



Research Article

**EFFECT OF GROWTH HORMONE ON THE GASTROCNEMIUS MUSCLE:  
ULTRASTRUCTURAL ANALYSIS**

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**Abstract**

The objective of this present study was to identify the ultrastructural changes of the gastrocnemius muscle, promoted by subcutaneous administration of growth hormone. We used 06 rats (Wistar Rats) randomly divided into 02 groups: sedentary rats without administration of GH (RSSH), rats with a sedentary lifestyle with administration of GH (RSCH). The subcutaneous administration of GH occurred in the period of eight weeks after the animals were sacrificed and the transverse section of the gastrocnemius muscle removed and prepared according to routine analysis transmission electron microscopy (TEM). The cuts were performed using Ultramicrotome sorvall Porter Blum MT2 and analyzed in the CM 100 Philips. The results of the administration of GH showed an increase in the diameter of the sarcoplasmic reticulum and accumulation of glycogen. Thus, it was concluded that the administration of GH accompanied causes of morphological alterations of muscle tissue examined, such as the changes in the dimensions and shape of the muscle fibers, increased thickness of the sarcoplasmic reticulum, accumulation of glycogen in the muscle fibers of the muscle gastrocnemius.

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**1. Introduction**

The growth hormone (GH) is produced in the pituitary anterior pituitary. Through, its action the cells in the tissues begin to develop. Its release causes the inhibition of the action of insulin by reducing the use of glucose. Powers *et al.* (1990) reported that GH also increases the mobilization of fatty acids from the adipose tissue to save the glucose from the plasma. The GH plays a

fundamental role in the metabolism of proteins, fats and carbohydrates (Keizer and Rogol, 1990).- + The concentration of circulating GH is a crucial factor for the growth and development of tissues, especially muscle, even if its concentration is not equal in all phases maturational. Exists in multiple isoforms and one, which differs from the produced in the liver (hypertension - IGF-1Ea), seems to be particularly sensitive to mechanical signals and to wear muscle. This isoform is called mechano

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growth factor (MGF). The actions of IGF-1 and MGF stimulate protein synthesis as well as the activation, proliferation and differentiation of cells. (Harridge, 2003). The effects of the administration of this hormone on normal muscle, hypertrophied and atrophied demonstrates the increase in weight and muscular size. Thus, the objective of this study was to identify ciliary ultrastructural changes of the *gastrocnemius* muscle in rats with administration of GH.

## 2. Materials and Methods

For this study, we used six (06) male rats (Wistar  $\pm$  90 days) in the initial phase of the experiment were placed in collective cages (04 animals per cage), where they were kept at room temperature (23 °C) and controlled lighting. The rats were kept in a vivarium of experimental laboratory. The power was controlled through balanced feed and water ad libitum. In animals RSCH were made subcutaneous administrations of 0.2 UI/kg (Taaffe *et al.*, 1994) of growth hormone 3 times per week for eight (08) weeks animals were sacrificed after this period.

The transverse section of the *gastrocnemius* muscle was removed for analysis Transmission electron microscopy (TEM). The fragments of the transverse section of the muscle were fixed in glutaraldehyde 2.5 % in cacodylate buffer solution at 0.1 m during 2 hours. After the end of this interval, time fragments were washed three times for 5 minutes in the phosphate buffer 0.1 M and then will post - fixed in osmium tetroxide 1 % for 2 hours in the dark. Then, they were washed in distilled water and placed for 2 hours in uranyl for that after the end of that time the fragments were dehydrated in acetone.

After dehydration, the material was placed in plastic resin - acetone for 24 hours and then in resin so that, finally, there was the inclusion of the same. The material was cut in the Ultramicrotome sorvall Porter Blum MT2 and then contrasted with uranyl acetate and lead citrate. Thus the material after contrasted was analyzed and photographed by Transmission electron microscope CM 100 Philips.

## 3. Results and Discussion

The experiment showed alterations in morphology of the structures of the muscle fiber. When it was observed the RSCH in relation to RSSH noticed an increase in the thickness of the sarcoplasmic reticulum (Figure 1-A and 1-B). The growth hormone (GH) has its physiological effect toward the stimulation of growth and somatic development of tissues, thus taking the anabolic effect. To this effect may occur it is connected to protein carriers called GHBPs (GH bindings proteins), which can modulate the biological activity of the hormone (Dove *et al.*, 2001).

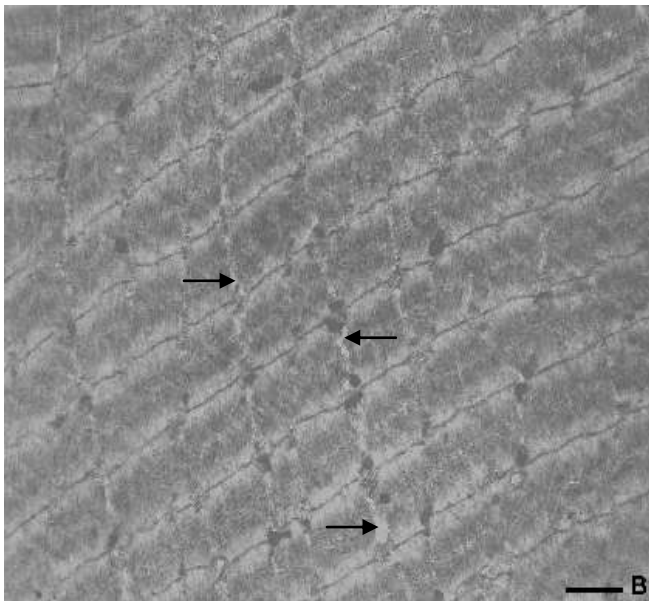
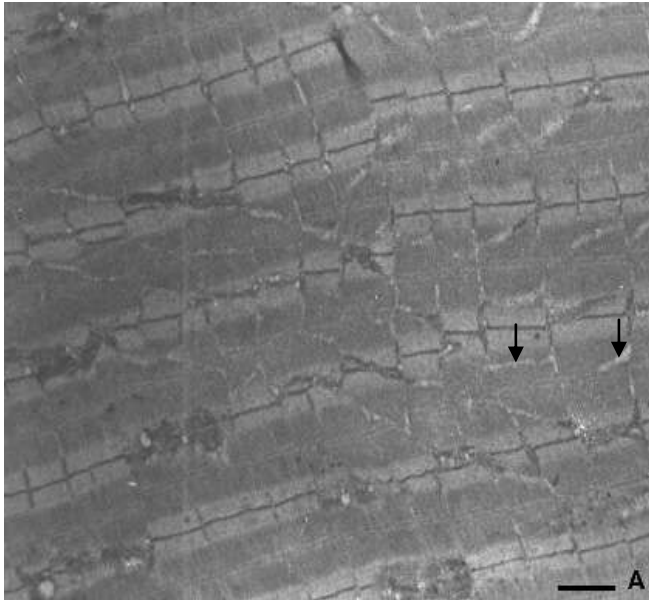
An increase in muscle mass is one of the anabolic effects of GH (Clarkson, 1991). The GH is a hormone anabolic steroids can increase muscle mass (Salomon *et al.*, 1991). In humans, it is known that the administration promotes an increase of total body mass and protein synthesis (Marcus *et al.*, 1990; Russell *et al.*, 1996; Butterfield *et al.*, 1997) and increases lean mass and decreases fat mass (Crist *et al.*, 1988; Richelsen *et al.*, 1994; Holloway *et al.*, 1994; Lange *et al.*, 2000; Rudman *et al.*, 1990; Jørgensen *et al.*, 1989; Yarasheski *et al.*, 1995).

An increase in the thickness of the sarcoplasmic reticulum (T-tubules) was observed in this study (Figure 1) may demonstrate that the increase of fluid retention the intercellular of muscle fiber. The use of GH causes, on body composition of the fluid retention in muscle tissue (Marcus *et al.*, 1990). According to studies of Lange *et al.* (2002) the administration of GH does not promote an increase in the cross-sectional area of muscle or the muscle hypertrophy.

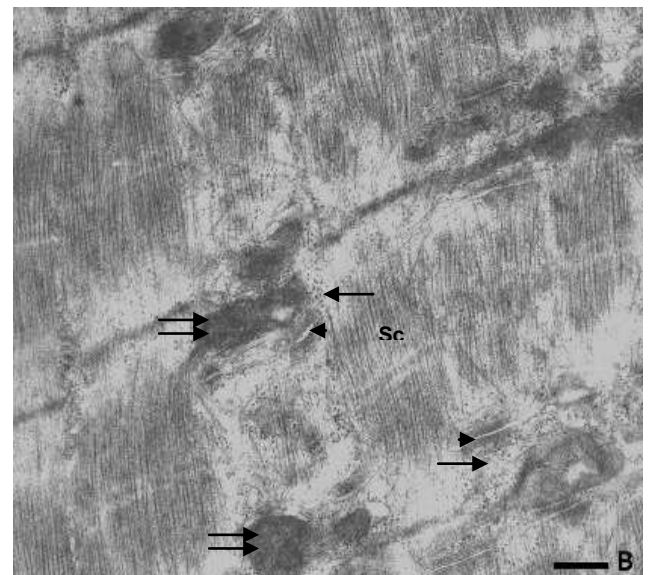
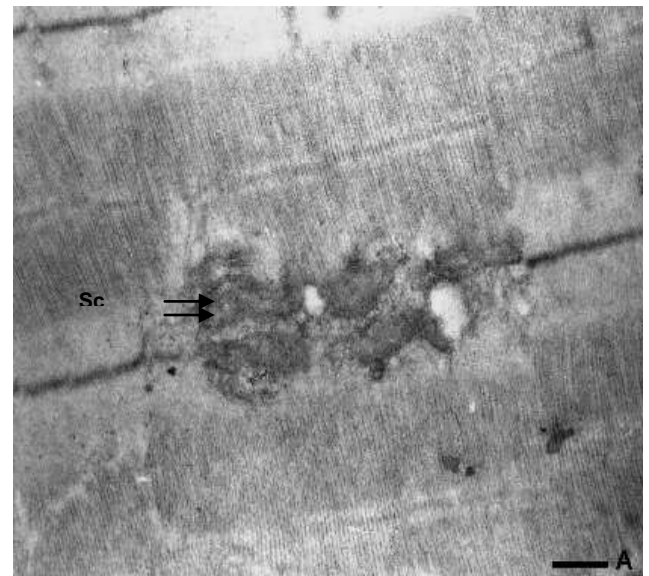
The main effects of GH are increased protein synthesis, decreased degradation of proteins, increasing the mobilization of lipids, the decreased glucose oxidation and increasing the storage of glycogen (Kopple, 1992; Revhaug and Mjaaland, 1993).

This buildup of glycogen was observed in the results of the analysis of MET (Figure 2) demonstrated the buildup of glycogen. Therefore, we can conclude that the GH promotes an increase

in the thickness of the T-tubeles and accumulation of glycogen, as well as, ciliary ultrastructural changes in muscle fiber (morphological disarrangement of the myofilaments).



**Fig - 1: Electronic micrographs of muscle fiber of RSSH (A) and RSCH (B) demonstrating the increase in the diameter of the sarcoplasmic reticulum (arrow) (Scale = 1, 5  $\mu$ m).**



**Fig - 2: Electronic micrographs of muscle fiber of the muscle gastrocnemius of RSSH (A) and RSCH (B), the sarcomere (Sc) disorganized and mitochondria more eletrondensas (double arrow), buildup of glycogen (arrow) next to the triad (tip of the arrow) are best viewed at RSCH. (Scale = 0, 5  $\mu$ m)**

#### 4. References

- 1) Butterfield GE, Thompson J, Rennie MJ, Marcus R, Hintz RL and Hoffman AR. 1997. Effect of rhGH and rhIGF-I treatment on protein utilization in elderly women. *American Journal of Physiology*, 272: E94 – E99.

- 2) Clarkson PM. 1991. Nutritional ergogenic aids: chromium, exercise and muscle mass. *International Journal of Sports and Nutrition*, 1 (3): 289 - 293.
- 3) Crist DM, Peake GT, Egan PA and Waters DL. 1988. Body composition response to exogenous GH during training in highly conditioned adults. *Journal of Applied Physiology*, 65: 579 – 584.
- 4) Harridge, SD. 2003. Ageing and local growth factors in muscle. *Scand Journal of Medical Science and Sports*, 13 (1): 34 - 39.
- 5) Holloway L, Butterfield G, Hintz RL, Gesundheit N and Marcus R. 1994. Effects of recombinant human growth hormone on metabolic indices, body composition, and bone turnover in healthy elderly women. *Journal of Clinical Endocrinology and Metabolism*, 79: 470 – 479.
- 6) Jørgensen JO, Pedersen SA, Thuesen L, Jørgensen J, Ingemann-Hansen T, Skakkebaek NE and Christiansen JS. 1989. Beneficial effects of growth hormone treatment in GH-deficient adults. *Lancet*, 1: 1221 – 1225.
- 7) Keizer HA and Rogol AD. 1990. Physical exercise and menstrual cycle alterations: What are the mechanisms? *Sports Medicine*, 10 (4): 218 - 235.
- 8) Koppole JD. 1992. The rationale for the use of growth hormone or insulin-like growth factor I in adult patients with renal failure. *Miner Electrolyte Metabolism*, 18: 269 - 275.
- 9) Lange KHW, Isaksson F, Juul A, Rasmussen MH, Bulow J and Kjaer M. 2000. Growth hormone enhances effects of endurance training on oxidative muscle metabolism in elderly women. *American Journal of Physiology, Endocrinology and Metabolism*, 279: E989 – E996.
- 10) Lange KHW, Andersen JL, Beyer N, Isaksson F, Larsson B, Rasmussen MH, Juul A, Bülow J and Kjaer M. 2002. GH administration changes myosin heavy Ca<sup>2+</sup> isoforms in skeletal muscle but does not augment muscle strength or hypertrophy, either alone or combined with resistance exercise training in healthy elderly men. *Journal of Clinical Endocrinology and Metabolism*, 87: 513 - 523.
- 11) Marcus R, Butterfield G, Holloway L, Gilliland L, Baylink DJ, Hintz RL and Sherman BM. 1990. Effects of short term administration of recombinant human growth hormone to elderly people. *Journal of Clinical Endocrinology and Metabolism*, 70: 519 – 527.
- 12) Powers SK and Howley ET. 1990. Exercise Physiology: Theory and application to fitness and performance. Brown & Benchmark: Madison, Wisconsin, Iowa, 94 - 95.
- 13) Revhaug A and Mjaaland M. 1993. Growth hormone and surgery. *Hormone Research*, 40: 99 - 101.
- 14) Richelsen B, Pedersen SB, Børglum JD, Møller-Pedersen T, Jørgensen J and Jørgensen JO. 1994. Growth hormone treatment of obese women for 5 wk: effect on body composition and adipose tissue LPL activity. *American Journal of Physiology*, 266: E211 – E216.
- 15) Rudman D, Feller AG, Nagraj HS, Gergans GA, Lalitha PY, Goldberg AF, Schlenker RA, Cohn L, Rudman IW and Mattson DE. 1990. Effects of human growth hormone in men over 60 years old. *English Journal of Medicine*, 323: 1 – 6.
- 16) Russell - Jones DL and Umpleby M. 1996. Protein anabolic action of insulin, growth hormone and insulin-like growth factor I. *European Journal of Endocrinology*, 135: 631 – 642.
- 17) Salomon F, Cuneo R and Sönksen PH. 1991. Growth hormone and protein metabolism. *Hormone Research*, 36 (Suppl 1): 41 – 43.
- 18) Yarasheski KE, Zachwieja JJ, Campbell JA and Bier DM. 1995. Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *American Journal of Physiology*, 268: E268 – E276.