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Effects of Hypercaloric Diet and Physical Exercise on Zebrafish (*Danio rerio*)

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Abstract

Zebrafish are becoming a valid model for investigating obesity and its associated disorders within a scientific context. The comprehension of the metabolic parameters linked to heightened body mass, adiposity, and energy expenditure stands as an indispensable endeavor in unraveling metabolic requisites among vertebrates. In this particular investigation, a cohort of 48 zebrafish specimens was scrutinized to determine the impact of physical exercise when subjected to a hypercaloric diet. The subjects were categorized into four distinct groups, each group respectively assigned to maintenance diets (GM, n=12, and GMex, n=12) or hypercaloric diets (GH, n=12, and GHex, n=12), with or without concurrent engagement in physical exercise. Applying a one-way ANOVA, with a significance level of $P \leq 0.05$, the results revealed noteworthy findings. Among the GMex group, regarding body mass, a statistically significant variance was observed during the initial week (0.30g ± 0.10). This disparity demonstrated a reduction of 30.83% in the subsequent week, 33.59% in the fourth week, and 40.01% in the sixth week. Similarly, within the GH group, the analysis of variance unveiled significant deviations in body mass (in grams) from the inception of the study (0.32g ± 0.07), in comparison to measurements from the sixth through the thirteenth weeks. The GHex group exhibited significant differences in body mass between the first week (0.27g ± 0.08) and the subsequent weeks, extending until the thirteenth week. Furthermore, discernible variations in the length (in centimeters) of the zebrafish specimens were recorded, with statistical significance observed from the ninth (3.29cm ± 0.33) to the thirteenth weeks (3.61cm ± 0.33). The implementation of a hypercaloric diet yielded an augmentation in body mass. Additionally, the combination of this dietary regimen with physical exercise further contributed to an augmentation in the length of the zebrafish specimens. Collectively, the proposed model exhibited the capability to induce discernible metabolic effects, thereby enhancing our understanding of the intricate interplay between diet, exercise, and metabolic dynamics.

Keywords: Fish; metabolism; body mass.

* Artículo de investigación

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Efectos de la dieta alta en calorías y el ejercicio físico en el pez cebra (*Danio rerio*)

Resumen

El pez cebra se está perfilando como un modelo válido para investigar la obesidad y sus trastornos asociados en un contexto científico. La búsqueda de comprender los parámetros metabólicos relacionados con el aumento de la masa corporal, la adiposidad y el gasto energético es un esfuerzo indispensable para desentrañar los requisitos metabólicos entre los vertebrados. En esta investigación en particular, se analizó una cohorte de 48 especímenes de pez cebra para determinar el impacto del ejercicio físico cuando se los sometía a una dieta hipercalórica. Los sujetos fueron categorizados en cuatro grupos distintos, cada grupo asignado respectivamente a dietas de mantenimiento (GM, n=12 y GMex, n=12) o dietas hipercalóricas (GH, n=12 y GHex, n=12), con o sin participación simultánea en ejercicio físico. Al aplicar un enfoque estadístico, concretamente ANOVA unidireccional, con un nivel de significancia de $P \leq 0,05$, los resultados revelaron hallazgos dignos de mención. Entre el grupo GMex, en relación con la masa corporal, se observó una variación estadísticamente significativa durante la semana inicial (0,30 g ± 0,10). Cabe destacar que esta disparidad demostró una reducción del 30,83% en la semana siguiente, del 33,59% en la cuarta semana y del 40,01% en la sexta semana. De manera similar, dentro del grupo de GH, el análisis de varianza reveló desviaciones significativas en la masa corporal (en gramos) desde el inicio del estudio (0,32 g ± 0,07), en comparación con las mediciones adquiridas desde la sexta hasta la decimotercera semana. El grupo GHex exhibió diferencias significativas en la masa corporal entre la primera semana (0,27 g ± 0,08) y las semanas posteriores, extendiéndose hasta la decimotercera semana. Además, se registraron variaciones discernibles en la longitud (en centímetros) de los ejemplares de pez cebra, observándose significación estadística desde la novena semana (3,29 cm ± 0,33) hasta la decimotercera semana (3,61 cm ± 0,33). La implementación de una dieta hipercalórica produjo un aumento de la masa corporal. Además, la combinación de este régimen dietético con ejercicio físico contribuyó aún más a un aumento en la longitud de los ejemplares de pez cebra. En conjunto, el modelo propuesto exhibió la capacidad de inducir efectos metabólicos discernibles, mejorando así nuestra comprensión de la intrincada interacción entre la dieta, el ejercicio y la dinámica metabólica.

Palabras clave: pez; metabolismo; masa corporal.

Introduction

In recent years, Zebrafish have been accepted and used as a multidisciplinary model, in the areas of genetics, reproduction, physiology, aging, and disease. They are animals similar to humans and other mammals, in the digestive organs, adipose tissue, skeletal muscle and their biochemical and cognition functions (1). Aiming at replicating and adopting an acceptable model for the study of obesity and obesity-related disorders, these fish are emerging as a system similar to humans and have systematically shown preservation of appetite and insulin regulation, lipid storage, and an appropriate response to diet modification (2).

Understanding the metabolic parameters related to the increase in body mass, adiposity, and energy expenditure proves to be vital for understanding the metabolic demands in vertebrates. To this end, Zebrafish point to evidence similar to that of mammals regarding the molecular and morphological aspects of adipocytes in their ability to store lipids with the function of providing nutrients (2). Enabling the replication and deepening of studies related to energy mobilization in vertebrates, through the establishment of an increase in body mass, induction of obesity, hyperglycemia, and hepatosteatosis (3). The comprehension of obesity and its conditions associated with physical exercise is paramount. In mammals, exercise makes it possible to improve numerous metabolic parameters, such as insulin and leptin resistance; ensuring the reduction of adiposity associated with the incidence of liver disease, heart disease, nephropathy, and neurodegenerative diseases (4, 5).

Aiming at expanding the understanding of the influence of diets related to the different caloric intakes of physical exercise, the present work evaluated the effects promoted by the offer of a hypercaloric diet in Zebrafish, doing or not to physical exercise.

Materials and Methods

Experimental Design

Forty-eight fish of the *Danio rerio* species, of both sexes, adults, approximately 10 to 18 months old, were used in the experiment. They were acclimatized and quarantined, over 15 days, divided into two groups in an aquarium with four divisions of 19 liters. The study was approved as it followed the Ethical Principles of Animal Experimentation, adopted by the National Council for Control of Animal Experimentation (CONCEA) and the Ethics Committee on Animal Use of the Federal University of Paraná–Palotina (Protocol number 32/2020).

During the acclimatization and quarantine periods, the animals were submitted to a cycle of 14 hours of light and 10 hours of darkness. They were fed twice a day with commercial flake food (Alcon Basic ®) (5% fat, 45% protein, 5% fibrous material, and 15% mineral material) and reared in aquariums with a closed circulation system, at a constant temperature of 26 +/- 2 °C controlled by a thermostat (Atman ®), measured using a thermometer (Aquadene ®). The water in the aquariums was circulated using a submerged pump for oxygenation (HBO-300) and external filtration (Alife 500). Each of the aquariums was subjected to a weekly change of 10% of its total water volume, as well as manual cleaning. Additionally, throughout this period, the quality of the water was evaluated, as follows: every day, pH = 6.8-7.0 (Tropical pH, LabconTest), weekly ammonia 0.1 ppm (Amônia Toxic, 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).

Diets and Physical Exercise

Keeping the same quarantine and acclimatization conditions, the animals were submitted to a gradual diet modification, for two weeks, receiving frozen artemia (AquaSmart–22% of lipids, 16% of carbohydrates, and 44 % of proteins), until they reached the following amount, according to Table 1:

– Maintenance (GM): Maintenance diet (5 mg artemia cysts /fish/day, once a day), (n=24). For thirteen weeks.

– Hypercaloric diet (GH): Hypercaloric diet (60mg artemia cysts /fish/day, twice a day), (n=24). For thirteen weeks.

Table 1–Absolute data of the different diets (Kcal/day per animal) provided to the *Danio rerio* over thirteen weeks. According to the protocol proposed by (3). Maintenance diet (GM); Diet maintenance + physical exercise (GMex); Hypercaloric diet (GH); Hypercaloric diet + physical exercise (GHex)

Diet (artemia)	GM / GMex	GH / GHEx
Energy intake (Kcal/day per animal)	20	240

Aiming at inducing overweight, the protocol was adopted (3). From then on, each group was again divided into two new groups n=12, aiming at the implementation of the physical exercise protocols. The aquariums were kept under the same conditions as previously exposed. The groups correspondingly received maintenance (GM and GMex) and hypercaloric (GH and GHex) diets, subject or not to physical exercise. These groups were kept for thirteen weeks and then submitted to euthanasia by immersion in cold water, 4 degrees Celsius, for 15 minutes, according to the protocol (4).

– GM: Maintenance diet (n=12);

– GMex: Maintenance diet + physical exercise (n = 12);

– GH: Diet hypercaloric acid (n =12);

– GHex: Hypercaloric diet + physical exercise (n = 12).

The modified physical exercise protocol was performed based on the work of (6), for the animals: GMex: Maintenance diet + physical exercise (n = 12) and GHex: Hypercaloric diet + physical exercise (n = 12). These animals were subjected to six hours of forced physical exercise, through a controlled flow of water at a velocity of five centimeters (cm) per second (5 cm/sec), for thirteen weeks in an aquarium with a tube for swimming. To reduce stress and optimize the imposed protocol, every day the water flow was gradually increased until it reached the expected speed in about 5 minutes, table 2.

Table 2–Adaptation protocol of the physical exercise of the animals over the weeks for the GMex animals: Maintenance diet + physical exercise and GHex: Hypercaloric diet + physical exercise (n=12)

Day/Week	Time (h/day)	Speed (cm/s)
1st	1	5
2nd	2	5
3rd	3	5
4th	4	5
5th	5	5
6th	6	5
2nd to 13th week	6	5

Throughout the entire period of the experiment, each of the animals was weekly weighed, when they were removed and placed in a 100ml glass beaker (Nalgon). In a precision balance (Mars AD 1000), the weights obtained in grams were compiled and presented for each of the groups in Figure 1.

All the animals, from each of the groups, over the 13 weeks, were measured (longitudinal length), using the measurement in centimeters of the distance obtained from the head. Using a universal caliper (Starret) for both. The data is expressed in Figure 2.

Statistical Analysis

Data, 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 Tukey's multiple comparison post-test, considering the P value ≤ 0.05, as statistically significant. Statistics were performed using the GraphPad Prism program (Prism version 6.0c for Mac, GraphPad Software, La Jolla, CA).

Results

GM–Maintenance diet group

For the GM animals (Figure 1), it was observed that over the 13 weeks, the body mass (grams) remained constant, with no statistical difference between the weeks (P ≤ 0.05). The data obtained about the longitudinal length of the animals, in centimeters, also demonstrated the maintenance of length over the period (Figure 2). Without the occurrence of statistical difference (P ≤ 0.05).

GMex–Maintenance diet and physical exercise group

In the GMex group, regarding body mass (grams), a statistical difference was observed between the animals in the first week (0.30g ± 0.10), when applying ANOVA one way, Dunnett's multiple comparison post-test (P ≤ 0.05), revealing a decrease: of 30.83 % for those in the second week (P ≤ 0.001); 33.59% for the fourth week (P ≤ 0.001) and 40.01% for the sixth week (P ≤ 0.001). Regarding the longitudinal length (cm) of the animals in this group, no statistical difference was observed over the evaluated period (Figure 2).

GH–Hypercaloric or rich diet group

It was observed that the hypercaloric diet promoted an increase in body mass (grams) in the animals over the weeks. The analysis of variance revealed statistical differences between the body mass (grams) in the first week (0.32g ± 0.07) when compared with the data obtained from the sixth to the beginning third week (Figure 1). Revealing a relative increase of: 45.68% for animals in the sixth week (P ≤ 0.001), 53.27% for those in the last week (P ≤ 0.001), 40.08 % for those in the eighth week (P ≤ 0.001), 47.59% for those in the ninth week (P ≤ 0.001), 63.94% for those in the d and above week (P ≤ 0.001), 49.80% for those in the d and above first week (P ≤ 0.001), 43.13% for those in the d and above second week (P ≤ 0.001) and 28.89% for those from *d* are up the third week (P ≤ 0.001). No statistical difference was observed in the length of the animals (cm), over the weeks (P ≤ 0.05) (Figure 2).

GHex – Hypercaloric diet and physical exercise group

Statistical differences were observed in the body mass (grams) of the animals in the GHex group, from the first week (0.27g ± 0.08) about the body mass from the sixth to the third week. This is proved by the application of the analysis of variance, one-way ANOVA, and Dunnet's multiple comparison post-test (P ≤ 0.05). The values increased by 45.75% for the sixth-week animals (P ≤ 0.001), 65.66% for the seventh-week animals (P ≤ 0.001), 85.98% for the eighth-week animals (P ≤ 0.001), 96.88 % for the ninth-week animals week (P ≤ 0.001), 104.48% for the animals from the d is top week (P ≤ 0.001), 94.59% for the animals from the d is up to the first week (P ≤ 0.001), 87.16% for the animals from the d is up to the second week (P ≤ 0.001) and 79.69% for the animals from the top third week (P ≤ 0.001) (Figure 1). A statistical difference was observed in the length (cm) of the animals (P ≤ 0.001), from the ninth (3.29cm ± 0.33) to the first third week (3.61cm ± 0.33). From the application of the same test used to assess body mass (P ≤ 0.05) (Figure 2).

Discussion

As an adaptive measure, a commercial diet consisting of 5% fat, 45% protein, 5% fibrous material, and 15% mineral material was offered to all animals in the first two weeks. After the adaptive period, the diet was replaced by brine shrimp, characterized by containing 22% fat, 16% carbohydrates, and 44% protein. The standardization of the diet had as its purpose the possibility of being highly caloric, capable of promoting an increase in body mass. For this purpose, data was recommended by (3), when he used a high-fat diet for Zebrafish, inducing obesity. coinciding with (7, 8), when they evaluated the effect of high-fat content in the diet. That is a diet featuring 20% corn oil or lard on body fat accumulation in Zebrafish. In the evaluated animals, it was clear that an effective weight gain was established and maintained. However, given the data generated, it was not possible to determine that they

Figure 1–Assessment of body weight gain. Evolution of body mass, in grams (g), of Zebrafish submitted to A. Maintenance diet (GM); B. Maintenance diet and physical exercise (GMex); C.hypercaloric diet (GH), and D. Hypercaloric diet and physical exercise (GHex), over 13 weeks. All values are mean day ± standard deviation, asterisks (*) represent the occurrence of statistical difference about the first week, one-way ANOVA, and Tukey's post-test (P ≤ 0.05).

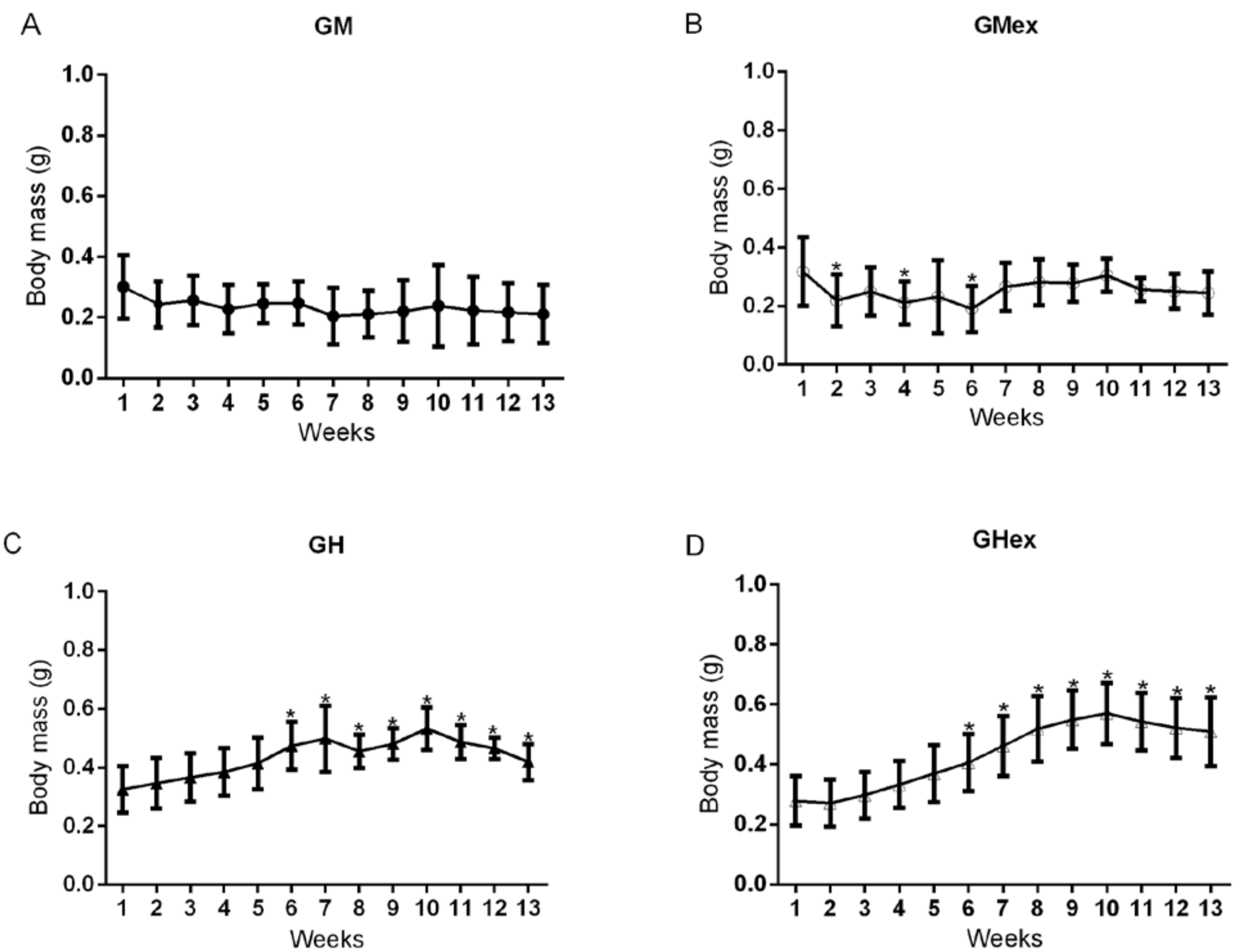
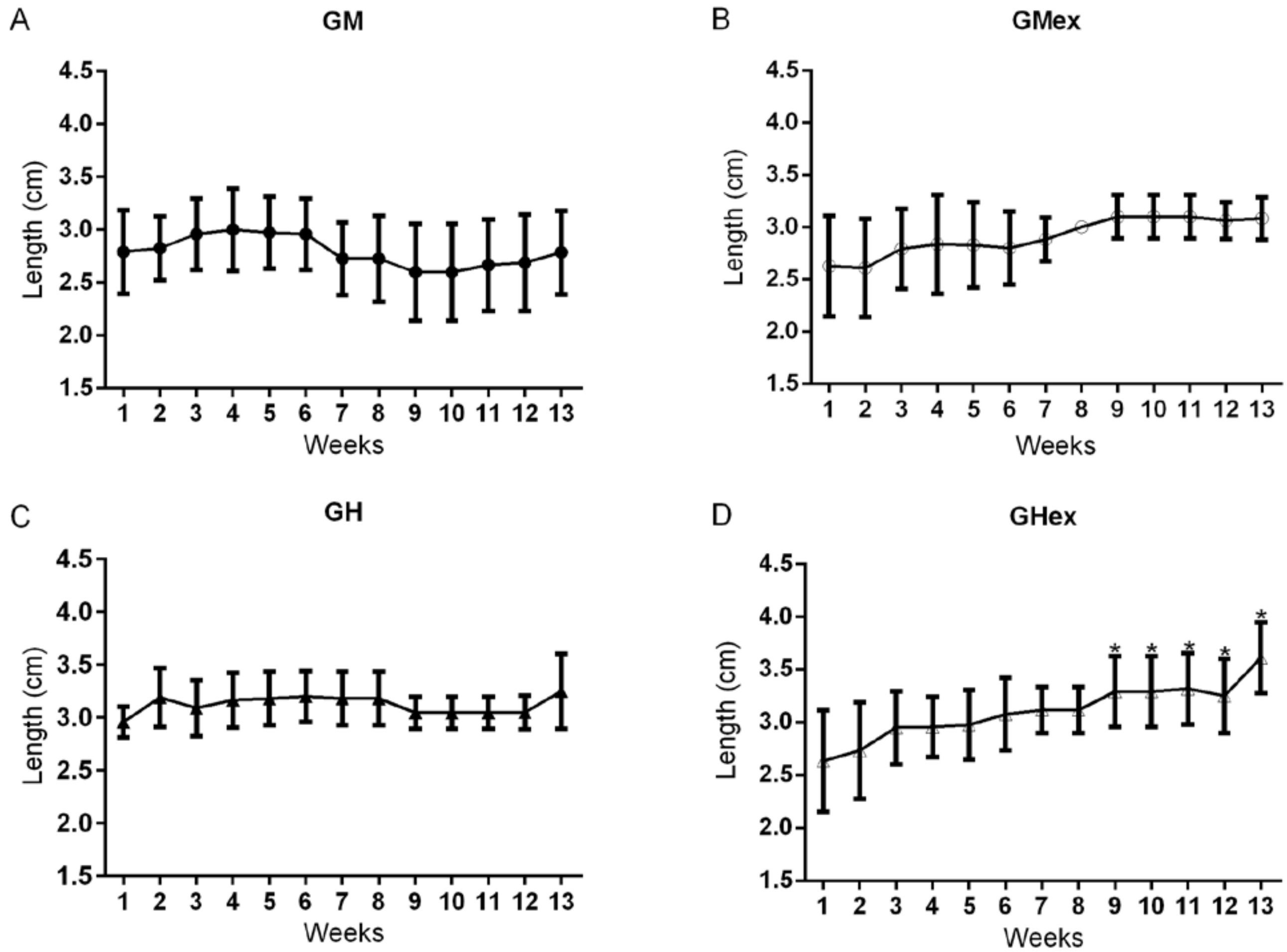


Figure 2–Assessment of body length. Evolution of body length, in centimeters (cm), of Zebrafish submitted to A. Maintenance diet (GM); B. Maintenance diet and physical exercise (Gmex); C. Hypercaloric diet (GH) and D. Hypercaloric diet and physical exercise (GHex), over 13 weeks. All values are mean day ± standard deviation, asterisks (*) represent the occurrence of statistical difference about the first week, one-way ANOVA, and Tukey's post-test (P ≤ 0.05).



had become obese since the results evaluated did not have the appropriate amplitude to affirm this condition. Above all, the nature of the diet was an important step, due to the adoption of artemia as the basis of the diet, revealing the possibility of increasing body mass even with the imposition of physical exercise.

Diet composition is critically important in pinpointing health or pathology (2). A combined diet with high sugar and fat content offered to mice led to the establishment of hyperglycemia, hypercholesterolemia, and high levels of inflammatory mediators, as well as the occurrence of low levels of T cells (9). Already (10), assessed fat supplementation by associating 5mg of brine shrimp with 30mg of egg yolk powder (59% fat, 32% protein, 2% carbohydrate), in comparison with 60mg of brine shrimp (22% fat). And found that both groups were overweight. Despite these quotes, it was possible to infer that the nature of the diet by itself could not promote weight gain, as observed in the maintenance groups (GM and GMex).

Zebrafish fed 60mg of artemia daily reached obesity in eight weeks, also revealing increased levels of triglycerides in blood plasma and hepatosteatosis (3). In other species, the diet actively promoted a positive energy balance capable of promoting metabolic disorders resulting from obesity, as verified in mice (11). Given the results, the effect promoted by the availability of artemias on body mass was confirmed, inducing overweight, due to the greater availability of intrinsic energy, from a diet rich in fat or carbohydrate.

In GMex, the imposition of physical exercise manifested itself significantly ($P \leq 0.05$), so the animals' body mass decreased in the second, fourth, and sixth weeks. Due to the characterization of the diet, which was hyperlipidemic, with 22% fat, but not hypercaloric. This is a determining factor for the understanding of the results obtained since a ketogenic diet promoted weight loss in rodents subjected to resistance exercise, including obese animals with a high-fat and high-calorie diet (12).

About the Zebrafish of the GH and GHex groups, the action determined by the amount of artemia made available through the diet was positive. Characterizing for that, these animals had an increase in their weights from the sixth week, revealing that there is still an evolutionary period of approximately five weeks. This weight gain was confirmed by the occurrence of a statistical difference when comparing the animals in the first week, in both groups ($P \leq 0.05$). Even with the imposition of exercise, there was a significant variation in the mass and length of the animals. This coincided with what was observed by (12), in Zebrafish for an exercise training model, which verified adaptive muscular hypertrophy and even an increase in angiogenesis.

Regarding longitudinal growth, the GHex group showed, from the sixth week onwards, the statistical difference when compared with the first week ($P \leq 0.05$). This result corroborates with (12) when they found growth-stimulating effects, in front of an intense exercise protocol. This fact was also observed in our study when the greater supply of food together with exercise promoted greater longitudinal growth of the animals from the sixth week of the experiment. In contrast, previous studies indicated that exercise-stimulated growth occurred only early in development 21-24 days after fertilization (6). This ambiguity could be associated with the diet offered, and its constitution, which responds to the metabolic demand, allowing the longitudinal growth of adult animals. However, in such a process, the active role promoted by exercise was not disregarded, exerting an efficient stimulus in establishing growth, probably due to the stretching of the skeletal striated muscle fibers.

References

1. Meshalkina DA, Kizlyk MN, Kysil E V., Collier AD, Echevarria DJ, Abreu MS, et al. Understanding zebrafish cognition. Behavioural Processes. 2017;141(Part 2):229-41. https://doi.org/10.1016/j.beproc.2016.11.020
2. Faillaci F, Milosa F, Critelli RM, Turola E, Schepis F, Villa E. Obese zebrafish: A small fish for a major human health condition. Animal Model Exp Med. diciembre de 2018;1(4):255-65. https://doi.org/10.1002/ame2.12042
3. Oka T, Nishimura Y, Zang L, Hirano M, Shimada Y, Wang Z, et al. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. BMC Physiol. 2010;10(1):21. https://doi.org/10.1186/1472-6793-10-21
4. van der Meulen T, Schipper H, van den Boogaart JGM, Huising MO, Kranenbarg S, van Leeuwen JL. Endurance exercise differentially stimulates heart and axial muscle development in zebrafish (*Danio rerio*). Am J Physiol Regul Integr Comp Physiol. 2006;291(4):R1040-8. https://doi.org/10.1152/ajpregu.00116.2006
5. Gupta T, Mullins MC. Dissection of Organs from the Adult Zebrafish. J Visualized Experiments. 2010;(37):e1717. https://doi.org/10.3791/1717

6. Meguro S, Hasumura T, Hase T. Body Fat Accumulation in Zebrafish Is Induced by a Diet Rich in Fat and Reduced by Supplementation with Green Tea Extract. PLoS One. 2015;10(3):e0120142. https://doi.org/10.1371/journal.pone.0120142
7. Stanley WC, Dabkowski ER, Ribeiro RF, O'Connell KA. Dietary Fat and Heart Failure: Moving From Lipotoxicity to Lipoprotection. Circ Res. 2012;110(5):764-76. https://doi.org/10.1161/CIRCRESAHA.111.253104
8. Maioli TU, Gonçalves JL, Miranda MCG, Martins VD, Horta LS, Moreira TG, et al. High sugar and butter (HSB) diet induces obesity and metabolic syndrome with decrease in regulatory T cells in adipose tissue of mice. Inflammation Research. 2016;65(2):169-78. https://doi.org/10.1007/s00011-015-0902-1
9. Landgraf K, Schuster S, Meusel A, Garten A, Riemer T, Schleinitz D, et al. Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity. BMC Physiol. 2017;17(1):4. https://doi.org/10.1186/s12899-017-0031-x
10. Li J, Wu H, Liu Y, Yang L. High fat diet induced obesity model using four strains of mice: Kunming, C57BL/6, BALB/c and ICR. Exp Anim. 2020;69(3):326-35. https://doi.org/10.1538/expanim.19-0148
11. Ma S, Huang Q, Yada K, Liu C, Suzuki K. An 8-Week Ketogenic Low Carbohydrate, High Fat Diet Enhanced Exhaustive Exercise Capacity in Mice. Nutrients. 2018;10(6):673. https://doi.org/10.3390/nu10060673
12. Palstra AP, Tudorache C, Rovira M, Brittijn SA, Burgerhout E, van den Thillart GEEJM, et al. Establishing Zebrafish as a Novel Exercise Model: Swimming Economy, Swimming-Enhanced Growth and Muscle Growth Marker Gene Expression. PLoS One. 2010;5(12):e14483. https://doi.org/10.1371/journal.pone.0014483