

**CREATINE SUPPLEMENTATION IN THE
TREATMENT OF MUSCULAR DYSTROPHIES**

Luke Lairson

0082957

&

Mark Wilke

0082939

CHEM*452

December 10th, 2001

BACKGROUND

Skeletal muscle must deliver mechanical work on demand, adapting to situations of rest, light activity, or heavy activity. As its immediate source of energy, metabolism in skeletal muscle is specialized to generate ATP. Depending on the degree of muscular activity, skeletal muscles will use free fatty acids, ketone bodies, or glucose as a fuel source. In resting muscle, the primary fuel molecules are free fatty acids from adipose tissue and ketone bodies from the liver. These are oxidized and degraded to yield acetyl-CoA, which enters the citric acid cycle for oxidation to CO₂. The subsequent transfer of electrons to O₂ provides the energy for ATP synthesis by oxidative phosphorylation. During situations of light activity, skeletal muscle uses blood glucose in addition to fatty acids and ketone bodies. The glucose is phosphorylated and then degraded by glycolysis to pyruvate, which is converted to acetyl-CoA and oxidized via the citric acid cycle.

Under conditions requiring intense muscular activity, aerobic respiration is insufficient to meet the muscle's demand for ATP due to the fact that O₂ cannot be provided quick enough by the blood. As a means of accommodating, muscle glycogen stores are metabolized to lactate by fermentation. Each glycogen-derived glucose unit yields three molecules of ATP because phosphorylation of glycogen produces glucose-6-phosphate, bypassing the ATP-consuming hexokinase reaction. In this way, lactic acid fermentation responds quickly to provide additional ATP energy, supplementing the production of ATP from aerobic respiration. The secretion of epinephrine from the adrenal medullae further facilitates the production of readily utilizable fuels for muscular activity. Besides the direct effect of increasing glycogenolysis in muscle tissue, blood glucose concentrations are heightened through stimulation of glycogenolysis in the liver,

and plasma fatty acid concentrations are increased via activation of a hormone-sensitive lipase, which has a direct lipolytic effect on adipose tissue (1). However, the consequential accumulation of H^+ resulting from anaerobic metabolism reduces the efficiency of glycolysis and leads to muscle fatigue. Following a period of intense muscular activity, heavy breathing replenishes depleted O_2 stores in the body. Much of the O_2 obtained in this manner is used for the production of ATP by oxidative phosphorylation in the liver. This ATP is used for gluconeogenesis from lactate, carried in the blood from the muscles to replenish their glycogen reserves.

In addition to ATP, skeletal muscle contains phosphocreatine, a source of high-energy phosphate bonds that is three to eight times as abundant as ATP and can rapidly regenerate ATP from ADP by the creatine kinase reaction (1) (Figure 1). This reaction is driven rapidly towards the synthesis of ATP by a net free energy change of -12.5 kJ/mol (2). The greater number of stabilizing resonance forms for creatine over phosphocreatine (Figure 2) provide a chemical basis for the relatively large, negative, standard free energy of hydrolysis of phosphocreatine ($\Delta G^{\circ} = -43.0$ kJ/mol). As a consequence, phosphocreatine acts as an *ATP buffer*, rapidly responding to conditions that consume ATP in

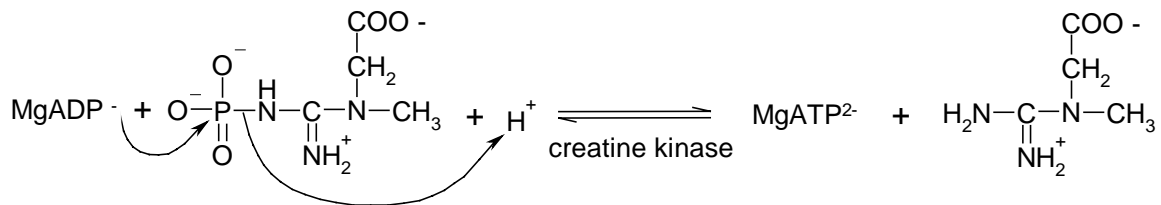


Figure 1: Regeneration of ATP from ADP and phosphocreatine, catalyzed by creatine kinase.

order to maintain nearly constant levels of ATP in myocytes until phosphocreatine stores are depleted. When excess ATP is available, phosphocreatine stores are replenished by the reverse reaction.

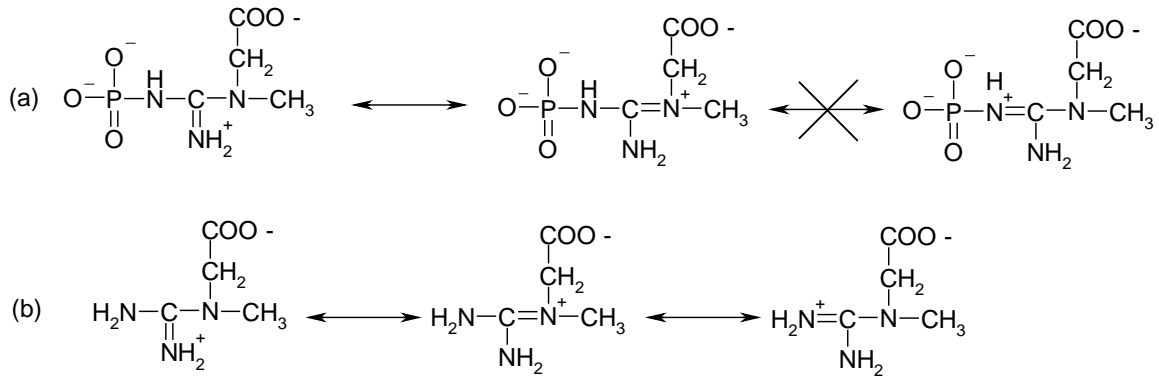


Figure 2: Resonance forms of (a) phosphocreatine and (b) creatine, demonstrating the greater resonance stabilization of creatine over phosphocreatine. Additional resonance forms exist for the phosphate group of phosphocreatine, but since this same phosphate group is transferred to ADP in the formation of ATP, its resonance has been ignored for clarity.

Creatine is a nonessential dietary element found in high abundance in meat and fish (3). In the body, it is synthesized primarily in the liver from three amino acids: glycine, arginine, and methionine (2) (Figure 3). Although about 95% of the body's creatine stores reside in skeletal muscle, muscle cells do not synthesize creatine, but are dependent on creatine uptake from the circulation by a sodium dependent membrane transporter (4). Once inside the myocyte, creatine is phosphorylated by the activity of creatine kinase. The distribution between creatine and phosphocreatine is determined by the energy state of the cell (*i.e.* ATP levels). The final end product of creatine catabolism is creatinine, which is formed by the nonenzymatic degradation of creatine and phosphocreatine (1).

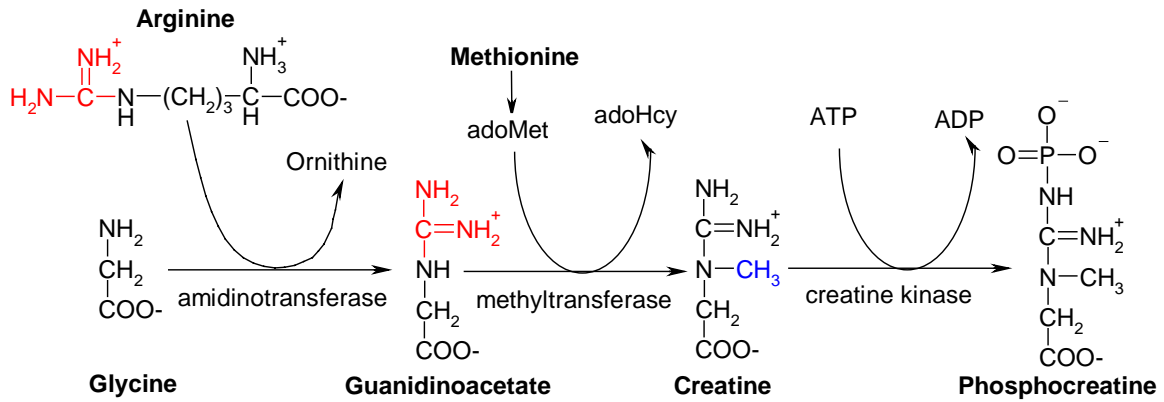


Figure 3: Biosynthetic pathway for phosphocreatine.

The quantity of ATP present in the muscles is sufficient to sustain maximal muscle power for only about three seconds (1). The energy transfer from phosphocreatine to ATP occurs within only a small fraction of a second and thus all the energy stored in muscle phosphocreatine is instantaneously available for muscle contraction, just as is the energy stored in ATP. The combined quantities of cellular ATP and phosphocreatine are known as the *phosphagen energy system*. Stores of ATP and phosphocreatine, together, can typically provide maximal muscle power for eight to ten seconds (1). For this reason, energy derived from the phosphagen system is used for short maximal bursts of muscle power. Used in combination with the phosphagen system, the glycogen-lactate system can sustain about 62.5% of maximal muscle activity for 1.3 to 1.6 minutes (1). Prolonged high-intensity, muscular activity requires the aerobic system, which can supply about $\frac{1}{4}$ th the ATP energy of the phosphagen system (1).

The role of phosphocreatine in rapidly rephosphorylating ADP to ATP has been exploited in recent years in the form of creatine dietary supplements for enhancing exercise performance. The basis for consuming supplemental creatine is to increase the

skeletal muscle creatine content, leading to an increased resting phosphocreatine content by the activity of creatine kinase and the law of mass action. An increase in the resting phosphocreatine store should increase the total quantity of ATP resynthesis and thus improve performance in brief bouts of high-intensity exercise. Since phosphocreatine levels become depleted after about ten seconds of high-intensity exercise, maximal muscle power cannot be maintained beyond ten seconds, and glycolysis becomes the sole contributor of anaerobic ATP. Thus, if creatine supplementation increases the concentration of phosphocreatine in resting muscle, high power outputs may be sustained longer, leading to improved exercise performance. Additionally, many sports require athletes to perform repeated bouts of high-intensity muscle activity, allowing various amounts of recovery time between bouts. Since most of the required energy for such activity is derived from anaerobic sources, the ability to recover during rest periods is essential for optimal performance. Phosphocreatine is resynthesized quickly when exercise stops; this rate of resynthesis will determine how much phosphocreatine is available for the next exercise bout. Moreover, due to the acidosis that accompanies anaerobic metabolism, the ability to reactivate the glycolytic pathway during repeated bouts of exercise is compromised, leaving phosphocreatine as the major source of anaerobic ATP during repeated short bouts of high-intensity exercise.

An additional benefit of creatine supplementation may be an increased buffering potential of H^+ produced during lactate fermentation. As mentioned, the energy contribution from the glycogen-lactate system is limited primarily by the accumulation of H^+ as a byproduct of glycolysis. The resulting acidosis exerts its effects directly by impeding glycolysis through inhibition of phosphofructokinase, and may inhibit electrical

and contractile processes involved in muscular contraction (4). For these reasons, cellular buffering of H^+ is considered an important process that delays muscular fatigue. Since each transfer of a high-energy phosphate to ADP from phosphocreatine consumes a free H^+ (Figure 1), increased phosphocreatine levels translate into an increased H^+ buffering potential. It has also been suggested that phosphocreatine plays a central role in the “shuttling” of ATP from the mitochondria to cytoplasmic locations for hydrolysis during predominately aerobic exercise (5), however, no conclusive evidence has ever been provided to demonstrate that this mechanism is functional in skeletal muscle (4).

It is generally accepted that creatine supplementation can increase muscle phosphocreatine content in some individuals, depending on the extent of the individual’s initial muscle total creatine content (3). Most, but not all, studies have indicated that creatine supplementation significantly enhances the ability to produce higher muscular force and/or power output during short bouts of maximal exercise in healthy, young, male adults (3). Although investigations into the safety of creatine supplementation are ongoing, there is presently no definitive evidence linking creatine supplementation with potential health risks (3). Moreover, few studies have been performed on the benefits of creatine supplementation considering alterations in age, sex, or patients suffering from diseases related to muscular dysfunction.

Muscular dystrophy is a group of hereditary myopathy diseases characterized by abnormal muscle wasting and weakness (6). The fundamental defect in many dystrophies is thought to reside in the muscle fiber sarcolemma membrane (7). It is believed that the muscle fiber membranes of affected individuals become “leaky” and allow materials to pass through the membrane with decreased regulation (7). Creatine

kinase is often found at elevated concentrations in the urine of patients affected by muscular dystrophy and is believed to leak out into circulation from the cells of affected muscle tissues (6). It is also known that intracellular $[Ca^{+2}]$ is elevated in the muscle cells of affected individuals (8). This is also believed to be the result of ions leaking into the cells, resulting in the observed deleterious effects.

As mentioned, muscular dystrophy is not a single disease but rather a group of genetic disorders, which lead to the abnormal wasting and weakness of muscle tissues. The degree and distribution of muscle weakness is used to distinguish between the different types of dystrophies. As many as twenty different types have been described, including the following common forms: limb girdle dystrophy, facioscapulohumeral dystrophy, Duchenne dystrophy, and Becker muscular dystrophy.

Limb girdle dystrophy affects the muscles around the hips and shoulders. It often results in scoliosis and life expectancy is shortened due to decreased respiratory ability, although the heart is not affected (6). Facioscapulohumeral dystrophy results in facial weakness and often leads to speech impairment and an inability to properly close the eyes. Unlike most other dystrophies, this form does not effect muscles in a symmetric fashion, muscle weakness often occurs on one side of the body more than the other (6). Duchenne muscular dystrophy is the most common and severe form of the dystrophies (6). It is caused by a frameshift mutation in the dystrophin gene, located on chromosome Xp21 (9). It occurs with a frequency of 1 in 3500 newborn males (7). The first signs of the disease appear at the age of 3 or 4 years. Most affected boys are restricted to a wheel chair by age 12 and do not survive past the age of 20 years. The milder Becker muscular

dystrophy form is associated with in-frame mutations on the dystrophin gene. Onset does not occur until 16 years of age and a more normal life span is usually observed.

Dystrophin is a 427 kDa protein which is believed to link the underlying cytoskeletal microfilaments with extracellular matrix components in skeletal muscle (10). This association occurs via a large oligomeric complex of membrane glycoproteins called the dystrophin-associated proteins (10). The generally accepted model of the dystrophin-glycoprotein complex is one in which dystrophin exists as a bent anti-parallel dimer. The C-terminus of dystrophin is linked to the transmembrane component of the oligomeric complex and the N-terminus binds the filamentous actin cytoskeleton (11). Thus dystrophin is located on the extracellular side of the sarcolemma. Mechanical instability of the sarcolemma leading to increased activity of Ca^{+2} leak channels results from a loss of this complex (10).

Absence of full-length dystrophin results in observations of increased total muscle calcium (12). Its absence also leads to significant elevation of intracellular free calcium concentrations ($[\text{Ca}^{+2}]_i$) in isolated myofibers (13) and cultured myotubes (14). However, calcium resequestration rates following stimulation are not different in cells which lack dystrophin, indicating that Ca^{+2} influx rates and not efflux rates are altered in dystrophic cells (14). Specifically, a three-fold higher open probability has been observed in the Ca^{+2} -specific leak channels of dystrophin-deficient cells (14). Elevated $[\text{Ca}^{+2}]_i$ leads to the activation of Ca^{+2} -dependent proteases, which have been shown to increase the activity of Ca^{+2} leak channels (15) and decrease the function of plasma-membrane Ca^{+2} -ATPases (15), leading to a feed forward cascade of Ca^{+2} influx. The *mdx* mouse contains a mutation in the gene for dystrophin and has been shown to be a good animal model for

Duchenne muscular dystrophy. Myotubes derived from mdx mice that have been transgenically engineered to contain dystrophin recover normal Ca^{+2} homeostasis (16). The basic mechanism underlying the pathology of muscular dystrophy is therefore believed to involve the deregulation of intracellular $[\text{Ca}^{+2}]$ control.

Creatine kinase is involved in the tight regulation of intracellular $[\text{Ca}^{+2}]$ by providing energy for the Ca^{+2} -sequestering sarcoplasmic reticulum (SR)-bound Ca^{+2} -ATPase (17). The muscle-type creatine kinase isoform is localized in the vicinity of the SR Ca^{+2} -ATPase, which has been shown to preferentially utilize creatine kinase-generated ATP (17). Transgenic mice lacking muscle creatine kinase have been shown to display muscle relaxation difficulties due to elevated intracellular $[\text{Ca}^{+2}]$ (18). Furthermore, decreased SR Ca^{+2} uptake has been observed in cells in which SR-bound creatine kinase has been inhibited (19). The concentrations of creatine and phosphocreatine in muscle cells thus influence the control of intracellular $[\text{Ca}^{+2}]$.

In the following, research articles by Pulido *et al.* (20) and Walter *et al.* (21) are summarized and evaluated. Each of these investigations assessed creatine supplementation as a treatment for muscular dystrophies. However, whereas the investigation of Pulido *et al.* involved an *in-vitro* analysis of intracellular $[\text{Ca}^{2+}]$ in cultured muscle cells of the *mdx* model of Duchenne muscular dystrophy, the research of Walter *et al.* studied the effects of creatine in a controlled, double-blind, clinical trial with human patients suffering from various forms of muscular dystrophy.

CREATINE SUPPLEMENTATION: A TREATMENT FOR MUSCULAR DYSTROPHIES?

IN-VITRO INVESTIGATION (20)

Dystrophic skeletal muscle myotube cells from *mdx* mice were used to show that creatine supplementation improves intracellular Ca^{+2} handling. Incubation of *mdx* myotubes with 20 mM creatine for 6-12 days resulted in a decrease of elevated intracellular $[\text{Ca}^{+2}]$, induced by hypo-osmotic stress or high extracellular $[\text{Ca}^{+2}]$, to control levels. Using $^{45}\text{Ca}^{+2}$ measurements it was suggested that creatine decreased the levels of intracellular Ca^{+2} by stimulating the sarcoplasmic reticulum Ca^{+2} -ATPase. It was therefore concluded that creatine supplementation might be used therapeutically to treat Duchenne muscular dystrophy.

The mdx mouse model. The *mdx* mouse is a model system used to study Duchenne muscular dystrophy. The X-chromosome-linked mouse mutation (*mdx*) was first described in 1984 (22) and in 1989 it was shown that *mdx* mice possess a mutation in the same gene that is abnormal in humans suffering from Duchenne muscular dystrophy (23). Dystrophic skeletal muscle cells from *mdx* mice exhibit elevated intracellular $[\text{Ca}^{+2}]$ as found in similar cells from Duchenne muscular dystrophy patients. Specifically, dystrophic skeletal muscle cells from *mdx* mice have been shown to possess impaired intracellular $[\text{Ca}^{+2}]$ regulation when exposed to hypo-osmotic or high extracellular $[\text{Ca}^{+2}]$ stress (20).

Treatment of Control and mdx Cell Cultures. The hind leg muscles of 1-4 day control C57BL/10 and *mdx* mice were used to prepare primary cell cultures. By day three, in culture myoblast fusion into myotubes had begun; myotube formation was

completed within 24 hours. Following the commencement of myoblast fusion, 20 mM creatine was added to the cultures. 1 μ M α -methylprednisolone (PDN), which has been shown to prevent stress-induced increases in intracellular $[Ca^{+2}]$ (24), was also added to equivalent myoblasts as a positive control. Medium was replaced following 5 days in culture. Myotubes were finally used for experiments on days 12 and 14 in culture.

Experimental Design. Changes in intracellular $[Ca^{+2}]$ following hypo-osmotic or high extracellular stress were measured in day 13 control and *mdx* myotubes. Cells were exposed to a 5 minute incubation with control buffer, followed by 100 mOsm buffer for 10 minutes, followed by control buffer for 5 minutes, then 40 mM $CaCl_2$ buffer for 2 minutes, and finally by control buffer for 10 minutes. Alternatively, the sequence for introduction of high osmolarity buffer and high Ca^{+2} buffer was reversed. The measurement of cytosolic calcium concentration was performed using the fluorometric probe, Fura-2/AM.

Calcium influx rates were also determined using $^{45}Ca^{+2}$. Briefly, cells were exposed to $^{45}Ca^{+2}$ under control or stress conditions. Influx of $^{45}Ca^{+2}$ was stopped with EGTA (ethylene glycol-bis[β -aminoethyl ether]-N,N,N',N'-tetraacetic acid), the cells were lysed, and the $[^{45}Ca^{+2}]$ was determined by scintillation counting.

Finally, ATP and phosphocreatine measurements were performed on 12 day *mdx* and control myotubes, exposed to both stress treatments and treated with 20 mM creatine from day three. Following extraction of ATP and phosphocreatine using trichloroacetic acid, their respective concentrations were measured using luciferase-catalyzed chemiluminescence assay (25).

Results. Pre-treatment of cell cultures with creatine or PDN did not effect basal intracellular $[Ca^{+2}]$ in either the control or *mdx* myotubes. Hypo-osmotic shock of *mdx* myotubes resulted in increased intracellular $[Ca^{+2}]$ from 45 to 118 nM. Pre-treatment with creatine or PDN yielded levels of 62 nM in *mdx* cells, similar to those found in control cells exposed to the same hypo-osmotic shock. Exposure to high levels of extracellular $[Ca^{+2}]$ resulted in *mdx* myotubes with intracellular Ca^{+2} levels that were more than double those of untreated *mdx* or control cells. Both creatine and PDN pre-treatment inhibited this increase to levels similar to those of the controls. Interestingly, it was found that inhibition of stress-induced increases in intracellular $[Ca^{+2}]$ was not observed if creatine was added to myotubes for periods shorter than 6 days before experimentation.

Both conditions of stress resulted in more than doubled Ca^{+2} influx in *mdx* myotubes. Control myotubes showed only a mild influx of Ca^{+2} . Creatine pre-treatment did not affect Ca^{+2} influx in the *mdx* myotubes, however, PDN pre-treatment produced a reduction of Ca^{+2} influx in *mdx* myotubes, indicating that the method of inhibiting stress-induced increases in intracellular $[Ca^{+2}]$ is different than that of creatine.

ATP concentrations in control and *mdx* myotubes were not significantly different, and in both cases, neither stress treatment was found to have any affect. Phosphocreatine levels in *mdx* myotubes, however, were about two times lower than those found in control myotubes. A stress treatment consisting of high extracellular $[Ca^{+2}]$, but not hypo-osmotic-related stress, resulted in even further decreases in phosphocreatine levels. Pre-treatment with creatine resulted in higher phosphocreatine levels in both *mdx* and control myotubes.

Significance of Results. Hypo-osmotic stress as well as hypercalcemic stress both induced elevated levels of intracellular $[Ca^{+2}]$ and significantly elevated levels of Ca^{+2} influx in *mdx*, but not in control myotubes. This is consistent with chronic Ca^{+2} overload and dysfunctional calcium homeostasis in skeletal muscle cells derived from Duchenne muscular dystrophy and *mdx* mice (13). It is known that elevated levels of intracellular calcium activate calcium dependant pathways, which eventually lead to muscle cell necrosis or apoptosis (26). Therefore, a method for the treatment of the chronic elevated levels of intracellular calcium in the skeletal muscle cells of muscular dystrophy patients is desirable.

The stress-induced increases in intracellular Ca^{+2} levels in *mdx* myotubes were significantly inhibited by pre-treatment with creatine. Because creatine pre-treatment did not affect the rate of Ca^{+2} influx, it can be concluded that creatine pre-treatment does not decrease leak channel-mediated influx. Rather, the creatine effect is thought to occur by increasing the rate of cytosolic Ca^{+2} uptake by the sarcoplasmic reticulum. The SR Ca^{+2} -ATPase is the most likely target of the creatine effect. As previously mentioned, the SR Ca^{+2} -ATPase is closely coupled to creatine kinase. Specifically, the SR-bound Ca^{+2} pump preferentially utilizes ATP molecules generated by the activity of SR-bound creatine kinase (17). The SR-bound creatine kinase generates a localized region of high ATP content in the vicinity of the SR Ca^{+2} -ATPase and therefore plays a crucial role in Ca^{+2} homeostasis (17). The phosphocreatine content of *mdx* myotubules was significantly lower than control cells, as is found in human dystrophic cells. Sufficient exposure to creatine led to significantly higher levels of phosphocreatine in *mdx* myotubules. Creatine supplementation, leading to elevated levels of cellular

phosphocreatine and increased local concentrations of ATP in the vicinity of the SR Ca^{+2} -ATPase, therefore appears to increase the ability of *mdx* myotubes to handle intracellular Ca^{+2} . Because oral creatine supplementation has been shown to lead to elevated levels of both creatine and phosphocreatine in muscle cells (27), creatine dietary supplementation is a potential therapy for the treatment of Duchennes muscular dystrophy that requires further investigation.

CLINICAL STUDY (21)

Walter *et al.* investigated the therapeutic efficacy and side effects of creatine supplementation in patients with clinically, morphologically, and genetically defined muscular dystrophies in a placebo-controlled, double-blind clinical trial.

Experimental Design. A total of 36 patients were included in the study: 12 patients with facioscapulohumeral dystrophy, 10 patients with Becker dystrophy, 8 patients with Duchenne dystrophy, and 6 patients with sarcoglycan-deficient limb girdle muscular dystrophy. Only 32 of these patients were treated for the entire study period; these consisted of 21 adults (mean age, 35 ± 15 years) and 11 children (mean age, 10 ± 4 years). There were no significant differences between these groups, according to age, gender, or disease duration. Patients that were confined to bed or wheelchair, on corticosteroid medication, or suffered from renal insufficiency were excluded from the study.

Subjects were randomized using a double-blind, placebo-controlled, crossover design, using creatine monohydrate or placebo (microcrystalline cellulose) for 8 weeks, with a washout period of 3 weeks. Creatine and placebo were administered in identical

powder form, in equal quantities (adults, 10 g/day; children, 5 g/day), dissolved in non-heated fluid with a small meal. Before and following each treatment period, the patient's response was assessed using the *Medical Research Council* (MRC) scale, the *Neuromuscular Symptoms Score* (NSS), *vital capacity* (VC), and the patient's own assessment of improvement. Physical examinations, routine blood chemistries, and laboratory tests, such as blood cell count and creatine kinase levels, were conducted during each patient contact.

Patient muscle strength was examined bilaterally according to the MRC scale. The MRC scale is a qualitative six-point grading scale that assesses muscles for strength on a muscle-by-muscle basis by assigning values (0→5) according to an evaluation performed by an experienced neurologist (0 = no movement; 1 = flicker of movement seen or felt in the muscle; 2 = muscle moves joint when gravity eliminated; 3 = muscle moves joint against gravity but cannot tolerate resistance; 4 = muscle moves joint against gravity and maintains resistance; 5 = normal strength [28]). In the present study, MRC scores for a single assessment were summed to a maximum score of 150 and converted to a percent change in average MRC score.

Fourteen daily-life activities were evaluated by the NSS, including: arm elevation over head plane, opening twist-top jars, buttoning, chewing, whistling, writing, taking off a sweater, turning a key, rising to a sitting position, swallowing, lifting head, taking on shoes and socks, and exhaustion following both slight and distinct strain. Ratings ranged from 0 (severe disability) to 3 (no disability) for each item, to yield a maximum summed score of 42 (29).

In addition, forced VC and percent-predicted VC were assessed. In typical measurements of forced VC, the patient first inspires maximally to total lung capacity, then exhales into a spirometer, a device which plots lung volume versus time (1). Various statistical analyses were performed to assess test-retest reliability, to compare baseline scores for the various qualitative measurements, and to evaluate the efficacy of creatine treatment. Due to the presence of small crossover effects, only the placebo-creatine crossover sequence was included in the final evaluation.

Results. Significant mean improvements of 3% in muscle strength and 10% in daily-life activities were observed during creatine treatment, when the beginning and end of each treatment cycle was compared. Both severely and less affected muscles appeared to improve, with children eliciting a better response as compared to adults. Moreover, significant differences between the effects of placebo versus creatine supplementation on muscle strength and daily-life activity performance were observed. Comparing the start and end of placebo treatment, there was a trend toward a decline in muscle strength. VC analyses revealed no significant changes for treatment with creatine versus placebo. As for the patient's own assessment of improvement, 60% of patients assigned improvement to creatine supplementation, 31% of patients did not feel a considerable difference between treatments, and 9% of patients described improvement during placebo administration. Physical examinations, blood chemistries, and laboratory tests revealed no significant changes and no side effects were reported as a result of creatine administration.

Significance of Results. The aim of Walter *et al.* was to evaluate the efficacy and safety of creatine supplementation in patients suffering from muscular dystrophy. As

such, muscular strength and the ability to perform daily-life activities were used as the primary measures of assessing responsiveness to the treatment. A mild, but significant improvement in both of these measures was observed. The better responsiveness of children with muscular dystrophy versus adult patients was suggested to result from lower muscle phosphocreatine storage and more rapid phosphocreatine depletion during exercise in boys with Duchenne muscular dystrophy when compared with healthy age-matched control subjects. The authors also pointed out that, at present, the only efficacious therapy for Duchenne muscular dystrophy is treatment with corticosteroids, which are known to possess severe side effects. Thus, creatine supplementation may be of benefit as a relatively safe alternative treatment for muscular dystrophy patients.

However, the authors admit that these findings are preliminary. The MRC grading system is limited in that it is not an interval scale. Interval measurement scales permit not only the construction of a rank order of the items that are being measured, but also allow quantification and comparison of the sizes of differences between those items, *i.e.* one unit on the interval scale represents the same magnitude of the characteristic being measured across the whole range of the scale. For this reason, future studies that make measurements using an interval scale, such as quantitative force measurement, would yield a more convincing data set. To critique the article of Walter *et al.* itself, some details concerning the experimental design were neither included or referenced, such as which particular muscles were assessed using the MRC scale. For this reason, the study cannot be reproduced from the article alone.

The decision to administer a daily oral dose of creatine monohydrate (adults, 10 g/day; children, 5 g/day) is relatively consistent with the dosage shown to be effective in

healthy adults for athletic performance enhancement (20 g/day for ~5 days followed by 2 g/day to sustain the elevated levels [3]). The initial 20 g/day dosage is used as a “loading” dose to speed the increase in skeletal muscle creatine and phosphocreatine concentrations. A 3 g/day dose has been shown to reach the same elevated levels, but over a longer period of time, reaching maximal levels after about 30 days (27). For this reason, a 10 g/day dose in adults and 5 g/day dose in children is reasonable to saturate creatine and phosphocreatine levels at a relatively early phase of an eight week study.

As noted by the authors, further investigation is warranted to evaluate more fully the putative benefits of creatine supplementation as a therapy for muscular dystrophy. The present study assessed four relatively small groups of patients with different varieties of muscular dystrophy for a mere eight-week period. Furthermore, the study cannot be reproduced due to exclusion of certain experimental details. It is thus recommended that controlled, clinical, long-term investigations be performed separately for each type of muscular dystrophy using more quantitative measures than the MRC scale for assessing responsiveness. Moreover, as data becomes available, comparisons with existing treatments, namely administration of corticosteroids such as prednisone (30), must be performed to assess whether creatine supplementation is a suitable alternative with comparable efficacy. Key to the judgment of creatine’s suitability as an alternative treatment will be the results of long-term studies evaluating the potential side effects of creatine. At present, there is only anecdotal evidence supporting any side effects resulting from short-term creatine supplementation (3). However, few data exist on the long-term risks associated with ingesting high levels of creatine and further investigation is thus necessary.

CONCLUSION

The decrease in intracellular creatine levels in Duchenne muscular dystrophy may contribute to the deterioration of intracellular energy homeostasis and may thus be one of the factors aggravating muscle weakness and degeneration in the disease. Pulido *et al.* demonstrated that pre-treatment of cultured mouse cells derived from an accepted Duchenne muscular dystrophy animal model with creatine decreased the elevation in intracellular Ca^{+2} , induced by either high extracellular Ca^{+2} or hypo-osmotic stress, to the level of controls. Based on radioactive $^{45}\text{Ca}^{+2}$ investigations, Pulido *et al.* suggested a mechanism for this effect involving creatine-dependent stimulation of the SR Ca^{+2} -ATPase. Moreover, it was observed during these experiments that myotube formation and survival were substantially enhanced by creatine pre-treatment. Considering the positive response of human muscular dystrophy patients to creatine supplementation in the controlled, double-blind study of Walter *et al.*, it is certainly feasible that oral creatine supplementation may have a potential use in alleviating clinical symptoms. However, the extent to which creatine supplementation will be effective as a treatment for muscular dystrophies remains to be seen. Further investigation, on both a cellular and clinical level, is necessary to properly assess creatine's efficacy as an alternative to existing treatments. The seeming lack of side effects, at least in short term treatments, simplifies investigations with human subjects immensely, ensuring that the putative benefits of creatine supplementation will be made accessible to patients in a timely fashion. Still, mechanistic data derived from in-vitro cell-based studies will reveal a clearer picture of the disease itself, leading to even newer therapeutic leads.

REFERENCES

1. Guyton, A.C. and Hall, J.E. *Textbook of Medical Physiology, Tenth Edition*. W.B. Saunders Company. Toronto, ON. (2000) pp. 486, 816, 891, 969-970.
2. Nelson, D.L. and Cox, M.M. *Lehninger Principles of Biochemistry, Third Edition*. Worth Publishers. New York, NY. (2000) p. 503.
3. Terjung, R.L. *et al. Med. Sci. Sports Exerc.* **32**, 706-717 (2000).
4. Spriet, L.L. *Ergogenic Aids: Recent Advances and Retreats*. In *Perspectives in Exercise Science and Sports Medicine, Volume 10: Recent Advances in the Science and Medicine of Sport*, Lamd, D.R. and Murray, R. (eds.). Cooper Publishing Group, Carmel, IN. (1997) pp. 186-201.
5. Bessman, S.P. and Savabi, F. *The role of the phosphocreatine energy shuttle in exercise and muscle hypertrophy*. In *Biochemistry of Exercise VII*, Taylor, A.W. *et al.* (Eds.). Champaign, IL. Human Kinetics (1990) pp. 167-178.
6. Emery, A.E.H. *Oxford Medical Publications: Muscular Dystrophy the Facts*. Oxford University Press. New York, NY. (1994) pp. 3-38.
7. Worton, R. *et al. The Nature of Mutation and the Prospect for Therapy in Duchenne Muscular Dystrophy*. In *Raven Press Series on Molecular and Cellular Biology Volume 2: Neuromuscular Development and Disease*, Kelly, A.M. and Blau, H.M. (eds.). Raven Press. New York, NY. (1992) pp. 303-328.
8. Franco, A.J. *et al. Nature* **344**, 670-673 (1990).
9. Monaco, *et al. Adv. Hum. Genet.* **17**, 61-98 (1988).
10. Ibraghimov-Beskrovnaya, O., *et al. Nature* **355**, 696-702 (1992).
11. Ervasti, J.M., *et al. Cell* **66**, 1121-1131 (1991).
12. Dunn, J.F., *et al. J. Neurol. Sci.* **103**, 226-231 (1991).
13. Turner, P.R., *et al. Nature* **335**, 735-738 (1988).
14. Turner, P.R., *et al. J. Cell Biol.* **115**, 1701-1712 (1991).
15. Fong, P.Y., *et al. Science* **250**, 673-676 (1990).
16. Denetclaw, W.H.J. *et al. Mol. Biol. Cell* **5**, 1159-1167 (1994).
17. Walliman, T., *et al. Biochem.* **281**, 21-40 (1992).

18. Steeghs, K., *et al. Cell* **89**, 93-103 (1997).
19. Rossi, A.M., *et al. J. Biol. Chem.* **265**, 5258-5266 (1990).
20. Pulido, S.M., *et al. FEBS Lett.* **439**, 357-362 (1998).
21. Walter, M.C., *et al. Neurology* **54**, 1848-1850 (2000).
22. Bulfield, G., *et al. P.N.A.S.* **81**, 1189-1192 (1984).
23. Sicinski, P., *et al. Science* **244**, 1578-1580 (1989).
24. Giumbal, C., *et al. J. Mol. Biol.* **241**, 317-324 (1994).
25. Dunant, Y. *et al. J. Neurochem.* **50**, 431-439 (1988).
26. O’Gorman, E., *et al. FEBS Lett.* **414**, 253-257 (1997).
27. Hultmann, E., *et al. J. Appl. Physiol.* **81**, 232-237 (1996).
28. LSU Health Sciences Center (2001) Medical Research Council Muscle Grading Scale. [Online] Available: <http://www.sh.lsumc.edu/neurology/schwen/Neurochart.html>. [2001, December 9]
29. Soueidan, S.A. and Dalakas, M.C. *Neurology* **43**, 876-857 (1993).
30. Griggs, R.C. *et al. Neurology* **43**, 520-527 (1993).