

## The therapeutic potential of oral creatine supplementation in muscle disease

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**Abstract** — The decrease in intracellular creatine concentration observed in a number of muscle diseases may deplete energy homeostasis and may, therefore, be one of the factors determining and/or aggravating muscle weakness and degeneration. Two hypotheses are put forward in the present communication to explain: (i) the mechanisms leading to the disturbances in creatine metabolism found in various muscle diseases; and (ii) the potential of oral creatine supplementation in alleviating the clinical symptoms.

### Introduction

Creatine kinase (CK, EC 2.7.3.2), phosphorylcreatine (PCr) and creatine (Cr) are involved in the energy metabolism of cells and tissues with high and fluctuating energy demands like skeletal and cardiac muscle, brain, retina and spermatozoa (for reviews see refs 1,2). CK isoenzymes catalyze the reversible transfer of the  $\gamma$ -phosphate group of adenosine 5'-tri (ATP) to the guanidino group of Cr to yield PCr and adenosine 5'-diphosphate (ADP). Since, in skeletal muscle, PCr and Cr are present in much higher concentration and are smaller and less negatively charged molecules than ATP and ADP, they serve both as a 'reservoir' for 'high-energy phosphates' during short periods of intense work and as a 'transport device' allowing for a higher flux of high-energy phosphates within the cells during endurance exercise.

Several lines of experimental evidence indicate that derangements in the proper function of the CK/

PCr/Cr system – either on the enzyme or on the substrate level – are associated with impaired muscle function. Transgenic mice lacking the cytosolic CK isoenzyme in muscle are unable to perform burst activity, i.e. they have lost the ability to sustain maximal muscle output during short periods of high-resistance work (3,4). Furthermore, the rate constant for tension recovery after stretching of the muscle fibers is greatly decreased in these transgenic animals (5). Injection of the CK inhibitor 2,4-dinitrofluorobenzene into the aorta of rats causes a metabolic myopathy characterized by spontaneous contractures in the hindlimbs, involving mainly the soleus muscle, and by selective type I muscle fiber degeneration (6). Finally, the intracellular Cr pools can be depleted by feeding of rats or mice with the Cr analogue 3-guanidinopropionic acid (3-GPA), which competes with Cr for uptake into muscle cells. 3-GPA feeding results in various pathological muscle changes (for a review see ref 7) and aggravates thyrotoxic myopathy in mice (8).

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On the other hand, many (neuro-) muscular diseases are associated with disturbances in Cr metabolism. Examples are Duchenne (DMD) and Becker (BMD) muscular dystrophy, facioscapulohumeral dystrophy, limb-girdle muscular dystrophy, myotonic dystrophy, spinal muscular atrophy, amyotrophic lateral sclerosis, myasthenia gravis, poliomyelitis anterior, or myositis (9–16; for reviews see refs 17–20). Common findings are increased serum CK activities, increased Cr concentrations in serum and urine, stimulation of creatinuria by oral supplementation with glycine or Cr, decreased urinary creatinine (Crn) excretion and, in particular, depressed muscle levels of Cr, PCr, P<sub>i</sub>, glycogen and ATP.

All these findings suggest a close relationship between the intracellular levels of Cr, PCr and CK activity on one hand and muscle function and integrity on the other hand. Consequently, disturbances in Cr metabolism, either as a primary defect or secondary to other metabolic derangements, may critically influence muscle performance and eventually result in muscle fiber degeneration.

### Hypotheses

Two hypotheses shall be considered that explain how disturbances in Cr metabolism may contribute to the clinical symptoms and to the progression of muscle disease:

a. Muscle cells lack Cr biosynthesis. Instead, Cr is synthesized in the liver, transported through the blood, and taken up into muscle cells – against a large concentration gradient – through a saturable Cr transporter (for a review see ref. 7). This Cr transporter has recently been cloned and is driven by the electrochemical gradients of Na<sup>+</sup> and Cl<sup>-</sup> across the plasma membrane (21). In both human and rat myoblasts and myotubes, Cr transport activity was shown to be down-regulated progressively by increasing extracellular concentrations of Cr (22).

In various muscle diseases, the intracellular concentrations of Cr and PCr are decreased (Fig. 1). The muscle cells should respond to this deficit by increased Cr uptake across the plasma membrane. However, due to the increased extracellular (serum) concentration of Cr that is frequently observed in these diseases, the Cr transporter activity may become depressed rather than increased. The net results would be further depletion of the intracellular Cr stores, progressive impairment of energy metabolism and thus of ATPases relevant for muscle integrity, and, consequently, continued release of CK and Cr from muscle cells.

b. Duchenne muscular dystrophy (DMD) patients

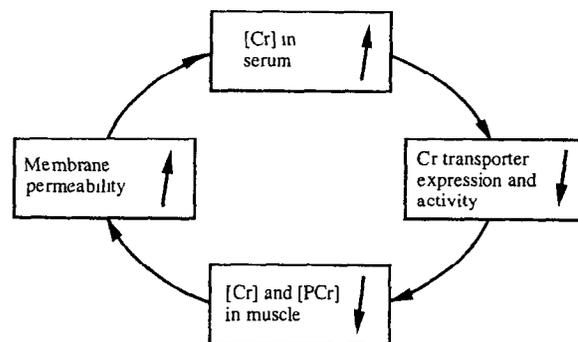


Fig. 1 Diagrammatic representation of a potential 'vicious cycle' operating in muscle disease that results in progressive muscle fiber degeneration.

and *mdx* mice share the same primary defect, namely dystrophin deficiency. Dystrophin is thought to play a crucial role in the stabilization of the plasma membrane, and its lack is associated with enhanced fragility and conductance of the plasma membrane. As a matter of fact, the intracellular concentrations of Na<sup>+</sup> and Ca<sup>2+</sup> were reported to be increased in skeletal muscle of both DMD patients and *mdx* mice, while the levels of K<sup>+</sup> and inorganic phosphate are decreased. In turn, the serum concentrations of K<sup>+</sup>, Ca<sup>2+</sup> and P<sub>i</sub> are increased whereas [Na<sup>+</sup>] and [Cl<sup>-</sup>] are decreased (19,23–28).

Since the Cr transporter is driven by the electrochemical gradients of Na<sup>+</sup> and Cl<sup>-</sup> across the plasma membrane, a decrease in these gradients as seen in DMD and in the *mdx* mouse should result in diminished uptake of Cr into muscle cells. Similar to the situation described under (a), this diminished Cr uptake may cause partial depletion of the intracellular Cr and PCr stores, progressive impairment of energy metabolism and thus of ion transport ATPases and, consequently, further deterioration of ion concentrations. Because disturbances in ion homeostasis were also observed for other myopathies (29–31), the scenario just described may not be limited to dystrophin deficiency, but may apply to a broader range of muscle diseases.

If either of these purported vicious cycles (a) or (b) were in fact operative, oral Cr supplementation should represent a feasible way to alleviate the clinical symptoms and/or to slow or even halt disease progression. If only hypothesis (b) is correct, continuous supplementation with Cr is indicated. If, however, hypothesis (a) is valid, intermittent short-period supplementation with high doses of Cr is expected to provide superior results. In this latter case, continuous Cr supplementation would result in further

down-regulation of the Cr transporter, with reciprocal neutralization of the effects. During short periods of supplementation with high doses of Cr, however, extra Cr can be taken up into the muscle cells. Then, supplementation has to be discontinued in order to allow for recovery of Cr transporter expression and/or activity. In this way, the muscle cells are rendered capable of taking up extra Cr also in the next round of Cr supplementation.

Preliminary investigation of oral Cr supplementation over 155 days in a 9-year-old boy with DMD revealed a decrease in the serum activities of CK and lactate dehydrogenase, a slight and transient decrease in the ATP/PCr, P<sub>i</sub>/PCr and phosphodiester/PCr ratios in resting muscle as determined by <sup>31</sup>P-MRS, and an increase in muscle performance as evaluated by different criteria. These findings are in line with previous Cr supplementation studies on normal subjects and trained athletes, which have demonstrated significant (up to 40%) increases in the intracellular Cr and PCr concentrations as well as in anaerobic muscle performance (32–35).

Even though dystrophin is not directly involved in Cr metabolism, making a drastic effect of Cr supplementation on the clinical symptoms of DMD unlikely, and in spite of the fact that training effects cannot be excluded for the DMD subject mentioned, the improvements in exercise performance are promising. Together with the hypotheses raised, they demand further and more detailed investigations on the relationships between disturbances in Cr metabolism on one hand and the primary defects or clinical symptoms of muscle diseases on the other hand, as well as careful examination of the potential of Cr supplementation as a novel approach for the therapy of muscle diseases. Towards this end, the following efforts should be attempted: (i) the disturbances in Cr metabolism should be investigated in greater detail in a broad range of muscle diseases; (No such studies, performed with up-to-date technology, are currently available.) (ii) the mechanisms and kinetics of regulation of Cr transporter expression and activity have to be studied in order to corroborate the previous finding that extracellular Cr down-regulates Cr transport activity in muscle cells (22) and to get an idea on the impairment of this protein in human muscle disease; (iii) other agents were proposed or may be indicated for the therapy of DMD, e.g. glucocorticoids (36) or carnitine (37). It is tempting to speculate that combination therapies with Cr and these latter agents may reveal potentiating effects. All these efforts will provide a more detailed knowledge of Cr metabolism in general and, hopefully, also of the pathophysiology and therapy of human muscle disease.

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