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The effect of creatine supplementation on mass and performance of rat skeletal muscle

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Abstract

This study investigated the effect of dietary creatine supplementation on hypertrophy and performance of rat skeletal muscle. Male Sprague−Dawley rats underwent either tibialis anterior ablation or partial ablation of the plantaris/gastrocnemius to induce compensatory hypertrophy of the extensor digitorum longus (EDL) or soleus respectively, or sham surgery. Creatine (300 mg/kg) was administered to one half of each group for 5 weeks, after which force production was measured. With the leg fixed at the knee and ankle, the distal tendon of the EDL or soleus was attached to a force transducer and the muscle was electrically stimulated via the sciatic nerve. Synergist ablation resulted in a significant increase in EDL mass and in soleus mass relative to control muscles. However, no effect of creatine supplementation on muscle mass or performance was found between control and either group of creatine-treated rats. Despite an apparent increase in muscle creatine content, creatine supplementation did not augment muscle hypertrophy or force production in rat EDL or soleus muscle, providing evidence that the potential benefits of creatine supplementation are not due to a direct effect on muscle but rather to an enhanced ability to train.

Keywords: Ergogenic aid; Muscle hypertrophy; Electrical stimulation

Introduction

Phosphocreatine plays an important role in maintaining the energy state of the muscle cell. Dietary supplementation of creatine has been shown to increase muscle levels of both creatine and phosphocreatine by 20–50% (Balsom et al., 1995; Green et al., 1996a,b; Harris et al., 1992; Hultman et al., 1996). Creatine taken up by skeletal muscle is phosphorylated by creatine kinase and functions to buffer the changes in ATP during altered energy states such as the transition from rest to steady-state exercise (Meyer and Foley, 1996). Phosphocreatine, which also serves as a source of ATP during high-intensity, short-duration exercise, is usually depleted in the first 20 s of high-intensity exercise (Meyer and Foley, 1996). For this reason, creatine supplementation is thought to exert an ergogenic effect on activities that consist of short-duration, high-intensity muscular activity and activities that feature repeated bouts of high-intensity activity (Balsom et al., 1995; Casey et al., 1996; Greenhaff et al., 1993; Harris et al., 1992). In addition, increases in fat free mass and resistance exercise performance have been attributed to creatine supplementation (Balsom et al., 1995; Green et al., 1996b; Kreider et al., 1998; Vandenberghe et al., 1997; Volek et al., 1999). Recent studies on the ergogenic effects of creatine supplementation have resulted in mixed outcomes. As recently reviewed, several investigations have found that creatine supplementation enhances performance in cycling, swimming, running, kayaking, and weight lifting, while other studies have failed to show any difference between creatine supplementation and placebo in similar measures of performance (Terjung et al., 2000). The purpose of this study was to investigate the effect of creatine supplementation on muscle hypertrophy and performance, using an animal model of compensatory hypertrophy to avert potential placebo effects and the variability of athletic performance. Compensatory hypertrophy induced by synergist

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Table 1
Increase in body weight and final muscle weight after 5 weeks of dietary supplementation with creatine monohydrate following ablation surgery or sham surgery

	Sham surgery	Tibialis anterior ablation surgery	Plantaris/gastrocnemius ablation surgery
Increase in body weight (g)			
Control	211±8	218±6	222±7
Creatine	211±9	205±7	176±7*
EDL weight (mg)			
Control	189±6	273±25**	
Creatine	177±6	299±39**	
Soleus weight (mg)			
Control	177±8		505±77**
Creatine	173±8		419±64**

Values are mean±SE. *Significant difference from control, $P<0.05$; **$P<0.01$.

ablation has been proposed as the best model to study exercise-induced muscle enlargement (Timson, 1990). In this study, hypertrophy of the extensor digitorum longus (EDL) and soleus muscles in rats was induced by ablation of the synergist tibialis anterior muscle or partial ablation of the plantaris/gastrocnemius respectively (Young et al., 1992). Performance of the muscle was evaluated by electrical stimulation to fatigue via the sciatic nerve. It was hypothesized that creatine supplementation during functional overload would result in greater increases in muscle mass and contractile force compared with functional overload alone.

Methods

Animals

The UNLV Institutional Animal Care and Use Committee approved all protocols in this study. Thirty-six male Sprague−Dawley rats (183±2 g) were randomly divided into six equal groups. The groups were sham surgery with and without creatine supplemented diet, tibialis anterior ablation surgery with and without supplemented diet, and plantaris/gastrocnemius ablation with and without supplemented diet. Rats were housed individually and fed standard rodent chow ad libitum. Creatine supplementation was begun immediately after recovery from surgery. Creatine monohydrate (Sigma Chemical, St. Louis, MO) was fed in gelatin, at a dose of 300 mg/kg body wt, daily for 5 weeks. This dose of creatine is the same as that used in other animal studies (Brannon et al., 1997; Gagnon et al., 2002; McGuire et al., 2001, 2002) and is equivalent to the customary loading dose of 20 g/day in a 70 kg person which produces maximal effects in 5 days (Greenhaff et al., 1994; Harris et al., 1992; Hultman et al., 1996).

Table 2
Peak force (g) produced in response to electrical stimulation

Condition	EDL initial stimulation	EDL recovery stimulation	Soleus initial stimulation	Soleus recovery stimulation
Control	41±4	6±1	42±3	20±5
Control +creatine	36±9	11±4	37±9	21±6
Hypertrophy*	54±4	23±2	49±10	36±10
Hypertrophy +creatine*	51±8	31±10	56±6	31±8

Values are mean±SE. *Hypertrophied muscles were significantly different from control muscles for both initial and recovery stimulations, $P<0.05$.

Table 3
Size corrected peak force for EDL

Condition	Initial stimulation	Recovery
Control EDL	22.0±1.9	3.3±0.3
Control EDL+creatine	20.5±5.1	6.4±2.4
Hypertrophy EDL	21.0±1.7	8.9±1.0
Hypertrophy EDL+creatine	15.3±3.3	9.1±3.1

Peak force (g) per 100 mg muscle weight. Values are mean±SE.

Ablation surgery

Rats were anesthetized with pentobarbital sodium (5 mg/100 g body wt, ip) and bilateral ablations were preformed. The lateral aspect of each hindlimb was shaved and a longitudinal incision was made through the skin and fascia, along the lateral aspect of the tibia. To induce hypertrophy of the EDL, the distal tendon of the tibialis anterior muscle was cut and the muscle removed in toto. Hypertrophy in the soleus was induced by ablation of the plantaris and gastrocnemius muscles. The lateral and medial heads of the gastrocnemius were isolated with careful blunt dissection and the distal 2/3 of the gastrocnemius and plantaris were removed with care, leaving the nerve and vascular supply to the soleus intact. The fascia and skin were then sutured, distal to proximal, with 5–0 silk and the rats were returned to their individual cages. Rats were monitored during recovery for signs of pain, stress, or discomfort.

To compensate for variations in creatine concentration caused by contractile force measurements, urinary creatinine was measured in a subset of rats prior to electrical stimulation. 12 rats (6 control and 6 creatine supplemented) were randomly selected and placed in metabolic cages for 24 h. Urine was collected and 24 h creatinine output was determined spectrophotometrically by standard colorimetric assay.

Electrical stimulation

Five weeks following ablation surgery, rats were again anesthetized with pentobarbital sodium (5 mg/100 g body wt, ip) and the hindlimb prepared for electrical stimulation. The sciatic nerve was exposed in the region of the thigh, before the separation of the tibial and common peroneal branches, but not cut. A dastre electrode (Harvard Apparatus, Holliston, MA) was placed around the nerve, proximal to the bifurcation. The limb was fixed at the knee and ankle by steel pins under the tibiopatellar ligament and the achilles tendon, respectively. The distal tendon of the muscle was cut and tied to an isometric force