

IGF-1 MINIMIZES THE HARMFUL EFFECTS OF DISUSE ON RAT SOLEUS MUSCLE

CARLOS ALBERTO DA SILVA¹, CARLOS PETERMANN³, KARINA MARIA CANCELLIERO², JOÃO LUIZ QUAGLIOTTI DURIGAN²,
MARIA LUÍZA OZORES POLACOW¹

ABSTRACT

Objective: To evaluate the effect of IGF-1 treatment on the morphological and metabolic profile of the soleus muscle submitted to ankle joint immobilization. **Methods:** Wistar rats were divided into 3 groups (n = 6): control (C), immobilized (I) and immobilized treated with IGF (I + IGF; 40mg/kg) for 7 days. **Results:** Immobilization led to a reduction in the weight (34%), glycogen content (31.6%) and area of muscle fibers (44%), and increased the density of connective tissue (216%). Also,

the IGF-1 increased the glycogen by 234.6% compared to I, minimized the area of muscle fibers by 33.7% and increased the connective tissue by 76% compared to C (p <0, 05). **Conclusions:** Treatment with IGF has an anti-catabolic action, which can promote faster recovery in the post-immobilization phase. **Level of Evidence:** Level II, prospective comparative study.

Keywords: Insulin-like growth factor I. Immobilization. Glycogen. Skeletal, muscle.

Citation: Silva CA, Petermann C, Cancelliero KM, Durigan JL, Polacow ML. IGF-1 minimizes the harmful effects of disuse on rat soleus muscle. *Acta Ortop Bras.* [online]. 2011;19(3):137-40. Available from URL: <http://www.scielo.br/aob>.

INTRODUCTION

Several experimental models are proposed with the intention of identifying the events triggered by muscle disuse and generation of the precursor processes of atrophy, with denervation, tenotomy, prolonged bed rest, suspension of the animal's hind limbs or unilateral immobilization of limbs by orthosis meriting special emphasis.¹

Although often necessary, immobilization presents side effects such as atrophy of the muscle fibers, intramuscular fibrosis, loss of muscle extensibility and limitation of joint movement.² Various studies demonstrated the occurrence of major modifications in the homeostasis of the skeletal muscle concomitant to muscle atrophy, compromising the synthesis of myofibrillar or non-fibrillar proteins, affecting the contractile dynamics as well as the effectiveness of the metabolic pathways.²

Muscle disuse induces insulin resistance and a catabolic state in the skeletal muscles of humans,³ yet it is still not clear how chronic muscle disuse or immobilization alter insulin signaling.⁴ Aiming to minimize the events triggered by muscle disuse, various techniques have been used in an attempt to improve the homeostatic conditions of muscle fibers, with special emphasis on neuromuscular electrical stimulation, drugs such as clen-

buterol and supplements such as vanadyl sulfate, creatine, glutamine and CGT (creatine, glutamine and taurine).⁵ In literature, there are studies related to the signaling pathway whereby the mechanical stimulus and the activity of IGF-1 (growth factor similar to insulin 1) lead to functional changes in the satellite cells, quantity of muscle DNA, quantity of muscle protein, muscle mass and muscle fiber area.⁶ According to Barton-Davis *et al.*,⁷ the local expression of IGF maintains muscle mass and strength during aging. Moreover, in at least two models of disease in animal experiments, amyotrophic lateral sclerosis and Duchenne muscular dystrophy, local treatment with IGF proved to delay the onset and progression of the disease and to reduce its severity.⁸ However, there are few studies demonstrating the effects of IGF-1 in the acute phase of immobilization.

The hypothesis of this study was that treatment with IGF-1 minimizes the harmful effects promoted by soleus muscle immobilization. Thus the objective of this study was to evaluate the action of IGF treatment on the soleus muscle in the condition of joint immobilization, under the aspects of weight, glycogen content, muscle fiber area and density of intramuscular connective tissue.

All the authors declare that there is no potential conflict of interest referring to this article.

1- Physiotherapy – FACIS / UNIMEP – Piracicaba – SP.

2- Universidade Federal de São Carlos, SP, Brazil.

3- Universidade Estácio de Sá, Belo Horizonte – MG, Brazil

Study conducted at Universidade Metodista de Piracicaba - UNIMEP.

Mailing address Universidade Metodista de Piracicaba - Faculdade de Ciências da Saúde - PPG-FISIOTERAPIA. Rodovia do Açúcar, Km 156 – Taquaral, Piracicaba – São Paulo - Brazil CEP 13400-911 Email: grupopesquisacas@yahoo.com.br

METHODOLOGY

Wistar rats (3 to 4 months) were treated with feed and water ad libitum and kept under controlled biotery conditions (23 ± 2 °C; 24hr light/dark photoperiodic cycle). The work was approved by the Animal Experimentation Ethics Committee of Universidade Federal de Sao Carlos 015/2006.

The animals were randomly split into 3 experimental groups (n=6): control, immobilized and immobilized treated with IGF. For immobilization, the rats were anesthetized (sodium pentobarbital, 40mg/Kg of body weight) and the acrylic resin orthosis was adapted with the ankle kept in neutral position (90°).⁵ IGF-1 (Sigma, code I2656) was administered subcutaneously in the daily dose of 50mg/Kg⁹. The period both of immobilization and of treatment was seven days.

After the experimental period, the animals were euthanized by anesthetic overdose and the soleus muscle was removed and sent for analysis of glycogen content, muscle weight, muscle fiber area and density of the intramuscular connective tissue. To determine muscle glycogen the participants followed the proposal of Siu *et al.*¹⁰ that consists of the digestion of muscle samples in hot KOH, passage through ethanol, centrifugation and submission to acid hydrolysis in the presence of phenol. Analytical scales were used to assess wet muscle weight.

For morphometry, the tissue was fixed in 10% formaldehyde and processed for paraplast embedding. Non-serial transverse sections in a thickness of 7µm from the ventral portion of the soleus muscle were stained by Hematoxylin-Eosin (H:E).

Five cross sections were selected and five areas were selected for each one of them, using an image capturing and analysis system consisting of Image Pro-plus 4.0 software (Media Cybernects), and a digital camera (JVC) coupled to a microscope (Zeiss) with integration to a microcomputer. All the images were captured with a 10x lens and eyepiece.

The cross section areas of 375 soleus muscle fibers per animal were determined as follows: 15 fibers by area, with five areas by section, and five sections by animal. For the choice of fibers to be analyzed the participants used a reticle with squares measuring 12.100µm² in area, containing 20 straight line intersections, and t considered the fibers that coincided with 15 intersections in a random manner

The intramuscular connective tissue area density analysis was quantified using the point counting planimetry system,⁵ by means of a reticle with 2.500µm² squares containing 56 straight line intersections. Coincident points in the endomysium and perimysium were counted in five areas per section, with five sections per animal, totaling 1.400 points per animal. The relative area of the connective tissue (area density) was calculated by dividing the sum of the number of coincident points at the straight line intersections on the connective tissue (endomysium and perimysium) by the total number of points.

The Kolmogorov-Smirnov normality test and homocedasticity test (Bartlett criterion) were applied initially for the statistical data analysis. Afterwards, the participants conducted the variance analysis (ANOVA) followed by Tukey's test. A critical level of 5% ($p < 0.05$) was set in all the calculations.

RESULTS

Immobilization promoted a significant reduction ($p < 0.05$) in the soleus muscle glycogen reserves of 31.6%, as well as a 34% reduction in weight. A reduction of 44% ($p < 0.05$) was also observed in the area of the muscle fibers with an increase of 216% ($p < 0.05$) in the density of the intramuscular connective tissue when compared to the control group. (Table 1 and Figure 1) The IGF-1 treatment produced a significant increase ($p < 0.05$) in glycogen content (234.6%), without any change in muscle weight ($p > 0.05$) in relation to the immobilized group. In relation to the muscle fiber area, this was reduced by 33.7% ($p < 0.05$) and the area density of the connective tissue was increased by 76% ($p < 0.05$) when compared to the control group, yet with lower values ($p < 0.05$) than the immobilized group. (Table 1 and Figure 1)

Table 1. Glycogen content (mg/100mg), weight (mg), area of fiber (µm²) and area density of the connective tissue (%) of the soleus muscle of the control (C), immobilized 7 days (I) and immobilized treated with IGF 7 days (I+IGF) groups. N=6, mean value ± SEM.

Groups	Glycogen	Weight	Area of fiber	Connective tissue
C	0.35 0.07	125.5 1.1	2647 97	7.9 1.9
I	0.26 0.03	81 0.6*	1301 52*	28.8 0.9*
I + IGF	0.91 ± 0.05*†	77 ± 0.7*	1706 93*†	15.54 1.3*†

* Differs significantly ($p < 0.05$) from the control group

† Differs significantly ($p < 0.05$) from the respective immobilized group

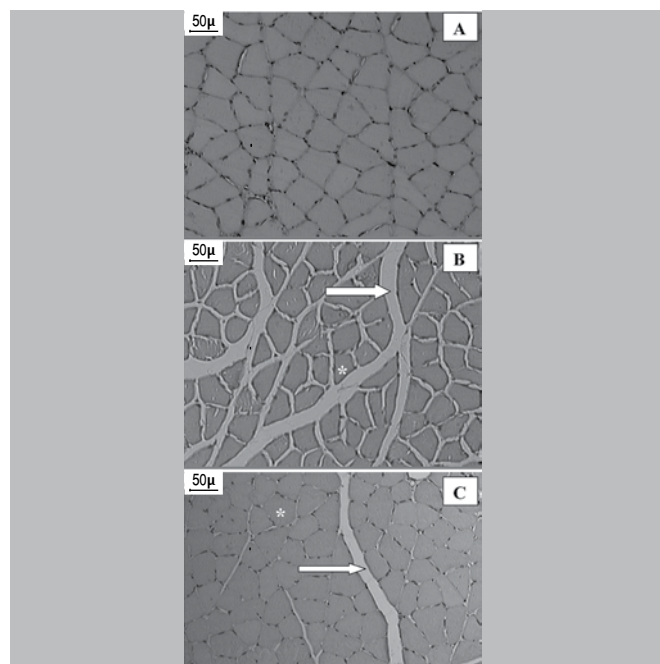


Figure 1. Soleus muscle fibers (Hematoxylin-Eosin, 100x). A- Control: Fibers with normal appearance (*) and hexagonal format. B- Immobilized: Presence of muscle fiber atrophy (*) and increase of connective tissue (arrow). C- Immobilized treated with IGF: Presence of muscle fiber atrophy (*) and increase of connective tissue (arrow), yet with lower values than the immobilized group.

DISCUSSION

This study was the pioneer in demonstrating the effect of IGF-1 on the immobilized soleus muscle after seven days on metabolic and morphometric variables. Our results demonstrated that IGF-1 can be used in the initial phase of muscle disuse, since it minimizes the reduction of muscle glycogen content, area of the fibers, and the increase of area density of the connective tissue. Such fact suggests a possible protective effect promoted by this growth factor against the atrophy observed in patients submitted to muscle disuse.

Muscle disuse provoked by conditions of lengthy confinement to bed, use of orthosis, fixation of limbs or microgravity induces the state of insulin resistance and consequently a catabolic state in the skeletal muscles.⁵ To this effect, Ploug *et al.*¹¹ refined the understanding of muscular metabolic behavior during a short period of immobilization (48 hours) in demonstrating significant decrease in the population of GLUT1 and GLUT4 transporters in the red fibers. The reduction in the number of insulin receptors and of the kinase activity of the receptor suggests, in a short period of time, resistance to glucose transport that is specific to the type of fiber and selective to the contractile process and to the action of insulin. This study demonstrated an expressive reduction in the glycogen reserves of the soleus muscle, besides a reduction in muscle weight, thus corroborating the hypothesis of multifactorial expression in the metabolic control of the skeletal musculature.

The use of IGF-1 as a therapeutic agent merits special attention, due to the molecule's ability to promote an increase in the tissue uptake of glucose, besides expressing powerful anabolic action.¹² It is known that the IGF-1 peptide exerts a potent trophic effect, participating efficiently in the development of the skeletal musculature and of modulation in the tissue uptake of metabolizable substrates, especially glucose.¹³ Although there are reports in literature proving that in the presence of this peptide there is a rise in the tissue uptake of glucose, this is less intense if compared to insulin, since the latter molecule is from 5 to 10 times less potent.¹⁴

It has been observed that glycogen reserves are directly linked to the endurance performance of the skeletal musculature, thus improving energy availability and delaying fatigue.⁴ Accordingly, this study demonstrated that IGF-1 promoted an increase of glycogen reserves of the immobilized soleus muscle. This fact may be related to the glycogen action of IGF triggered by the activation of specific receptors present in the muscle fiber membrane, which signal by promoting a rise in glucose uptake, translocation of GLUT4 from cytosolic reservoirs towards the membrane, thus favoring an increase in hexose uptake besides activation of the glycogen synthase enzyme.¹⁴

Moreover, it was demonstrated that IGF-1 treatment also increases insulin sensitivity, a phenomenon known as "insulin-saving IGF effect". The exact mechanism whereby IGF-1

increases insulin sensitivity remains unknown, but it may be involved in direct effects of IGF-1 on insulin target tissues, as well as an inhibition mediated by feedback from the IGF-1 in secretion of the growth hormone.¹⁵

As regards the morphometric analysis of the soleus muscle, a significant reduction in muscle weight accompanied by a reduction in the muscle fiber area was observed during the seven-day period, indicating a picture of muscle atrophy inherent to disuse, corroborating studies present in literature. Kannus *et al.*² reported a reduction of 69% in the fiber area of the soleus muscle immobilized with a plaster cast for 3 weeks. It is firmly established that anabolic hormones, as well as growth factors such as IGF-1, can preserve muscle mass in catabolic states.¹⁶ The results presented in this paper showed that IGF-1 treatment minimized the reduction of the cross section area of the immobilized soleus muscle, ratifying the result presented by Zdanowicz and Teichberg⁹, who reported that IGF-1 decreased intramuscular protein degradation as well as the density of the soleus muscle fibers of rats submitted to suspension for 10 days.

These results can be justified by the fact that IGF-1 promotes mitogenic and anabolic effects on the skeletal muscle. It has the ability to mediate both the differentiation and the proliferation of myoblasts.¹⁷ Analyses of the IGF type 1 (IGFR1) receptor indicate that the ability to promote cellular differentiation may be dissociated from the mitogenic effects of the IGFR1 bond, suggesting that different intracellular signaling pathways might mediate these processes.¹⁸ *In vitro*, the characterization of the intracellular signaling pathways that respond to the IGFR1 bond in the skeletal muscle has led to two-way focusing, one characterized by the increased activity of the Ras-ERK cascade (extracellular signal-regulated kinase), important in stimulating cellular proliferation, and a second that involves the signaling of phosphatidylinositol-3 kinase (PI3K), related to cellular differentiation.¹⁸

In relation to connective tissue analysis, immobilization for seven days promoted a significant increase of 216% in the connective tissue area density if compared to the control group. These results are consistent with literature, since to Jozsa *et al.*¹⁹, regardless of the model of muscular disuse studied (immobilization, tenotomy or denervation), the quantity of connective tissue in the endomysium and perimysium increases significantly, ranging from 50% to 700%.

IGF treatment minimized the connective tissue area density increase when compared to immobilization. Due to its anabolic action, an increase in the synthesis of extracellular matrix proteins was expected. As this was not observed, the differences in the renewal rate between the intramuscular proteins and the extracellular matrix proteins may have influenced the results. Since collagen proteins have a slower rate of renewal than those of the muscle tissue,²⁰ the seven-day period of treatment might not have been sufficient for the increase in area density of the connective tissue with the use of IGF-1, as an increase of fiber area was observed in this

period in comparison to the immobilized group. This finding is extremely important, yet more studies are necessary to determine the mechanism whereby IGF-1 reduced the area density of the connective tissue in the muscle immobilized for seven days, since such alterations determine elasticity, rigidity and muscle extensibility.

CONCLUSION

This study showed that the treatment with IGF for 7 days minimized the harmful effects triggered in the soleus muscle by acute joint immobilization, maintaining better energy conditions in the muscle, besides demonstrating anti-catabolic action, factors that can favor rehabilitation in the post-immobilization phase.

REFERENCES

1. Reardon KA, Davis J, Kapsa RM, Choong P, Byrne E. Myostatin, insulin-like growth factor-1, and leukemia inhibitory factor mRNAs are upregulated in chronic human disuse muscle atrophy. *Muscle Nerve*. 2001;24:893-9.
2. Kannus P, Jozsa L, Jarvinen TL, Kvist M, Vieno T, Järvinen TA, *et al*. Free mobilization an low to high-intensity exercise in immobilization-induced muscle atrophy. *J Appl Physiol*. 1998;84: 1418-24.
3. Ferrando AA, Lane HW, Stuart AA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol*. 1996;270(4 Pt 1):E627-33.
4. Hirose M, Kaneki M, Sugita H, Yasuhara S, Martyn JA. Immobilization depresses insulin signaling in skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000;279:E1235-41.
5. Cancelliero KM, Durigan JL, Vieira RP, Silva CA, Polacow ML. The effect of a low dose of clenbuterol on rat soleus muscle submitted to joint immobilization. *Braz J Med Biol Res*. 2008;41:1054-8.
6. Balagopal P, Rooyackers OE, Adey DB, Ades PA, Nair KS. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *Am J Physiol*. 1997;273(4 Pt 1):E790-800.
7. Barton-Davis ER, Shoturma DI, Sweeney HL. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *ActaPhysiol Scand*. 1999;167:301-5.
8. Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage, FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science*.2003;301:839-42.
9. Zdanowicz MM, Teichberg S. Effects of insulin-like growth factor-1/binding protein-3 complex on muscle atrophy in rats. *ExpBiol Med*. 2003;228:891-7.
10. Siu LO, Russeau JC, Taylor AW. Determination of glycogen in small tissue samples. *J Appl Physiol*. 1970;28:234-6.
11. Ploug T, Ohkuwa T, Handberg A, Vissing J, Galbo H. Effect of immobilization on glucose transport and glucose transporter expression in rat skeletal muscle. *Am J PhysiolEndocrinolMetab*. 1995;268:980-6.
12. Frick F, Oscarsson J, Vikman-Adolfsson K, Ottosson M, Yoshida N, Eden S. Different effects of IGF-I on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. *Am J PhysiolEndocrinolMetab*.2000;278:729-37.
13. Froesch ER, Schmid C, Schwander J, Zapf J. Action of insulin like growth factors. *Ann Rev Physiol*. 1985;47:443-67.
14. Hussain MA, Schmitz O, Christiansen JS, Zapf J, Froesch ER. Metabolic effects of insulin-like growth factor-I: a focus on insulin sensitivity. *Metabolism*. 1995;44(10 Suppl 4):108-12.
15. Ørskov L, Møller N, Bak JF, Pærksen N, Schmitz O. Effects of the somatostatin analog, octreotide, on glucose metabolism and insulin sensitivity in insulin-dependent diabetes mellitus. *Metabolism*. 1996;45:211-7.
16. Clemmons DR, Smith-Bank A, Underwood LE. Reversal of diet-induced catabolism by infusion of recombinant insulin-like growth factor-I in humans. *J ClinEndocrinolMetab*. 1992;75:234-8.
17. Singleton JR, Feldman EL. Insulin-like growth factor-I in muscle metabolism and myotherapies. *Neurobiol Dis*. 2001;8:541-54.
18. Kim JJ, Accili D. Signalling through IGF-I and insulin receptors: where is the specificity? *Growth Horm IGF Res*. 2002;12:84-90.
19. Jozsa L, Reffy A, Demel S, Thoring J, Rapcsak M, Szoor A, Hideg J. Quantitative alterations of intramuscular connective tissue in calf muscles of the rat during combined hypoxia and hypokinesia. *ActaPhysiol Hung*. 1989;73:393-40.
20. Järvinen TA, Józsa L, Kannus P, Järvinen TL, Järvinen M. Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles. *J Muscle Res Cell Motil*. 2002;23:245-54.