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Glycogen reserve behavior in denervated muscles of female rats treated with different estrogen doses

Comportamento das reservas de glicogênio no músculo desnervado de ratas tratadas com diferentes doses de estrógeno

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Abstract

Objective: To evaluate the action of estrogen on the glycogen profile of denervated skeletal muscle in female rats. **Methods:** The animals were divided into six experimental groups (n=6): control; denervated for 15 days; denervated and treated with estrogen at a concentration of 20μg/weight/day; denervated and treated with estrogen at a concentration of 40μg/weight/day; denervated and treated with estrogen at a concentration of 80μg/weight/day; and denervated and treated with estrogen at a concentration of 160μg/weight/day. The animals were treated with estradiol cypionate for 15 days. The following analyses were carried out: glycogen content of the soleus, white gastrocnemius and red (mixed) gastrocnemius, by means of the phenol-sulfuric acid method as well as body weight and soleus muscle weight. The statistical analysis included ANOVA and the *post-hoc* Tukey test ($p<0.05$). **Results:** The denervation induced a reduction in glycogen content in the soleus and the white and mixed gastrocnemius muscles. In contrast, there was a progressive elevation of glycogen content concomitant with dose elevation in the groups treated with estrogen, thereby reinstating the glycogen reserves. The most effective concentration was 160μg/weight/day, which showed the best chemometabolic responses and least weight loss. **Conclusion:** These results suggest that estrogen at the concentration of 160 μg/weight/day was the most efficient at minimizing metabolic impairment and that it may be an important pharmacological instrument for physical therapy practice during rehabilitation.

Key words: estrogen; denervation; muscle; physical therapy.

Resumo

Objetivo: Avaliar a ação estrogênica sobre o perfil glicogênico do músculo esquelético desnervado de ratas. **Métodos:** Os animais foram divididos em seis grupos experimentais (n=6): controle; desnervado durante 15 dias; desnervado tratado com estrógeno na concentração de 20μg/peso/dia; desnervado tratado com estrógeno na concentração de 40μg/peso/dia; desnervado tratado com estrógeno na concentração de 80μg/peso/dia e desnervado tratado com estrógeno na concentração de 160μg/peso/dia. Os animais foram tratados com a substância cipionato de estradiol durante 15 dias. As análises realizadas foram: conteúdo de glicogênio dos músculos sóleo, gastrocnêmio branco e gastrocnêmio vermelho (misto), realizadas por meio do método do fenol sulfúrico, além do peso corporal e do músculo sóleo. A análise estatística incluiu ANOVA e teste *post-hoc* de Tukey ($p<0,05$). **Resultados:** A desnervação promoveu redução no conteúdo de glicogênio dos músculos sóleo, gastrocnêmio branco e misto. Por outro lado, nos grupos tratados com estrógeno, foi observada uma elevação progressiva no conteúdo de glicogênio concomitante com a elevação da dose, restabelecendo as reservas glicogênicas, sendo que a concentração mais efetiva foi a de 160μg/peso/dia, em que foram observadas as melhores respostas químio-metabólicas e a menor perda de peso. **Conclusão:** Esses resultados sugerem que o estrógeno na concentração de 160μg/peso/dia foi a mais eficiente para minimizar o comprometimento metabólico e pode ser um importante instrumento farmacológico aplicado na prática fisioterapêutica durante a reabilitação.

Palavras-chave: estrógeno; desnervação; músculo; fisioterapia.

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Introduction

Skeletal muscle represents 40 to 45% of total body weight, and its actions focus on providing mobility to the skeletal system. The skeletal muscle also has an essential role in maintaining glucose homeostasis, due to its responsiveness to insulin. Thus, the maintenance of ideal energy conditions depends on the integration of metabolic pathways that are sequentially activated. This allows energy production according to demand[1].

The functional relationships between the integrity of motor innervation and energy homeostasis of muscle fibers have been the subject of different studies, especially on neuromuscular denervation, a condition that leads to chemometabolic changes which converge to reduce insulin sensibility as well as in the post-receptor signaling cascade, predisposing hypotrophy[2,3].

It is known that the energy homeostasis of muscle fibers is fine-tuned by hormones and neurotransmitters. In the 1990's, the presence of estrogen receptors in muscle fibers was described, and it was found that they influence cellular energy metabolism and modulate and regulate muscle strength[4,5]. Another important relationship is based on estrogen signaling, which indicates cytosolic action and activates signaling cascades. These signaling cascades modify cellular responsiveness to other hormones, e.g. insulin-like growth factor (IGF) and growth hormone (GH), changing the homeostasis of muscle fibers and interfering in fiber size, myosin composition and protein synthesis[6]. However, there is disagreement on the physiological answers linked to estrogen action. It has been described that orally administered estrogen triggers a reduction in the plasmatic concentration of IGF-1 followed by GH concentrations three times higher after 24h. In contrast, if estrogen is subcutaneously administered, it will elevate the plasma concentration of IGF-1 without changing the GH plasma concentration. Thus, this form of administration was chosen for the present study[7,8].

In the area of women's health, the study of estrogen actions is essential to understanding the organic changes that take place in periods of lower hormonal production, causing various symptoms such as hot flashes, mood swings and loss of bone and muscle mass, which hormone replacement therapy seeks to minimize[9]. With regard to the unwanted side effects associated with hormone therapy with high doses of estrogen, data show an increase in the relative risk of some hormone-dependent breast and endometrial neoplasias, as well as an increase in the risk of cardiovascular diseases, thromboembolism, mastalgia, weight gain and change in lipid profile[10]. It should be pointed out that a recent study indicates that estrogen treatment can modify antioxidant capacity in fibers, decreasing changes generated by muscle injuries and accelerating the regeneration process. However, there is a wide range in the doses administered, varying from 20μg/weight/day (low dose) to 200μg/ weight/day (high dose) with different actions. A stimulating effect is referred in low doses and an inhibitory effect in high doses[11].

A recent study evaluated the effect of high doses of estrogen (200μg/weight/day) on the glycogen content of the denervated skeletal muscle of female rats and found an improvement in the formation of this reserves[12]. The importance of studying the behavior of muscle glycogen reserves in females is based on two important aspects: the first one refers to the metabolic rate because this reserve is an indication of endurance and/or fatigue[13], and the other is based on the lack of information on the behavior of these reserves during menopause[14]. However, it has not been determined whether a high dose is required to increase glycogen reserve production or whether low doses can also be used, minimizing the possible side effects. New studies are needed to clarify and indicate an efficient dose that promotes energy and functional balance in disused muscles. Therefore, the aims of this study were to evaluate the effect of different estrogen doses, ranging from 20 to 160μg/weight/day, on the glycogen content of denervated skeletal muscle in female rats.

Methods

We used female Wistar rats weighing 180 to 200 grams, aged three to four months. They received food and water *ad libitum* and were submitted to a photoperiodic cycle of 12h light/dark and controlled temperature of 23±2ºC. The animals were divided into six experimental groups (n=6): control; denervated; denervated and treated with estrogen at 20μg/weight/day; denervated and treated with estrogen at 40μg/weight/day; denervated and treated with estrogen at 80μg/weight/day; and denervated and treated with estrogen at 160μg/weight/day. The animals were treated according to the Guide for Care and Use of Laboratory Animals. The project was approved by the Animal Research Ethics Committee of Universidade Federal de São Carlos (UFSCar), protocol CEEA n. 011/2006.

For denervation, the female rats were anesthetized with sodium pentobarbital at 50mg/Kg. The posterior portion of the thigh was shaved, and a 0.7 cm portion of the sciatic nerve was sectioned and removed[2] as shown in Figure 1. The hormonal treatment with estradiol cypionate was performed by subcutaneous administration in the concentrations mentioned above, every morning for 15 days from the first day of denervation.

After the experimental period, the soleus, white gastrocnemius and mixed gastrocnemius muscles were isolated, removed and immediately submitted to glycogen content assessment by means of the phenol-sulfuric acid method[15]. The

weight of the soleus muscle and body weight of the female rats were also measured. After verifying the homogeneity of the samples, the statistical data analysis was performed using ANOVA, followed by the Tukey test. For all calculations, the critical level was set at 5%.

Results

The denervation decreased the glycogen reserves by 50% in the soleus muscle, 54% in the white gastrocnemius and 13% in the mixed gastrocnemius muscles ($p<0.05$) as shown in Table 1. Sequentially, the glycogen reserves of the denervated skeletal muscle treated with different estrogen concentrations were assessed. The same table shows that the reserves were significantly high compared with the untreated group. In the estrogen concentration of 20μg/weight/day, the values were 76% higher in the soleus muscle and 204% in the white gastrocnemius muscle ($p<0.05$), with no changes in the mixed gastrocnemius muscle. In the treatment with estrogen at 40μg/weight/day, there was an increase of 212% in the soleus muscle, 195% in white gastrocnemius and 25% in mixed gastrocnemius muscles ($p<0.05$). In the concentration of 80μg/weight/day, there was also an increase reaching 260% in the soleus muscle, 60% in the white gastrocnemius and 150% in the mixed gastrocnemius muscles ($p<0.05$). Finally, in the concentration of 160μg/weight/day, the glycogen concentrations were the highest across all muscles, reaching 260% in the soleus, 282% in the white gastrocnemius and 218% in the mixed gastrocnemius ($p<0.05$).

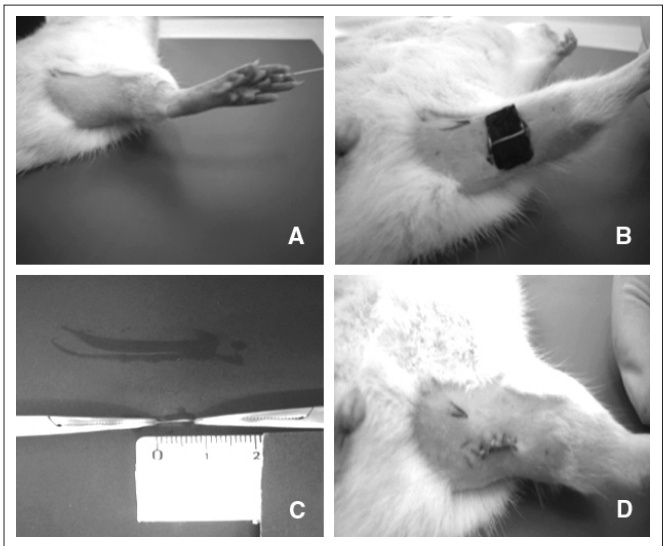
With respect to the soleus muscle weight, it should be noted that this muscle was chosen because of greater precision in its borders and its isolation. With denervation, there was a 52.6% decrease in weight, changing from 132±4mg in the control group to 62.5±3mg in the denervated group. In contrast, only the group treated with estrogen at 160μg/weight/day lost less weight than the untreated group, with values 41% lower than the control group, i.e. 77.8±3mg in the denervated and treated group. Thus, the treated group showed weight values 24% higher than the untreated group ($p<0.05$).

Discussion

The assessment of the glycogen content of denervated muscles showed a significant decrease in the reserves with greater intensity in the soleus and white gastrocnemius muscles, composed of type I and II fibers, respectively, and with less intensity in the mixed gastrocnemius muscle. These data are consistent with the hypothesis of a strong functional correlation in the triad of neuromuscular junction/energy profile/insulin sensitivity. Thus, concomitant with the nerve section, there is a disruption in the functional integration that sustains energy homeostasis. This causes a decreased translocation of glucose transporter isoform GLUT 4 from cytosolic stores to the membrane, increased glycolytic enzyme activity, increased concentration of cAMP-dependent protein kinase, reduced glycogen synthetase activity and increased glycogen phosphorylase enzyme activity, compromising the energy balance and predisposing the individual to insulin resistance[16,17].

The assessment of denervated soleus muscle weight, however, showed that only the muscle treated with estrogen at 160μg/weight/day had a significant reduction in weight loss. This may indicate that the expression of estrogen signaling interferes in the processes linked to the proteolysis that accompanies hypotrophy[18,19].

Regarding the effect of different doses of estradiol, assessment began with the concentrations of 20 and 40μg/weight/day, which are considered low doses with little effect on various



A – Shaved area; B – Sciatic nerve; C- 0.7cm portion removed; D - Suture.

Figure 1. Denervation method.

Table 1. Skeletal muscle glycogen content (mg/100mg) of female rats from the control group, denervated group (D) and group treated with different estrogen concentrations (E). Means±epm, n=6.

	Soleus	White Gastrocnemius	Mixed Gastrocnemius
Control	0.49±0.05	0.50±0.04	0.37±0.04
D	0.25±0.04*	0.23±0.02*	0.32±0.04*
D+E 20μg/weight/day	0.44±0.05#	0.47±0.05#	0.32±0.03
D+E 40μg/weight/day	0.53±0.05#	0.45±0.05#	0.40±0.02#
D+E 80μg/weight/day	0.65±0.09#	0.60±0.06#	0.48±0.03#
D+E 160μg/weight/day	0.65±0.08#	0.65±0.08*#	0.70±0.04*#≈

Denervated (D); Estrogen (E); *p<0.05 compared to control; #p<0.05 compared to denervated groups, ≈p<0.05 compared to group treated with 80μg/weigh/day;

biological systems[10,20,21]. This analysis showed a significant increase in glycogen reserves, although with a lower expression in the mixed gastrocnemius muscles, indicating that microdoses can activate metabolic parameters but not homogeneously. There may be a triad that depends on fiber type, hormonal concentration and current metabolic pattern. It is important to note that some studies suggest that the variation in circulating estrogen concentration does not have an influence on muscle strength[22,23].

When estrogen was administered at a concentration of 80μg/weight/day, there was also a significant and generalized increase in the muscular glycogen reserves, inducing a new pattern also in the mixed gastrocnemius muscle. Finally, the assessment of the treatment with estrogen at a concentration of 160μg/weight/day showed a significant homogeneous increase in the glycogen reserves across the different muscle fiber types, thus establishing a new and improved energy pattern in the denervated fibers. The results demonstrated that the white gastrocnemius muscle which showed the highest glycogen concentrations. It is known that this fiber type has the highest number of estrogen receptors and it is a site of estrogen action[14]. This information is very important because it has been shown that the number of type II fibers is greatly reduced during menopause and they can be preserved concomitantly with hormone replacement therapy[14].

A recent study by Severi[12] showed that estrogen treatment (200μg/weight/day) was also efficient in minimizing the proliferation of connective tissue and keeping the denervated fibers in better physiological conditions. This reinforces the importance of estrogen for balancing the histophysiological functions of muscle tissue. The benefits to muscle tissue described here probably reflect the estrogen modulation that acts on the interface between the genomic and non-genomic pathways which involve the hormonal sites of action[22,23].

Finally, the present study showed that estrogen administered at a dose 20% lower than the one used by Severi[12] promoted benefits in the energy homeostasis of the denervated muscle fibers. It is possible that, because it is a lower dose, there is a minimization in the occurrence of side effects.

Conclusion

The data demonstrated that the best estrogen concentration to promote improvement or restore the energy conditions of denervated muscle fibers was 160μg/weight/day. This concentration deserves especial attention because it may be an important pharmacological tool used/applied in rehabilitation in the field of physical therapy.

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