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THE ESTROGEN EFFECT ON GLYCOGEN RESERVES OF DENERVATED SKELETAL MUSCLES OF FEMALE RATS

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ABSTRACT

Objective: To evaluate the effects of estrogen on muscles in female rats subjected to hindlimb denervation. Methods: Female Wistar rats were divided into five groups (n= 6): control; denervated 7 days; denervated 15 days; denervated treated with estrogen (200µg/rat, subcutaneously, daily) for 7 days; and denervated treated with estrogen for 15 days. After the experimental periods, glycogen (GLY) evaluations were performed on the soleus (S), white gastrocnemius (WG) and red gastrocnemius (RG), and the soleus was weighed. The statistical analysis was performed using the normality test, ANOVA and Tukey test ($p< 0.05$). Results: The denervation caused a reduction ($p< 0.05$) in GLY over a 7-day period (S: 44%, WG: 32%; RG: 32%) and 15-day period (S: 62%, WG: 44%; RG: 53%), and also S weight reduction (7 days: 29.7%; 15 days: 36.6%). However, the estrogen treatment caused elevation ($p< 0.05$) of GLY under this condition, both over 7 days (S: 19%; WG: 60%; RG: 18%) and over 15 days (S: 52%; WG: 51%; RG: 11%), but it was not enough to minimize the muscle weight reduction. Conclusions: The treatment with low doses of estrogen minimized the metabolic alterations induced by denervation, but it was not effective in interfering in the weight loss of the soleus muscle. This suggests that the hormone acts by enabling chemical-metabolic protection that acts like the insulin route, but the effect is multifactorial and depends on the dose, manner and duration of the treatment, as well as the time since denervation.

Key words: denervation, estrogen, skeletal muscle, physical therapy.

INTRODUCTION

The contractile dynamic functional capability of the skeletal musculature depends on the integrity of several factors, such as the generation of electrical potentials at the interfaces of the neuromuscular junctions; variations in ion concentrations, created by the activity of the ion channels; metabolic activity; and modulation of the systems participating in the metabolic adjustments. Thus, the metabolic patterns of the muscle fibers are constantly being adjusted, according to fluctuations in the availability of substrates that can be metabolized. They are influenced by insulin receptor sensitivity and populations, specific system activity connected with glucose uptake and the activity of key enzymes for carbohydrate metabolism. These associated events emphasize the importance of the glycogen content as a performance-limiting or fatigue energy reserve[1].

Under resting conditions, the skeletal muscle fibers take up small quantities of glucose. However, with increased contractile activity or in the presence of insulin, processes facilitating greater hexose uptake are triggered. Hexose can be promptly oxidized to generate energy, or it can be directed towards glycogen formation[3].

Studies aimed at evaluating the alterations triggered in denervated muscles have come to the consensus that, after complete discontinuation of motor innervation, there are losses of voluntary and reflex activity in the muscle, strength and mass losses and reductions in fiber diameter, followed by progressive muscle atrophy[4]. It has been demonstrated that, concomitant to sectioning of the motor innervation, there are also significant modifications relating to carbohydrate metabolism and particularly with regard to insulin resistance, which is triggered by reduction in the activity of the regulating enzymes for the metabolic pathways connected with the post-

receptor interface of the insulin. There are reductions in GLUT4 population (glucose transporter type 4), in cytosol concentration of GLUT4 RNAm (ribonucleic acid messenger), in gene expression of GLUT 1 (glucose transporter type 1) and GLUT 4, in the activity of enzymes participating in glycolysis and of the enzyme glycogen synthetase, and in the ability of insulin to activate this[5]. These associated events participate in the metabolic alterations that predispose the muscle fibers to hypotrophy[6].

Recent studies have demonstrated the functional relationships between motor innervation and the metabolic homeostasis of muscle fibers, in which there is a consensus that the reduction in glycogen reserves that is induced by denervation can be minimized by drugs such as metformin[7] and clenbuterol[8], energy supplements such as glutamine[9] and CGT (creatine-glutamine-taurine)[8], as well as by neuromuscular electrical stimulation[10].

The importance of estrogen in homeostasis of the reproductive, cardiovascular and central nervous systems is highlighted in the scientific literature[11]. On other hand, pioneering studies that started in the 1990s have also found that estrogen receptors are present in muscle fibers, thus suggesting that many systems may be linked to its actions, such as muscle strength maintenance, tissue protection factor and the agent inhibiting free radical generation, since the molecule presents similarities with vitamin E[11].

Estrogen receptors are divided in two types, named ERα (estrogen receptor α) and ERβ (estrogen receptor β). The latter detects the presence of receptors of ERβ type in the skeletal muscles of humans, bovines, mice and rats[12, 13].

A recent contribution was made towards the understanding of the action of estrogen receptors in muscle fibers by using immunocytochemistry, from which the presence of ERβ receptors was observed both in muscle fibers and in the capillarization of the muscles[14].

Concerning the ERβ estrogen receptor, *in vivo* studies have suggested that there is a functional relationship with carbohydrate metabolism, in which an insulinotropic effect was observed because of both inducing insulin secretions and minimizing the insulin resistance[15].

Studies on chemical-metabolic neuromuscular relationships have shown that, besides the neurotransmitter/receptor relationship responsible for muscle contraction, there is an interrelation between the innervation and the mechanisms that allow the nutritional homeostasis of muscle fibers, given that the denervated fibers show strong metabolic alterations, as well as presenting insulin resistance and atrophy.

Thus, the objective of this study was to evaluate the glycogen reserves of denervated soleus and gastrocnemius muscles subjected to treatment with estradiol cypionate for 7 and 15 days.

MATERIAL AND METHODS

Female Wistar rats (180 to 200 grams) of mean age three to four months were used. They were fed with animal feed and water *ad libitum*, and were subjected to 12-hour photoperiodic cycles of light and dark, at a controlled temperature of 23 ± 2°C. The animals were divided randomly into the following experimental groups (n= 5): Control, denervated 7 days, denervated 15 days, denervated treated with estrogen for 7 days and denervated treated with estrogen for 15 days. This study was approved by the ethics committee for animal experimentation of the Federal University of São Carlos, under CEEA protocol No. 011/2006.

For denervation, the rats were anesthetized with pentobarbital sodium at a concentration of 50 mg/kg of body weight, the posterior part of the thighs was shaved, and from this a 5-mm portion of the sciatic nerve was sectioned and removed, in accordance with the model proposed by Coderre et al.[5]

The treatment consisted of administration of estradiol cypionate subcutaneously, at a concentration of 200 μg/rat, daily at the same time of day[16], for 7 days and 15 days, according to the groups described above.

After the time periods of the experiment, the soleus and white and red gastrocnemius muscles were isolated, removed and promptly sent for the glycogen content to be evaluated by means of the sulfuric phenol method[17]. The weight of the soleus weight was also evaluated.

The statistical analysis of the data started with the normality test (Kolmogorov-Smirnov). Since the data presented normal distribution, the ANOVA (analysis of variance) and Tukey parametric tests were used, in which the critical level set for all calculations was 5% ($p < 0.05$).

RESULTS

The result from denervation was a significant decrease in muscle glycogen content, which in the soleus muscle decreased by 44% after the first 7 days and 62% after 15 days ($p < 0.05$). In the white portion of the gastrocnemius muscle, the decrease was 32% after the first 7 days and 44% after 15 days ($p < 0.05$) and in the red portion the decrease was 32% after the first 7 days and 53% after 15 days (Figures 1, 2 and 3).

In evaluating the glycogen content of the denervated muscle, it could be seen that, after 7 days of daily estrogen treatment, the soleus muscle presented an increase of 19%, while the white portion of the gastrocnemius presented an increase of 60% elevation and the red portion presented an increase of 18% ($p < 0.05$).

After treatment with estrogen for 15 days, the reserves were also higher, by 52% in the soleus, while the white

portion of the gastrocnemius presented an increase of 51%, and the red portion presented an increase of 11% ($p< 0.05$).

Regarding the muscular weight of the soleus, after 7 days the denervation produced a significant reduction ($p< 0.05$) of 29.7% in relation to the control and, after 15 days, this reduction was greater, reaching 36.6% (Table 1).

The treatment with estrogen was insufficient to minimize the muscle weight reduction over any of the time periods analyzed, showing a non-significant increase ($p> 0.05$) after 7 days (18.3%) and no change after 15 days, in relation to the respective denervated group.

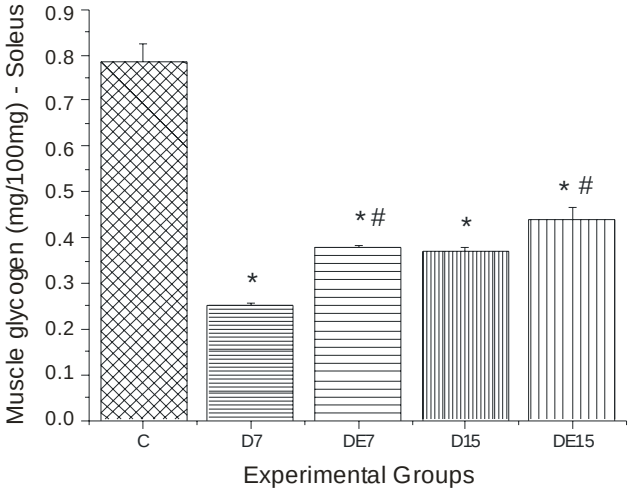

Figure 1. Soleus (S) muscle glycogen concentration (mg/100mg) in the groups: Control, Denervated 7 days, Denervated 15 days, Denervated treated with estrogen for 7 days and Denervated treated with estrogen for 15 days. The values correspond to the mean ± S.E.M, n= 5. *p< 0.05 compared with control and # compared with the respective denervated group.

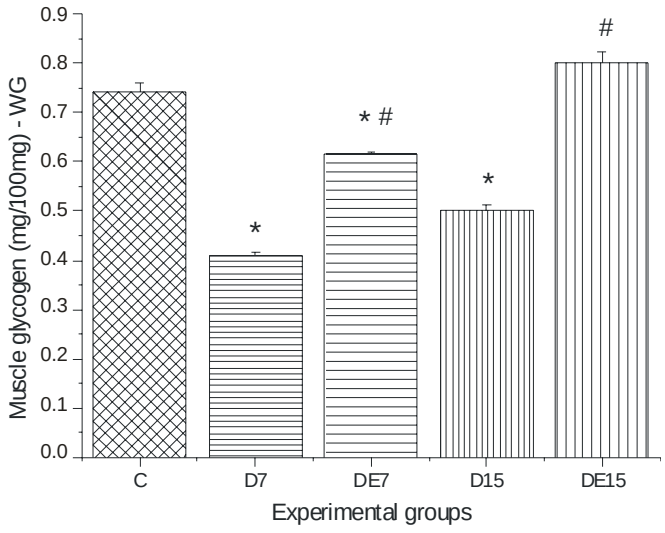

Figure 2. White gastrocnemius (WG) muscle glycogen concentration (mg/100mg) in the groups: Control, Denervated 7 days, Denervated 15 days, Denervated treated with estrogen for 7 days and Denervated treated with estrogen for 15 days. The values correspond to the mean ± S.E.M, n= 5. *p< 0.05 compared with control and # compared with the respective denervated group.

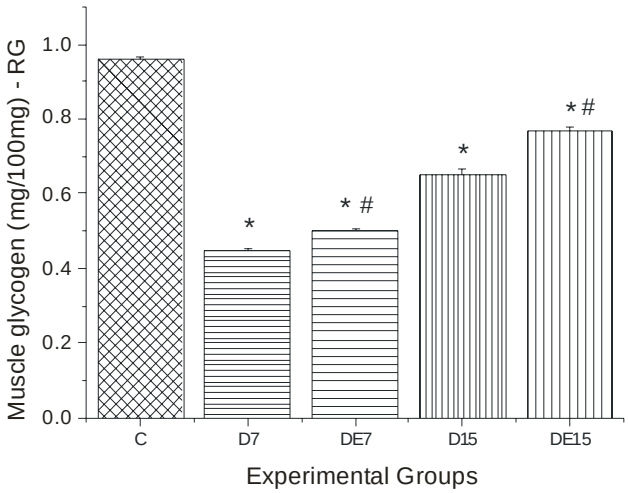

Figure 3. Red gastrocnemius (RG) muscle glycogen concentration (mg/100mg) on the groups: Control, Denervated 7 days, Denervated 15 days, Denervated treated with estrogen for 7 days and Denervated treated with estrogen for 15 days. The values correspond to the mean ± S.E.M, n= 5. *p< 0.05 compared with control and # compared with the respective denervated group.

Table 1. Soleus muscle weight (mg) in the groups: Control, Denervated 7 days, Denervated 15 days, Denervated treated with estrogen for 7 days and Denervated treated with estrogen for 15 days. The values correspond to the mean ± S.E.M, n= 5. *p< 0.05 compared with control and # compared with the respective denervated group.

Groups	Muscle weight (mg)
Control	101 ± 1.7
Denervated 7 days	71 ± 2.9*
Denervated treated with estrogen for 7 days	84 ± 8.9*
Denervated 15 days	64 ± 1.4*
Denervated treated with estrogen for 15 days	63 ± 2.4*

DISCUSSION

The functional capability of the skeletal musculature has strong multifactorial relationships, represented by its sensitivity to insulin, tissue metabolic activity and contractile activity. The glucose energy supply may be enabled both through insulin action and through increased contractile activity, in which the mechanism consists of translocation of type 4 glucose transporters (GLUT4), from cytosolic tubule-vesicular deposits to the membrane, thus assisting in increasing the glucose uptake for energy generation and/or glycogen formation. In this respect, it has been found that 70 to 85% of the glucose taken up by muscles at rest is probably stored in the form of glycogen[18].

The growing interest in the morphophysiological changes stimulated by denervation of the skeletal musculature has instigated a more detailed investigation of the neuromuscular relationships. These studies have revealed that, concomitant

to sectioning of the motion innervation, significant functional and metabolic modifications occur, which predispose the muscle fibers to atrophy[7].

Initially, we evaluated the effect of denervation on the glycogen concentration in the soleus and the white and red portions of the gastrocnemius and demonstrated that the deposits were drastically reduced over 7 and 15 days, because of the denervation. This finding corroborates the hypotheses of other authors who postulated that, concomitant to the sectioning of the motor innervation, there is a reduction in the activity of the cascade of events relating to insulin action. This impairs glucose uptake and metabolism, and also the sensitivity to insulin, which thereafter stimulates a condition of resistance, followed by atrophy[5].

It must be taken into consideration that, concomitant to the denervation, there was a significant loss of mass in the soleus muscle, thus suggesting that there is a close neurotrophic relationship that plays a part in the contractile-metabolic modulation. Therefore, it is considered that the events triggered by denervation correspond to changes of a trophic/metabolic/functional nature.

These chemical-metabolic relationships of the skeletal musculature are expressed in both a modulating and an activating manner, depending on the functional status of the muscle tissue. One agent that has been arousing increasing interest in the literature is estrogen, for which receptor populations have now been described both in muscle fibers and in the surrounding tissues, such as vascular endothelium[14].

The results from the present study are pioneering in that they demonstrate that the metabolic alterations triggered by denervation were minimized by the treatment with estrogen for 7 days, since the glycogen reserves were elevated in relation to the denervated group. However, the treatment over 15 days was only sufficient to raise the white gastrocnemius deposits, while emphasizing that the content in the 15-day denervation group was already elevated in relation to the 7-day denervation group, thus demonstrating that there is a modulating action that is limited by the mass action effect.

This may be directly related to the low concentration of estradiol used in this study, or the capacity for action in an insulin-resistant state, as observed in denervation, or it may even be due to an indirect effect as an insulinotropic agent[15,19]. Brussaard et al.[20] suggested that estrogen action elevates insulin secretion and reduces the state of resistance to insulin.

Kadi et al.[21] observed that the reduction in estrogen production was related to the deleterious effects on the muscle system, with a significant reduction in the production of muscle strength and a direct relationship with the functional capability of crossed bridges.

The insulinotropic action of estrogen has been evaluated in detail, and it has been demonstrated in rats that this hormone acts on beta pancreas cells as a potassium channel blocker that is modulated by the ATP/ADP ratio. This in itself stimulates the secretion process and, at the same time, boosts the secretion dynamic that is induced by glucose[22,23]. In this sense, it has been verified that there is a functional convergence in the signaling network that integrates estrogen and insulin action, with a direct relationship with the metabolic cascade that involves AMPc, MAP kinase and the PI3-K/Akt system. These mechanisms allow estrogen to act rapidly through phosphorylation and activation of preexisting protein systems, thereby integrating the signalizing action with the genome coding[24].

It is important to emphasize that estrogen, as an insulinotropic agent, can also over the long term trigger a secondary adaptive response that is represented by a possible state of insulin resistance triggered by constant exposure to therapy-induced insulin[25]. However, it has recently been demonstrated that the tissue sensitivity to insulin goes through a functional adjustment in which the insulin sensitivity is raised in the presence of estrogen, thereby reiterating our results[19].

Although there is evidence that treatment with estrogen promotes an insulin-inhibiting action, the functional dynamics of estrogen and its relationship to insulin action have been demonstrated. There is a direct relationship with glycemic homeostasis and the maintenance of adequate metabolic conditions, bearing in mind that low doses of hormone are effective in promoting an increase in the number of insulin receptors, especially in muscle tissue, and the maximum effect is observed from the sixth day onwards[25].

Our results corroborate this study, since the dose administrated was low and the time periods analyzed were 7 and 15 days, thereby showing that the estrogen acted to elevate the glycogen deposits over a 7-day period in all muscles analyzed, and only in white fibers, represented by the white gastrocnemius, over a 15-day period. Gonzalez et al.[32] observed that the effect of estradiol on elevating the sensitivity to insulin was manifested in the liver and in the muscles from the 6th to the 11th day of treatment, although after the 16th day, its action was manifested only in muscle tissue.

CONCLUSION

Treatment with low doses of estrogen minimized the metabolic muscle alterations triggered by denervation, but it was ineffective in interfering with the weight loss of the soleus muscle, thus suggesting that the hormone acts towards allowing chemical-metabolic protection with similarities to the action of the insulin route. However, this effect is multifactorial and depends on the dose, its form and the length of treatment, as well as the length of time with denervation.

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