Strength loss after eccentric contractions is unaffected by creatine supplementation

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Warren, Gordon L., John M. Fennessy, and Melinda L. Millard-Stafford. Strength loss after eccentric contractions is unaffected by creatine supplementation. *J Appl Physiol* 89: 557–562, 2000.—This study's objective was to determine whether 14 days of dietary creatine supplementation preceding an injurious bout of eccentric contractions affect the in vivo strength loss of mouse anterior crural muscles. Three groups of nine mice each were fed a meal diet for 14 days, one group at each of three levels of creatine supplementation (i.e., 0, 0.5, and 1% creatine). Electrically stimulated concentric, isometric, and eccentric contraction torques produced about the ankle were measured both before and after a bout of 150 eccentric contractions. Tibialis anterior muscle creatine concentration was significantly increased by the supplementation, being 12% higher in the mice fed the 1% creatine diet compared with control mice. After the bout of eccentric contractions, the reductions in torque (i.e., 46–58%) were similar for the isometric contraction, all eccentric contractions, and the slow (i.e., ≤ 200 °/s) concentric contractions; above 200 °/s, the percent reduction in concentric torque increased progressively to 85–88% at 1,000–1,200 °/s. However, there was no effect of creatine supplementation on the isometric torque loss or on the torque loss at any eccentric or concentric angular velocity ($P \geq 0.62$). In conclusion, a moderate increase in muscle creatine concentration induced by dietary supplementation in mice does not affect the strength loss after eccentric contractions.

muscle; torque; damage; angular velocity

BECAUSE OF ITS ERGOGENIC POTENTIAL, oral creatine supplementation is in widespread use among athletes, particularly those competing in sports with brief bouts of intense muscular exercise (e.g., track and field, swimming, American football). A recent National Collegiate Athletic Association study revealed that 13% of all US intercollegiate athletes used creatine supplementation in the last 12 mo (1). However, considerable speculation exists that creatine supplementation may be linked with a greater incidence of muscle injury (e.g., Refs. 11, 12). Creatine supplementation is associated with intramuscular water retention, potentially leading to increased muscle compartment pressures and tissue dysfunction (11). In addition, relatively rapid increases in muscle strength and body weight can occur in athletes using creatine supplementation,

and it is speculated that the additional stress may predispose muscle and other connective tissues to injury (12). However, there is no peer-reviewed research supporting these views, and there are even preliminary data showing decreased muscle injury incidence rates among collegiate football players taking creatine supplements (8, 13).

It has been hypothesized that creatine supplementation may in fact protect against the strength loss and degeneration associated with several skeletal muscle diseases (26). Data supporting this hypothesis come from dystrophic and normal muscle cells cultured in a creatine-containing media (19). The cells were found to be protected from stress induced by hypoosmotic conditions or a high extracellular Ca^{2+} concentration. The protection was attributed to a better maintenance of intracellular Ca^{2+} homeostasis in the stressed cells, presumably resulting from a phosphocreatine concentration-dependent stimulation of the sarcoplasmic reticulum Ca^{2+}-ATPase. A protective effect of creatine supplementation against injury appears not to be limited to skeletal muscle. The improved intracellular energy status after supplementation has been proposed to explain the protection from injury observed in a central nervous tissue model of Huntington's disease (17) and in myocardial tissue subjected to metabolic stress (6).

The purpose of the present study was to assess the effect of creatine supplementation on the susceptibility of muscle to contraction-induced injury. Changes in muscle functional capacity are considered the best means of quantifying contraction-induced skeletal muscle injury (7, 22). Thus the specific objective was to determine whether the in vivo strength loss of mouse anterior crural muscles after a bout of eccentric contractions is affected by 14 days of dietary creatine supplementation preceding the bout. We sought to test the hypothesis that an increase in muscle creatine concentration would attenuate the strength loss associated with eccentric contractions. The hypothesis is based on the limited data in the literature indicating a protective effect of creatine supplementation against tissue injury in general. To our knowledge, the present

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study is the first to directly test for an effect of creatine supplementation on muscle injury susceptibility. The findings are important because of their implications for athletes' safety and for the efficacy of creatine supplementation.

METHODS

Animals

Twenty-seven female ICR mice (8 wk old) were purchased from Harlan Laboratories. The mice were individually housed in polycarbonate cages ($27 \times 18 \times 13$ cm) with a 12:12-h light-dark cycle and at a temperature of 23–25°C. Five days after arriving at the animal care facility, the mice were weighed and divided into three groups of nine mice each such that the mean body weights of the three groups were identical [i.e., 27.3 ± 0.9 (SD), 27.3 ± 0.9, and 27.4 ± 1.0 g]. For a 14-day period, each group was fed ad libitum with Purina 5001 rodent diet in meal form. The diet of two groups of mice was supplemented with creatine monohydrate powder (Nature's Best), one at 0.5% creatine and the other at 1.0% creatine. The rationale for selecting the supplement dosages was based on data showing *1*) increased treadmill performance but relatively small increases in muscle creatine content in rats supplemented at 0.33% creatine (4) and *2*) near-maximal neuroprotective effects in rats supplemented with 1% creatine (17). For each mouse, feed and water consumption as well as body weight were measured on a daily basis.

In preparation for the experimental protocol, each mouse was anesthetized with intraperitoneal injections of 0.33 mg/kg fentanyl, 16.7 mg/kg droperidol, and 5.0 mg/kg diazepam. This anesthetic regimen is the regimen we previously determined to be optimal for in vivo measurements of anterior crural muscle torque production about the ankle (9). At the conclusion of each experiment, the mouse was euthanized with an overdose of pentobarbital sodium (200 mg/kg ip). All animal care and experimental procedures were approved by the Georgia State University institutional animal care and use committee and were in compliance with the guidelines set by the American Physiological Society.

Experimental Procedures

In vivo strength measurements and injury protocol. After the 14-day dietary regimen, contractile properties of the mouse left anterior crural muscles were studied by using the miniature isokinetic dynamometer as described previously (e.g., Refs. 20, 21, 23). First, the lower lateral left hindlimb was shaved and aseptically prepared. Two percutaneous needle electrodes were inserted adjacent to the common peroneal nerve as it passes over the lateral gastrocnemius muscle. By using 200-ms trains of 0.1-ms pulses at 300 Hz, stimulation voltage was adjusted to yield the maximal isometric tetanic torque of the anterior crural muscles.

Next, a sequence of seven concentric, one isometric, and seven eccentric contractions (in that order) was performed. Concentric and eccentric contractions were done at seven angular velocities about the ankle joint (i.e., 100, 200, 400, 600, 800, 1,000, and 1,200 °/s). The concentric and eccentric contractions were done over a 40° arc (i.e., ±20° about the ankle angle in which the plantar surface is perpendicular to the tibia longitudinal axis). The muscles were stimulated at 300 Hz only for a duration necessary to complete the movement (i.e., 33.3 ms at 1,200 °/s to 400 ms at 100 °/s). Contractions in this sequence were done at 45-s intervals. The con-

centric contractions were done in order of decreasing angular velocity, whereas the eccentric contractions were done in order of increasing velocity. Such an order results in a progressive increase in peak torque over the 15 contractions and minimizes the influence of any possible injury induced by preceding high-force contractions. Furthermore, the contraction sequence itself does not induce fatigue because the anterior crural muscle group is quite difficult to fatigue (20, 21).

An injury-inducing protocol consisting of 150 eccentric contractions was then done as previously described in studies from our laboratory characterizing the injury model histologically, biochemically, and physiologically (e.g., Refs. 10, 14, 21, and 23). Eccentric contractions of the anterior crural muscles were done by moving the foot from 20° of dorsiflexion to 20° of plantar flexion at 2,000 °/s. A 100-ms isometric stimulation immediately preceded the plantar flexion movement; thus total stimulus duration for the contraction was 120 ms. Contractions were done at 12-s intervals, making the protocol ~30 min long.

Beginning 3 min after the 150th eccentric contraction, the sequence of seven concentric, one isometric, and seven eccentric contractions was performed again. In this experimental model, the greatest isometric torque loss occurs immediately after the injury protocol with a slow recovery occurring thereafter; the recovery in isometric torque is, however, not complete even after 2 wk (10, 14, 21). Because we were most interested in how muscle creatine concentration influences the peak strength loss and not the recovery of strength per se, strength measurements were not done at later times after the injury.

After the last contraction in the sequence of 15 contractions, both the exercised and contralateral control tibialis anterior (TA) muscles were excised. The muscles were weighed and frozen in liquid nitrogen as rapidly as possible. Muscles were stored at −80°C until the time the total creatine concentration assays were performed. Only the TA muscles were excised and assayed because the TA muscle comprises 82% of the mass of the anterior crural muscle group and contributes 89% of the maximal isometric tetanic torque produced by the muscle group (Ref. 14; unpublished observations). In addition, the fiber-type composition of the TA muscle is similar to that of the entire anterior crural muscle group (i.e., ≥95% fast-twitch fibers).

Total creatine concentration assays. Total creatine concentrations were determined in the extracts of contralateral control TA muscles by using a fluorometric procedure (5). A muscle extract was prepared by homogenizing the TA muscle (~50 mg wet wt) in 0.75 ml of ice-cold 0.6 M perchloric acid followed by a centrifugation for 5 min at 5,000 g and 4°C. An aliquot (400 μl) of the supernatant was then neutralized with 90 μl of ice-cold 2 M K_2CO_3. After sitting on ice for 15 min, the extract (minus the potassium perchlorate precipitate) was removed and filtered in a 1.5-ml filter microcentrifuge tube for 5 min at 5,000 g and 4°C. Aliquots (13 μl) of the extract were then frozen in borosilicate glass tubes and stored at −80°C until the time the assays were run.

To assay for total creatine concentration, residual phosphocreatine in the muscle extract had to be hydrolyzed. A 13-μl aliquot of extract was mixed with 487 μl H_2O and 50 μl 1 M HCl and heated at 60°C for 20 min. This procedure completely hydrolyzes all phosphocreatine as determined from muscle extracts spiked with phosphocreatine. The procedure also does not result in any measurable degradation of free creatine. After the phosphocreatine hydrolysis, sample volume was brought to 2 ml with H_2O. The rest of the assay was conducted as described by Conn (5).

Statistical Analyses

The effects of diet (i.e., 0, 0.5, and 1% creatine) and contraction number on the torque loss during the injury protocol were analyzed by using a two-way ANOVA with repeated measures on the contraction number factor. The effects of diet, injury (i.e., before vs. after the injury protocol), and angular velocity on torque were analyzed by using a three-way ANOVA with repeated measures on injury and angular velocity factors. The effects of diet and muscle type (i.e., exercised vs. contralateral control muscle) on muscle wet weight were analyzed by using a two-way ANOVA with repeated measures on the muscle-type factor. The effects of diet and time (before vs. after 14-day dietary regimen) on mouse body weight were analyzed by using a two-way ANOVA with repeated measures on the time factor. The effect of diet on other variables was analyzed by using a one-way ANOVA. When significant main effects or interactions were found, post hoc testing was done by using Student-Newman-Keuls tests or single-degree-of-freedom contrasts in the case of the repeated-measures ANOVAs. All repeated-measures ANOVAs were run by using Systat 8.0 (SPSS Science); the remaining statistics were run by using Sigma-Stat 1.02 (Jandel Scientific). An α-level of 0.05 was used for all statistical analyses. Values presented in RESULTS are means $\pm$ SE.

RESULTS

The presence of creatine monohydrate in the diet of the mice did not alter their feed or water intake. There were no significant differences among the three diet groups in feed ($P = 0.43$) or water ($P = 0.24$) consumption over the 14-day period. For all mice, mean feed and water consumption were 4.8 ± 0.1 g/day and 8.8 ± 0.3 ml/day, respectively. The mean creatine intake of the 0.5% creatine and 1% creatine groups was 0.84 ± 0.02 and 1.74 ± 0.06 g $\cdot$ kg$^{-1} \cdot$ day^{-1}, respectively. Body weight of the mice increased on average by 8% over the 14-day period, i.e., from 27.3 ± 0.3 to 29.5 ± 0.3 g. However, there were no differences among the three diet groups in this weight gain or in the final body weights of the mice ($P \geq 0.52$).

As shown in Fig. 1, TA muscle creatine concentration was significantly increased by the creatine supplementation ($P = 0.01$). However, only the concentration of the 1% creatine group was significantly greater (i.e., by 12%) than that of the 0% creatine group (Fig. 1A). TA muscle creatine concentration was significantly correlated to creatine intake on a mouse-by-mouse basis ($r = 0.61$; $P = 0.001$; Fig. 1B).

Before the injury protocol, maximal isometric tetanic torque for all mice averaged 3.89 ± 0.06 N $\cdot$ mm (Fig. 2). There was a strong effect of angular velocity on concentric torque, with torque decreasing almost linearly with increasing velocity; at 1,200 °/s, concentric torque averaged 22% of that measured isometrically. On the other hand, angular velocity had little effect on eccentric torque, with eccentric torque ranging from 56 to 64% above the isometric level. There was no significant effect of the creatine supplementation on concentric, isometric, or eccentric torque measured before the injury protocol ($P = 0.76$ for a main effect and $P = 0.98$ for a diet $\times$ angular velocity interaction; Fig. 2).
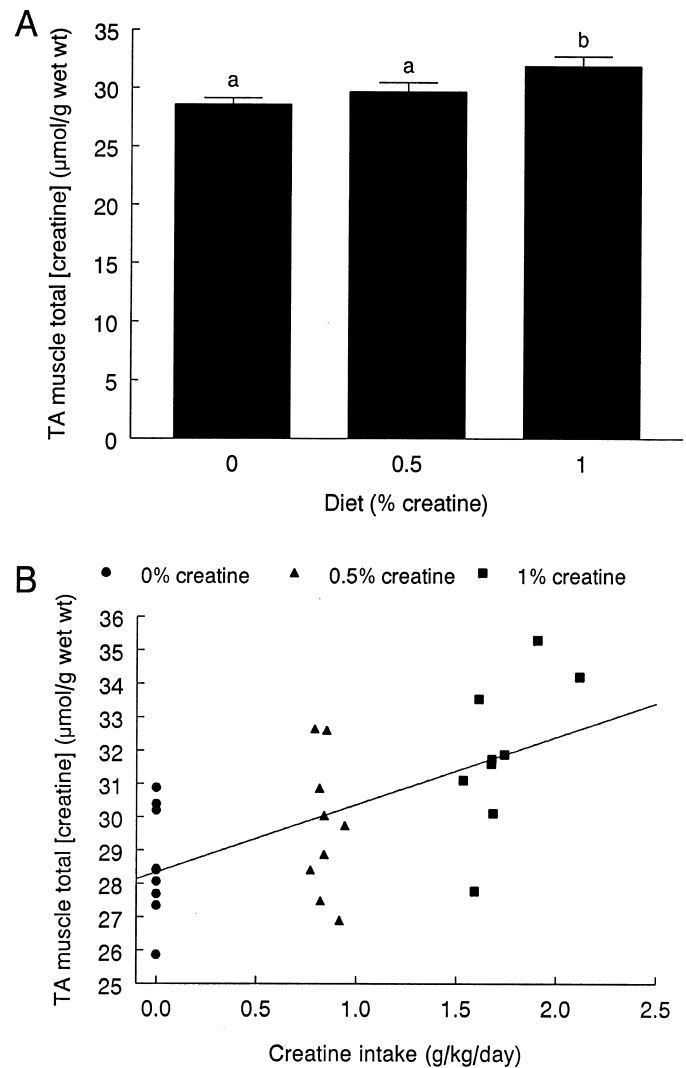

Fig. 1. Effect of dietary creatine supplementation on tibialis anterior (TA) muscle total creatine concentration ([creatine]). *A*: comparison among the 3 diet groups (i.e., 0, 0.5, and 1% creatine) of total [creatine] in control TA muscle. Values with the same letter are not significantly different. *B*: relationship of TA muscle total [creatine] to daily creatine intake among the 27 mice. Solid line, least squares fit between the 2 variables (i.e., $r = 0.61$, $P = 0.001$).

During the injury protocol, peak torque fell sharply over the first 50 contractions but more gradually thereafter; by the 150th contraction, peak torque had decreased by 47% (Fig. 3). There was no effect of creatine supplementation on the peak torque during the first eccentric contraction or on the decline in torque over the protocol ($P \geq 0.82$).

After the injury protocol, the decrease in torque was dependent on the angular velocity at which the contractions were done (Fig. 2). The isometric contraction, all eccentric contractions, and the slow (i.e., ≤ 200 °/s) concentric contractions had similar percent reductions in torque (i.e., 46–58%). As concentric angular velocities increased above 200°/s, the percent reduction in torque increased progressively to 85–88% at 1,000–1,200°/s. The greater torque reductions at higher angular velocities are most likely due to a reduction in
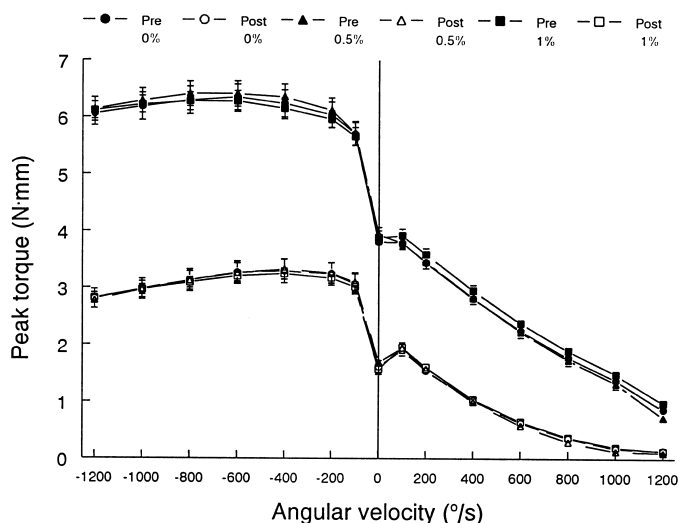
Fig. 2. Effect of dietary creatine supplementation (i.e., 0 vs. 0.5 vs. 1% creatine) on peak torque production by anterior crural muscles during the sequence of 7 concentric, 1 isometric, and 7 eccentric contractions measured both before (Pre) and after (Post) injury-inducing eccentric contraction protocols. Positive and negative angular velocities indicate concentric and eccentric contractions, respectively. There was no significant effect of creatine supplementation on torque measured before the injury protocol ($P \geq 0.76$) or on torque loss after the protocol ($P \geq 0.62$).

muscle maximal shortening velocity that occurs in addition to the loss of intrinsic force-producing capacity (24). In contrast to our hypothesis, there was no effect of creatine supplementation on the isometric torque loss or on the torque loss at any eccentric or concentric angular velocity ($P = 0.62$ for a diet × injury interaction and $P = 1.00$ for a diet × injury × angular velocity interaction). Furthermore, when examined across all animals, there were no significant correlations be-

between muscle creatine content and the protocol-induced torque reduction as measured with concentric, isometric, or eccentric contractions ($P \geq 0.13$).

Immediately after the final strength measurements, both the exercised and contralateral control TA muscles were excised, and wet weights were obtained. The mean weight of the exercised TA muscles from the 0% creatine mice was 4.5% greater than that of the contralateral control muscles (Fig. 4). The mean weight of the exercised muscles from the 0.5% creatine and 1% creatine mice was 1.9–3.8% greater than that of the contralateral control muscles, but these differences were not statistically significant ($P \geq 0.07$).

DISCUSSION

The results of the present study show that 14-day dietary creatine supplementation has no protective effect on the strength loss induced by a bout of injurious eccentric contractions. These findings do not support our hypothesis that an increased muscle creatine concentration would have a protective effect because of previous observations showing protection from injury in neural and myocardial tissues and in cultured muscle cells (6, 17, 19). Our findings also do not support the speculation that creatine supplementation may be linked with a greater incidence of muscle injury (11, 12), at least when the injury assessment is based on function. It is, however, possible that the creatine supplementation might have been found to attenuate (or exacerbate) the injury if other injury markers (e.g., histopathology, blood levels of myocellular proteins) had been employed.

Our negative findings cannot be attributed to an insensitive experimental design. The repeated-measures design we employed was quite sensitive to changes in muscle strength. For example, if the strength loss in the 1% creatine group alone had differed by 6.6% on average, then a significant effect of
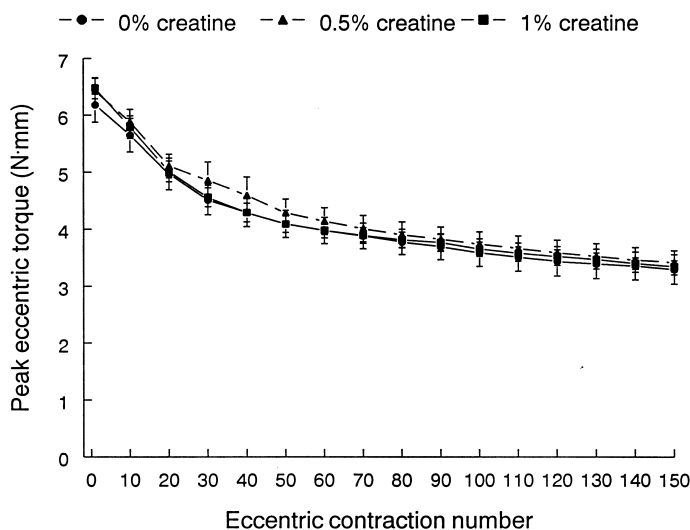

Fig. 3. Effect of dietary creatine supplementation on peak torque production by anterior crural muscles during the injury protocol consisting of 150 eccentric contractions. Data are shown for the 1st eccentric contraction and every 10th contraction thereafter. There was no significant effect of creatine supplementation on decline in torque over the injury protocol ($P \geq 0.82$).
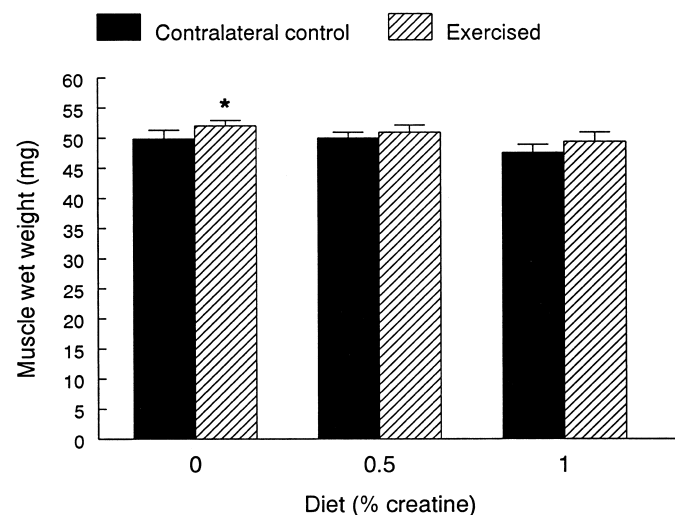

Fig. 4. Effect of injury protocol on TA muscle wet weight in each of the 3 diet groups. *Significant difference between exercised and contralateral control muscle values in the 0% creatine diet group, $P < 0.05$.

creatine supplementation on the strength loss would have been found. This is despite the fact that the postinjury torque values were virtually identical for all three groups of mice (Fig. 2).

The negative findings also cannot be attributed to a failure of the creatine supplementation. TA muscle creatine concentration in the 1% creatine group was 12% greater than that measured in the 0% creatine group. Although muscle phosphocreatine concentrations were not measured because of a decision not to freeze clamp the muscles in situ, one would predict from the literature that the percent increase in phosphocreatine concentration would have exceeded that observed for total creatine concentration (4, 25). The 12% increase in muscle creatine concentration is similar to the 11% increase previously reported for the fast plantaris muscle of rats provided a 0.33% creatine diet for 14 days (4). Although an 11% increase seems quite modest, Brannon and co-workers (4) reported significant improvements by the rats in sprint performance on the treadmill.

It appears that dietary creatine supplementation may be more effective in elevating muscle creatine concentration in humans than in small animals (present study; Refs. 4, 15, 25). In a recent review by Williams and colleagues (25) of 21 creatine supplementation studies in humans, muscle creatine concentration after ~1 wk of supplementation increased 18.5% on average, ranging from 15 to 22%. This is despite the fact that, compared with the rodent studies, the human studies typically supplemented at lower creatine intakes (i.e., ~0.3 $g \cdot kg^{-1} \cdot day^{-1}$) and for shorter periods. The greater accumulation of creatine in human muscle may reflect intrinsic species differences in creatine metabolism, but it is may also reflect the fact that normal rodent feed (e.g., Purina 5001) is high in arginine and glycine. It has been shown that a rodent diet high in arginine and glycine is as effective in increasing muscle creatine concentration as is a creatine-supplemented diet (18). This may explain why the high creatine intake rates used in the present study (i.e., ~3- to ~6-fold greater than that typically used in human studies) elicited only moderate increases in muscle creatine concentration. It may be advisable for future creatine supplementation studies with rodents to use a control diet with arginine and glycine concentrations at the lower end of the range recommended for rodents rather than at the higher end (e.g., 0.7 vs. 1.4% for arginine and 0.6 vs. 1.2% for glycine).

One could argue that the higher muscle creatine accumulation observed in human studies might have a protective (or adverse) effect on the susceptibility of skeletal muscle to contraction-induced injury. Contraction-induced injury is associated with elevated free cytosolic Ca^{2+} levels (2, 3, 10, 16), and this elevation has been proposed to result in a failure of excitation-contraction coupling causing most of the initial strength loss (2, 10). It is possible that the modest elevation in the mouse TA creatine concentration was insufficient to stimulate sarcoplasmic reticulum Ca^{2+}-ATPase activity and help maintain a lower free cytosolic Ca^{2+}

levels in the injured muscle cells, as was found in the work of Pulido and co-workers (19). However, we do not think that higher TA muscle creatine concentrations in the mouse, if achievable, would have been protective. When the data were analyzed on a mouse-by-mouse basis, there were no significant correlations (or systematic trends) found between muscle creatine content and the injury protocol-induced decrease in torque under any eccentric, isometric, or concentric condition.

A protective effect of creatine against the strength loss might have been found if we had employed an injury-inducing protocol that also induced fatigue. Creatine supplementation has been shown to ameliorate the strength losses associated with high-intensity fatiguing events [see review by Williams et al. (25)]. However, the injury-inducing protocol we used did not have a fatigue component. A bout of equivalent concentric contractions has no adverse effect on maximal isometric torque in this experimental model; torque actually increases 5–6% after a bout of 150 concentric contractions (20, 21).

The only beneficial effect of creatine supplementation in the present study was a small reduction in the swelling induced by the injury protocol. The exercised muscles from the 0% creatine mice weighed 5% more than did their contralateral unexercised muscles. However, in the creatine-supplemented mice, the wet weights of the exercised muscles were not significantly different from those of their contralateral muscles. The explanation for this effect is not clear. One might have expected the muscles from the supplemented mice to weigh more on the basis of preliminary data from creatine supplementation studies on humans that have shown increased muscle water content and/or growth after creatine supplementation (27, 28). Control muscle weights in the supplemented mice were not greater than those in the unsupplemented mice ($P = 0.23$); therefore, creatine supplementation apparently did not induce fluid retention.

In conclusion, a moderate increase in muscle creatine concentration induced by dietary supplementation in mice does not increase or decrease a muscle's susceptibility to contraction-induced injury. This conclusion is based on changes in muscle function observed after injury and not on changes in other markers of injury (e.g., histopathology, blood levels of myocellular proteins) that might have been affected by the creatine supplementation. This distinction is of great practical importance because it is the change in muscle function that is of most importance to the athlete and coach, from both performance and general health standpoints.

REFERENCES

1. **Armsey TD and Green GA.** Nutrition supplements: science vs. hype. *Physician Sportsmed* 25: 76–92, 1997.
2. **Balnave CD and Allen DG.** Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *J Physiol (Lond)* 488: 25–36, 1995.
3. **Balnave CD, Davey DF, and Allen DG.** Distribution of sarcomere length and intracellular calcium in mouse skeletal muscle

following stretch-induced injury. *J Physiol (Lond)* 502: 649–659, 1997.

4. **Brannon TA, Adams GR, Conniff CL, and 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.

5. **Conn RB.** Fluorometric determination of creatine. *Clin Chem* 6: 537–548, 1960.

6. **Constantin-Teodosiu D, Greenhaff PL, Gardiner SM, Randall MD, March JE, and Bennett T.** Attenuation by creatine of myocardial metabolic stress in Brattleboro rats caused by chronic inhibition of nitric oxide synthase. *Br J Pharmacol* 116: 3288–3292, 1995.

7. **Faulkner JA, Brooks SV, and Opiteck JA.** Injury to skeletal muscle fibers during contraction: conditions of occurrence and prevention. *Phys Ther* 73: 911–921, 1993.

8. **Hunt J, Kreider R, Melton C, Ransom J, Rasmussen C, Stroud T, Cantler E, and Milnor P.** Creatine does not increase incidence of cramping or injury during pre-season college football training. II (Abstract). *Med Sci Sports Exerc* 31: S355, 1999.

9. **Ingalls CP, Warren GL, Lowe DA, Boorstein DB, and Armstrong RB.** Differential effects of anesthetics on in vivo skeletal muscle contractile function in the mouse. *J Appl Physiol* 80: 332–340, 1996.

10. **Ingalls CP, Warren GL, Williams JH, Ward CW, and Armstrong RB.** E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol* 85: 58–67, 1998.

11. **Juhn MS and Tarnopolsky M.** Potential side effects of oral creatine supplementation: a critical review. *Clin J Sport Med* 8: 298–304, 1998.

12. **Kreider RB.** Creatine supplement: analysis of ergogenic value, medical safety, and concerns. *J Exerc Physiol* [Online] 1(1), 12 p., 1998. http://www.css.edu/users/tboone2/asep/jan3.htm. [1999, Dec. 18].

13. **Kreider R, Melton C, Hunt J, Rasmussen C, Ransom J, Stroud T, Cantler E, and Milnor P.** Creatine does not increase incidence of cramping or injury during pre-season college football training. I (Abstract). *Med Sci Sports Exerc* 31: S355, 1999.

14. **Lowe DA, Warren GL, Ingalls CP, Boorstein DB, and Armstrong RB.** Muscle function and protein metabolism after initiation of eccentric contraction-induced injury. *J Appl Physiol* 79: 1260–1270, 1995.

15. **Lowe JA, Murphy M, and Nash V.** Changes in plasma and muscle creatine concentration after increases in supplementary dietary creatine in dogs. *J Nutr* 128: 2691S–2693S, 1998.

16. **Lynch GS, Fary CJ, and Williams DA.** Quantitative measurement of resting skeletal muscle $[Ca^{2+}]_i$ following acute and long-term downhill running exercise in mice. *Cell Calcium* 22: 373–383, 1997.

17. **Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, and Beal MF.** Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* 18: 156–163, 1998.

18. **Minuskin ML, Lavine ME, Ulman EA, and Fisher H.** Nitrogen retention, muscle creatine and orotic acid excretion in traumatized rats fed arginine and glycine enriched diets. *J Nutr* 111: 1265–1274, 1981.

19. **Pulido SM, Passaquin AC, Leijendekker WJ, Challet C, Wallimann T, and Ruegg UT.** Creatine supplementation improves intracellular Ca^{2+} handling and survival in mdx skeletal muscle cells. *FEBS Lett* 439: 357–362, 1998.

20. **Warren GL, Ingalls CP, and Armstrong RB.** A stimulating nerve cuff for chronic in vivo measurements of torque produced about the ankle in the mouse. *J Appl Physiol* 84: 2171–2176, 1998.

21. **Warren GL, Ingalls CP, Shah SJ, and Armstrong RB.** Uncoupling of in vivo torque production from EMG in mouse muscles injured by eccentric contractions. *J Physiol (Lond)* 515: 609–619, 1999.

22. **Warren GL, Lowe DA, and Armstrong RB.** Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43–59, 1999.

23. **Warren GL, Lowe DA, Inman CL, Orr OM, Hogan HA, Bloomfield SA, and Armstrong RB.** Estradiol effect on anterior crural muscles-tibial bone relationship and susceptibility to injury. *J Appl Physiol* 80: 1660–1665, 1996.

24. **Warren GL, Williams JH, Ward CW, Matoba H, Ingalls CP, Hermann KM, and Armstrong RB.** Decreased contraction economy in mouse EDL muscle injured by eccentric contractions. *J Appl Physiol* 81: 2555–2564, 1996.

25. **Williams MH, Kreider RB, and Branch JD.** *Creatine: The Power Supplement*. Champaign, IL: Human Kinetics, 1999, p. 46–58.

26. **Wyss M, Felber S, Skladal D, Koller A, Kremser C, and Sperl W.** The therapeutic potential of oral creatine supplementation in muscle disease. *Med Hypotheses* 51: 333–336, 1998.

27. **Ziegenfuss T, Lemon PWR, Rogers MR, Ross R, and Yarasheski KE.** Acute creatine ingestion: effects on muscle volume, anaerobic power, fluid volumes, and protein turnover (Abstract). *Med Sci Sports Exerc* 29: S127, 1997.

28. **Ziegenfuss TN, Lowery LM, and Lemon PWR.** Acute fluid volume changes in men during three days of creatine supplementation. *J Exerc Physiol* [Online] 1 (3), 10 p., 1998. http://www.css.edu/users/tboone2/asep/jan13d.htm. [1999, Dec. 18].