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Journal of Cystic Fibrosis 2 (2003) 177–182

Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

Effects of creatine supplementation in cystic fibrosis: results of a pilot study

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Received 19 September 2002; accepted 28 July 2003

Abstract

Background: The CF transmembrane conductance regulator (CFTR), whose mutations cause cystic fibrosis (CF), depends on ATP for activation and transport function. Availability of ATP in the cell and even more in specific cellular microcompartments often depends on a functional creatine kinase system, which provides the 'energy buffer' phosphocreatine. Creatine supplementation has been shown to increase phosphocreatine levels, thus promoting muscle growth and strength in athletes and having protective effects in neuromuscular disorders. *Aim*: To test clinically, if creatine supplementation improves maximal isometric muscle strength (MIMS), lung function and CFTR channel activity in patients with CF, and to determine enzymatic activity of creatine kinase in respiratory epithelial cells. *Methods:* In an open-label pilot study 18 CF patients (8–18-year-old) with pancreatic insufficiency and mild to moderate lung disease received daily creatine supplementation during 12 weeks. Patients were monitored during 24–36 weeks. Enzymatic activity of creatine kinase was measured in primary epithelial cell cultures. *Results*: After creatine supplementation, there was no change in lung function and sweat electrolyte concentrations, possibly due to the very low creatine kinase activities detected in respiratory epithelia. However, the patients consistently showed signicantly increased MIMS (18.4%; $P < 0.0001$), as well as improved general well-being, as assessed by a standardized questionnaire. Except for one patient with transient muscle pain, no side effects were reported. *Conclusions*: Our pilot study suggests, that creatine supplementation should be further evaluated as a possible clinically beneficial adjuvant therapy for patients with CF to increase muscle strength, body-weight and well-being

Keywords: Creatine kinase; Cystic fibrosis transmembrane conductance regulator; Energy metabolism; Muscle strength; Creatine supplementation

1. Introduction

Creatine (Cr) is a central compound for the energy metabolism of many tissues with high energy turnover like muscle or brain. In humans, Cr is mainly synthesized by the liver or ingested in food, especially meat and fish, and taken up into cells via a specific Cr transporter [1]. Cr then serves as a substrate for creatine kinase (CK) to generate phosphocreatine (PCr) [2]. In the cell, isoenzymes of CK are partially associated with ATP providing processes (e.g. glycolytic enzymes, mitochondrial adenylate translocator) to synthesize PCr, or with ATP consuming reactions to use PCr for the regeneration of ATP pools [2,3]. A close association of cytosolic CK with ATPases allows the direct exchange of ADP and ATP, known as 'functional coupling' [4–8]. These properties make the CK/PCr system a key factor for cellular energetics in the human body. Although CK has not yet been analyzed in epithelia of the respiratory tract, which are relevant to cystic fibrosis (CF), a functional CK/PCr system has been reported

Abbreviations: CF, Cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; CK, Creatine kinase (EC 2.7.3.2); Cr, Creatine; FEV1, Forced expiratory volume in 1 s; FMG, Functional muscle group; MEF 25/75, Mean expiratory flow at 25–75 forced vital capacity; MIMS, Maximal isometric muscle strength; PCr, Phosphocreatine; RV, Residual volume; TLC, Total lung capacity

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doi:10.1016/S1569-1993(03)00089-4

for a number of epithelia in the urogenital system, the digestive tract, skin and some other tissues [7–15]. Here, CK seems to sustain high proliferation rates, ion pumps and active transport processes [7–9].

Recently, the clinical application of Cr supplementation has attracted considerable attention. It was found to increase intracellular Cr and PCr pools [16,17], thus improving muscle growth and strength not only of athletes [18], but also of patients with certain myopathies and neuromuscular diseases [19–21]. Cr also exerts marked protective effects in a number of animal models for neuromusular and neurodegenerative diseases and in some other pathologies [22–26]. Cr supplementation could be beneficial for CF patients through different mechanisms. First, Cr may generally improve the cellular energy status through elevated PCr/ATP ratios, leading to the known positive effects on muscle and brain functions and thus increasing the general well-being of CF patients. In fact, an improved energy balance inducing weight gain has been shown to correlate well with an improved clinical long-term prognosis in patients with CF [27]. Second, Cr could directly act on the CF transmembrane conductance regulator (CFTR) channel in respiratory epithelia. This epithelial chloride transporter, which is rendered non-functional in CF by mutations in the CFTR gene [28], has a high ATP requirement. ATP is needed for activation via phosphorylation by cAMP-dependent protein kinase, and also for channel gating and chloride transport, which depends on a dual ATP hydrolysis cycle [29,30]. Thus, an increased cellular PCr pool together with CFTR or cAMP-dependent protein kinase functionally coupled to CK could maintain higher local ATP/ADP ratios [2] for channel opening or CFTR phosphorylation. Such a functional coupling is well known for H^+/K^+-ATPase in kidney and gastric epithelia [7,8] or chloride transport in dogfish epithelia [31]. It could rescue those CFTR mutants that are correctly localized and still partially functional. In fact, close interactions of CFTR with other energy-related kinases like AMP-activated protein kinase [32] and nucleoside diphosphate kinase [33] suggest that CFTR is part of a multi-enzyme complex that may also comprise CK.

The aim of the present pilot study was to determine potential effects of Cr supplementation on CFTR-related pathological symptoms, muscle strength and general well-being [34]. We have studied 18 CF patients with different mutations in the CFTR protein in respect to their lung-function, cellular ion transport (sweat electrolytes), muscle strength, body weight and overall wellness. In parallel, we have determined CK activity in primary cell cultures from respiratory epithelia. This pilot study should be useful to evaluate whether such a simple intervention with a nutritional supplement could improve CFTR functioning and/or increase muscle parameters and thus lead to a better general well-being that would be fundamentally useful to improve life quality of these patients [34].

2. Methods

2.1. Patients

For this pilot study eighteen patients with CF (11 boys and seven girls, age 8–18 years) were recruited attending the outpatient clinic of the University Children's Hospital Zurich. All patients had pancreatic insufficiency and mild to moderate lung disease by clinical and radiographic criteria. Anthropometrical data of weight and height were recorded. Exclusion criteria were renal, cardiac, musculoskeletal diseases; and significant undernutrition defined as current weight below 85% of weight equivalent to current height. Patients volunteered and informed consent was obtained. Protocol and consent form were approved by the Ethics Committee of the University Children's Hospital Zürich.

2.2. Study design

The patients were supplemented with a loading dose of 12 g Cr (Podium®–Creatine was a gift from Synergen, Switzerland) daily for one week and a dose of 6 g for another 11 weeks. The patients were monitored four times: before supplementation (baseline); after 4, and 12 weeks (during supplementation); and after 24–36 weeks. With a standardized questionnaire they were asked for general well-being and dyspnea. Body-weight, maximal isometric muscle strength (MIMS) and lung function (FEV1, RV%TLC, MEF 25/75) were measured. MIMS was scaled with the force gauge instrument AFG (Mecmesin Limited, West Sussex RH12 3JR, UK) on the following seven functional muscle groups (FMGs): shoulder-flexion, shoulder-extension, elbow-flexion, elbow-extension, hip-extension, knee-flexion, ankle-flexion. All FMGs were evaluated on both left and right side of the body. The methods used is described in detail elsewhere [35–37]. Briefly, each joint was evaluated three times sequentially at each evaluation visit, interspersed by short rest periods of 1 min. The maximum strength result was monitored and used. The evaluation visits were carried out between 2 and 3 h after the last meal (breakfast or lunch). All children completed the tests successfully. During the 24–36 week study period the patients did not do any additional exercise. The laboratory investigations at each evaluation session included sweat electrolytes, serum electrolytes, liver function tests, serum creatinine, creatine kinase (CK), blood count, blood gases and CRP. Chest X-rays (Shwachman score) were done three times: Before supplementation (baseline) and after 12 and 24–36 weeks.

2.3. Cell cultures and CK enzymatic activity

Primary fetal bovine cell lines were established from respiratory tract and bronchial epithelium in our laboratory as described elsewhere [38,39] with minor modifications. Macroscopically normal respiratory organs were taken from 7-month-old bovine fetuses 2–4 h after death in the slaughterhouse (Zürich). Mucosal tissue was dissected immediately after removal. Epithelial strips were incubated at 4 °C over night in a dissociation buffer containing dispase type I 0.1% (Roche Molecular Biochemicals). Suspensions were collected, centrifuged and the cells were plated in collagen type I coated culture dishes (Falcon BIOCOAT). Growth of epithelial cells was stimulated with Ham's F12 modified medium [39], supplemented with 15 mM Cr-monohydrate and cholera toxin as growth inhibitor of fibroblasts and melanocytes. After 8–11 days of culture in a humidified CO_2 incubator, whole cell extracts of approximately 10^6 cells/sample were obtained by sonication of cell pellets in phosphate buffer pH 7.8, 1 mM β-mercaptoethanol, 1 mM PMSF and 0.2 mM EDTA. Extracts of bovine myocardium were used as positive control. After centrifugation of lysates, clear supernatants and pellets were separately analyzed for enzymatic activity of CK with an electrochemical assay (pH-stat) using PCr and ADP as substrates [40]. Protein was determined with the Bio–Rad reagent according to Bradford [41], using bovine serum albumin as standard.

Table 1
Average of maximal isometric muscle strength of all investigated functional muscle groups in kp (1 kp = 9.81 N) before, during and after creatine supplementation; pairwise time differences were tested by paired t-test for statistical significance

MIMS (kp)	Mean		SD[1]
Before supplementation	12.5		4.5
After 4 weeks	13.5	p=0.014	4.3
After 12 weeks	14.3	p=0.04	4.8
After 24–36 weeks	14.8	p=0.2 (n.s.)	4.7
Relative difference[2]	18.2%	p<0.0001	

[1] SD of individual means over all functional muscle groups
[2] Difference in muscle strength before supplementation after 24-36 weeks

3. Result

3.1. Creatine supplementation—a clinical study

During the Cr supplementation period, MIMS of the patients increased in all of the investigated FMGs (Fig. 1). The increase was on average 8% after 4 weeks, 14.3% after 12 weeks, and 18.2% after 24–36 weeks (Table 1), which was statistically highly significant ($P < 0.0001$). The most pronounced change was seen in the MIMS of the FMG of the right ankle flexion, which increased from 14.5 kp at baseline to 17.4 kp at week 4, 19.4 kp at week 12, and 21.0 kp at week 24–36,

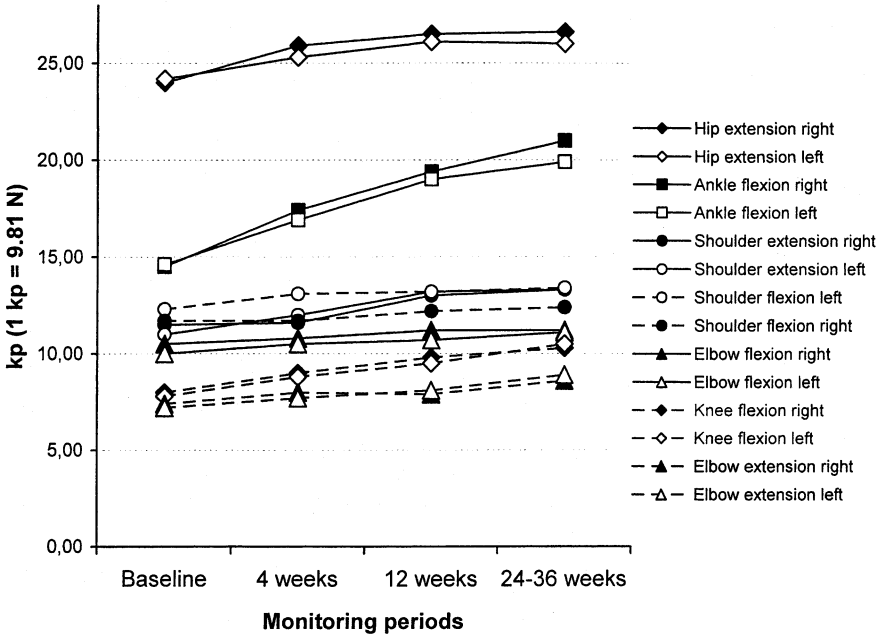

Fig. 1. Maximal isometric muscle strength of different functional muscle groups before, during and after creatine supplementation

which is an increase of 45% (Fig. 1). Six patients (33%) reported an improved general well-being, nine patients (50%) observed no change, and in three patients (17%) general well-being decreased during the study period. However, there was no change in lung-function and chest X-rays (assessed by the Shwachman score). There was also no change of the sweat electrolytes and other laboratory parameters (data not shown). Except for one patient who complained about transient muscle pain in his legs, no other adverse effects were noted.

3.2. CK enzymatic activity in epithelial cells

We have established bovine primary cell cultures of different epithelia from the respiratory tract, including nasal, tracheal, and bronchial epithelia. Enzymatic activity of CK in soluble and insoluble fractions of crude cell lysates was then compared to bovine myocardium, an organ with high expression levels of cytosolic and mitochondrial CK that relies on a functional CK/PCr system. All epithelial cells showed very low specific CK activity in the soluble fraction and no detectable activity in the insoluble fraction. While specific activity of soluble CK in myocytes was 2.75 U/mg protein, we measured only 0.21 U/mg in nasal, 0.02 U/mg in tracheal and 0.01 U/mg in bronchial epithelial cells. Supplementation of epithelial cell cultures with 15 mM Cr didnot increase CK activity nor affect significantly the growth rate or general morphology of these cells (data not shown). Thus, the CK/PCr-system is present in epithelial cells, albeit at a much lower extent as compared to muscle or brain and, at least in the primary cell cultures, it is not inducable by Cr supplementation.

4. Discussion

In order to judge the possible significance of an improved CK/PCr-system for CF-patients, we have conducted a clinical pilot study on Cr-supplementation of CF-patients and analyzed CK activity in different epithelia of the respiratory tract. Although disturbances in the respiratory system constituted the preponderant pathology of the CF patients participating in our study, there was no evidence of a direct effect of Cr on epithelial cells or CFTR-function in these patients. The lack of such direct beneficial effects may be due to low CK expression in respiratory tract epithelial cells and thus a probably rather low-key CK/PCr system at work. Alternatively, CK may not associate with CFTR as it does with different Na^+/K^+-ATPases [7,8] or other chloride transporters [31]. However, considering the physiological functions of these epithelial cells, which need substantial amounts of ATP for ion pumping, their specific CK activity was lower than anticipated from the literature [12,42]. Possibly, these earlier studies overestimated CK because of either a contamination of epithelial preparations by underlying smooth muscle, or due to a concentration of CK at specific subcellular sites, giving rise to strong positive signals in immunohistochemical stainings.

In contrast to respiratory organs, Cr supplementation had clear beneficial effects on the skeleto–muscular system, leading to an improved general well-being of the patients. Such improvements of muscle strength, as well as a moderate anabolic effect, are known from Cr supplementation studies in sports physiology [43,44]. These are linked to the generally improved cellular energetics in muscle cells [45], more efficient calcium homeostasis [4] leading to faster muscle relaxation [46], as well as to an increase in muscle mass affecting all fiber types [47]. However, such effects have so far not been shown in children and immobilized patients unable to follow an entrainment program in parallel to Cr uptake. Here we report a more than 20% increase in muscle strength in CF patients by just ingesting Cr as a supplement without exercise schedule. This effect compares favorably with the increases in muscle strength of well trained athletes undergoing Cr supplementation plus heavy exercise (usually amounting to 5–10%) or of patients with neuromuscular diseases (with an increase of just a few percent in muscle force) [19,21]. In a recent publication it was shown that Cr ingestion significantly improves rehabilitation after immobilization atrophy, possibly by enhancing myogenic transcription factors, like MRF4 [48]. This can happen to a certain extent even without exercise. Such a mechanism could explain the improvement of MIMS in a number of FMGs in our CF patients.

As long as the basic defect of CF cannot be corrected [49,50], symptomatic treatment alleviating symptoms and thus improving quality of live of CF patients is highly welcome. So far no serious side-effects have been reported for Cr supplementation [44]. Since our pilot study showed beneficial effects on MIMS and quality of life parameters in CF patients, it seems well founded to further investigate the beneficial effects of Cr supplementation on these parameters in a future randomized placebo-controlled study, preferably in combination with an exercise schedule specifically adapted to CF patients.

Acknowledgments

We thank Luciano Molinari for statistical analysis, and Catherine Figarella and Marc Mertain for their contributions and critical reading of the manuscript, as well as Els Zanolla and Robert Tschudin for technical assistance. This work was in part supported by a grant of the Swiss Society for Cystic Fibrosis (to C.B. and F.S.), Swiss National Science Foundation (grant No. 3100-50824.97, to T.W. and U.S.), a grant from the Swiss Society for Research on Muscle Diseases (to

T.W.), as well as by private sponsoring from PreVention Health Concepts, Switzerland. Podium®–Creatine was a gift from Synergen, Switzerland.

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