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Creatine supplementation attenuates corticosteroid-induced muscle wasting and impairment of exercise performance in rats

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Menezes LG, Sobreira C, Neder L, Rodrigues-Júnior AL, Martinez JAB. Creatine supplementation attenuates corticosteroid-induced muscle wasting and impairment of exercise performance in rats. *J Appl Physiol* 102: 698–703, 2007. First published October 19, 2006; doi:10.1152/japplphysiol.01188.2005.—The objective of the present study was to investigate whether creatine (Cr) could attenuate the deleterious effects of high doses of dexamethasone (Dexa) on body mass, exercise performance, and respiratory variables of rodents. Forty-four Wistar rats performed incremental maximal exercise tests. They were then assigned to four groups: G1: subcutaneous (SC) and intraperitoneal (IP) saline; G2: SC saline and IP Cr (250 $mg \cdot kg^{-1} \cdot day^{-1}$); G3: SC Dexa (7.5 $mg \cdot kg^{-1} \cdot day^{-1}$) and IP saline; G4: SC Dexa and IP Cr. New exercise tests and analysis of the respiratory pattern under resting conditions and after stimulation with doxapram (2 mg/kg IP) were performed after 18 days. Post- minus pretreatment differences were compared between groups. G3 and G4 showed a significant impairment in body mass gain compared with G1 and G2 ($P < 0.05$) (G1: 65.3 ± 26.1, G2: 93.1 ± 27.4, G3: −18.4 ± 20.1, G4: 9.8 ± 23.1 kg × 10^{-3}). Similar results were observed for maximal oxygen consumption (G1: 9.5 ± 8.5, G2: 25.8 ± 14.5, G3: −25.5 ± 6.0, G4: −4.8 ± 9.5 $ml \cdot kg^{-1} \cdot min^{-1}$) and test duration (G1: 43.0 ± 45.0, G2: 72.0 ± 59.5, G3: −165.0 ± 60.6, G4: −48.0 ± 48.5 s). Simultaneous use of Cr significantly attenuated the Dexa-induced impairment of the last two variables. Cr attenuated Dexa-induced gastrocnemius and diaphragm muscle weight losses and the atrophy of gastrocnemius type IIb fibers. Cr supplementation had only small effects on Dexa-induced respiratory changes. These results suggest that Cr may play a role in the prophylaxis or treatment of steroid-induced myopathy.

steroids; toxicity; exercise tolerance; muscular atrophy; respiration; drug effects

CORTICOSTEROIDS (CS) ARE FREQUENTLY used for the treatment of several medical conditions, including autoimmune disorders, malignancies, and organ transplantation. However, when employed for a long time or at high doses, they may induce significant muscle weakness and myopathy (31).

Animal studies and clinical evidence have shown that CS induce muscle weight loss and preferential type II fiber atrophy (7, 17). Although skeletal muscles are primarily attacked, some studies suggest that respiratory muscles may be similarly affected (14, 15). One of the various metabolic effects of CS is the induction of a negative protein balance mediated by an increase in protein degradation and a decrease in protein synthesis (21).

Prophylactic and therapeutic actions against CS-induced myopathy may include exercise training and nutritional supplementation (9, 13, 31). Over the last few years, there has been an increasing interest in creatine (Cr) metabolism and its pathways. Cr monohydrate supplementation has been described to improve fat-free mass and muscle function in healthy humans (27, 30). Cr supplementation can enhance exercise performance, mainly regarding high-intensity, short-term tasks (5, 6, 40, 41). In addition, clinical investigations have also suggested a potential role for Cr supplementation in medical conditions, such as heart failure, chronic obstructive pulmonary disease, and neuromuscular disorders (1, 16, 19, 38, 39).

Clinical data suggest that patients at risk for steroidal myopathy show substantial urinary losses of Cr (2). However, only one study has investigated the effects of Cr supplementation in an animal model of CS-induced myopathy (34). Roy et al. (34) found that a diet containing 2% Cr prevented the growth attenuation of young rats induced by a 6-wk treatment with high doses of methyl-prednisolone (34). Muscle total Cr, phosphocreatine (PCr), and mean fiber area of type II fibers were increased in the extensor digitorum longus of the groups that had received Cr alone or in combination with CS. Although these results strongly suggest a role for Cr in the prevention or treatment of CS-induced myopathy, functional evidence of its usefulness is still missing. Furthermore, the role of Cr supplementation in respiratory muscle CS-myopathy has not been investigated.

Therefore, the purpose of the present study was to investigate whether simultaneous supplementation with Cr could attenuate the deleterious effects of high doses of CS on body mass, exercise performance, and respiratory function variables of rodents. Our hypothesis was that Cr supplementation would prevent the deleterious effects of dexamethasone (Dexa) on both peripheral muscles and diaphragm, improving exercise and respiratory performance.

METHODS

Animal care. Forty-four male Wistar rats, 12–14 wk of age, were obtained from the Central Animal Facility of the Medical School of Ribeirão Preto, University of São Paulo. The local Animal Ethics Committee approved the research protocol, and the rats were handled according to the APS' *Guiding Principles in the Care of Animals*. The specific pathogen-free animals were housed in individual cages in the Animal Facility of the Department of Internal Medicine and maintained on a 12:12-h light-dark cycle at ~25°C, with free access to a standard rodent diet and water throughout the study.

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The animals gained familiarity with the new environment during the first week while they learned how to run on a motorized treadmill (model 0184–003L, Columbus Instruments, Columbus, OH). The rats ran for 15 min/day for 7 consecutive days, at an incline of 10%. The initial speed of 14 m/min was kept for 5 min; between the 6th and the 9th min, the speed was progressively increased to 28 m/min, and this value was kept for the last 5 min of training. After this initial period of learning, the animals were submitted to an incremental maximal exercise test and randomly assigned to one of four groups of 11 rats each: *group 1* (G1): treated with subcutaneous (SC) and intraperitoneal (IP) saline; *group 2* (G2): treated with SC saline and IP Cr (250 $mg \cdot kg^{-1} \cdot day^{-1}$); *group 3* (G3): treated with SC Dexa (7.5 $mg \cdot kg^{-1} \cdot day^{-1}$) and IP saline; and *group 4* (G4): treated with SC Dexa and IP Cr. Micronized Cr monohydrate was available from Nutrilatina (São Paulo, Brazil) and Dexa from Schering-Plough (São Paulo, Brazil). Every morning, the drugs were freshly diluted in warm saline to respective concentrations of 25 and 1 mg/ml. The amounts of saline administered SC and IP were corrected for body weight in volumes analogous to those of the drug dilutions. Based on previous work from our laboratory, the duration of treatment was set at 18 days (Campos AR, unpublished observations). During the treatment period, all animals ran ~28 m/min, 5 min/day, 3 days/wk, to keep their running ability.

Twenty-four hours after the last drug administration, the animals were submitted to a second incremental maximal exercise test. One day after this test, the animals were anesthetized with 1.3 mg/kg of urethane ethyl carbamate (Sigma Chemical, St. Louis, MO), and their respiratory pattern was analyzed under basal conditions and 5 min after stimulation with 2 mg/kg IP doxapram hydrochloride (Fort Dodge, Campinas, Sao Paulo, Brazil). Finally, the animals were killed by decapitation while still anesthetized, and their diaphragm and the right gastrocnemius muscle were removed and weighed. Muscles from representative animals of each group were also frozen in liquid nitrogen for later histochemical analysis.

Maximal exercise studies. Maximal exercise tests were performed on a treadmill in an air-tight plastic wall chamber connected to an open-circuit calorimeter (Eco-Oxymax, Columbus Instruments, Columbus, OH). A standard exercise protocol was applied on all occasions as follows: *1*) the treadmill incline was kept constant at 10°; *2*) after a resting period of 5 min, the rats ran for an equal period at 14 m/min; and *3*) the speed was then increased every 2 min in increments of 7 m/min until animal exhaustion. Exercise tests were always performed at the same temperature (18°C) and at the same time of day (between 2:00 and 3:00 PM). Oxygen consumption ($\dot{V}O_2$) was measured every 10 s with a gas analyzer connected to a computer system, while rats were running on the treadmill. The analyzer was calibrated with gases of standard concentrations before each test. Exhaustion was defined as the point when the rat seemed unable to maintain the pace and to avoid the shock grid at the rear of the treadmill. The duration of the test and the highest speed reached were recorded. Resting (basal $\dot{V}O_2$) and maximal $\dot{V}O_2$ ($\dot{V}O_{2\,max}$) were also determined.

Respiratory function studies. The breathing pattern was analyzed using a computerized spirometer for rodents (Quadra t, Scireq, Montreal, Canada). This system contains a large-pore pneumotachograph mounted on the wall of a plastic chamber. A bias flow ensures that the air in the chamber is adequately refreshed. The animals spontaneously breathe through an orifice located on one plastic wall. The computer clamps the bias flow at regular intervals, forcing the animal to breathe through the pneumotachograph and collecting the respiratory data. The animals were anesthetized with urethane and then tracheostomized, and a plastic catheter was placed inside the tracheal lumen. Next, a thin liquid-filled catheter was placed in the lower esophagus to record the transthoracic pressure. Both catheters were connected to the system, and the respiratory rate (RR), tidal volume (V_T), and esophageal pressure (Pes) were measured following a short period of stabilization. The same variables were reevaluated 5 min after IP injection of doxapram.

Histochemical analysis. The muscle biopsies were cut with a cryostat into 10-μm-thick transverse sections, which were initially stained with hematoxylin and eosin. The differentiation of three fiber types was possible using a modification of a previously described histochemical staining technique for myosin ATPase (11). The modification consisted of preincubation at pH 4.5 before incubation at pH 9.4. Measurements of fiber-type proportions and mean minimum diameters (MMD) were obtained from myosin ATPase-stained cross sections using the computer-based image processing system Image-Pro Plus 4.0 (Media Cybernetics, Silver Spring, MD) connected to a light microscope. The MMD were calculated for each fiber type. About 50 fibers of each type were measured per muscle.

Statistical analysis. All results are reported as means ± SD. The statistical analysis considered the combinations of Dexa and Cr treatments as factors in the four experimental groups, according to a completely randomized design. These factors were evaluated by ANOVA, and, when indicated, post hoc analysis was done using the Bonferroni test (8, 29). The variables measured before the experimental treatments are reported as raw values. The same is true for posttreatment muscle weights and MMD and respiratory measurements before doxapram stimulation. The effects of the treatments on total body weights and exercise variables are shown and were analyzed as the differences between post- and pretreatment values. The same approach was adopted to examine the respiratory variables after doxapram stimulation. The histological data were analyzed statistically by the Kruskal-Wallis test followed by Dunn's posttest when indicated, because of the small number of samples available. Significance was set at $P < 0.05$. All statistical analyses were performed using the statistical software R 2.2.1 (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The four groups had similar mean body mass at the beginning of the study. There were significant differences in mean weight variations between the groups that received Dexa and the others after 18 days of treatment (Table 1). Controls [*group 1* (G1)] and the Cr alone group [*group 2* (G2)] showed comparable amounts of increases in body mass after treatment (Table 1). Although there was no significant difference between *group 3* (G3) and *group 4* (G4) regarding total body weight variations, it is worth noting that, whereas G3 showed body mass loss, G4, as a whole, exhibited body mass gain.

The groups did not differ regarding the exercise variables observed before treatment. The posttreatment minus pretreatment changes for the exercise variables are listed in Table 1. Compared with controls (G1), the administration of Dexa alone (G3) led to decreases in exercise duration, maximum speed, basal $\dot{V}O_2$, and $\dot{V}O_{2\,max}$, while the use of Cr alone (G2) led to a significant increase in the last variable. The supplementation of Cr with simultaneous administration of Dexa (G4) significantly attenuated the impairments in maximum speed and $\dot{V}O_{2\,max}$ induced by the use of the CS alone.

The results of the respiratory variables obtained after treatment and predoxapram stimulation are listed in Table 2. Treatment with Dexa alone (G3) had a significant effect on RR and V_T, stimulating both under basal conditions. The simultaneous administration of Cr led to a minor but statistically significant reduction in the Dexa-induced V_T elevation (G4). Neither Cr nor Dexa showed significant effects on Pes under basal conditions. Table 2 also shows the respiratory responses to doxapram stimulation as the differences post- minus pre-

Table 1. *Total body weight and exercise test variables along the study*

		G1	G2	G3	G4
Weight, kg × 10^{-3}	Pretreatment	165.6±17.0[a]	166.5±9.8[a]	166.9±19.4[a]	167.4±19.7[a]
	Δ	65.3±26.1[a]	93.1±27.4[a]	−18.4±20.1[b]	9.8±23.1[b]
Test duration, s	Pretreatment	831±46.9[a]	832±63.7[a]	832±56.4[a]	823±54.6[a]
	Δ	43±45.0[a]	72±59.5[a]	−165±60.6[b]	−48±48.5[c]
Highest speed, m/s × 10^{-3}	Pretreatment	743.3±58.3[a]	743.3±78.3[a]	753.3±61.7[a]	753.3±61.7[a]
	Δ	53.3±61.6[a]	63.3±80.0[a]	−148.3±75.0[b]	−75.0±58.3[c]
$\dot{V}O_{2basal}$, ml·kg^{-1}·min^{-1}	Pretreatment	32.2±3.3[a]	30.8±3.8[a]	34.0±4.5[a]	32.0±3.2[a]
	Δ	7.2±5.5[a]	13.7±6.8[a]	−6.3±4.8[b]	0.2±5.2[b]
$\dot{V}O_{2max}$, ml·kg^{-1}·min^{-1}	Pretreatment	70.0±4.7[a]	69.5±5.0[a]	70.9±3.3[a]	71.51±4.8[a]
	Δ	9.5±8.5[a]	25.8±14.5[b]	−25.5±6.0[c]	−4.8±9.5[d]

$\dot{V}O_{2basal}$, resting oxygen consumption; $\dot{V}O_{2max}$, maximal oxygen consumption; Δ, posttreatment minus pretreatment values; G1–G4, *groups 1–4,* respectively. [a,b,c,d]Different letters indicate statistically significant differences at the 5% level by the Bonferroni test.

doxapram measurements. The use of Dexa alone (G3) led to responses showing more pronounced elevations of RR and less intense elevations of Pes compared with G1 and G2. Cr supplementation alone (G2) or in combination with Dexa (G4) did not show any statistically significant effect on the RR and VT responses after doxapram stimulation. The simultaneous use of Cr did not significantly interfere with the pattern of Pes response to doxapram produced by Dexa.

The mean muscle weights for all animals are shown in Table 3. There were statistically significant differences between all groups for the analyses of both muscles. Compared with controls (G1), Dexa alone (G3) led to significant gastrocnemius and diaphragm mass losses, while the administration of Cr alone (G2) led to muscular gain. The simultaneous use of Cr attenuated the muscle weight losses induced by the CS.

Unfortunately, technical problems related to poor material preservation precluded histological analysis of the muscles from all animals. Eight right gastrocnemius and four diaphragm muscles were available from representative animals of each group. The mean number of fibers analyzed in six optical fields for each muscle did not differ between groups (gastrocnemius: G1 = 264 ± 114, G2 = 259 ± 109, G3 = 268 ± 124, G4 = 291 ± 09; diaphragm: G1 = 267 ± 22, G2 = 250 ± 25, G3 = 260 ± 38, G4 = 264 ± 24). There were no statistical differences regarding the proportions of fiber types for the gastrocnemius (type I: G1 = 27.0 ± 7.3%, G2 = 27.0 ± 7.2%, G3 = 25.7 ± 7.9%, G4 = 24.8 ± 5.0%; type II: G1 = 73.0 ± 7.3%, G2 = 73.1 ± 7.2%, G3 = 74.3 ± 7.9%, G4 = 75.2 ± 5.0%) or for the diaphragm (type I: G1 = 25.5 ± 3.7%, G2 = 28.9 ± 3.7%, G3 = 24.5 ± 5.6%, G4 = 25.8 ± 3.1%; type II: G1 = 74.5 ± 3.7%, G2 = 71.1 ± 3.7%, G3 =75.6 ± 5.6%, G4 = 74.2 ± 3.1%).

The MMD of the gastrocnemius and diaphragm muscle fibers are presented in Fig. 1. Concerning the gastrocnemius muscle, the mean diameters of all fiber types were significantly lower in G3 than in G1 and G2. In addition, the MMD of type IIb fibers was also significantly lower in G3 than in G4. There were no statistically significant differences in the comparisons involving the MMD of diaphragmatic muscle fibers, although the mean value of type IIb fibers was markedly reduced in G3.

DISCUSSION

The present study shows that simultaneous supplementation with Cr monohydrate protected rats from the deleterious effects of high doses of Dexa on muscle mass and maximal exercise performance. Cr supplementation had only modest effects on the changes in breathing pattern induced by Dexa under resting conditions.

Administration of Dexa for 18 days led to significant impairment of body mass growth in young rats. Indeed, at the end of the study, while G3 showed an approximate 10% reduction in mean weight, G1 exhibited a 38% gain. The muscle mass reflected this phenomenon, since the G3 weights of both gastrocnemius and diaphragm were lower than those of G1. Histochemical studies performed on a small number of animals in each group indicated that Dexa induced some degree of atrophy of all three fiber types in the gastrocnemius. The histochemical evidence of diaphragmatic steroidal myopathy was absent, but this result may reflect the small number of muscles available for analysis. The present findings are not unexpected, since the catabolic effects of CS on body and muscle metabolism are well recognized (2, 21). The mechanisms associated with the development of CS-induced atrophy are not completely known, but elevations of glutamine synthase activity and reduction of intracellular glutamine levels appear to play an important role in this process (23, 25). In addition, recent data have shown that CS may reduce the

Table 2. *Posttreatment respiratory variables measured under basal conditions and after stimulation with doxapram*

		G1	G2	G3	G4
RR, inhalations/min	Basal	111.8±7.9[a]	110.9±13.5[a]	150.6±17.6[b]	136.4±6.8[b]
	Δ	28.6±3.9[a]	28.6±16.5[a]	45.6±10.0[b]	37.8±5.9[a,b]
VT, ml	Basal	1.2±0.1[a]	1.4±0.3[a,b]	1.7±0.3[b]	1.5±0.3[a]
	Δ	0.2±0.1[a]	0.3±0.1[a]	0.3±0.3[a]	0.3±0.3[a]
Pes, cmH_2O	Basal	1.3±0.2[a]	1.3±0.4[a]	1.2±0.5[a]	1.5±0.6[a]
	Δ	2.2±0.4[a]	1.9±0.9[a]	0.3±0.8[b]	0.6±1.3[b]

Δ, Postdoxapram minus predoxapram stimulation values; RR, respiratory rate; VT, tidal volume; Pes, esophageal pressure. [a,b]Different letters indicate statistically significant differences at the 5% level by the Bonferroni test.

Table 3. *Posttreatment gastrocnemius and diaphragm weights*

	G1	G2	G3	G4
Gastrocnemius, mg	1660±150[a]	1870±140[b]	770±100[c]	960±130[d]
Diaphragm, mg	650±60[a]	800±130[b]	290±50[c]	420±90[d]

[a,b,c,d]Different letters indicate statistically significant differences at the 5% level by the Bonferroni test.

expression of IGF-I, inhibiting its anti-apoptotic effects at the muscle level (18, 36, 37).

The alterations in the measurements of the maximum exercise tests induced by Dexa accompanied the changes in muscle mass composition. Although the best explanation for the present findings rests on the losses of muscle mass, the chronic use of CS may elicit mitochondrial enlargement and aggregation and also reduce mitochondrial oxidative capacity (24, 28). In addition, humans treated with CS have shown higher levels of serum lactate at rest and during exercise and evidence of muscular oxidative damage. Complex I respiratory chain activities of muscle mitochondrial fractions were also reduced in CS-treated subjects (28). Therefore, since the $\dot{V}O_2$ results were corrected according to body weight, we may suppose that disorders of muscle energy metabolism could have contributed to these results as well.

A significant detrimental effect of Dexa on Pes was only observed after doxapram stimulation. However, the animals treated with CS showed a breathing pattern of hyperventilation at rest and after chemical stimulation. This pattern was characterized by significant increases of both RR and V_T in G3 compared with G1 and G2. Although these findings might be explained, at least in part, by weakness of inspiratory muscles, V_T elevations would not be expected in this situation. Systemic acidosis could have developed as a consequence of the use of high doses of Dexa and would certainly justify the results. However, arterial blood-gas analysis performed in a few rats did not support this explanation (data not shown). Another possibility would be related to the potential stimulatory actions of CS at the central nervous system level. Most probably, these findings resulted from a combination of these phenomena.

Although there was no significant difference between G3 and G4 regarding total body weight variations, while the former group exhibited a negative change of 11%, the latter showed a positive gain of 5.8%. This suggests that Cr could

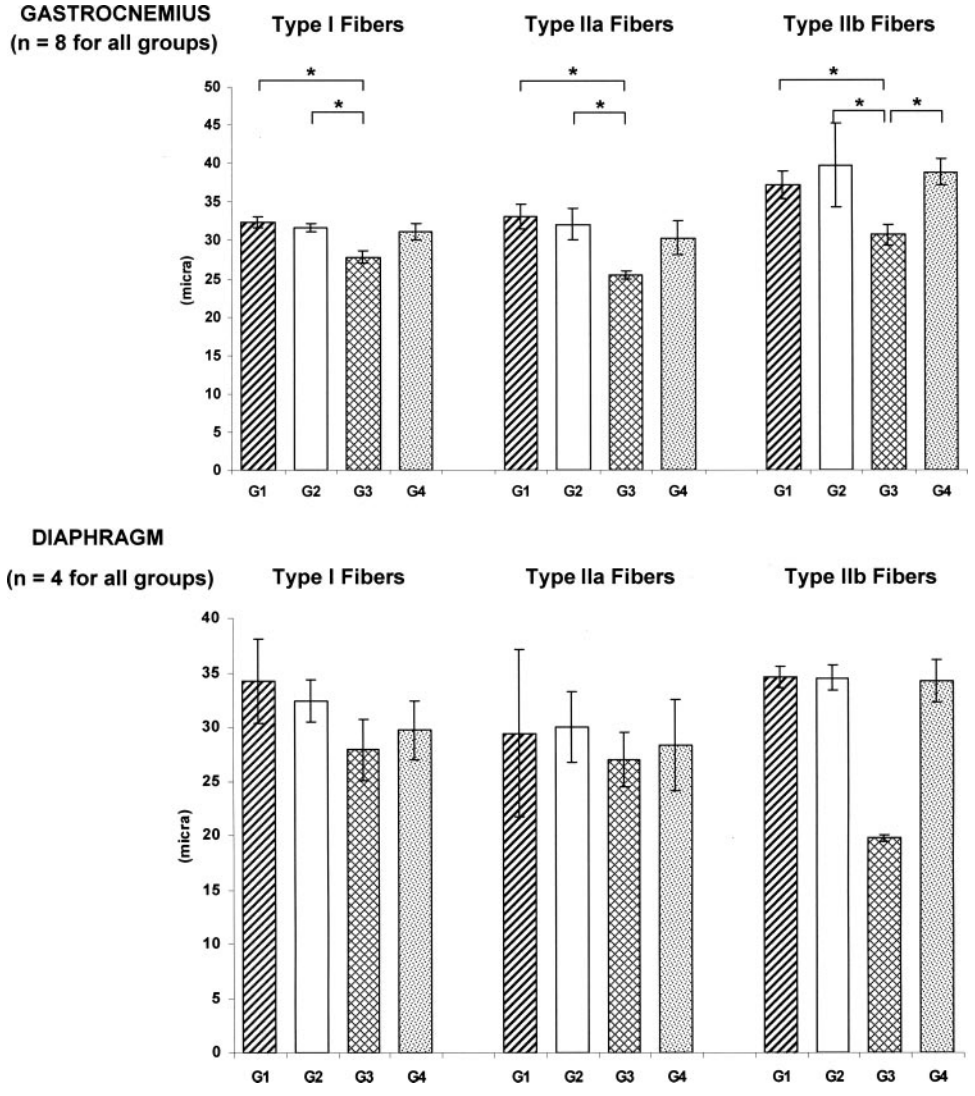

Fig. 1. Mean minimum diameter of different fiber types of muscle samples. G1–G4, *groups 1–4*, respectively. *$P < 0.05$ by the Kruskal-Wallis test and Dunn's posttest.

really have a protective effect against Dexa-induced body mass losses that was not clearly evident due to the small number of animals included in each group. In addition, Cr use significantly attenuated the losses of gastrocnemius and diaphragm muscle mass associated with CS administration. Histochemical studies have shown that Cr inhibited to some extent the CS-induced muscle fiber atrophy. This effect was evident for IIb fibers of the gastrocnemius, a finding similar to previously published results (34). The small number of muscles available for analysis may have precluded a clear histochemical demonstration of the effects of Cr on CS-induced muscle fiber atrophy.

It is well known that Cr supplementation may induce increases in body mass (29, 41). This has been attributed to increases in total body water content and to increased protein synthesis, conceivably stimulated by cell swelling (3, 22). Another way by which Cr may increase muscle mass is related to improvements in satellite cell activity (10). However, studies in humans have suggested that Cr does not increase whole body muscle protein synthesis but, most probably, exhibits anticatabolic actions restricted to some proteins (32). Although all of the above mechanisms may be related to the present findings, another explanation is the simple replacement of lost proteins. In fact, a previous study has shown that the development of CS myopathy is associated with important urinary losses of Cr (2).

The administration of Cr also attenuated the falls in exercise duration, maximum speed, and $\dot{V}O_{2\,max}$ observed after Dexa administration. G4 animals showed a better exercise performance than G3 animals, a finding characterized, among other factors, by a less marked drop in $\dot{V}O_{2\,max}$.

The better exercise performance of G4 compared with G3 may be largely explained by the preservation of muscle mass. However, since a previous study showed that Cr supplementation leads to attenuation of the CS-induced decreases of muscular PCr contents, this supplementation may also probably lead to improvements in energy metabolism (32). Cr and ATP form PCr and ADP in a reversible reaction catalyzed by Cr kinase. This system works as a temporal and spatial energy and pH buffer (4, 26). As a spatial energy buffer, Cr and PCr are involved in the shuttling of ATP from the inner mitochondria to the cytosol. Large negative charges on PCr prevent diffusion across the membranes and "lock" Cr in the muscle cell (20). At times, in the presence of low pH, as is the case during lactic acid accumulation in exercise, the reaction will favor the generation of ATP. The PCr levels will increase during periods of recovery from exercise, when ATP is generated aerobically. The way and the extent to which this physiological system may be affected by high doses of CS are still unknown. A recent paper has reported that a 6-day Dexa treatment in rats led to altered resting gastrocnemius metabolism by decreasing oxidative phosphorylation, producing ATP at the expense of PCr (12). We speculate that the simultaneous administration of Cr may modify this picture, at least in part. A greater availability of Cr to the muscles could bring the tissue content of PCr to more physiological levels. Since anaerobic, glycolytic, short-acting type IIb fibers frequently show CS-induced histological abnormalities, we can also guess that these fibers would probably exhibit the greatest benefit with Cr supplementation (5, 41). Since our results indicated a beneficial effect of Cr on maximal exercise performance, the function of other fiber types may probably have been improved as well. Finally, another potentially useful action of Cr on CS-induced myopathy may be related to the stabilization of cell membranes (33, 35).

The CS-induced changes in animal breathing pattern showed only little reversal with the use of Cr. A significant effect of Cr on V_T could be observed only before doxapram stimulation. These poor functional results agree with the lack of morphological differences between groups observed in the diaphragm after treatment.

Although the present results indicate that Cr may play a beneficial role in steroidal myopathy, this study has limitations. The researchers who performed the functional evaluations and morphological analysis were not blinded to the treatments administered. In addition, since the methodology for the measurement of muscle levels of Cr was not available at our institution, such measurements could not be obtained.

In conclusion, long-term administration of high steroid doses led to significant decreases in muscle mass and maximal exercise performance of rats. Respiratory changes included increases in RR and V_T under resting conditions and impairment of Pes elevations after a chemical stimulus. Simultaneous supplementation with Cr attenuated the changes in muscle mass and exercise disorders, but practically had no effect on breathing disturbances. Clinical studies are necessary to clarify the potential role of Cr supplementation in subjects with, or at risk for, steroidal myopathy.

GRANTS

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REFERENCES

1. **Andrews R, Greenhaff P, Curtis S, Peny A, Cowley AJ.** The effect of dietary creatine supplementation on skeletal muscle metabolism in congestive heart failure. *Eur Heart J* 19: 617–622, 1998.
2. **Askari A, Vignos PJ Jr, Moskowitz RW.** Steroid myopathy in connective tissue disease. *Am J Med* 61: 485–491, 1976.
3. **Berneis K, Ninmis R, Haussinger D, Keller U.** Effects of hyper and hypo-osmolality on whole body protein and glucose kinetics in humans. *Am J Physiol Endocrinol Metab* 276: E188–E195, 1999.
4. **Bessman SP, Carpenter CL.** The creatine-creatine phosphate energy shuttle. *Annu Rev Biochem* 54: 831–862, 1985.
5. **Brannon TA, Adams GR, Conniff CL, Baldwin KM.** Effects of creatine loading and training on running performance and biochemical properties of rat skeletal muscle. *Med Sci Sports Exerc* 29: 489–495, 1997.
6. **Casey A, Constantin-Teodosiu D, Howell S, Hultman E, Greenhaff PL.** Creatine ingestion favorably affects performance and muscle metabolism during maximal exercise in humans. *Am J Physiol Endocrinol Metab* 271: E31–E37, 1996.
7. **Clark AF, Vignos PJ Jr.** Experimental corticosteroid myopathy: effect on myofibrillar ATPase activity and protein degradation. *Muscle Nerve* 2: 265–273, 1979.
8. **Cochran WG, Cox GM.** *Experimental Designs.* New York: Wiley, 1950, p. 148–155.
9. **Czerwinski SM, Kurowski TG, O'Neill TM, Hickson RC.** Initiating regular exercise protects against muscle atrophy from glucocorticoids. *J Appl Physiol* 63: 1504–1510, 1987.
10. **Dangott B, Schultz E, Mozalziak PE.** Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *Int J Sports Med* 21: 13–16, 2000.
11. **Dubowitz V.** *Muscle Biopsy. A Practical Approach* (2nd Ed.). London: Bailleré Tindall, 1985.
12. **Dumas JF, Bielicki G, Renou JP, Roussel D, Ducluzeau PH, Malthièry Y, Simard G, Ritz P.** Dexamethasone impairs muscle energetics, studied by ^{31}PNMR, in rats. *Diabetologia* 48: 328–335, 2005.

13. **Falduto MT, Young AP, Hickson RC.** Exercise interrupts ongoing glucocorticoid-induced muscle atrophy and glutamine synthetase induction. *Am J Physiol Endocrinol Metab* 263: E1157–E1163, 1992.
14. **Fergunson GT, Irvin CG, Cherniack R.** Effect of corticosteroids on diaphragm function and biochemistry in the rabbit. *Am Rev Respir Dis* 141: 156–163, 1990.
15. **Ferguson GT, Irvin CG, Cherniack RM.** Effect of corticosteroids on respiratory muscle histopathology. *Am Rev Respir Dis* 142: 1047–1052, 1990.
16. **Fuld JP, Kilduff LP, Neder JA, Pitsiladis Y, Lean MEJ, Ward SA, Cotton MM.** Creatine supplementation during pulmonary rehabilitation in chronic obstructive pulmonary disease. *Thorax* 60: 531–537, 2004.
17. **Gardiner PF, Edgerton VR.** Contractile responses of rat fast-twitch and slow-twitch muscles to glucocorticoid treatment. *Muscle Nerve* 2: 274–281, 1979.
18. **Gayan-Ramirez G, Vanderhoydone F, Verholven G, Decramer M.** Acute treatment with corticosteroids decreases IGF-1 and IGF-2 expression in the rat diaphragm and gastrocnemius. *Am J Respir Crit Care Med* 159: 283–289, 1999.
19. **Gordon A, Hultmann E, Kauser L, Kristjansson S, Rolf CJ, Nyquist A, Sylvén C.** Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphate and muscle performance. *Cardiovasc Res* 30: 413–418, 1995.
20. **Greenhaff P.** The nutritional biochemistry of creatine. *J Nutr Biochem* 8: 610–618, 1997.
21. **Hasselgren PO.** Glucocorticoids and muscle catabolism. *Curr Opin Clin Nutr Metab Care* 2: 201–205, 1999.
22. **Haussinger D, Lang F, Gerok W.** Regulation of cell function by the cellular hydration state. *Am J Physiol Endocrinol Metab* 267: E343–E355, 1994.
23. **Hickson RC, Czerwinski SM, Wegrzyn LE.** Glutamine prevents downregulation of myosin heavy chain synthesis and muscle atrophy from glucocorticoids. *Am J Physiol Endocrinol Metab* 268: E730–E734, 1995.
24. **Marolda M, Padua V, Camporeale FS, Lioffi M, Orsini AV, Gentile A.** Steroid miopathy: clinical and immunohistochemical study of a case. *Ital J Neurol Sci* 12: 409–413, 1991.
25. **Max SR, Mill J, Mearow K, Konagaya M, Konagaya Y, Thomas JW, Banner C, Vitkovic L.** Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles. *Am J Physiol Endocrinol Metab* 255: E397–E402, 1988.
26. **Meyer RA, Sweeney HL, Kushmerick MJ.** A simple analysis of the "phosphocreatine shuttle". *Am J Physiol Cell Physiol* 246: C365–C377, 1984.
27. **Mihic S, MacDonald R, McKenzie S, Tarnopolsky MA.** Acute creatinine loading increases fat-free mass, but does not affect blood pressure, plasma creatine, or CK activity in men and women. *Med Sci Sports Exerc* 32: 291–296, 2000.
28. **Mitsui T, Ozuma H, Nagasawa M, Iuchi T, Akaike M, Odorni M, Matsumoto T.** Chronic corticosteroid administration causes mitochondrial dysfunction in skeletal muscle. *J Neurol* 249: 1004–1009, 2002.
29. **Montgomery DC.** *Design and Analysis of Experiments*. New York: Wiley, 1997, p. 290–301.
30. **Nissen SL, Sharp RL.** Effect of dietary supplements on lean mass and strength gains with resistance exercise: a meta-analysis. *J Appl Physiol* 94: 651–659, 2003.
31. **Owczarek J, Jasinska M, and Orszulak-Michalak D.** Drug-induced myopathies. An overview of possible mechanisms. *Pharmacol Rep* 57: 23–34, 2005.
32. **Parise G, Mihic S, MacLennan D, Yarasheski KE, Tarnopolsky MA.** Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed muscle protein synthesis. *J Appl Physiol* 91: 1041–1047, 2001.
33. **Rawson E, Gunn B, Clarkson P.** The effects of creatine supplementation on exercise-induced muscle damage. *J Strength Cond Res* 15: 178–184, 2001.
34. **Roy BD, Bourgeois JM, Mahoney DJ, Tarnopolsky MA.** Dietary supplementation with creatine monohydrate prevents corticosteroid-induced attenuation of growth in young rats. *Can J Physiol Pharmacol* 80: 1008–1014, 2002.
35. **Sharov VG, Sacks VA, Kupriyanov VV, Lakomkin VL, Kapelko V, Steinschneider A, Javadov SA.** Protection of ischemic myocardium by exogenous phosphocreatine. I. Morphologic and phosphorus 31-nuclear magnetic resonance studies. *J Thorac Cardiovasc Surg* 94: 749–761, 1987.
36. **Singleton JR, Baker BL, Thorburn A.** Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis. *Endocrinology* 141: 2945–2950, 2000.
37. **Singleton JR, Dixit VM, Feldman EL.** Type I insulin-like growth factor receptor activation regulates apoptotic proteins. *J Biol Chem* 271: 31791–31794, 1996.
38. **Tarnopolsky MA, Martin J.** Creatine monohydrate increases strength in patients with neuromuscular diseases. *Neurology* 52: 854–857, 1999.
39. **Tarnopolsky MA, Mahoney DJ, Vajsar J, Rodriguez C, Doherty DJ, Roy BD, Biggar D.** Creatine monohydrate enhances strength and body composition in Duchenne muscular dystrophy. *Neurology* 62: 1771–1777, 2004.
40. **Williams MH, Branch JD.** Creatine supplementation and exercise performance: an update. *J Am Coll Nutr* 17: 216–234, 1998.
41. **Volek JS, Duncan ND, Mazzetti SA, Staron RS, Putukian M, Gomez AL, Pearson DR, Fink WJ, Kraemer WJ.** Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc* 31: 1147–1156, 1999.