Biochemical and Biophysical Research Communications **290,** 47–52 (2002)
doi:10.1006/bbrc.2001.6164, available online at http://www.idealibrary.com on IDEAL®

Direct Antioxidant Properties of Creatine

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Received November 21, 2001

Creatine is the most popular supplement proposed to be an ergogenic aid. There is some evidence in the literature that creatine supplementation increases lean body mass, muscular strength, and sprint power. However, the efficacy of creatine has not been consistent, and the potential mechanisms are unresolved. While limited evidence that suggests that creatine could possess an antioxidant effect this has not been tested directly. Because oxidants such as free radicals can affect muscle fatigue and protein turnover, it is important to know whether creatine can neutralize free radicals and other reactive oxygen species. We tested the hypothesis that creatine would remove superoxide anions ($O_2^{\cdot-}$), peroxynitrite ($OONO^-$), hydrogen peroxide, and lipid peroxides (t-butyl hydroperoxide). We also determined whether creatine displayed a significant antioxidant scavenging capacity (ASC) using 2,2′-azino-bis(3-ethylbenzothiazolamine-6-sulfonic acid) ($ABTS^+$) quenching as a marker. Creatine did not significantly reduce levels of hydrogen peroxide or lipid peroxidation. In contrast, creatine displayed a significant ability to remove $ABTS^+$, $O_2^{\cdot-}$, and $OONO^-$ when compared with controls. Creatine quenching of $ABTS^+$ was less than physiological levels of reduced glutathione (0.375 mM). To our knowledge, this is the first evidence that creatine has the potential to act as a direct antioxidant against aqueous radical and reactive species ions.

Key Words: **creatine; antioxidants; superoxide anion; hydrogen peroxide; lipid peroxidation; peroxynitrite; $ABTS^+$.**

Creatine is the most widely used supplement advocated as an ergogenic aid (20). Claims for increased lean body mass, skeletal muscle strength, muscle power, and muscle endurance are scattered throughout the literature. However, direct supporting evidence for ergogenic claims has been inconsistent at best (5, 34), and many of studies have not focused critically on potential biochemical mechanisms that could effect fatigue or muscle protein turnover. Demant and Rhodes (7) proposed that people might be categorized as "responders" or "non-responders" to a wide variation of creatine dosage, formula, and frequency of administration (34). It is also possible that alterations in negative feedback systems such as downregulation of the creatine transporter (4) or mitochondrial respiration could confound the efficacy of creatine supplementation.

Creatine supplementation does appear to increase creatine levels in muscle cells as determined by biochemical and magnetic resonance imaging (MRI) techniques, although loading is not consistent (11, 21, 27). Creatine supplementation can cause weight gain through an increase in lean body mass with no effect on fat mass (36). Muscle mass gain could be a product of increased water content via elevated intracellular osmolality, increased myofibrillar content, or both (7, 16, 17, 31). If enhanced muscle protein and myofibrillar protein occur with creatine supplementation, the mechanisms are uncertain (17). Recently, Dangott *et al.* (6) demonstrated increased satellite cell mitotic activity with creatine supplementation, as determined through thymidine analog (5-bromo-2′-deoxyuridine) labeling, during compensatory hypertrophy following synergistic muscle ablation.

Creatine is a downstream product of the amino acids glycine and arginine producing guanidinoacetate and also ornithine, which feeds back into the urea cycle. Arginine is also a substrate for the nitric oxide synthase family and can increase production of nitric oxide, a free radical that modulates metabolism, contractility and glucose uptake in skeletal muscle (25, 30). A number of amino acids such as histidine, methionine, and cysteine are especially susceptible to free radical oxidation (10). Indeed, cysteine's sulfhydryl groups are redox modulators for the turnover and function of many proteins, able to protect protein sulfhydryls, and may increase muscular performance (22, 30). It is possible that creatine promotes antioxidant function as well. For example, Vergnani *et al.* (35) demonstrated a

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0006-291X/02 $35.00

protective role for the creatine precursor arginine against oxidative stress imposed by oxidized LDL in endothelial cells. Additional data indicate that arginine may be able to quench free radicals such as superoxide anions ($O_2^{\cdot -}$) (38). Matthews *et al.* (26) proposed that creatine supplementation provides direct or indirect antioxidant protection against metabolic damage with Huntington's disease. It is possible that creatine supplementation may blunt increased hypoxanthine efflux from muscle observed with repeated bouts of high intensity exercise (2, 13) and increased flux through xanthine oxidase (12), an enzyme that generates $O_2^{\cdot -}$ and hydrogen peroxide.

Since oxidants such as free radicals can affect muscle fatigue, protein turnover, atrophy, and growth (30), it is important to know whether creatine has the ability to neutralize free radicals and reactive oxygen species (ROS). Previously, *in vitro* and *in vivo* models demonstrated a contribution of reactive oxygen species to skeletal muscle fatigue and protection of antioxidants against fatigue (23, 30, 31). To our knowledge, no research articles exist which test the hypothesis that creatine acts directly as an antioxidant. To test this important hypothesis, we designed a series of experiments to determine the effect of creatine on five ROS systems: xanthine oxidase for superoxide anions ($O_2^{\cdot -}$), hydrogen peroxide, peroxynitrite ($OONO^-$) lipid peroxidation, and removal of $ABTS^+$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical. We chose to use a highly controlled paradigm to ensure that any antioxidant effect was directly due to creatine.

METHODS

All reagents were of the highest purity available. Creatine, cytochrome *c*, xylenol orange, superoxide dismutase (Cu,Zn isoform), and catalase were purchased from Sigma Chemical. Reduced glutathione and other reagents were purchased from ICN. We chose a creatine range (20–60 mM wet) that represents typical maximum levels of creatine found within muscle cells with and without creatine supplementation (27). Creatine had no effect on the pH of any of the assay systems.

To determine the direct creatine antioxidant ability, we tested the ability of creatine to quench 4 oxidant systems: 3 charged free radicals and reactive species ($O_2^{\cdot -}$, peroxynitrite, and $ABTS^+$) and uncharged, non-radical ROS (H_2O_2 and t-butyl hydroperoxide). This allowed our assessment of a broad range of radicals and ROS with emphasis on 4 physiological ROS systems: superoxide generation (via xanthine oxidase), hydrogen peroxide, peroxynitrite (via 3-morpholinosydnonimine known as SIN-1) and lipid peroxidation (t-butyl hydroperoxide) as well as a marker of antioxidant scavenging capacity that has proved to be sensitive across a broad array of biochemicals (28).

For comparative purposes, we also tested the antioxidant scavenging capacity of (a) physiological levels of reduced glutathione (0.375 mM) for skeletal muscle as well as (b) whole muscle homogenates. Gastrocnemius muscles ($n = 7$) were extracted from young adult-Fischer (4 month old) following anesthesia with 100 mg · kg^{-1} Na pentobarbital. Use of animals had been approved by the University Laboratory Animal Care Committee. Muscle samples were homogenized in 100 mM K^+ phosphate buffer (pH 7.4) prior to chemical assays (24).

Antioxidant assays. The antioxidant scavenging capacity (ASC) was determined using our modification of the technique of Pelligrini *et al.* (28). The principle involves the decolorization of the blue/green free radical chromophore $ABTS^+$ by a compound with antioxidant scavenging properties. Briefly, 660 μg/ml of potassium persulfate was added to 7 mM ABTS stock producing the stable radical cation $ABTS^+$. $ABTS^+$ was diluted to 91 μM and 600 μl was added to each cuvette. Then 600 μl of creatine (final [ ] = 0, 20, 40, or 60 mM), distilled water, or Trolox standard was added to the cuvettes. Absorbance was determined at 734 nm and recorded for 4 min. ASC was calculated and equated against a Trolox standard curve (0, 0.1, 0.22, 0.3, and 0.4 mM). This assay is sensitive to either singular or complex antioxidant systems (28). In a follow-up set of experiments, the ability of 40 mM creatine to remove $ABTS^+$ was compared to 0.375 mM reduced glutathione (GSH) and whole gastrocnemius homogenates.

Superoxide anion quenching ability was determined using xanthine oxidase and xanthine as substrate as a superoxide generator. We modified a procedure outlined by Ysebaert-Vanneste and Vanneste (40) using cytochrome *c* was used as an electron acceptor. 500 μl of solution containing 16 mM xanthine and 160 μM cytochrome *c* were added to a 1.5 ml cuvette. 500 μl of creatine solution (final [ ] = 40 mM), distilled water, or standard were then added. The reaction commenced with the addition of xanthine oxidase (final [ ] = 1 U · ml^{-1}). Cytochrome *c* reduction was followed spectrophotometrically at 540 nm. The ability of an antioxidant to quench $O_2^{\cdot -}$ was quantified as the percentage inhibition of cytochrome *c* reduction. A standard curve was also established by comparing the ability of creatine to remove $O_2^{\cdot -}$ with superoxide dismutase (0.2, 0.5, 1, 2, 5 U · ml^{-1}).

The ability to remove hydrogen peroxide was tested directly. We used a modification of the procedure described by Aebi (1) for catalase determination. 500 μM of hydrogen peroxide (final [ ] = 12 mM) was added to a quartz cuvette. 500 μl of creatine solution (final [ ] = 40 mM), distilled water, or standard was then added and incubated for 5 min. Hydrogen peroxide concentrations were followed directly using a spectrophotometer set at a wavelength of 240 nm for 90 s. A rate constant was calculated as a function of the log ratio between in final and initial absorbance readings at 240 nm. A standard curve was established via the ability of catalase (0.1, 0.2, 0.5, 1 U · ml^{-1}) to remove hydrogen peroxide

The ability to quench lipid hydroperoxides was performed using a modification of the technique of Hermes-Lima *et al.* (14). Final concentrations for t-butyl hydroperoxide of1, 2, 5, 10, and 20 μM were used as lipid peroxidation donors. Xylenol orange forms a complex with a Fe(III) iron source ($FeSO_4$) in a sulfuric acid environment when exposed to hydroperoxide products. Thus this reaction was used as a marker of reaction with lipid peroxides. Oxidation of xylenol orange results in a purple chromophore with the absorbance detected at 580 nm. An antioxidant compound that competes or quenches hydroperoxides would reduce the oxidation of xylenol orange and consequently absorbance. Solutions were added in the following order: 300 μl of 1.2 mM $FeSO_4$, 100 μl of 0.25 M H_2SO_4, 100 μl of 1 mM xylenol orange, and 300 μl of creatine (final [ ] = 40 mM), or distilled H_2O. Then 200 μl of t-butyl hydroperoxide solution was added in concentrations described above to initiate the reaction. The samples were incubated at 23°C for 1 h and the absorbance read at 580 nm.

Peroxynitrite ($OONO^-$) quenching was measured directly as adapted from Beckman *et al.* (1994). Briefly, we used 200 μM of SIN-1 as a $OONO^-$ generator. 100 μl of creatine solution of controls were placed in a cuvette and 1000 μl of SIN-1 solution added. After 2 min of equilibration, changes in absorbance were followed for 5 min in a spectrophotometer at a wavelength of 302 nm. Peroxynitrite removal was determined by subtracting A_{302} differences from controls and multiplying by the extinction coefficient.

Statistics. Analysis of variance was used to determine if creatine exhibited antioxidant properties for our four markers: superoxide

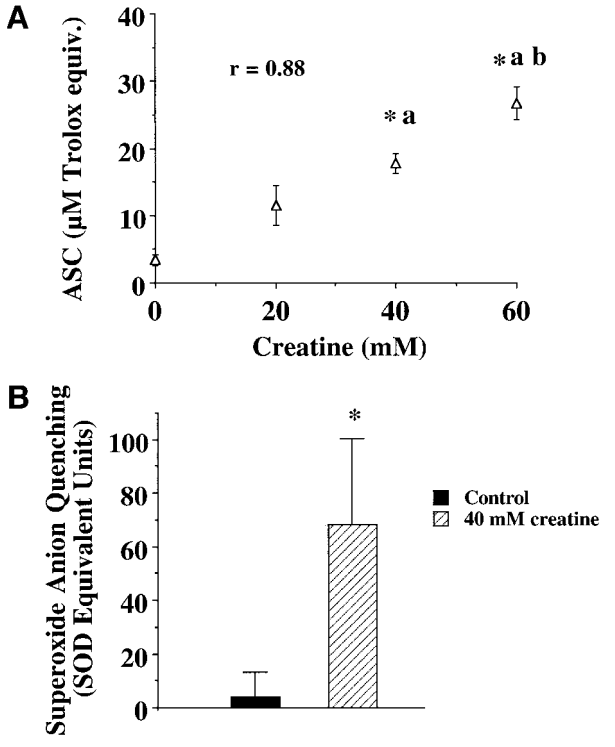

FIG. 1. (A) Effect of 0, 20, 40, and 60 mM creatine vs control buffer in total antioxidant scavenging capacity (ASC) using $ABTS^+$ as an oxidizing agent. Values are expressed per μmole Trolox equivalents/g.w.w. (*) indicates significantly higher than controls. (a) Indicates significantly higher than 20 mM. (b) Indicates significantly higher that 40 mM. (B) Effect of 40 mM creatine vs control buffer to quench superoxide anions using cytochrome *c* as an electron acceptor. Values are expressed as a function of superoxide dismutase equivalents. (*) indicates significantly higher than controls.

anion quenching, hydrogen peroxide removal, hydroperoxide quenching, and ASC. Pearson moment correlation coefficients between creatine concentration and ASC in Trolox equivalents were also conducted.

RESULTS

We found a direct dose–response relationship between creatine concentration and ASC as determined from $ABTS^+$ removal (Fig. 1A). Mean ± SEM values were 3.46 ± 0.78, 11.47 ± 2.96, 17.79 ± 1.49, and 26.69 ± 2.41 μmol of Trolox equivalents for the 0, 20, 40, and 60 mM creatine groups, respectively. A significant, direct correlation (r = 0.88) was calculated between increasing creatine concentration and ASC. Thus, creatine displayed a direct ability to reduce $ABTS^+$ cation in the ASC test.

Results of the experiments designed to determine if creatine (40 mM) would quench $O_2^{\bullet -}$ are included in Fig. 1B. Creatine resulted in a significantly greater difference in the rate of cytochrome c reduction, as detected via change in absorbance, when compared with controls. Mean ± SEM values were 68.0 ± 32.5 and 4 ± 9 superoxide dismutase equivalents for creatine and control groups, respectively. To our knowledge, this is the first direct testing and evidence that creatine can quench a biological radical.

Results of experiments testing the ability of 40 mM creatine to quench 10 mM hydrogen peroxide are presented in Fig. 2A. While H_2O_2 decomposition trended higher with creatine, there were no significant differences when compared to controls. The influence of creatine on lipid peroxidation is summarized in Fig. 2B. The reaction of t-butyl hydroperoxide with Fe^{2+} was not significantly slowed by 40 mM creatine in relation to control buffer. In fact, the lipid peroxidation measured via oxidation of xylenol orange as a marker tended to increase with creatine treatment. This is inconsistent with creatine possessing a significant antioxidant capacity for lipid peroxidation.

The ability of 20, 40, and 60 mM creatine to quench $OONO^-$ generated by SIN-1 are presented in Fig. 3. Thus creatine displayed a significant, modest ability to quench $OONO^-$. The highest creatine concentrations (60 mM) was most effective in removing $OONO^-$. This is consistent with an ability of creatine to act as an antioxidant agent against charged reactive species.

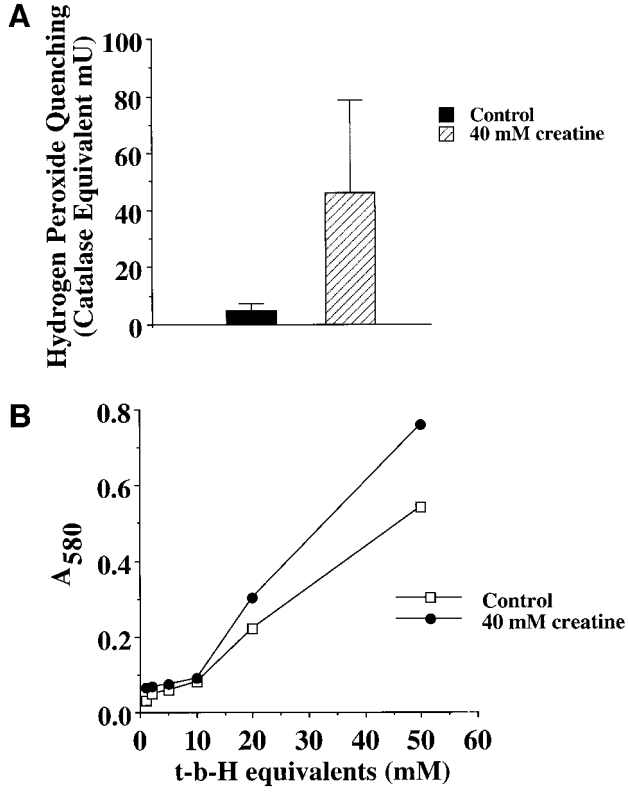

FIG. 2. (A) Effect of 40 mM creatine vs control buffer to quench hydrogen peroxide. Values are expressed as a function of catalase equivalents. (B) Ability of 40 mM creatine to quench lipid peroxidation as detected by absorbance of a xylenol orange–Fe(III) complex. Lipid peroxidation was expressed as a curve for t-butyl hydroperoxide (t-b-H) equivalents.

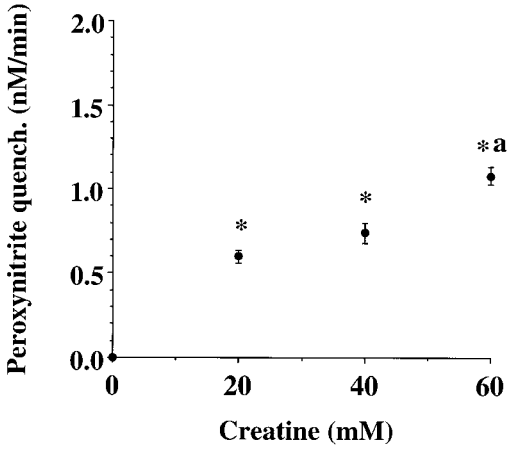

FIG. 3. Effect of 0, 20, 40, and 60 mM creatine to quench peroxynitrite produced by 100 μM SIN-1 (3-morpholinosydnonimine). (*) Indicates significantly different than controls. (a) Indicates significantly different from 20 mM.

We compared the antioxidant scavenging capacity of 40 mM creatine with 0.375 mM reduced glutathione, typical for skeletal muscle, and gastrocnemius homogenates. Results are found in Fig. 4. While creatine again displayed a significant antioxidant scavenging capacity, creatine ASC was significantly less than for reduced glutathione or gastrocnemius homogenates. We also conducted additional experiment to determine if creatine and 0.375 mM GSH acted in a synergistic manner. Results from that experiment indicated a simple additive effect between creatine and GSH (data not shown).

DISCUSSION

Our results demonstrated that creatine displays novel, direct antioxidant properties. Creatine showed an ability to remove $ABTS^+$, $O_2^{\cdot-}$, and $OONO^-$. To our knowledge, this is novel data that identify the potential of creatine to act as a direct antioxidant against radical and reactive species that are aqueous ions. In contrast, creatine was unable to significantly quench the non-radical oxidant sources for hydrogen peroxide and t-butyl hydroperoxide. Thus creatine did not demonstrate a significant antioxidant capacity against non-charged, non-radical hydroperoxides. These data demonstrate that the antioxidant properties of creatine are clearly selective. Finally, the ability of creatine to act as an antioxidant scavenger for $ABTS^+$ was significantly less than for physiological levels of reduced glutathione and acted in an additive manner with GSH. As many ionic radicals are highly reactive, our data are consistent with a supportive role of creatine against oxidative stress.

While the hypothesis that creatine may possess antioxidant properties has never been directly tested until now, it is not completely surprising that creatine does display some ability to remove or reduce radical and reactive species ions. All essential amino acids are oxidized via metabolism and many are also particularly sensitive to free radicals and reactive oxygen species. For example, arginine, a substrate for creatine formation can scavenge $O_2^{\cdot-}$ generated via xanthine oxidase, impede copper-induced lipoprotein oxidation, and slow $O_2^{\cdot-}$ release by endothelial cells and aortic rings (37, 38). Wu and Meininger (38) proposed that the antioxidant effect of arginine might protect against heart disease. The preceding findings are consistent with the potential function of creatine as a superoxide anion quencher and antioxidant since creatine is produced from arginine and glycine.

Our data indicate that the ability of creatine to act as a direct antioxidant may be limited to radicals and/or charged reactive species. As $ABTS^+$, $O_2^{\cdot-}$, $OONO^-$ are different in structure, it raises the possibility that this is a broad effect that could quench a wide range of radical or reactive species ions. Since creatine displayed a significantly lower antioxidant scavenging capacity than reduced glutathione and an additive effect with GSH, it would seem probable that creatine plays a supportive, rather than a primary antioxidant role. As creatine in solution is widely dispersed throughout the sarcoplasm, creatine may be readily accessible to radicals and reactive oxygen and nitrogen species generated during exercise and could play an important supportive role.

Any support of cellular antioxidant systems could have pathophysiological relevance by reducing fatigue as well as excess protein oxidation and degradation. Both *in vivo* and *in vitro* models have associated reactive oxygen species with increasing fatigue (23, 30). Indeed, pharmacological antioxidants have reduced muscle fatigue *in vitro* and *in vivo* (30). Oxidative stress, due to excess oxidant production and/or insufficient antioxidant capacity, increases protein degradation (30). For example, the ubiquitin proteolysis sys-

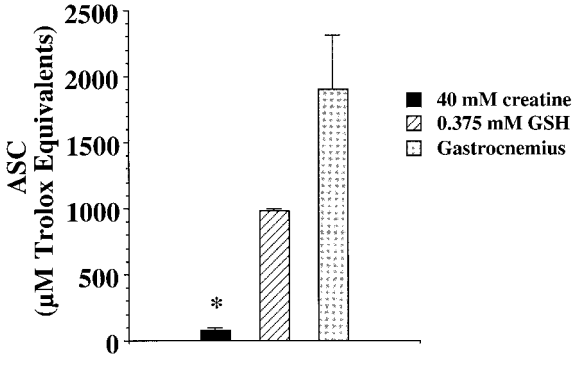

FIG. 4. Comparison of 40 mM creatine, 0.375 mM reduced glutathione, and gastrocnemius muscle homogenate to quench $ABTS^+$. (*) Indicates significantly different that reduced glutathione and muscle homogenates.

tem, now believed to be critical in degradation of myofibrillar proteins, is sensitive to oxidative stress (8, 30, 33).

Health status may be a critical factor in the efficacy of creatine. For example, it has been postulated that patients with neuromuscular disease, often characterized by increased oxidative stress (29) might benefit to a greater extent from creatine supplementation than healthy subjects (31). Creatine supplementation may alleviate symptoms of Huntington's Disease (26). Creatine supplementation has been shown potential to be efficacious in treatment of oxidative stress and damage with pathologies that result in muscle wasting including amyotrophic lateral sclerosis (ALS) (33) and motor neuron disease in Wobbler mice (15).

It is also possible that creatine may act indirectly to promote antioxidant function. For example, if creatine levels in muscle cells were increased, then less arginine would be required for energy metabolism and more available for nitric oxide production by nitric oxide synthase. Creatine supplementation may also increase intracellular levels of arginine, which also can act as an antioxidant (36, 37). It is also possible that increased cellular creatine might have indirect antioxidant effects if ATP and phosphocreatine levels are better maintained with rigorous exercise resulting in more effective maintenance of Ca^{2+} homeostasis. Recently, reactive oxygen and nitrogen species have been shown to inhibit creatine kinase (19, 39). The antioxidant effects of creatine and arginine following creatine supplementation might be beneficial in ameliorating cardiovascular disease (38). However, these hypotheses are untested in a rigorous manner and should be addressed in future studies.

In conclusion, creatine displayed a significant ability to act as an antioxidant scavenger primarily against radical ions. These data are novel as it is the first demonstration of a direct antioxidant property for creatine. The direct antioxidant effect for creatine was lower than physiological levels of reduced glutathione, but was additive with reduced glutathione. Thus, there is potential for creatine to exert a protective effect against neuromuscular diseases that cause muscle wasting and cardiovascular disease.

ACKNOWLEDGMENT

The authors thank Jason Koop for technical assistance.

REFERENCES

1. Aebi, H. (1984) Catalase. *Methods Enzymol.* **105,** 121–126.
2. Balsom, P. D., Soderlund, K. Sjodin, B., and Ekblom B. (1995) Skeletal muscle metabolism during short duration high-intensity exercise: Influence of creatine supplementation. *Acta Physiol. Scand.* **154,** 303–310.
3. Beckman, J. S., Chen, J., Ischiropoulos, H., and Chow, J. P. (1994) Oxidative chemistry of peroxynitrite. *Methods Enzymol.* **233,** 229–240.
4. Benzi, G. (2000) Is there a rationale for the use of creatine either as nutritional supplementation or drug administration in humans participating in sport? *Pharmacol. Res.* **41,** 255–264.
5. Cooke, W. H., Grandjean, P. W., and Barnes, W. S. (1995) Effect of oral creatine supplementation on power output and fatigue during bicycle ergometry. *J. Appl. Physiol* **78,** 670–673.
6. Dangott, B., Schultz, E., and Mozdziak, P. E. (2000) Dietary creatine monohydrate supplementation increases satellite cell mitotic activity during compensatory hypertrophy. *Int. J. Sports Med.* **21,** 13–16.
7. Demant, T. W., and Rhodes, E. C.. (1999) Effects of creatine supplementation on exercise performance. *Sports Med.* **28,** 49–60.
8. Figueiredo-Pereira, M. E., and G. Cohen, G. (1999) The ubiquitin/proteosome pathway: Friend or foe in zinc, cadmium, and H_2O_2-induced neuronal oxidative stress. *Mol. Biol. Rep.* **26,** 65–69.
9. Gilliam, J. D., Hohzorn, C., Martin, D., and Trimble, M. H. (2000) Effect of oral creatine supplementation on isokinetic torque production. *Med. Sci. Sports Exer.* **32,** 993–996.
10. Grune T., Reinheckel, T., and Davies, K. J. A. (1997) Degradation of oxidized proteins in mammalian cells. *FASEB J.* **11,** 526–534.
11. Harris, R. C., Soderlund, K., and Hultman, E. Elevation of creatine in resting and exercise muscle of normal subjects by creatine supplementation. *Clin. Sci.* **83,** 367–374.
12. Hellsten, Y., Frandsen, U. Orthenblad,, N. Sjödin, B., and Richter, E. A. (1997) Xanthine oxidase in human skeletal muscle following eccentric exercise: A role in inflammation. *J. Physiol* **498,** 239–248.
13. Hellsten-Westing, Y., Kaijser, L., Ekblom, B., and Sjödin, B. (1994). Exchange of purines in human liver and skeletal muscle with short-term exhaustive exercise. *Am. J. Physiol.* **266,** R81–R86.
14. Hermes-Lima, M., Wilmore, W. G., and Storey, K. B. (1995) Quantification of lipid peroxidation in tissue extracts based on Fe(III) xylenol orange complex formation. *Free Radical. Biol. Med.* **19,** 271–280.
15. Ikeda, K., Iwasaki, Y., and Kinoshita, M. (2000) Oral administration of creatine monohydrate retards progression of motor neuron disease in the wobble mouse. *Amyotrophic Lateral Sclerosis Other Motor Neuron Disorders* **1,** 207–212.
16. Ingwall, J. S. (1976) Creatine and control of muscle-specific protein synthesis in cardiac and skeletal muscle. *Circ. Res.* **38,** I1115–I1123.
17. Jones, A. M., Atter, T., and Georg, K. P. (1999) Oral creatine supplementation improves multiple sprint performance in elite ice-hockey players. *J. Sports Med. Phys. Fitness.* **39,**189–196.
18. Juhn, M. S., and Tarnopolsky, M. (1998) Oral creatine supplementation and athletic performance: A critical review. *Clin. J. Sports Med.* **8,** 286–297.
19. Konorev, E. A., Hogg, N., and Kalyanaraman, B. (1998) Rapid and irreversible inhibition of creatine kinase by peroxynitrite. *FEBS Lett.* **427,** 171–174.
20. Kraemer, W. J., and Volek, J. S. (1999) Creatine supplementation. Its role in human performance. *Clin. Sports Med.* **18,** 651–666.
21. Kreis, R., Kamber, M., Koster, M., Felblinger, J., Slotboom, J., Hoppeler, H., and Boesch, C. (1999) Creatine supplementation. II. *In vivo* magnetic resonance imaging. *Med. Sci. Sports Exer.* **31,** 1770–1777.
22. Lands, L. C., Grey, V. L., and Smountos, A. A. (1999) Effect of

supplementation with a cysteine donor on muscular performance. *J. Appl. Physiol.* **87,** 1381–1385.

23. Lawler, J. M., C. C. Cline, C. C., Hu, Z., and Coast, J. R. (1997) Effect of oxidative stress and acidosis on diaphragm contractile function. *Am. J. Physiol.* **273,** R630–R636.
24. Lawler, J. M., and Demaree, S. R. (2001) Relationship between NADP-specific isocitrate dehydrogenase and glutathione peroxidase in aging rat skeletal muscle. *Mech. Age. Dev.* **122,** 291–304.
25. Lawler, J. M., and Powers, S. K. (1998) Oxidative stress, antioxidant capacity, and the contracting diaphragm. *Can. J. Appl. Physiol.* **23,** 23–55.
26. Matthews, R. T., Yang, L., Jenkins, B. G., Ferrante, R. J., Rosen, B. R., Kaddurah-Dauok, R., and Beal, M. F. (1998) Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J. Neurosci.* **18,** 156–163.
27. McKenna, M. J., Morton, J., Selig, S. E., and Snow, R. J. (1999) Creatine supplementation increases muscle total creatine but not maximal intermittent exercise performance. *J. Appl. Physiol.* **87,** 2244–2252.
28. Pelligrini, R. N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radicals Biol. Med.* **26,** 1231–1237.
29. Rando, T. A., Crowley, R. S., Carlson, E. J., Epstein, C. J., and Mohaptra, P. K. (1998) Overexpression of copper/zinc superoxide dismutase: A novel cause of murine muscular dystrophy. *Ann. Neurol.* **44,** 381–386.
30. Reid, M. B. (2001) Redox modulation of skeletal muscle contraction: What we know and what we don't. *J. Appl. Physiol.* **90,** 724–731.
31. Reid, M. B., Stokic, D. S., Koch, S. M., Khawli, F. A., and Leis, A. A. (1994) *N*-Acetylcysteine inhibits muscle fatigue in humans. *J. Clin. Invest.* **94,** 2468–2474.
32. Shang, F., Gong, X., and Taylor, A. (1997) Activity of ubiquitin-dependent pathway to oxidative stress. Ubiquitin-activating enzyme is transiently upregulated. *J. Biol. Chem.* **272,** 23086–23093.
33. Strong, M. J., and Pattee, G. L. (2000) Creatine and coenzyme Q10 in the treatment of ALS. *Amyotrophic Lateral Sclerosis Other Motor Neuron Disorders* **1,** 17–20, 2000.
34. Terjung R. L., Clarkson, P., Eichner, E. R., Greenhaff, P. L., Hespel, P. J., Israel, R. G., Kraemer, W. J., Meyer, R. A., Spriet, L. L., Tarnopolsky, M. A., Wagenmakers, A. J., and Williams, M. H. (2000) American College of Sports Medicine roundtable. The physiological and health effects of oral creatine supplementation. *Med. Sci. Sports Exer.* **32,** 706–717.
35. Vergnani, L., Hatrick, S., Ricci, F., Passaro, A., Manzoli, N., Zuliani, G., Brokovych, V., Fellin, R., and Malinski, T. (2000) Effect of native and oxidized low-density lipoprotein on endothelial nitric oxide and superoxide production: key role of L-arginine availability. *Circulation* **101,** 1261–1266.
36. Volek, J. S., Duncan, N. D., Mazzetti, S. A., Staron, R. S., Putukian, M., Gomez, A. L., Pearson, D. F., Fink, W. J., and Kraemer, W. J. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med. Sci. Sports Exer.* **31,** 1147–1156.
37. Wascher, T. C., Posch, K., Wallner, S., Hermetter, A., Kostner, G. M., and Graier, W. F. (1997) Vascular effects of L-arginine: Anything beyond a substrate for the NO-synthase? *Biochem. Biophys. Res. Commun.* **234,** 35–38.
38. Wu, G., and Meininger, C. J. (2000) Arginine nutrition and cardiovascular function. *Rec. Adv. Nutr. Sci.* **130,** 2626–2629.
39. Yatin SM., Aksenov, M., and Butterfield, D. A. (1999) The antioxidant vitamin E modulates amyloid beta-peptide-induced creatine kinase activity inhibition and increased protein oxidation: Implications for the free radical hypothesis of Alzheimer's disease. *Neurochem. Res.* **24,** 427–435.
40. Ysebaert-Vanneste, M., and Vanneste, W. H. (1980) Quantitative resolution of Cu,Zn and Mn-superoxide dismutase activities. *Anal. Biochem.* **107,** 86–95.