ABSTRACT: The effect of creatine (Cr) supplementation on muscle function and body composition of 12 boys with Duchenne muscular dystrophy and three with Becker dystrophy was evaluated by a randomized double-blind cross-over study (3 g Cr or maltodextrin daily for 3 months, with wash-out period of 2 months). After placebo, no change was observed in maximal voluntary contraction (MVC) and resistance to fatigue, whereas total joint stiffness (TJS) was increased by ~25% ($P < 0.05$). The patients receiving Cr did not show any change in TJS, improved MVC by 15% ($P = 0.02$), and almost doubled their resistance to fatigue ($P < 0.001$). In patients still independent of a wheelchair ($n = 5$), bone mineral density increased by 3% ($P < 0.05$), and urinary excretion of collagen type I cross-linking N-telopeptide declined to about one third ($P < 0.001$) after Cr. No adverse effect was observed. Thus, Cr may provide some symptomatic benefit in these patients.

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BENEFICIAL EFFECTS OF CREATINE SUPPLEMENTATION IN DYSTROPHIC PATIENTS

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Patients with Duchenne (DMD) or Becker muscular dystrophy (BMD) have muscle weakness and wasting, as well as a progressive contracture of the joints.[21] The level of creatine kinase (CK) in blood is higher than normal, reflecting muscle degeneration.[16] Reduction in bone mineral density also occurs.[15] In most patients, shortened life is mainly due to failure of the respiratory musculature. No curative treatment is available for this myopathy.

The improvement of muscle performance observed in athletes and healthy subjects after creatine (Cr) supplementation[24] has prompted interest in its potential to limit muscle degeneration or even restore muscle function in neuromuscular diseases.[31] Strength was reportedly enhanced after Cr supplementation in patients with various neuromuscular diseases,[23,30]but the heterogeneity of the population made it difficult to draw any conclusion regarding a specific myopathy. A preliminary investigation in a 9-year-old boy with DMD showed improved muscle performance after 155 days of Cr supplementation.[9]

In this study, we investigated the effects of Cr supplementation in dystrophic patients over a 3-month period.

Abbreviations: ATP, adenosine triphosphate; BCE, bone collagen equivalent; BMD, Becker muscular dystrophy; CK, creatine kinase; Cr, creatine; DMD, Duchenne muscular dystrophy; IGF, insulin-like growth factor; IGF-BP, insulin-like growth factor binding protein; MVC, maximal voluntary contraction; NMR, nuclear magnetic resonance; NTx, collagen type I cross-linking N-telopeptide; TJS, total joint stiffness

Key words: bone mineral density; dietary supplement; Duchenne muscular dystrophy; myopathy; stiffness

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METHODS

Subjects. Fifteen boys with muscular dystrophy, aged from 6 to 16 years (10.8 ± 2.8 years), took part in a double-blind cross-over study. The characteristics of each subject are presented in Table 1. Patients were diagnosed by clinical examination and identification of dystrophin gene mutation after muscle biopsy, which was the inclusion criterion for the study. Patients receiving another drug therapy or unable to develop a torque of at least 1 Nm during elbow flexion were excluded. The limit was set by the sensitivity of the ergometer used in the study. Subjects were randomly divided into two groups: one group ($n = 8$) first received oral Cr monohydrate (Flamma, Chignola d'Isola, Italy), 3g daily over a 3-month period, whereas the second group ($n = 7$) received a placebo (maltodextrin) during the same period. After a wash-out period of 2 months, subjects crossed from one treatment group to the other. Before and after each supplementation period, subjects underwent several tests, including muscle function analyses during elbow flexion, densitometry, tests of hepatic and renal functions, and ^{31}P nuclear magnetic resonance spectroscopy. The tests of muscle function included determination of maximal voluntary contraction (MVC), fatigue resistance, and evaluation of total joint stiffness (TJS). The Ethical Committee of the Medicine Faculty of the Université Catholique de Louvain approved this experimental protocol, which was in accord with the Helsinki Declaration of 1975. The written consents of the patients and of both of their parents were obtained.

Muscle Function. Muscle mechanical performance was studied using an ergometer specifically designed to test properties of the muscle groups and joint during elbow flexion in children. The ergometer is described in detail elsewhere.[5] Briefly, it consists of a platform supporting a power unit and a seat associated with a driving unit. The seat is adaptable to the shape and size of the child and is adjustable three dimensionally to optimize the subject's position. A fixed column supports a direct-drive actuator (NSK Megatorque, Tokyo, Japan), a forearm support directly fixed onto the rotor, and a specific transducer for the angular position.

The subject was seated and maintained by a special belt system to keep the body and head against the seat back and headrest. The seat was adjusted near the fixed column so that the subject's forearm rested on the support. The motor rotation axis was aligned with the epitrochlear–epicondyle axis of the humerus. The hand was placed in the prone position holding a horizontal grip, the distance of which was adjusted from the motor rotation axis. During measurements, the subject remained with the arm and forearm in the horizontal plane, with an angle of 45° between the frontal plane and arm, and with the elbow joint angle set at 90°.

Maximal voluntary contraction of elbow flexion was first measured under isometric conditions. Three tests were performed and the highest score was accepted. Sinusoidal perturbations were imposed on the right elbow (4–12 Hz, 3° peak-to-peak harmonic angular displacement) while the subject maintained a mean constant torque equal to 25 or 50% of the MVC. The expected torque level was displayed on an oscilloscope, in front of the subject, who was instructed to try to maintain it. Each trial lasted 4 s. The trials were separated by a rest period

Table 1. Individual characteristics of the patients.

Subjects	Age (yr)	Height (cm)	Body mass (kg)	Disease	Wheelchair dependency
1	10.5	130.4	35.0	DMD	Yes
2	8.3	131.0	50.0	DMD	Yes
3	11.2	144.4	40.0	DMD	Yes
4	8.1	110.0	17.0	DMD	No
5	12.9	147.2	37.4	BMD	No
6	13.6	139.0	39.0	DMD	Yes
7	12.2	152.0	60.0	DMD	Yes
8	6.0	122.1	29.6	BMD	No
9	7.7	120.6	26.0	DMD	No
10	16.1	163.0	60.0	BMD	Yes
11	12.7	146.0	32.0	DMD	Yes
12	11.7	144.8	39.0	DMD	Yes
13	7.8	125.1	28.0	DMD	No
14	11.2	160.0	31.4	DMD	Yes
15	9.8	134.8	25.6	DMD	Yes

Results are presented as means ± SE (n = 15). Statistical significances were assessed by Student's paired t-test.

of about 2 min to prevent muscle fatigue. The time courses of angular displacement and torque were recorded during each trial. For each maintained torque, averaged position-to-torque amplitude ratios and position-to-torque phases were calculated and plotted on a Bode diagram. Frequency-dependent phase shifts reflect a mixed mechanical contribution from the inertia, viscosity, and stiffness of the musculo-articular system.[5] A second-order model including these parameters was adjusted to the Bode diagram using identification techniques, and the stiffness ($Nm.rad^{-1}$) was determined for 25 and 50% of the MVC. The procedure and calculations are described in detail elsewhere.[5]

After a recovery period of 8 min, the subjects were asked to maintain 75% of MVC for as long as possible in order to measure resistance to fatigue.

^{31}P NMR. ^{31}P Nuclear magnetic resonance (NMR) spectra were acquired from the calf muscle of the leg of six subjects, at rest, using a Bruker Biospec spectrometer (horizontal bore) working at 4.7 Tesla and 81 MHz for ^{31}P. The magnet was 33 cm in width, allowing only the slimmest subjects ($n = 6$) to enter far enough into the spectrometer to have the calf appropriately positioned at the center of the magnet. The leg was firmly immobilized over a 50-mm diameter surface coil. After fine tuning of the probe with the subject properly installed, the magnetic field was shimmed. The ^{31}P NMR spectrum was then recorded. The signal was acquired on 2048 points (spectral width, 3484 Hz) after a 100-μs excitation pulse, corresponding to an angle of 90° in the center of the coil. To improve the signal/noise ratio, 64 free induction decays were accumulated with a repetition time of 10 s. Different peaks (inorganic phosphate, phosphorylcreatine, α-, 107- and β-adenosine triphosphate [ATP]) were quantified by fitting in the time domain.[26] The β-ATP peak was used as an internal reference.

Bone Mineral Density. Lumbar spine (L1–L4), and whole body bone mineral density were determined by dual energy X-ray absorptiometry (QDR 4500 Elite; Hologic, Inc., Bedford, Massachusetts). Prior to the measurements, calibration was performed according to the recommendations of the manufacturer.

Blood and Urine Samples. Subjects came to the hospital four times: before and after each Cr or placebo supplementation for 3 months. Each time, blood samples were drawn from the antecubital vein or hand, and a 24-h urine sample was collected. Plasma was separated by centrifugation at 1000*g* for 12 min, at 4°C. Creatine and creatinine were determined in plasma and urine using an enzymatic colorimetric test (PAP; Boerhinger, Mannheim, Germany). Albumin in urine was assayed by an immunological turbidimetric method (Turbiquant Albumin Urine, Marburg, Germany). Serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase and CK activity were analyzed by enzymatic colorimetric tests (Roche Diagnostics, Mannheim, Germany, and Sigma, St Louis, Missouri, respectively). The collagen type I cross-linking N-telopeptide (NTx), a biochemical marker of bone resorption, was measured in urine by an enzyme-linked immunosorbent assay (ELISA) method (Ostermark; Ostex International, Inc., Seattle, Washington).

Statistics. The results are expressed as means ± standard error. Statistical evaluation of the data was performed by Student's paired *t*-test on the differences between pretreatment and posttreatment values. The level of statistical significance was set at $P < 0.05$.

RESULTS

A summary of the results is given in Table 2.

Muscle Function. Under placebo supplementation, no change was observed in MVC and time to exhaustion, whereas TJS increased by about 25% ($P < 0.05$), revealing the evolution of the dystrophy over a 3-month period (Fig. 1). When the patients consumed a daily load of 3 g of Cr over the same period of time, MVC increased by 15% ($P = 0.02$) and the time to exhaustion was almost doubled ($P < 0.001$; Fig. 1). In contrast to the placebo group, the patients receiving Cr did not show any change in TJS values measured at 50% of MVC. At the end of the wash-out period following Cr treatment, the effect on MVC and time to exhaustion persisted and regained values similar to the initial levels only after the next 3 months of placebo (Fig. 1).

^{31}P NMR. The PCr/β-ATP ratio increased by about 8% after Cr supplementation and remained unchanged in the placebo group. However, the paired *t*-test applied on the difference between pretreatment and posttreatment values did not reach the significance threshold. The PCr/ATP ratios were similar before the Cr and after the wash-out periods (3.71 ± 0.34 and 3.68 ± 0.31, respectively; NS).

Table 2. Effects of a 3-month period of oral Cr supplementation or placebo in dystrophic patients.

	Placebo		Cr		*P*-value
	Pre	Post	Pre	Post	Placebo vs Cr
MVC (N.m)	5.01 ± 1.5	4.9 ± 1.6	5.4 ± 2.1	6.1 ± 2.2	0.02
Time to exhaustion (s)	4.9 ± 1.8	4.2 ± 1.2	4.1 ± 1.3	7.2 ± 1.4	< 0.001
TJS at 50% MVC (N.m.rad^{-1}) (n = 6)	17.7 ± 4	21.9 ± 4.5	19.8 ± 6.1	19.3 ± 4.7	0.03
PCr/β-ATP (n = 6)	3.8 ± 0.2	3.9 ± 0.2	3.6 ± 0.3	3.9 ± 0.2	0.04
Body mass (kg)	38 ± 2.9	39.8 ± 2.9	40.1 ± 3.7	41.6 ± 3.5	NS
Lumbar spine bone density (g/cm^2)	0.601 ± 0.06	0.603 ± 0.07	0.596 ± 0.08	0.599 ± 0.08	NS
Whole-body bone density (g/cm^2)	0.758 ± 0.07	0.769 ± 0.07	0.757 ± 0.06	0.762 ± 0.07	NS
Urine NTx/creatinine ratio (mmol BCE/ mmol Crn)	1619 ± 224	1134 ± 143	2123 ± 417	1167 ± 175	NS
Daily urine Cr (mg/24 h)	846 ± 128	616 ± 121	600 ± 76	1671 ± 265	< 0.001
Daily urine creatinine (mg/24 h)	417 ± 82	370 ± 72	239 ± 38	356 ± 115	NS
Albumin excretion rate (μg/min)	11.3 ± 3.7	12.2 ± 2.7	15.2 ± 2.3	14.5 ± 3.2	NS
Plasma creatinine (mg/L)	1.3 ± 0.22	0.68 ± 0.17	1.42 ± 0.7	1.16 ± 0.3	NS
Plasma Cr (mg/L)	15.12 ± 0.67	14.87 ± 0.54	15.36 ± 0.87	43.31 ± 6.14	< 0.001
Serum aspartate aminotransferase (U/L)	114 ± 12	144 ± 47	117 ± 15	136 ± 23	NS
Serum alanine aminotransferase (U/L)	167 ± 20	149 ± 17	159 ± 18	166 ± 22	NS
Serum γ-glutamyltransferase (U/L)	9.8 ± 1.1	12 ± 1.1	10.5 ± 0.9	11.8 ± 1.2	NS
Serum Cr kinase (U/L)	5769 ± 976	4911 ± 818	5340 ± 1122	5211 ± 826	NS

Results are presented as means ± SE (n = 15, except when stated otherwise). Statistical significances were assessed by Student's paired t-test.

Densitometry. Mean body mass and bone mineral density did not change with either placebo or Cr. However, in five patients who were able to walk during the 8 months of the study, a significant increase (about 3%) was observed in bone mineral density values after treatment with Cr (Fig. 2). In these patients, bone density of the lumbar spine increased from 0.601 ± 0.0476 g/cm² to 0.624 ± 0.0498 g/cm² ($P < 0.05$) after Cr supplementation, whereas it was not modified during the placebo period (from 0.604 ± 0.0426 g/cm² to 0.604 ± 0.0442 g/cm²). Similarly, whole-body bone mineral density was increased with Cr (from 0.776 ± 0.0190 g/cm² to 0.792 ± 0.0227 g/cm², P < 0.05) but not with placebo (from 0.776 ± 0.0215 g/cm² to 0.781 ± 0.0189 g/cm², NS). The only patient (of five) whose bone mineral density remained stable was an 8-year-old DMD boy with the poorest walking capacity among the group of ambulant patients. The NTx/creatinine ratio, a marker of bone resorption,[6,13,20] significantly decreased during the Cr supplementation in these five patients (from 1365 ± 245 mmol bone collagen equivalent [BCE]/mmol creatinine to 599 ± 154 mmol BCE/mmol creatinine, $P < 0.001$), whereas no changes were observed during placebo periods (from 1144 ± 204 mmol BCE/ mmol creatinine to 1325 ± 264 mmol BCE/ mmol creatinine, NS). No effect was found in the wheelchair-dependent group.

Blood and Urine Studies. Oral Cr loading resulted in a threefold increase in plasma Cr, indicating correct supplementation of the patients. This observation was confirmed by the urinary Cr excretion rate over a 24-h period reaching a mean 56% of the dose administered daily. The creatinine excretion rates with placebo were comparable to those reported for healthy subjects of similar age.[19] Urinary excretion rate of albumin was not affected by Cr loading.

As expected, the activity values of liver and serum enzymes were not modified by Cr or placebo supplementation.

DISCUSSION

An elevated muscle Cr content assessed by PCr/β-ATP ratio has been reported in healthy subjects after Cr supplementation[10] and in a 9-year-old boy suffering from DMD.[9] However, no changes were reported in patients with McArdle's disease after 5 weeks of Cr supplementation (150 mg.kg^{-1}.d^{-1}).[28] Even if we observed a small increase in the PCr/ATP ratio in our patients receiving Cr, this change did not reach the significance threshold, possibly due to the small number of patients investigated. We calculated that 12 subjects would have been necessary to detect a statistical increase ($P < 0.05$) in PCr/ATP ratio. Moreover, dystrophic patients are known to accumulate less Cr than do healthy subjects, due to a reduction in their muscle Cr transporter.[32]

In healthy subjects, a few days of Cr loading improves muscle performance during high-intensity intermittent exercise,[12] but this beneficial effect

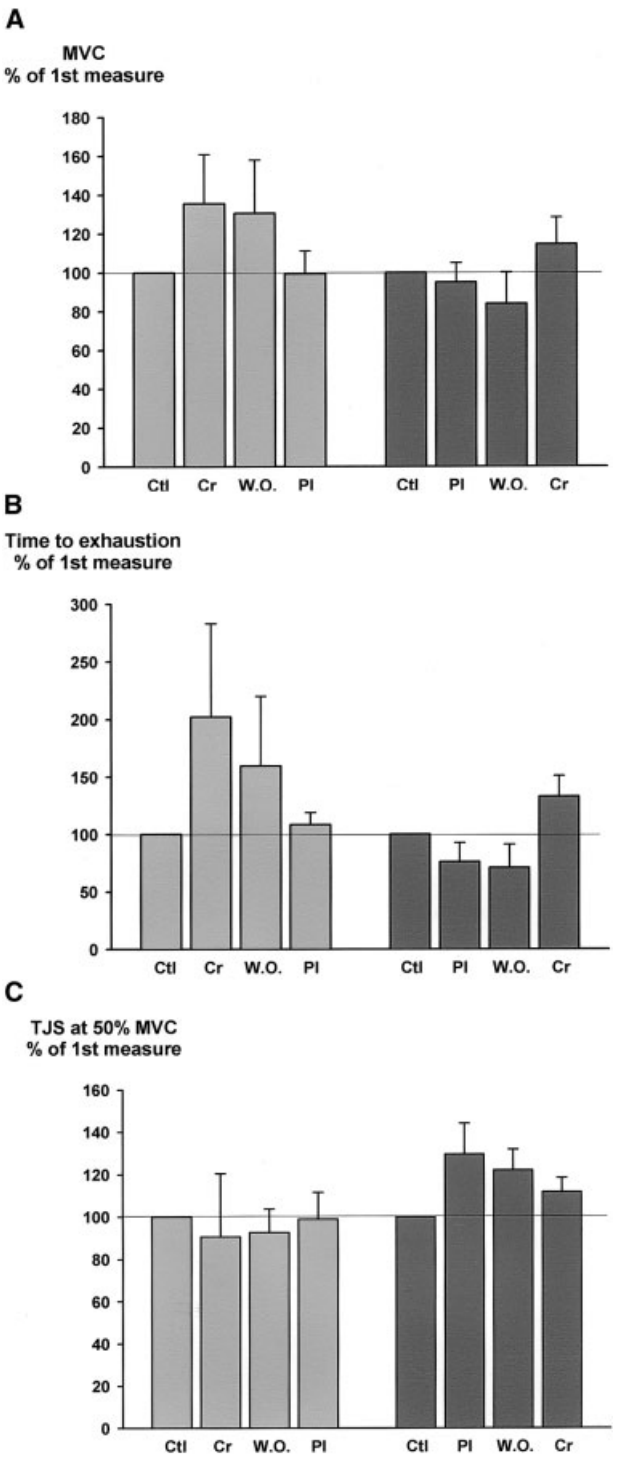

FIGURE 1. Mean values (± SE) for MVC **(A)**, time to exhaustion **(B)**, and TJS **(C)** for the two groups of subjects. The lightly shaded bars refer to patients receiving Cr initially, and the dark bars to those receiving placebo. (Abbreviations: Ctl, control; Cr, creatine; W.O., wash-out; Pl, placebo.)

does not appear after a single bout of maximal exercise.[2] Nevertheless, during a 10-week resistance-training protocol, a greater increment of maximal muscle strength has been observed in healthy young women receiving Cr compared with those receiving placebo.[25] This suggested that long-term Cr supplementation associated with exercise training was essential to improve strength. In the current study, the improvement of muscle strength observed in our patients suggests that exercise training is not necessary to induce beneficial effects of Cr in dystrophic patients. It is generally accepted that the greatest effect of Cr is observed during activities that highly

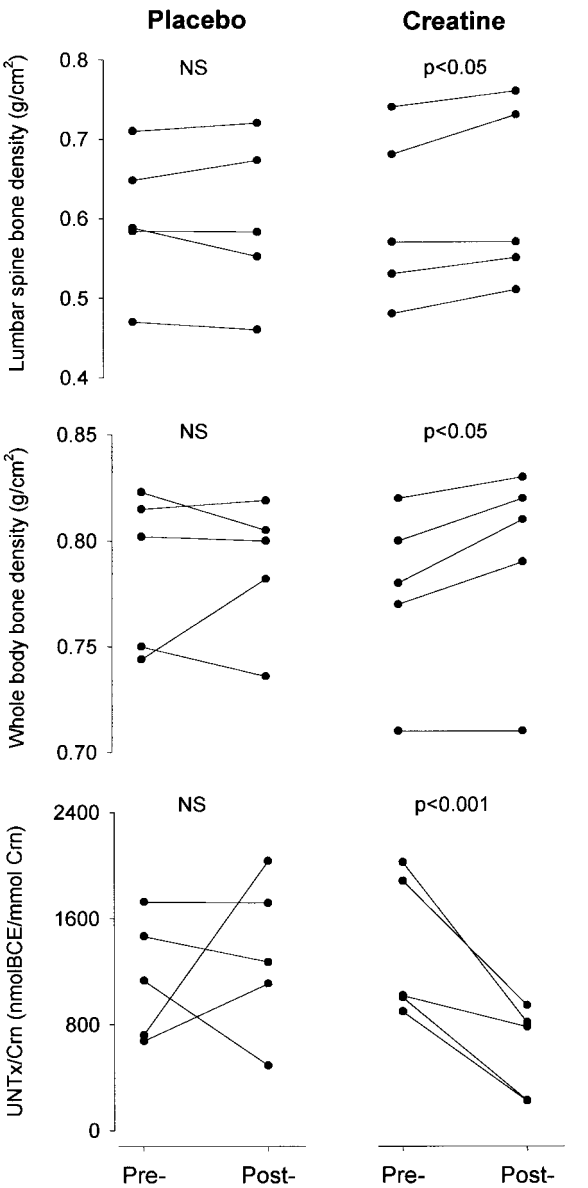

FIGURE 2. Individual changes observed in ambulatory dystrophic patients ($n = 5$) for lumbar spine bone mineral density, whole-body bone mineral density, and NTx/Crn (creatinine) ratio. Each solid line represents an individual change either during placebo (left) or during Cr supplementation (right). Statistical significances were assessed by Student's paired *t*-tests.

utilize the PCr system.[24] In our study, the greatest improvement of muscle performance was also observed during the fatigue test. After Cr loading, patients were able to maintain 75% of their MVC twice as long as otherwise.

Beside muscle atrophy and weakness, DMD and BMD patients also develop a progressive contracture of the joints.[21] In our group of dystrophic patients, we found TJS values well matched with those reported elsewhere for similar torque levels.[5] In a group of nine dystrophic boys followed over 6 months, Cornu et al.[5] reported an increase of about 35%. In the current study, the increased TJS during the placebo period revealed the progression of the disease over a period of 3 months. By contrast, the TJS did not change during the period of Cr supplementation. This finding suggests that Cr supplementation may slow the evolution of the disease. A slowing in the downhill course of the disease was also observed after steroid administration (prednisone and prednisolone[7]), but the side effects of steroids limit their long-term administration.

In contrast to most of the studies completed with healthy subjects,[11] we did not observe any increase of lean body mass in our myopathic patients after Cr supplementation (Table 2). Any significant change in lean body mass may have been masked because the young dystrophic boys were growing and gained an average of 0.72 kg of body mass each month, even during the placebo and wash-out periods.

Felber et al.[9] noted a reduced level of serum CK after 155 days of Cr supplementation in one dystrophic patient. Our results from a group of 15 dystrophic patients did not confirm this observation, suggesting that Cr is not part of a restorative treatment for the disease.

Our DMD and BMD patients showed a higher activity of serum γ-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase compared with a normal population.[16] However, Cr supplementation did not have any further impact on these enzymes. Similarly, albumin and creatinine excretion rates as well as plasma creatinine concentration were not affected by the supplementation. A slight increase in plasma creatinine concentration was reported after high-dose Cr supplementation ($21g \cdot d^{-1}$ for 7 days),[27] whereas no changes were observed when lower doses were ingested over a longer period of time.[18] In the current study, we did not observe any adverse effect of Cr supplementation in either hepatic or renal function. Moreover, patients did not report muscle cramps, diarrhea, or other side effects.

Our results suggest that Cr supplementation may act through a mechanism other than an enhancement of the energetic status of the cell. Indeed, the improvement of MVC and time to exhaustion persisted after the wash-out period of 2 months (Fig. 1), whereas the muscle Cr content returned to values similar to the initial levels. In humans, Cr changes the expression of myogenic factors during a rehabilitation period,[14] giving evidence for a modulation of muscle protein synthesis. Muscle and bone growth factors share several signaling pathways. In muscle, insulin-like growth factors (IGFs) stimulate both proliferation and differentiation of myoblasts[8] and IGF binding proteins (IGF-BPs) are involved in the regulation of phenotype changes,[1] whereas IGFs and IGF-BPs are also believed to play a key role in bone metabolism.[3] In this article, we report that Cr supplementation enhances bone mineral density and reduces urinary NTx excretion, a marker for bone resorption,[22,29] in wheelchair-independent patients. Therefore, we hypothesize that the increase of maximal strength, reduced progression of joint contractures, and increase in bone mineral density in wheelchair-independent patients could be initiated by a common signaling pathway that remains to be identified. The effect on bone mineral density seems strongly related to the patient's activity, whereas little change was noted in wheelchair-dependent patients. However, this effect could also be an indirect consequence of the increased mobility of the patients, resulting from the improvement of muscle function. Usually, a minimum of 6 months is required to detect a significant effect of any pharmacological intervention on bone mineral density of postmenopausal women.[17] However, a recent study reported that a resistance training of 12 weeks in elderly men increased whole-body bone mineral density and that Cr supplementation combined with this training provided a further increase.[4] Thus, it seems that Cr may potentiate the effect of muscle activity on bone mineral density in patients suffering from bone demineralization, for instance, our myopathy patients. If this effect can be confirmed, further studies will be required to understand the subsequent mechanisms of action.

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