

# Growth Hormone (GH) and Strength Training: Effects on Morphology of Aged Rats

Hormona del Crecimiento (GH) y Entrenamiento de Fuerza:  
Efectos Sobre la Morfología de Ratas Envejecidas

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**SUMMARY:** The objective of this study was to investigate the effects of growth hormone (GH) associated with a strength training (ST) protocol on body composition and muscle tissue in aged rats. Forty aged animals of the Wistar lineage were used, divided into 4 groups: Control (C) [n=10], GH Control (GHC) [n=10], Strength Training (T) [n=10], and Strength Training (n=10) with Growth Hormone (GHT) [n=10]. The intervention period was 4 weeks and consisted of 4 x 10 water jumps, performed 3 times a week, on non-consecutive days, with an overload of 50% of body mass. The GHC and GHT groups received 0.2 IU per kilogram of body mass (0.067 mg/kg) at the start of each training session. After the end of the experiment, the animals were euthanized with a combination of anesthetics and exsanguination. The variables Body Mass, Body Mass Index (BMI), and the Lee Index (Lee) were measured and visceral adipose tissue was collected. Furthermore, the medial gastrocnemius muscle was removed to measure the cross-sectional area. The Shapiro-Wilk normality test was performed. For the “body mass” variable, repeated measures ANOVA analysis of variance was used, with the Bonferroni post-test. For the BMI, Lee index, and visceral adipose tissue variables, analysis of variance ANOVA with Tukey's post-test was used. To analyze the “cross-sectional area”, the Kruskal-Wallis test with Dunn's post-test was used. All procedures adopted a significance value of 5% (p<0.05). There were no statistically significant differences for the variables body mass, visceral adipose weight, BMI, and Lee index (p>0.05). Increases in muscle were observed in all experimental groups (p<0.05). It is concluded that GH caused hypertrophy in the muscle, with or without training, however, there were no differences in the body composition of aged animals.

**KEY WORDS:** Aging; Growth Hormone; Strength training; Skeletal muscle; Wistar Rats.

## INTRODUCTION

Growth hormone (GH) is a substance produced by the anterior pituitary gland and controlled by hypothalamic trophic hormones. GH is known for its anabolic functions, including promoting protein synthesis and increasing muscle mass, as well as having significant effects on regulating growth, metabolism, and immune function (Laron *et al.*, 1991).

Its production is influenced by several factors, including nutrition, sleep quality, and physical exercise (Baumeister *et al.*, 2018) Although some treatments with synthetic hormones can be administered to improve or

compensate for the natural decrease in GH production, which occurs due to advancing age, these must be carried out under medical supervision in order to avoid unfavorable side effects (Caputo *et al.*, 2021).

Despite the importance of GH, with advancing age, this decrease in production is associated with a series of alterations in the body, such as decreased muscle mass, bone loss, and body weight gain (Roelfsema *et al.*, 2018). Strength training has been identified as a way to stimulate the release of GH and other anabolic factors, increasing muscle mass and strength. For this reason it is important to carry out

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research to understand how strength training associated with GH can influence morphological and health aspects, especially with advancing age (Magalhães & Ortêncio Filho, 2006).

Furthermore, studies with aged rats can provide important information about the impact of strength training (ST) on skeletal muscles during aging, and thereby lead to improvements in the health and well-being of older people, preventing health implications (González-Badillo & Sánchez-Medina, 2010; Pimenta *et al.*, 2019).

Therefore, the objective of the current study was to associate strength training with GH and analyze the responses to the intervention in the body composition and skeletal muscle tissue of aged rats.

## MATERIAL AND METHOD

**Animals.** Forty male animals (*Rattus norvegicus albinus*) aged 14 months (Aged) of the Wistar lineage were used, which were kept in groups of 4 animals per box (polyethylene), with a controlled ambient temperature of (22±2 °C) and luminosity (twelve-hour light cycle / dark), with free access to water and food (food for laboratory rats - SupraLab).

The study was developed in accordance with the ethical standards and principles of animal experimentation, after approval by the Ethics Committee for the use of animals (CEUA- 5682).

**Experimental Protocol.** The animals were randomly distributed into four groups: control (C) [n=10], GH control (GHC) [n=10], strength training (T) [n=10], and strength training with the application of GH (GHT) [n=10].

The animals in the T and GHT groups were subjected in advance to a period of adaptation to the liquid medium and equipment (1x10 jumps; 2x10 jumps; 3x10 jumps), for one week, with progressive increases in overload and duration. The animals in all experimental groups were weighed every training day, before each training session, in order to control the training load of the T and GHT groups (Castoldi *et al.*, 2023).

**C Group:** the animals remained free in their boxes, with *ad libitum* access to water and food.

**GHC Group:** the animals also remained free in their boxes, with *ad libitum* access to water and food; however, at the start of each training session, 0.2 IU per kilogram of body mass (0.067 mg/Kg) was administered (Castoldi *et al.*, 2020).

**T Group:** the animals also remained free in their boxes, with *ad libitum* access to water and food, but performed training consisting of four sets of 10 jumps, three times a week, on non-consecutive days (Mondays, Wednesdays and Fridays), in a cylindrical polyvinyl chloride (PVC) container, specially modified for jumping in water, with a depth appropriate to the animals' length (Fig. 1a). Between each series of jumps, a 1-minute break was given, monitored using a stopwatch. The overload used corresponded to 50 % of the body mass of each animal (Fig. 1b) and was accommodated in the anterior region of the thorax (torso) using a specific vest, made for this type of training (Castoldi *et al.*, 2017).

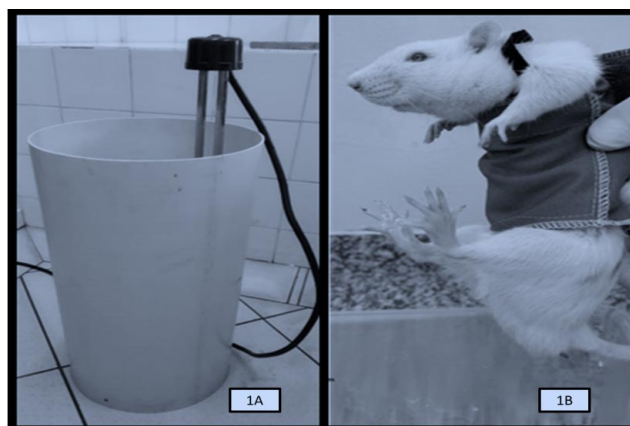


Fig. 1. Scheme illustrating the location of the strength training (1a) and a vest with weight overload and accommodation on the animal (1b).

**GHT Group:** the animals were subjected to the same procedures as the T group. However, as in the case of the GHC group, at the beginning of each training session, 0.2 IU per kilogram of body mass (0.067 mg/kg) was administered (Castoldi *et al.*, 2020). Immediately after application, the animals were induced to perform the weight training protocol.

The training period was four weeks (Fig. 2). Seventy-two hours after the final exercise session, the animals were anesthetized with the combination of two anesthetics, ketamine hydrochloride and xylazine hydrochloride, at 40 mg/kg of body weight, injected intraperitoneally (Marcelo *et al.*, 2021).

**Adipose tissue.** To weigh the adipose tissue, a Marte Científica® analytical scale (model AD500, São Paulo, Brazil) was used. In this case, the visceral fat of each animal was measured.

**Body mass index.** The Body Mass Index (BMI) was calculated using the formula of Novelli and collaborators (Novelli *et al.*, 2007; Machado *et al.*, 2014).

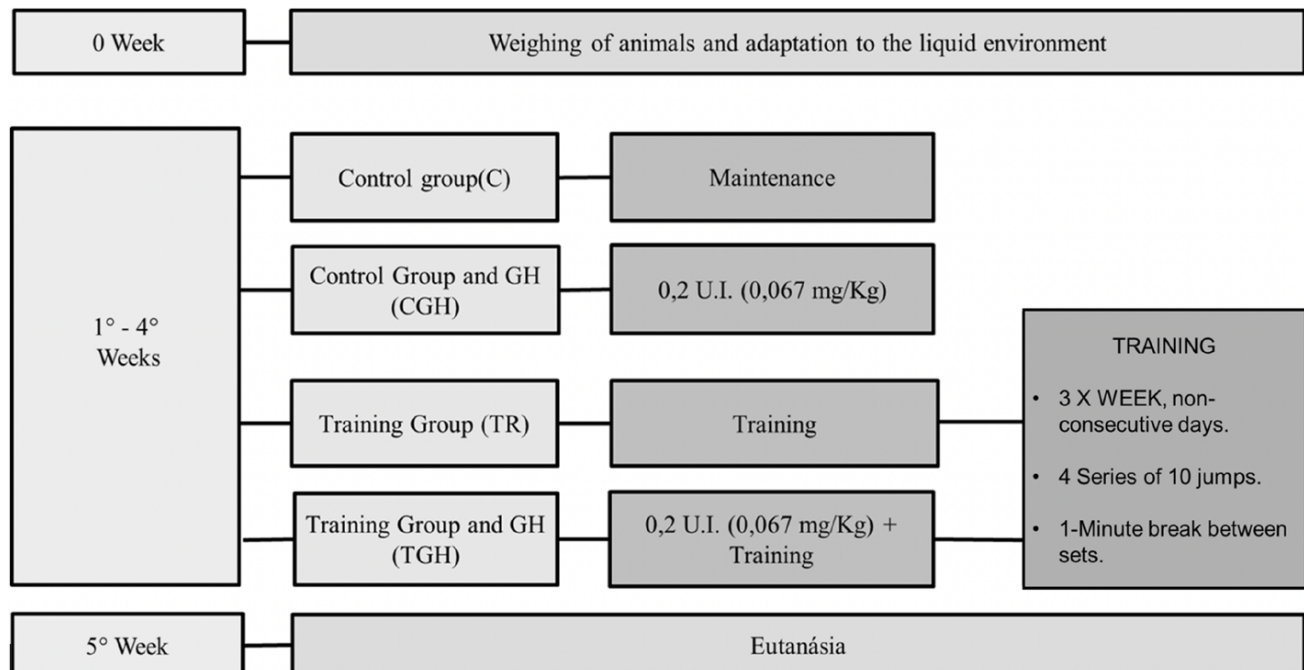


Fig. 2. Time periods and activities developed during the experiment.

**Lee Index.** The Lee index was calculated in all animals, using the relationship between the cubic root of body weight (BW), divided by the snout-coccyx length (CFC) (Novelli *et al.*, 2007).

**Striated skeletal muscle.** Samples were collected from the Medial Gastrocnemius muscle of the right limb, which has specific functions in the mechanics of the jumping movement. After this procedure, the muscle tissue was immersed in n-hexane cooled in liquid nitrogen using the unfixed tissue freezing method, and subsequently stored in an ultra-low temperature freezer (-80 °C). The 5 µm sections were produced transversely, in the ventral portion of the muscle in a cryostat microtome at -20 °C, collected on slides and then stained with hematoxylin-eosin (HE) for general analysis of the structure of the muscles.

**Muscle analysis.** The sections subjected to staining and histochemical reactions were observed in normal and polarized light and photomicrographed under a Nikon x microscope, model yh550s. To analyze the images, an “Infinity 1” camera was used. To determine the average cross-sectional areas (AST), interactive markings were made with the software (AUXIOvisionrel4.8 – Carl Zeiss x and Nis-elements d3.0x -sp7-nikonx). In total, 100 muscle fibers were observed in each slide, according to the established protocol (Castoldi *et al.*, 2020).

**Statistical analysis.** After obtaining the data, the Shapiro-

Wilk normality test was performed. In the case of the “body mass” variable, repeated measures ANOVA was used, with the Bonferroni post-test. For the variables of BMI, Lee index, and visceral adipose tissue, analysis of variance ANOVA with Tukey's post-test was used.

For the analysis of the “cross-sectional area”, the Kruskal-Wallis test with Dunn's post-test was used. All procedures adopted a significance value of 5% ( $p < 0.05$ ). The calculations were carried out with the statistical package program (SPSS 22.0 for Windows - IBM®).

## RESULTS

In the present study, no statistical differences were found for the variables body mass, visceral fat weight, body mass index, and Lee Index ( $p > 0.05$ ) (Fig. 3).

The gastrocnemius muscle presented different morphologies. It was possible to verify differences in the cross-sectional area between the different groups, indicating muscular hypertrophy (Fig. 4).

Figure 5 presents the analyses of the cross-sectional area of the muscles of the animals in the analyzed groups. Significantly higher median values were observed for animals in groups CGH, T, and GHT in relation to animals in group C. The GHT groups presented higher median gastrocnemius muscle cross-section values than those obtained for the T group.



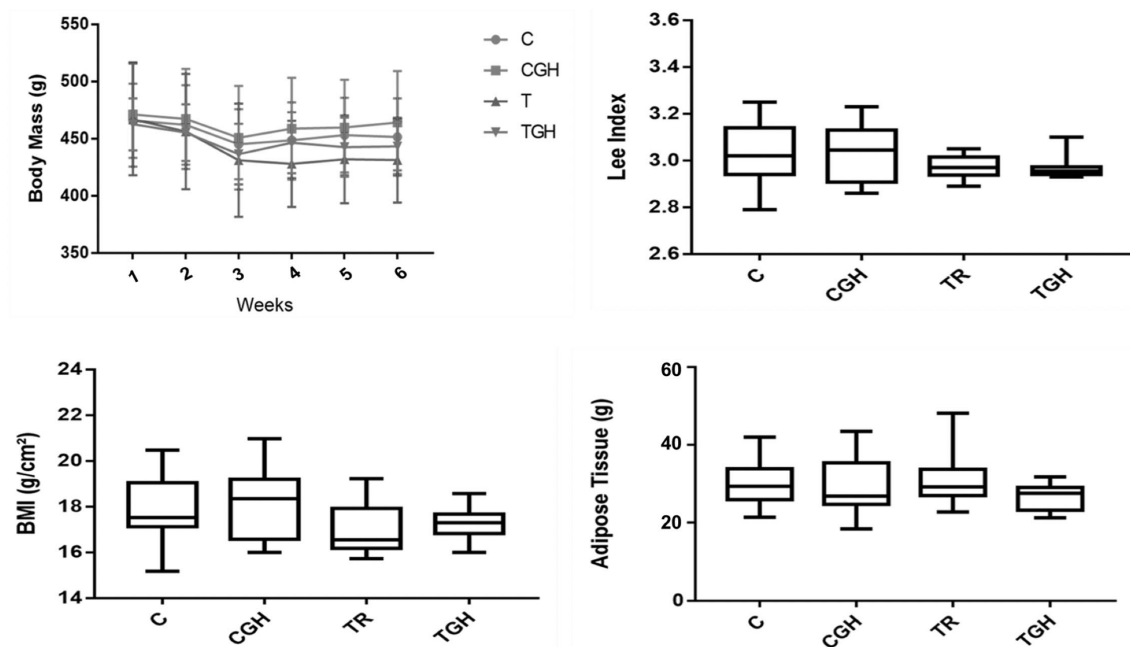


Fig. 3. Analysis of body mass (g), body mass index (g/m2), Lee index, and visceral adipose tissue (g) of the groups studied; Legend: (C) Control Group; (GHC) GH Control Group; (T) Training Group; (GHT) GH Training Group.

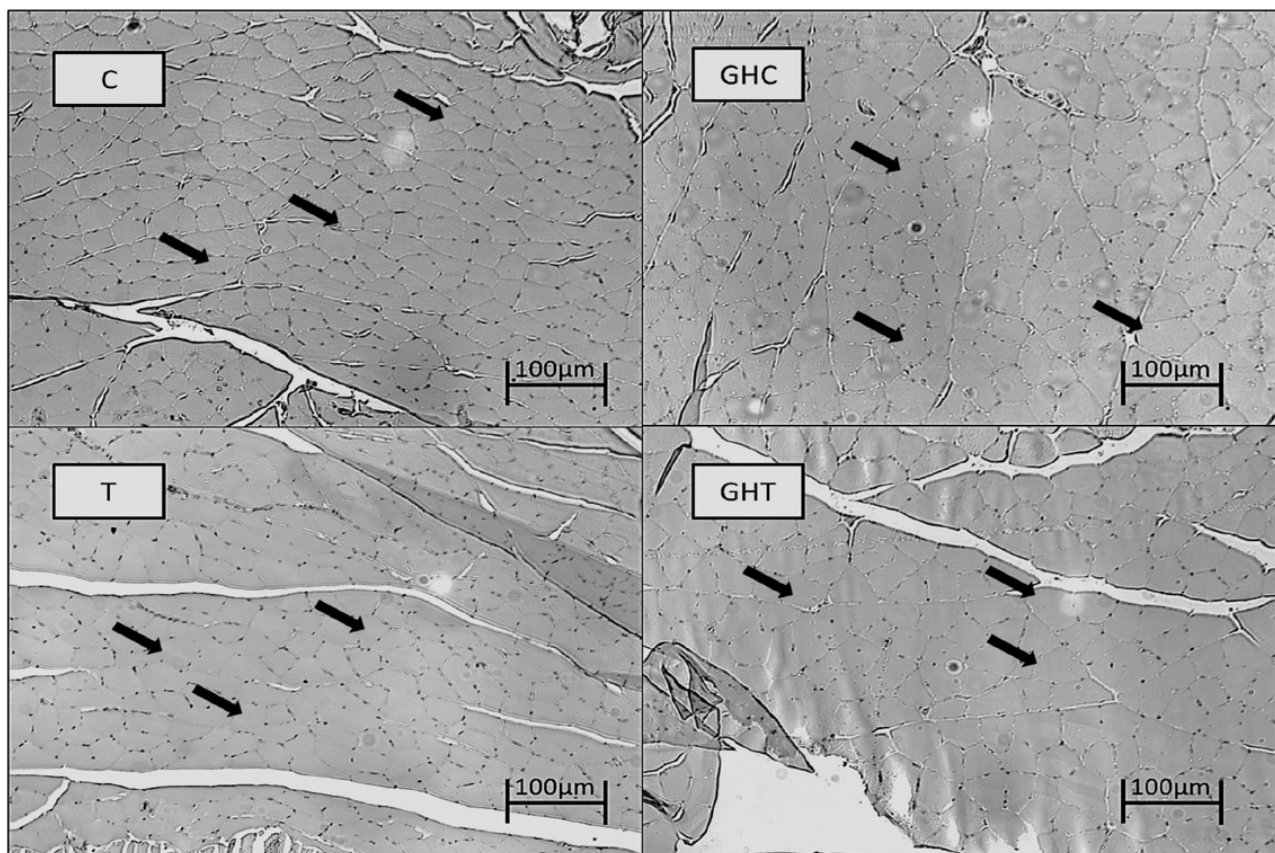


Fig. 4. Histological sections stained in HE on a 100µm scale. Arrows indicate muscle fibers. Legend: (C) Control Group; (GHC) GH Control Group; (T) Training Group; (GHT) GH Training Group.

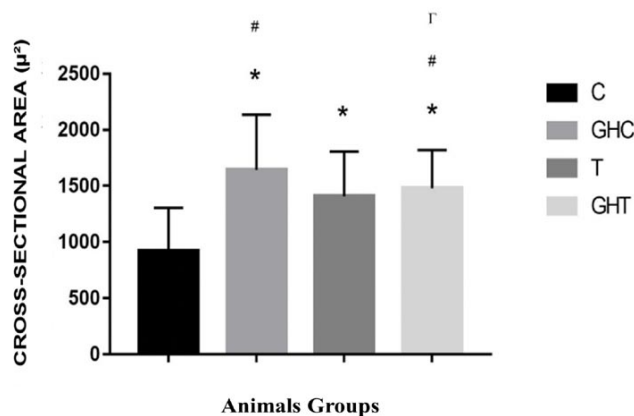


Fig. 5. (\*) Difference compared to the C group; (#) Difference compared to the GHC group. T; (r) Difference compared to the T group. Significance values  $p < 0.05$ . Scale of 100µm. Kruskal-Wallis test and Dunn post-test. Significance of 5% ( $p < 0.05$ ). Legend: (C) Control Group; (GHC) GH Control Group; (T) Training Group; (GHT) GH Training Group.

## DISCUSSION

After the analysis performed in the present study, considering the use of the substance somatropin (GH) and strength training (ST), no difference was observed for the variables body mass, BMI, the Lee Index, and visceral tissue between the groups. However, there was an increase in the cross-sectional area of the gastrocnemius muscle in all groups, which characterizes muscular hypertrophy.

In agreement with our findings, Bronstein (2003), showed that in addition to aforementioned benefits, GH can present conflicting and inconclusive results or even negative effects in the treatment. These controversies may be related to age, the training model, and the amount of hormone administration used.

This finding is similar to that found in the protocol used in our study, in which the administration of GH showed higher values than the control group (GHC), when compared to the isolated training (T), and GH associated with training groups (GHT).

However, the benefits of GH administration are described in the literature, where Ullman & Oldfors (1989), studied the effects of administering 4UI of GH on the muscles of rats and observed hypertrophy in skeletal muscles and in the type of muscle fibers.

The diameter and type of muscle fibers related to training with GH use can also be seen in the study of Castoldi *et al.* (2017), in which the animals performed strength training and showed an increase in the diameter of muscle

fibers. Our findings corroborate some studies suggesting that treatment with GH slows down the loss of muscle mass during aging (Reis & Calsolari, 1997; Castoldi *et al.*, 2017). Strength training is considered a form of treatment against the decrease in muscle mass loss with age, as it improves body composition and bone mass. Furthermore, training associated with GH leads to improvements in skeletal muscle functions and structures (Gharahdaghi *et al.*, 2021), and also helps to stimulate the secretion of hormones, both growth (GH) and testosterone (male hormone) (Jambassi Filho *et al.*, 2015).

In the present study, the association of GH with strength training promoted an increase in the area of myocytes in skeletal muscle. This fact may be associated with the results of Cianforlini *et al.* (2020), who analyzed the treatment of muscle injuries in rats using GH and found a potential effect of GH with regard to the repair and regeneration of muscle tissue, through the activation of satellite cells.

Several studies have indicated that the controlled use of GH does not enhance gains in strength and muscle mass in individuals. However, some individuals use this hormone in combination with other types of substances. For this reason, the administration of GH is controlled by national and international agencies, however, individuals still take risks in administering such substances, trying to optimize gains (Holt & Ho, 2019).

A study of Rønnestad *et al.* (2011), applied strength training to individuals and measured GH, testosterone, and cortisol at different times and combinations of training. Following the principle that strength training is a GH stimulator, individuals from different disciplines use this type of training, with the intention of improving gains. In high-performance sports, the use of anabolic hormones is increasingly common, even with increasingly strict anti-doping controls (García-Arnés & García-Casares, 2023).

It is important to highlight that the use of recombinant hormones should only be performed under medical guidance and after careful assessment of the risks and benefits. Furthermore, lifestyle changes, such as regular physical activity and exercise, and a balanced diet, can also help to improve health in older adults and compensate for the natural loss of GH with age (Huayllas *et al.*, 2001).

The present study collaborates with the literature, reinforcing that strength training associated with GH may not lead to alterations in the body composition of aged rats.

In short, the results of the current study

demonstrate that the use of GH, together with the ST protocol presented, did not lead to anthropometric differences in aged animals. Although strength training associated with the use of GH resulted in hypertrophy of muscle cells in aged rats, new studies are necessary to better understand the responses in the muscle tissue of healthy adult and aged animals, associated with different forms of training, hormonal dosages, or combinations of hormones.

## CONCLUSION

The current study demonstrated that strength training associated with GH administration in aged rats did not lead to alterations in body mass, BMI, Lee index, and visceral adipose tissue, however it promoted hypertrophy of skeletal muscle cells.

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**RESUMEN:** El objetivo de este estudio fue investigar los efectos de la hormona del crecimiento (GH) asociada a un protocolo de entrenamiento de fuerza (EF) sobre la composición corporal y el tejido muscular en ratas de edad avanzada. Se utilizaron 40 ratas Wistar de edad avanzada, divididas en cuatro grupos: Control (C) [n=10], Control con GH (GHC) [n=10], Entrenamiento de Fuerza (T) [n=10] y Entrenamiento de Fuerza (n=10) con Hormona del Crecimiento (GHT) [n=10]. El período de intervención fue de cuatro semanas y consistió en cuatro saltos de agua de diez saltos, realizados tres veces por semana, en días no consecutivos, con una sobrecarga del 50 % de la masa corporal. Los grupos GHC y GHT recibieron 0,2 UI por kilo de masa corporal (0,067 mg/kg) al inicio de cada sesión de entrenamiento. Tras finalizar el experimento, los animales fueron sacrificados mediante una combinación de anestesia y desangrado. Las variables Masa Corporal, Índice de Masa Corporal (IMC) e Índice de Lee (Lee) se midieron y se recolectó tejido adiposo visceral. Además, se retiró el músculo gastrocnemio medial para medir el área de la sección transversal. Se realizó la prueba de normalidad de Shapiro-Wilk. Para la variable “masa corporal”, se utilizó el análisis de varianza ANOVA de medidas repetidas, con el post-test de Bonferroni. Para las variables IMC, índice de Lee y tejido adiposo visceral, se utilizó el análisis de varianza ANOVA con el post-test de Tukey. Para analizar el “área de la sección transversal”, se utilizó la prueba de Kruskal-Wallis con el post-test de Dunn. Todos los procedimientos adoptaron

un valor de significancia del 5% ( $p < 0,05$ ). No hubo diferencias estadísticamente significativas para las variables masa corporal, peso adiposo visceral, IMC e índice de Lee ( $p > 0,05$ ). Se observaron aumentos en el músculo en todos los grupos experimentales ( $p < 0,05$ ). Se concluye que la GH causó hipertrofia muscular, con o sin entrenamiento; sin embargo, no se observaron diferencias en la composición corporal de los animales mayores.

**PALABRAS CLAVE:** Envejecimiento; Hormona del Crecimiento; Entrenamiento de fuerza; Músculo esquelético; Ratas Wistar.

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