.76, while the GM had 5.7%±0.5, with statistical difference. In relation to the animals in the GHex group, they had 20.39% less fat than the animals in the GH group. The animals in the GMex group had 72.47% less fat than those in the GM group. It is concluded that the different dietary constitutions and the imposition of physical exercise were able to modify the structural architecture of the liver of *Danio rerio*. These are acceptable criteria for modulations, thus aiming at the control and possible interferences directly related to the metabolism of the species and therefore the control of diseases.

Keywords: overfeeding, obesity, fishes, metabolism.

Resumo

O zebrafish têm sido usado em pesquisas há mais de 80 anos. Nas últimas 3 décadas, descobertas sobre as propriedades fundamentais do desenvolvimento, regeneração, câncer e outras doenças estabeleceram o peixe-zebra como um importante organismo modelo em pesquisas biomédicas. Este estudo teve como objetivo avaliar as alterações hepáticas em zebrafish, avaliando quantitativamente as áreas ocupadas por hepatócitos, bem como, tecidos conjuntivos e adiposos. Foram usados quarenta e oito *Danio rerio* (38 machos e 10 fêmeas), adultos, de aproximadamente 13 meses de idade. Foram divididos em quatro grupos, com 12 animais cada. Os peixes foram distribuídos aleatoriamente para formar os grupos, que receberam uma dieta de manutenção e/ou hipercalórica, com ou sem a adição de exercício físico. Os animais foram submetidos a seis horas de exercício forçado (5 cm/s) durante treze semanas. Os animais que praticaram exercício físico apresentaram maior densidade volumétrica da área ocupada pelos hepatócitos (65,92%±1,81 – GMex e 50,75%±2,24 GHex) dentre os grupos. Os animais do grupo GH apresentaram maior densidade volumétrica da área ocupada pelo tecido conjuntivo (15,12%±0,72), seguidos do grupo GHex (13,53%±1,43). Com relação densidade volumétrica da área ocupada pelo tecido adiposo, o grupo GH apresentou maior densidade (27,21%±1,36), seguido pelo grupo GHex (21,66%±1,11) com diferenças estatisticamente significativas. O GMex apresentou densidade volumétrica da área ocupada pelo tecido adiposo de 3,5%±0,76, enquanto o GM apresentou 5,7%±0,5, com diferença estatística. Relativamente os animais do grupo GHex apresentaram 20,39% menos gordura que os animais do grupo GH. Já os animais do grupo GMex apresentaram 72,47% menos gordura do que aqueles do GM. Concluiu-se que as diferentes constituições dietéticas e ainda a imposição de exercício físico foram capazes de modificar a arquitetura estrutural do fígado do *Danio rerio*. Sendo então critérios aceitáveis para que hajam modulações, visando assim, o controle e possíveis interferências relacionadas diretamente com o metabolismo da espécie e daí controle de doenças.

Palavras-chave: alimentação em excesso, obesidade, peixes, metabolismo.

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1. Introduction

To study the development of the vertebrate liver, zebrafish (*Danio rerio*), in the last decades, became the primary model. Zebrafish liver has similarities to humans and rodents in terms of functions and microanatomy (Cheng et al., 2019). The liver of these animals is also similar in the processing of lipids, vitamins, proteins, and carbohydrates and the synthesis of serum proteins (Oliveira et al., 2016).

Zebrafish there are several similarities in the characterization of Non-alcoholic fatty liver disease (NAFLD), which include increased hepatocytes and accumulation of triglycerides. As much as the zebrafish has been useful in the study of the disease, the interaction between the hepatocyte and other types of cells, progression, and severity of steatohepatitis, involving inflammation and fibrosis (Goessling and Sadler, 2015).

The link between obesity and the emergence of NAFLD is related to the hydrolysis of tryacylglycerol, present in adipose tissues, which are transformed into free fatty acids and glycerol, being sent to the liver, where they accumulate, favoring the development of this disease (Milić et al., 2014). Hepatic steatosis or fatty liver is defined as accumulation of triacylglycerol (TAG) intrahepatic of at least 5% of liver weight or 5% of hepatocytes containing lipid vacuoles in the absence of a secondary contributing factor such as excess alcohol intake, viral infection, or drug treatments.

The liver does not store TAG in normal conditions; however, under stressed settings such as in obesity or with high fat/high carbohydrate intake, abnormal lipid metabolism leads to ectopic hepatic lipid accumulation (Nassir et al., 2015). The permanence of fat in the liver leads to the development of steatosis. It is often considered a benign condition, but over time it promotes inflammatory processes and hepatocyte degeneration, with an increased risk of fibrotic progression and cirrhosis (Nassir et al., 2015). In the intervention and treatment of NAFLD, to induce lipophagia in the liver, the use of a calorie-restricted diet and the imposition of the practice of physical exercise has been efficient (Gao et al., 2020).

There is a knowledge gap concerning the physiology and biochemistry of exercise in fish (Moraes et al., 2004). Physical exercise yields beneficial effects on the efficient utilization of energy sources, such as adipose tissue. Fat is stored in adipocytes as triglycerides. When stimulated, adipocytes release triglycerides into the bloodstream as free fatty acids (FFAs) through lipolysis. In this process, each triglyceride molecule is hydrolyzed into glycerol and three fatty acids. The released FFAs enter the bloodstream and are transported to the muscles where they are utilized for energy. With increased blood flow in active muscles, there is a heightened delivery of FFAs to meet the energy demands (El-Zayat et al., 2019).

It is relevant to understand the physiological and morphological response of zebrafish exposed to diets and physical exercise. Therefore, this study aimed to evaluate the changes promoted by these factors, specifically the hepatic changes of the zebrafish, assessing through quantitative data the changes that occurred in the areas occupied by hepatocytes, as well as connective and adipose tissues.

2. Materials and Methods

2.1. Experimental design

Forty-eight fish of the *Danio rerio* species, thirty-eight males and ten females, adults, approximately 13 months old were used in the experiment. The animals were obtained from commercial breeding, without distinction of sex, but with an approximate age of 13 months. All fish were maintained in aquariums with constant temperature of 24 ± 2 °C and pH = 7.0-7.5 (Labcon test). Ammonia and nitrite levels were frequently checked and corrected. The animals were exposed to a daily cycle of illumination, 14 hours of light and 10 hours of darkness. Subjected to acclimatization and quarantine, over 15 days, then housed in four groups of 12 animals, in an aquarium with four divisions of 19 liters of stabilized water, maintained at the ideal temperature and adequately oxygenated. The authors declare that they are aware of the content of the resolutions of the National Council for the Control of Animal Experimentation and its regulations. In addition, all stages of the experiment were carried out by veterinarians, following the guidelines of the Ethics Committee on Animal Use of the Palotina Sector of UFPR (Protocol number 32/2020).

During the acclimatization and periods of the experiment, the animals were submitted to a cycle of 14 hours of light and 10 hours of darkness and were fed twice a day with commercial feed in flakes (Alcon Basic®). Throughout acclimatization, quarantine, and experiment, the animals were kept in aquariums with a closed circulation system, at a constant temperature of 26 ± 2°C controlled by a thermostat (Atman®) and measured with an aid of a thermometer (Aquadene®). The water in the aquariums was circulated using a submerged pump for oxygenation (HBO-300) and filtration using an external filter (Alife 500), this circulation permits the majority of these particulates to be circulated or suspended, allowing them to be filtered out by a mechanical filter. Aiding in the health of the tank inhabitants. There was a 10% water change per week, manual cleaning of the aquariums and filters, to prevent accumulation of residue from feeding and ensure the health of the fish.

During the whole period, the water quality was evaluated, as follows: every day, pH = 6.8-7.0 (Tropical pH, LabconTest), weekly ammonia = 0 ppm (Toxic Ammonia, LabconTest), and monthly dissolved oxygen = 6-8 mg/L (Dissolved Oxygen, LabconTest). When necessary, parameters were corrected using Discus Buffer pH corrector (Seachem) and Am Guard ammonia remover (Seachem).

2.2. Management of diets and physical exercise

Prior to the start of the experiment, all fish were kept for two weeks in acclimation, separated into two tanks with 24 animals each and adults. The fish were randomly distributed, Figure 1. Keeping the same acclimatization, the animals were submitted to a gradual modification of their diet, for two weeks, receiving frozen artemia (AquaSmart - 22% of lipids, 16% of carbohydrates, and 44% protein). At is to reach the amount proposed by Oka et al. (2010) - 1 mg of artemia contains approximately 5 calories, it was estimated that a zebrafish would obtain 20 calories from 5 mg of artemia at 80% consumption, and 150 calories from

60 mg of artemia at 50% consumption. Zebrafish were fed under these dietary protocols for 8 weeks:

a) GM and GMex: Maintenance diet (5mg artemia cysts/fish/day, once a day - 20Kcal/day) (n=24), nineteen males and five females. For thirteen weeks;
b) GH and GHex: Hypercaloric diet (60mg artemia cysts/fish/day, twice a day - 240Kcal/day) (n=24), nineteen males and five females. For thirteen weeks.

The animals were randomly divided into four groups of 12 animals each. The groups received a maintenance and/or high-calorie diet, with or without the addition of physical exercise. They were kept for 13 weeks and then sacrificed by immersion in cold water, 4 degrees Celsius, for 15 minutes (Gupta and Mullins, 2010), Figure 1.

- GMex: Maintenance diet + physical exercise (n =12), ten males and two females, 13 months of old;
- GM: Maintenance diet (n=12), nine males and three females, 13 months of old;
- GH: Hypercaloric diet (n=12), nine males and three females, 13 months of old;
- GHex: Hypercaloric diet + physical exercise (n =12), ten males and two females, 13 months of old.

Imposed physical exercise protocol following van der Meulen et al. (2006), for animals: GMex: Maintenance diet + physical exercise (n=12) and GHex: Hypercaloric diet + physical exercise (n = 12). The animals were submitted to six hours of forced physical exercise, stimulated through the flow of water controlled at a velocity of five centimeters (cm) of animals per second (5 cm/sec), for a total of thirteen weeks, in the aquarium with a swimming tube. During the first 3 weeks of training, the fish showed variable growth. To decrease stress and optimize the physical exercise protocol, every day the water flow was gradually increased until it reached the expected speed of about 5 minutes/per day Figure 1.

Throughout the entire period of the experiment, every animal, without individual marking, was weekly weighed. The weights were obtained in grams, on a precision scale (Mars AD 1000), Figure 2. In the same way, all the animals, from each of the groups, over the 13 weeks, were measured about their longitudinal length, from the head to the tail. Using a universal caliper (Starret) for both, Figure 3.

The body mass (BM) and body length were evaluated weekly with the animals. From these data, the Body Mass Index (BMI) of the fish was calculated by dividing the body weight (g) by the square of the body length measured (cm), BMI=BW(g)/BLM^2(cm) (Clark et al., 2018), Figure 4.

2.3. Histomorphometric evaluation

After the experiment was over, the fish were euthanized and fixed in 10% formalin in an aqueous solution for 24 hours. Then processed using conventional histological techniques and embedded in paraplast blocks (Sigma-Aldrich, St. Louis, MO, USA). Sections with a thickness of 4 µm were obtained along the entire animal with the aid of a manual microtome (Leica RM 2125RT). Slides were stained with Hematoxylin and Eosin and Masson's Trichrome.

Digital images of 21 random fields from each fragment were captured. The slides were evaluated under an optical microscope (Olympus ® BX51, Japan), increases of 10x and 40x. Photomicrographs of 10 fields chosen randomly for each slide were obtained using an optical microscope coupled to software for capturing and analyzing images, ProgRes ® Capture Pro 2.5

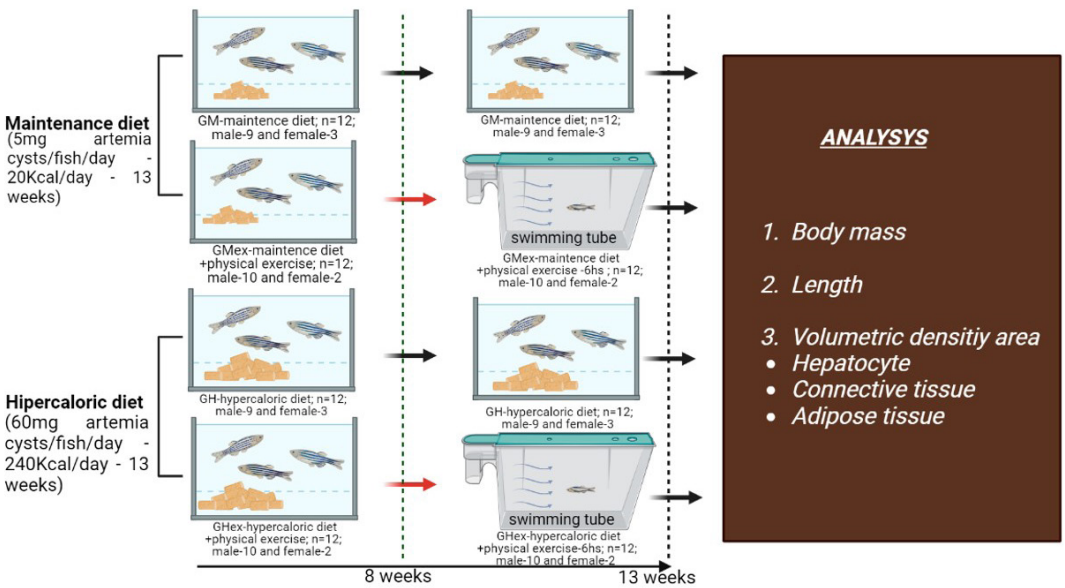

Figure 1. Schematic representation of the protocols adopted for the experiment execution, assessing Maintenance (5mg artemia cystis/fish/day-20Kcal/day-13 weeks) and Hypercaloric (60mg artemia cysts/fish/day-240Kcal/day-13 weeks) diets. GM = maintenance diet, n=12; male 9 and female 3); GMex = maintenance diet+physical exercise, n=12, male 10 and female 2; GH = hypercaloric diet, n=12, male 9 and female3; GHex = hypercaloric diet+physical exercise, n=12, male 10 and female 2. Red Arrows, emphasizing the imposition of physical exercise.

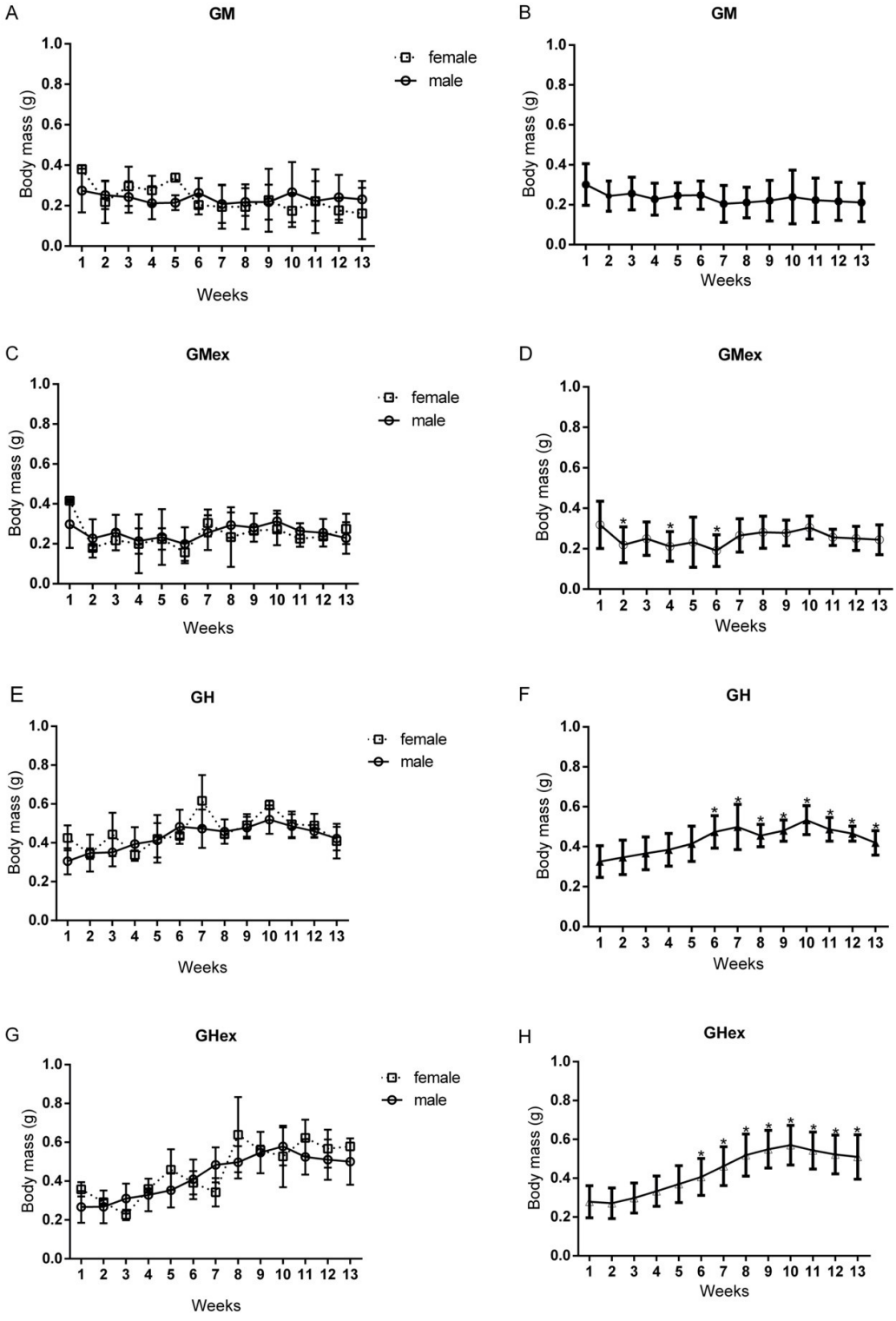

Figure 2. Representative graphs of the different groups GM-maintenance diet (A, B), GMex-maintenance diet+physical exercise (C, D), GH-hypercaloric diet (E, F) and GHex-hypercaloric diet+physical exercise (G, H). Mean and standard deviation, Body mass, grams (g), weeks. Asterisk between the weeks represent statistical difference compared to the first week. Differences between groups were tested by one-way analysis of variance (ANOVA), followed by Dunnett's Holm-Sidak multiple comparison post-test, $P \leq 0.05$, as statistically significant.

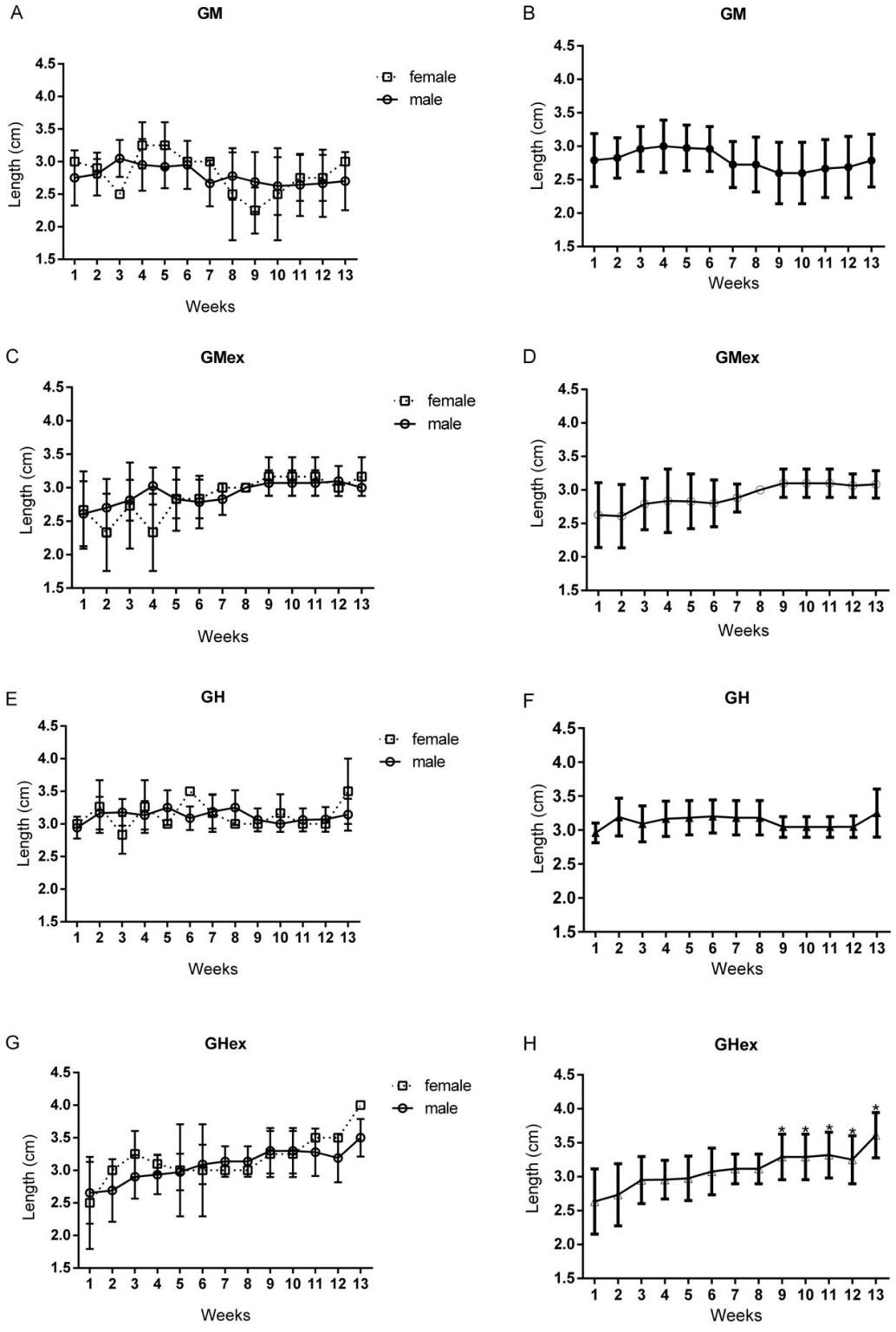

Figure 3. Representative graphs of the different groups GM-maintenance diet (A, B), GMex-maintenance diet+physical exercise (C, D), GH-hypercaloric diet (E, F) and GHex-hypercaloric diet+physical exercise (G, H). Mean and standard deviation, Length, centimeters (g), weeks. Asterisks(*) between the weeks represent statistical differences comparing the first week. Differences between groups were tested by one-way analysis of variance (ANOVA), followed by Dunnett's Holm-Sidak multiple comparison post-test, $P \leq 0.05$, as statistically significant.

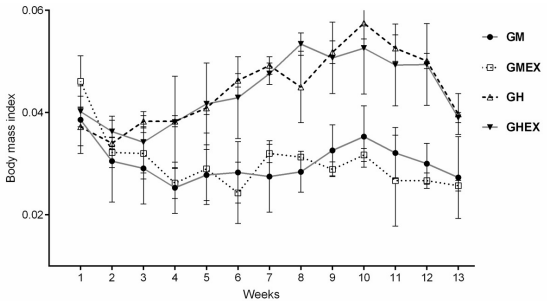

Figure 4. Body mass index (BMI) of zebrafish over the 13 weeks of the experiment. GM-maintenance diet, GMex-maintenance diet+physical exercise, GH-hypercaloric diet and GHex-hypercaloric diet+physical exercise. Mean and standard deviation. Asterisks(*) between the weeks, between the two groups, represent statistical differences comparing the first week. Application of Student's t-test, P ≤ 0.05, as statistically significant.

The animals' livers were analyzed for the area occupied by hepatocytes and connective and adipose tissue. For that, the STEPanizer© 1.0 software was used. The area occupied by hepatocytes, connective and adipose tissues was quantified using a point test system(Catta-Preta et al., 2011) in which the calculation of the total area was based on the principle Delesse's principle where A = Vv = Pp/Pt, where Pp is the points that affect the analyzed structure and Pt is the total number of points (36 points) and the Volume Density (Vv %) was used for the presentation of the results, as mean ± standard deviation of the mean. To calculate the volumetric densities of hepatocytes, six new serial sections of 4 µm thickness were obtained from each group and stained with Hematoxylin and Eosin. Digital images of 21 random fields from each slide fragment were then obtained, and 100 points in the 40x objective were used for quantification, with each point corresponding to an area of 16µ^2, resulting in a total area of 1600µ^2. The same total area was used to calculate the numerical density of hepatocytes. The numerical density of hepatocytes was calculated using the formula Nv(hepatocytes) = Q-A/ disector, where Q-A represents the number of nuclei seen in the upper plane of the section and disector is the test volume obtained by multiplying the section thickness by the area of the test system (Brüel, 2011).

The degree of hepatic steatosis was based on the classification of NAFLD (Sanyal, 2002; Hashimoto et al., 2015) with the first degree being mild (0-33%), the second degree being moderate (33-66%), and the third degree being severe (>66%).

2.4. Statistical analysis

Body mass and length were tested for normality by applying the Kolmogorov -Smirnov normality test, presented as mean±standard deviation. Differences between groups were tested by one-way analysis of variance (ANOVA), followed by Dunnett's Holm-Sidak multiple comparison post-test, P ≤ 0.05, as statistically significant. Statistics were performed using the GraphPad Prism program (Prism version 6.0c for Mac, GraphPad Software, La Jolla, California USA). The Body Mass Index (BMI) of the fish between the weeks and between the two groups was subjected to Student's t-test to verify the occurrence of statistical differences with the first week and between the groups, with a P value of ≤ 0.05 considered statistically significant. The results were presented as mean ± standard deviation of the mean. All data were submitted to the application of the Kolmogorov -Smirnov test, evaluating the normal distribution. The analyzes took place between the groups of animals that received a maintenance diet (GM) and that were submitted to physical exercise (GMex). Likewise, the Mann-Whitney test evaluated the animals in the groups that received a hypercaloric diet (GH) and underwent physical exercise (GHex), P≤0.05 was considered statistically significant. The analysis was performed using the GraphPad Prism program (Prism version 6.0c for Mac, GraphPad Software, La Jolla, CA).

3. Results

3.1. Biometrics

For GM animals, it was observed that over the 13 weeks, body mass (grams) remained constant, with no statistical difference between weeks (P≤ 0.05), Figure 2. The data obtained, about the longitudinal length of the animals, did not show statistical differences, Figure 3.

In the GMex group, in terms of body mass, there was a statistical difference between the animals in the first week (0.30g ± 0.10), with a decrease of: 30.83% for those in the second week; 33.59% for the fourth week and 40.01% for the sixth week, Figure 2. Regarding the longitudinal length of the animals in this group, no statistical difference was observed, Figure 3.

The hypercaloric diet in the GH group promoted an increase in body mass in the animals over the weeks. The statistical difference between the body mass of the first-week animals (0.32g ± 0.07) and relative increment of: 45.68% for animals in the sixth week, 53.27% for animals in the seventh week, 40.08% for animals in the eighth week, 47.59% for animals in the ninth week, 63.94% for animals from the tenth week, 49.80% for animals from the eleventh week, 43.13% for animals from the twelfth week and 28.89% for animals from the thirteenth week, Figure 2. No statistical difference was observed with the length of the animals (P ≤ 0.05), Figure 3.

Statistical differences were observed in the body mass (grams) of the animals in the GHex group, in the first week (0.27g ± 0.08), with an increment of 45.75% for the animals in the sixth week, 65,66% for the animals in the seventh week, 85.98% for the animals in the eighth week, 96.88% for the animals in the ninth week, 104.48% for the animals in the tenth week, 94.59% for the animals in the eleventh week, 87.16% for the animals in the twelfth week and 79.69% for the animals in the thirteenth week, Figure 2. To the length of the animals, from the ninth (3.29cm ± 0.33) to the thirteenth week (3.61cm ± 0.33), Figure 3. The data regarding the body mass index were presented in Figure 4. Throughout the period and also between groups, they were evaluated using statistical tests.

3.2. Histomorphometric analysis

Initially, the data related to the volumetric density of the area occupied by the hepatocytes, of the groups (GM; 57.35% ± 2.9), in comparison with those who underwent exercise physical exercise (GMex; 65.92% ± 1.81), showed an increase of 14.94% in the volumetric density of the area of the latter, with P<0.001. Concerning the volumetric density of the hepatocytes area, among the animals that received a hypercaloric diet (GH; 47.17% ± 1.28 and GHex; 50.75% ± 2.24). It was observed that the area occupied by the animals that practiced physical exercise (GHex) was 7.59% larger than the area of the group without physical exercise (GH), with a difference in statistics P <0.001, Figure 5. The numerical density of hepatocytes Nv(h) increased by approximately 14.9% between the groups (GM; 12.9μ3 ± 0.65) and (GMex; 14.82μ3 ± 0.41), with a statistically significant difference of P < 0.001, as shown in Figure 5. In contrast, the numerical density of hepatocytes Nv(h) in the groups (GH; 10.61μ3 ± 0.29) and (GHex; 11.42μ3 ± 0.5) increased by approximately 7.63% when compared to each other, also with a statistically significant difference of P < 0.001, as presented in Figure 5.

Regarding the volumetric density of the area occupied by the connective tissue, it was possible to state that when comparing the animals submitted to a maintenance diet (GM; 12.16% ± 0.81) with those that practiced exercise physical activity (GMex; 13.5% ± 0.76), with an increase of 11.02% in this area, with statistical difference P < 0.001. For the animals that received a hypercaloric diet, the difference was approximately 10.51% less, when comparing the different groups (GH; 15.12% ± 0.72 and GHex; 13.53 ± 1.43), with P<0.001, Figure 6.

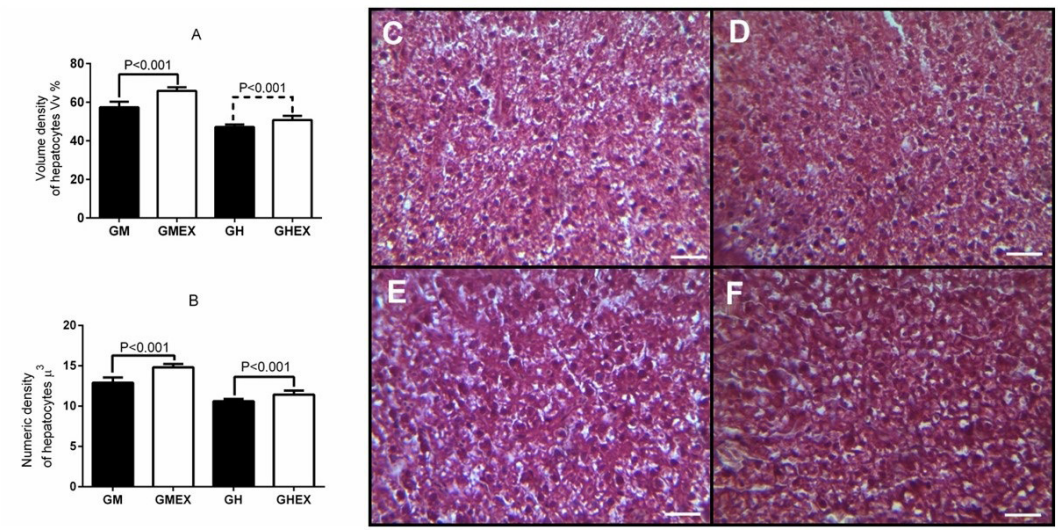

Figure 5. Quantification of Volume density of hepatocytes Vv% (A), Numeric density of hepatocytes μ^3 (B), all values are mean day ± standard deviation, represent the occurrence of statistical difference, by applying the Mann-Whitney test (P ≤ 0.05), over 13 weeks. Micrographs, stained with H&E, illustrating the different volumetric densities of zebrafish hepatocytes, males: (C) maintenance diet (GM), (D) maintenance diet and physical exercise (GMex), (E) hypercaloric diet (GH) and (F) hypercaloric diet and physical exercise (GHex). Bar = 30 micrometers.

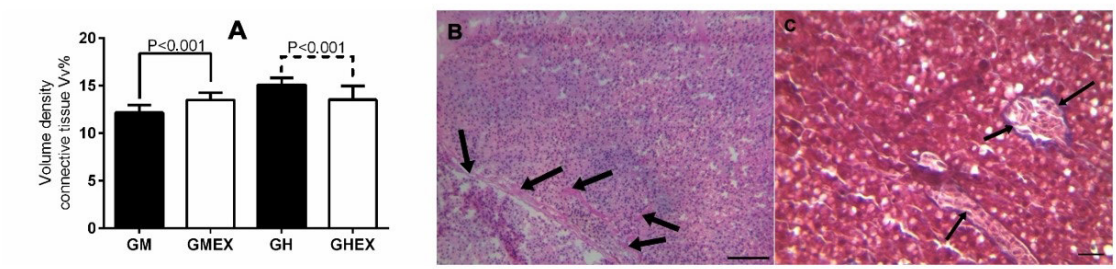

Figure 6. Quantification of Volume density connective tissue Vv% (A), all values are mean day ± standard deviation, represent the occurrence of statistical difference, by applying the Mann-Whitney test (P ≤ 0.05), over 13 weeks. Micrographs, stained with H&E (B) and Masson's Trichrome (C) illustrating the different of the area occupied by the connective tissue of zebrafish in female zebrafish: B- maintenance diet (GM), C- maintenance diet and physical exercise (GMex). Black arrows pointing to the connective tissue in the liver. Bar = 30 micrometers.

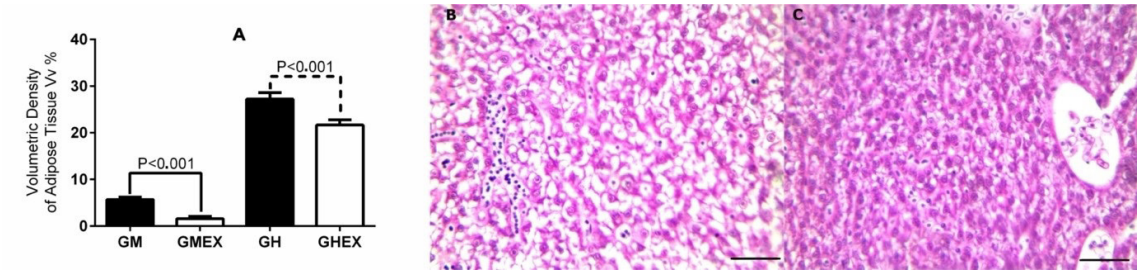

Figure 7. Adipose tissue of the zebrafish. Volumetric density of adipose tissue Vv% (A) from zebrafish submitted to maintenance diet (GM); maintenance diet and physical exercise (GMex); hypercaloric diet (GH) and hypercaloric diet and physical exercise (GHex), over 13 weeks. One-way ANOVA, by applying the Mann-Whitney test ($P \leq 0.05$). Micrographs, stained with H&E, illustrating the different volumetric densities of the area occupied by the adipose tissue of the zebrafish, different steatoses in male zebrafish: GH-hypercaloric diet (B), GHex-hypercaloric diet and physical exercise (C). Bar = 30 micrometers.

The values obtained for the volumetric density, of the adipose tissue area, disposed along the hepatic parenchyma, of the animals that received a maintenance diet and practiced physical exercise (GMex; 3.5% ± 0.76) was 72.47% lower than those kept on a maintenance diet (GM; 5.7% ± 0.5), with P<0.001. Otherwise, the volumetric density of the area of adipose tissue found in the livers of animals submitted to a hypercaloric diet (GH; 27.21% ± 1.36), when compared with animals that received the same diet, especially practiced physical exercise (GHex; 21.66% ± 1.11), decreased approximately 20.39%, with P<0.001, Figure 7. The quantitative evaluations allowed us to classify the animals of all groups as presenting a mild degree of steatosis, as shown in Figure 7.

4. Discussion

Animals in the GH and GHex groups showed a significant increase in body mass gain due to the ingestion of artemia from the sixth week of the experiment. This weight gain was confirmed by the occurrence of a statistical difference when comparing the animals in the first week, in both groups ($P \leq 0.05$). Obesity at eight weeks was shown in zebrafish fed a high-fat diet (Oka et al., 2010). In the GMex group, there was a decrease in body mass in the second, fourth and sixth weeks, compared to the first week. Due to the hyperlipidic characterization of the artemia, considering also the amount available for the group. Corroborating with a study that showed weight loss in rodents with a ketogenic diet when they are also subjected to resistance exercise (Ma et al., 2018). Even with the imposition of exercise, there was a significant variation in the mass and length of the animals.

Hepatocytes exhibited a reduction in the occupied area across different groups, indicating that the impact of the hypercaloric diet likely led to a decrease in the hepatic structure's occupied space. The fatty diet induced abnormalities such as steatosis to steatohepatitis, causing liver damage, inflammation, and fibrosis (Lahmi et al., 2023). This aligns, to some extent, with findings from (Forn-Cuní et al., 2015), who observed that high-fat diets triggered significant inflammation characterized by an apoptotic profile and a decrease in cell proliferation. In a study involving zebrafish maintained on a commercial diet for thirteen weeks, there was no variation in the volume and numerical density of hepatocytes, suggesting that factors affecting volumetric density also influence numerical density (Oliveira et al., 2016).

Fat diet was able to unbalance lipid homeostasis in the animals, causing accumulation of triglycerides in the hepatocytes, inducing inflammation, and oxidative stress, and causing hepatocyte damage, with or without fibrosis (Shimpi et al., 2017). These effects were incisive to establish hepatic steatosis and possibly promoted progression to NAFLD (Bellentani et al., 2010).

In obese humans, subject to high-intensity intermittent training, there was a reduction in liver fat (Houghton et al., 2017). Mice fed with a hyperlipidic diet developed hepatic steatosis and insulin resistance, and aerobic physical exercise acted as a non-pharmacological alternative to prevent and treat the deposition of adipose tissue in the liver (Cho et al., 2014). Exercise also caused a reduction in body mass and improves insulin sensitivity, with beneficial changes in the liver (Schultz et al., 2012).

The liver, influenced by physical exercise, could engage in lipogenesis, leading to the utilization of fat as an energy source for muscles and consequently decreasing the accumulation of hepatic intracellular fat (El-Zayat et al., 2019). High-performance exercise was found to enhance susceptibility to oxidative stress and contribute to hepatic damage (Vale et al., 2019). In the face of physical exercise, of moderate intensity and long duration, there was the suppression of fat deposition in the liver, in rats fed a high-fat diet and with moderate NAFLD (Qian et al., 2021). Already in obese zebrafish, according to (Zou et al., 2021), alterations from the high-fat diet were observed, such as lipid accumulation, hepatosteatosis, inflammation, fibrosis, and apoptosis. Different from what was observed in animals that practiced physical exercise, suggesting that exercise could mitigate the homeostasis of lipid disorders, due to excessive fat intake (Zou et al., 2021). Therefore, physical exercise and caloric restriction were beneficial to promote the regression of pathological lesions, and increased energy expenditure, reducing lipid overload and improving metabolic homeostasis (Gao et al., 2020).

Quantitative alterations related to connective and adipose tissues, in animals that received a high-fat diet,

generated a process of steatohepatitis, which could progress to liver cirrhosis. Otherwise, in animals that received a maintenance diet, their livers could be considered healthy (Nassir et al., 2015), since exercise and a balanced diet, in their contribution of macronutrients, attenuated the histopathological changes caused by a high-fat diet in zebrafish (Zou et al., 2021).

In rats, total collagen deposition increased in the livers of rats fed a hypercaloric diet (Qian et al., 2021). Simultaneously, inflammation, lipogenesis and collagen deposition in the liver decreased significantly after eight weeks of swimming (Qian et al., 2021). All these alterations are secondary to the inflammatory process, stimulated by the damage to the hepatocytes, mainly not evidenced in the evaluated animals.

Based on the results of this study, it was concluded that GH and GHex supplementation was beneficial for body mass gain and reduction of adipose tissue in the liver of zebrafish. For the GH animals, the provision of a high-fat diet without exercise increased fat accumulation, promoting inflammation, apoptosis, and fibrosis in the hepatic structure. The findings of this research contributed to the understanding of how different diets can be used to improve physical performance of zebrafish. Furthermore, the hypercaloric diet triggered reduction in the area occupied in the hepatic structure, suggesting that hypercaloric diet can also be used to improve physical performance. Providing new insights into the relationship between diets and physical exercise, as well as their effects on the physiological parameters of zebrafish.

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