EFFECT OF CREATINE SUPPLEMENTATION ON GLYCOGEN CONTENT IN RAT SKELETAL MUSCLE TREATED WITH DEXAMETHASONE

Keyla Regina da Silva Taliari[1]
Wanderley Albino Junior[1]
Carlos Alberto da Silva[2]

[1] *Graduation course on Physical therapy – UNIMEP*

[2] *Professor, Graduation course on Physical therapy – UNIMEP*

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ABSTRACT

The diabetogenic effects of glucocorticoid excess are due in part to peripheral resistance to insulin, reduction of glucose uptake and metabolism unleashed atrophy . The purpose of this study was to investigate the effect of oral creatine supplementation on muscle atrophy induced by dexamethasone, a rodent model of insulin resistance. Four groups of rats were treated 5 days with creatine (1,6 g/kg1. d1) and/or dexamethasone (1mg/kg-1.d-1, IP). Muscle glycogen was assessed in samples from soleus and gastronemius by phenol sulphuric method and plasmatic glucose, lactate and aspartate aminotransferase enzyme was evaluated by laboratory kit (Sigma diagnostic). Results indicate that creatine supplementation result in an increase in muscle glycogen without changing muscle mass. Although dexamethasone always promotes a significant increase in muscle glycogen, however, it induces a reduction in muscle weight. The associated treatment showed a potential benefit represented by a great glycogen reserves associated with reduction of muscle mass loss without promote a toxic effect. The data from this study suggest that treatment with creatine alters the metabolic muscle homeostasis and impedes the loss of mass induced by dexa-

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methasone. These findings support the hypothesis that creatine supplementation exerts a beneficial effect on glicocorticoid induced muscle atrophy.

KEY WORDS: creatine; glycogen reserves; desamethasone; rats skeletal muscles

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INTRODUCTION

Dexamethsone has been widely used due to its low mineralocorticoid activity, long run action and easiness of administration. At the same time it was observed some adverse effects such as intensification of hepatic glycogenolisis, lipolisis, resistance to insuline action due to an antagonistisc affect to its action, increase in the proteolicit activity of muscle tissue leading to weakness and atrophy (AMATRUDA et al., 1985; KANDA et al., 1999; 2001).

Several studies report that patients treated with glucocorticoids show expressive alteration in the energetic homeostasis leading to the so called "steroids myopathy", whose incidence varies from 7 to 60% (BATCHELOR et al., 1997). Such alterations are attributed to the direct action of the glucocorticoid and/or to the resistacne due to the reduction of the insulin signaling system (SAAD et al., 1993, 1994).

With respect to the action of insuline, it is well known its action in a variety of cells and tissue in which it promotes the influx of nutrients and blocks the release of other sourcers of stored energy, that is, in the skeletic and cardiac muscle it stimulates proteic synthesis and intake of glucose and glucogen. In fat tissue it activates the lipogenesis blocking lipolisis (TAYLOR, 1991).

Special attention has been payed to the signaling system of insuline, being a consensus the existence of an effective functional integration between the inlusine receptor and the cell intake of glucose. Presently different types of transportation – the GLUT - are known with a varied distribution in tissues. A remarkable one is GLUT4, a protein whose activity is regulated both by insuline and contractile activity being expressed solely in peripheral tissues sensitive to insuline as well as fat tissue, heart and skeletic muscle (LEIGHTON et al., 1987).

Recent metabolic studies revealed that hipercholesterolomy is associated to the decrease in the utilization and peripheral trasportation of glucose and an increase in the amount o insuline

required for the task of taking glucose and or glucogen (SESTI et al., 2001).

Vanstapel et al. (1982) studied the action of dexamethasoe in rats submitted to fasting and adrenalectomy and found that three hours after the administration of glucocorticoid there was an elevation in the muscular content of glucogen, revealing that glucogen promotes the dephosphorilation of the enzime glucogne synthetase from its b form (inactive) to the a form (active) favoring the formation of such stocks. In this regard the activation of the enzime glucogen synthetase can be used as an index for the glucocorticoid action.

Creatine is an efficient nutritional ergogenic agent that increases the performance being the main source of energy to the muscular tissue in high intensity stimulus favoring the early resynthesis of ATP. Originally it is synthetized in the liver and pancreas by means of arginin, glycin and methionin. In the muscular tissue the creatine is stored in the form of phophocreatine with the function of support the proteic synthesis and generation of energy (GREENHAFF,1997).

Undoubtely, it has been observed that phosphocreatine has a fundamental role in the energetic metabolism of mucualr contraction since it removes increased muscular glucogen (AMERICAN COLLEGE, 2000; SLATER; JENKINS, 2000).

The absorption of oraly ingested creatine is made in the bowel and enters the blood stream. After intestinal absorption the plasmatic creatine is distributed in many body tissues including heart, smooth muscular fibers, brain and tests. However, the major part is stored in the skeletic muscles (BUCCI, 1993).

Facing the homesostatic alterations leaded by the treatment with glucocorticoid (dexamethasone) and taking into consideration the benefits associated to the treatment with creatine, the objective of this study was to evaluate whether the supplementaion with creatine interferes in the atrophy induced by corticoids.

MATERIAL AND METHODS

ANIMALS

It where used male Wistar rates 3 to 4 months old provided by the animal farm of UNIMEP. Animals were feed and water “ad libitum”, submitted to photoperiodic cycles of 12h of light and 12h of darkness and divided in experimental groups according to TABLE 1.

TABLE 1 – Distribution of rats in the experiment.

Groups	N
Control	6
Control treated with creatine	6
Control treated with dexamethasone	6
Control treated with dexamethasone and creatine	6

TREATAMENT

Treatment consisted in the administration of creatine (1.6g/Kg) in the water available for drinking and dexamethasone (1mg/kg, IP) for five days (SAAD, et al., 1993; IPSIROGLU et al., 2001).

SAMPLING

For sample collection rats were anesthetised with SOdium Pentobarbital (40mg/Kg intra peritoneal); blood sample was collected in the renal vei, centrifugate during 10 minutes at 2,5000 rpm and plasma was separated. Soleus and gastrocnemius msucles (white and red parts) were removed and imediately digested in hot KOH 30% and the glucogen was precipitated by passing in hot ehtanol. Later, it was submitted to acid hydrolisis in the presence of phenol according to the proposal of Siu Lo et Taylor (1970). Values were recorded in mg/100 ml of humid weigth.

To the determination of the toxicity index it was evaluated the plasmatic concentration of glucose, lactate and the enxime aspartato aminotrasferase through a specific kit (Sigma Diagnosticos).

The statistical evaluation was made by Variance analysis followed by the Test of Turk. The critical level was set in $P<0.05$(5%) to all tests.

RESULTS

It was evaluated the effects of treatment with dexamethasone in the concentration of mucular glucogen. FIGURES 1, 2 and 3 shows that, in the presence of glucocorticoid, there was an elevation in the glucogen content predominantely in the red muscles since the content in the soleus muscle was elevated in 100% ($p<0.05$) whereas in the white portion of gastrocnemius the evelation was 66% ($p<0.05$) and in the red portion of the same muscle it was 64% ($p<0.05$) indicating the glucogenic effect of steroids. The figures also show that the treatment with creatine was effective in promoting elevation in the glucogen content. In this

regard, the elevation in the soleus was 48% ($p<0.05$), in the white protion of gastrocnemiuns 71% ($p<0.05$) and in its red portion 61% ($p<0.05$). These figures indicate a preferential effect towards white muscles.

It was then evaluated the association of creatine and dexamethasone. Findings reveal an additive effect on the glucogen stocks. In this regard the glucogen content in the soleus was elevated in 117% ($p<0.05$) whereas the white portionof soleus showed an elevation of 148% ($p<0.05$) and the soleus red protion an elevation of 84=3% ($p<0.05$).

As regards the muscular mass of soleus it can be seen in FIGURE 4 that the treatment with dexamethasone induced a reduction of 13% of the muscular mass ($p<0.05$) and that creatine has not promoted alteration in the muscular weight. However, when these substances were associated there was inhibition of proteolisis since there was no difference between the control group and the group with associated treatment.

It should be stressed that it was not observed toxicity associated to the treatment as can be seen in TABLE 2.

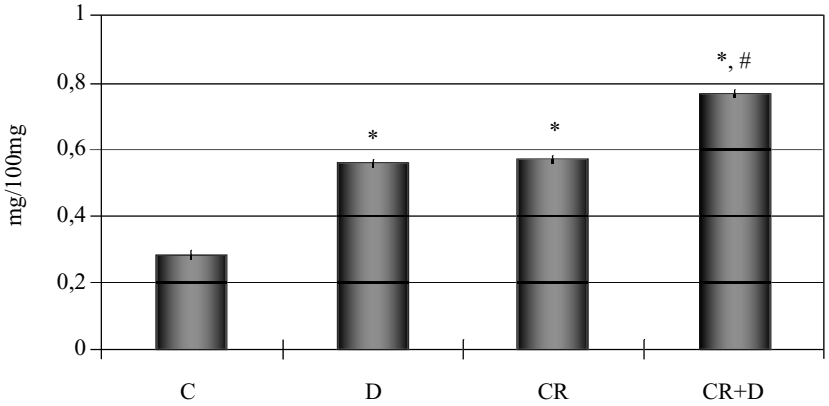

FIGURE 1 – Plasmatic concentration of glucogen (mg/100mg) in soleus muscle in the control group (C), dexamethasone (D), creatine (CR) and creatine + dexamethasone CR+D). Values are average ± epm, n=6. * $p<0.05$ if compared to control and # $p<0.05$ if compared to the group treated with dexamethasone.

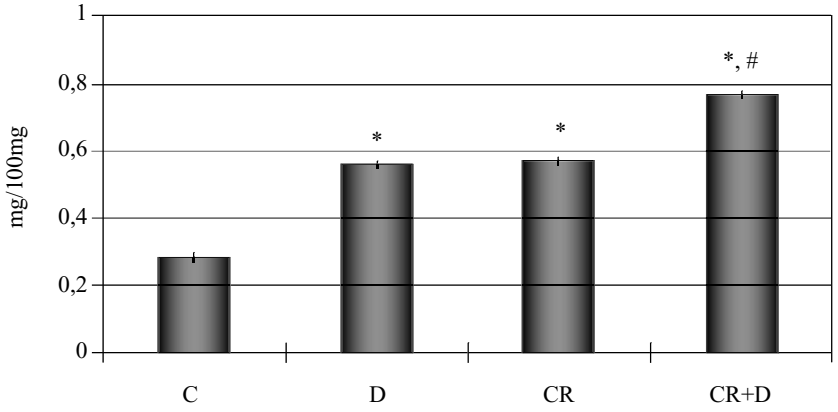

FIGURE 2 – Plasmatic concentration of glucogen (mg/100mg) in the white portion of gastrocnemius muscle of the control group (C), dexamethasone (D), creatine (CR) and creatine + dexamethasone CR+D). Values are average ± epm, n=6. * p<0.05 if compared to control and # p<0.05 if compared to the group treated with dexamethasone.

TABLE 2 – Biochemical profile of the control group, dexamethasone treated group, creatine treated groups and creatine + dexamethasoen treated group. Values are average ± epm, n=6.

Groups	Glucose (mg/dl)	Lactate (mmol/L)	AST (U/ml)
Control	104.93 ± 5.1	1.07 ± 0.8	39.54 ± 3.3
Dexamethasone	128.13 ± 2.6	1.19 ± 1.6	41.22 ± 1.1
Creatine	110.21 ± 1.0	1.06 ± 2.3	36.40 ± 2.4
Dexamethasone + Creatine	108.21 ± 2.2	1.00 ± 0.8	31.06 ± 0.9

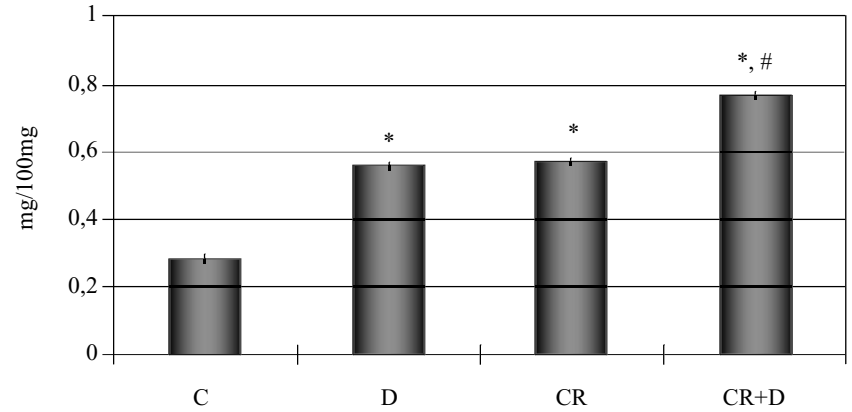

FIGURE 3 – Plasmatic concentration of glucogen (mg/100mg) in the red portion of gastrocnemius muscle of the control group (C), dexamethasone (D), creatine (CR) and creatine + dexamethasone CR+D). Values are average ± epm, n=6* p<0.05 if compared to control and # p<0.05 if compared to the group treated with dexamethasone.

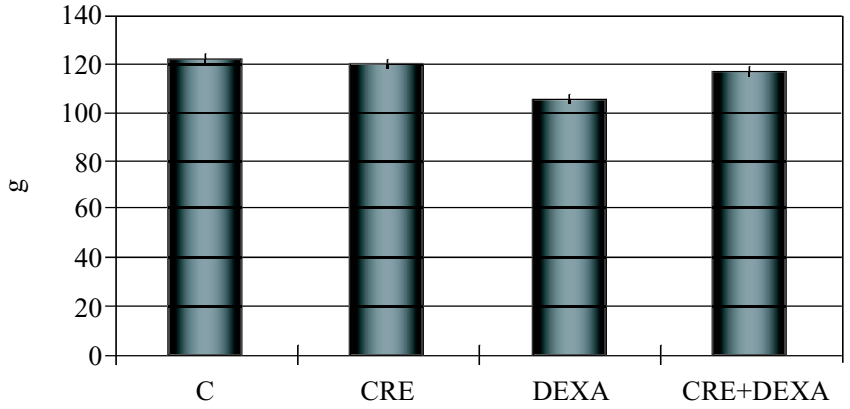

FIGURE 4 – Weight in grams of the soleus in the control group (C), dexamethasone (D), creatine (CR) and creatine + dexamethasone CR+D). Values are average ± epm, n=6. * $p<0.05$ if compared to control.

DISCUSSION AND CONCLUSION

Many physiological, biochemical, psychological and nutritional factors can limit the energetic homeostasis of the muscle tissue. In this regard, in the search for metabolic adaptations, it has been advocated the use of nutritional strategies that varies in its efficiency. Recently creatine has been established as a popular nutritional supplement in which the permissive effect of insuline acts as an integrator factor in the absorption and intake of creatin by musuclar fibers (FITCH; SHIELDS, 1996).

It has been noted that during treatment with glucocorticoids there is a depression in protein synthesis, activation of proteolysis and reduction in the effectiveness of the signaling paths of insuline that compromisses the metabolic hemostasis of the muscle tissue (VANSTAPEL et al., 1982). In this connection, the present study shows that dexamethasone interferes in the muscular synthesis of glucogene promoting an increase in the deposit. This fact is connected to the ability of glucocorticoids to induce secretion of insulin, indirectely enhancing the glucogenic properties of the musuclar tissue (BOSQUEIRO et al., 2001; ROONEY et al., 2002). It is important to note that, despite the findings of improved metabolic profile of mucular fibers, there was proteolysis expresssed by the markerd redution of the muscular mass along the development of signs of weaknes and atrophy (SAAD et al., 1994; KAYALI, et al., 1987).

An important fact it that, during treatment with creatine, it was observed a high level of cell hydratation favoring the synthesis of musuclar glucogen taking into consideration that, due to molecu-

lar hydratation, there is facilitation of the paths responsible for glucogenesis (IPSIROGLU et al., 2001; NEWSHOME et al., 1998).

Recent studies revealed that a supplementation of creatine induce elevation in the cytosolic concentration of creatine phospahte in the mouse muscular tissue leading to an improvement of the performance and efficiency of the muscular work directely connected to an increased disponibility of energetic substrate and indirectely by enchancing the sensibility to insuline (HARRIS et al., 1992).

To test the ergogenic effect of creatine a group of rats were supplement for five days. The result was an elevation in the glucogen content. This glucogenic effect is due to the capacity of creatine to facilitate the muscular intake of glucose and facilitate the formation of stocks as it was observed in hepatocytes in which it estimulates the glucogen synthetase enzime (VARNIER, et al., 1995; TEIJNDE et al., 2001).

It was decided to associate both treatments after the identification of proteolysis induced by steroid was made. In this regard it was observed an additive effect amidst the substances inducing a marked increase in the glucogen content with an sythesis index above those observed in the isolated treatements similarly to the observation in hepatocytes (ROBINSONS et.al., 1999).

It should be stressed that there was a reduction in proteolysis leading to preservation of the tissue mass. This represents benefits to the function of the muscular tissue and assures the protective capacity of fibers facing miophaty induced by steroids therapy.

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