

Appendix

Accepted with minor revisions – *J. Gerontology A*, Sept., 2002.

Creatine Supplementation Enhances the Isometric Strength and Body Composition Improvements Following Strength Exercise Training in Older Adults.

Brose, A¹, MSc, Parise, G¹, MSc, Tarnopolsky, M.A.^{1,2}, MD, PhD.

Department of Kinesiology¹, and Medicine², McMaster University, Hamilton, Ontario, CANADA.

Running Title: Creatine increases strength gains following training in the elderly.

Word Count: 3998 (including headings).

Correspondence: Dr. M. Tarnopolsky, Dept. of Neurology,
Rm 4U4, McMaster University Medical Center,
1200 Main St. W., Hamilton, Ontario,
CANADA, L8N 3Z5.
1-905-521-2100 (75226),
FAX = 1-905-521-2656,
Email = tarnopol@mcmaster.ca

Abstract

In the current investigation, we sought to determine whether creatine monohydrate (CrM) supplementation would enhance the increases in strength and fat-free mass developing during resistance exercise training in older adults. Twenty-eight healthy men and women over the age of 65 years participated in a whole-body resistance exercise program 3 days per week for 14 weeks. The study participants were randomly allocated, in a double-blind fashion, to receive either CrM (5 g/d + 2 g dextrose; n=14) or placebo (7 g dextrose; n=14). The primary outcome measurements included: total body mass, fat-free mass, 1- repetition maximum strength for each body part, isometric knee extension, hand- grip, and dorsi- flexion strength, chair stand performance, 30m walk test, 14- stair climb performance, muscle fiber type and area, and intramuscular total creatine. Fourteen weeks of resistance exercise training resulted in significant increases in all measurements of strength and functional tasks and muscle fiber area for both groups (P<0.05). CrM supplementation resulted in significantly greater increases in fat-free mass and total body mass, as compared to placebo (P<0.05). The CrM group also showed a greater increase in isometric knee extension strength in men and women, as compared to placebo (P<0.05), and also greater gains in isometric dorsi- flexion strength (P<0.05), in men only. There was a significant increase in intramuscular total creatine in the CrM group (P<0.05). Finally, there were no significant side-effects of treatment or exercise training. This study confirms that supervised heavy resistance exercise training can safely increase muscle strength and functional capacity in older adults. The addition of CrM supplementation to the exercise stimulus enhanced the increase in total and fat-free mass, and gains in several indices of isometric muscle strength.

Key Words: sarcopenia, creatine monohydrate, exercise, strength training

Introduction

Aging is associated with a reduction in total muscle mass and an increase in intramuscular fat and connective tissue. These changes are correlated with reduced strength, type II fiber area (1, 2) and number (3), motor unit number (4), and circulating anabolic hormones (5, 6, 7). Aging also results in a progressive decline in functional capacity leading to impaired mobility, increased risk of falls, a loss of independence, disability, and increased consumption of health care resources (8).

Countermeasures designed to maintain or enhance muscle mass and strength in aging may have important functional implications for older adults. The most effective non-pharmacological intervention identified is resistance exercise-training, which has been consistently shown to partially reverse age-associated decrements in muscle strength and mass in older males and females (9, 10, 11, 12, 13). Importantly, improvements in functional capacity and independence have been documented following resistance training, even in very old men and women (14).

Creatine monohydrate (CrM) supplementation has been shown to accentuate gains in fat free mass and strength in response to resistance training in young men and women (15, 16, 17). Intra-muscular creatine concentrations are ~25% lower in older (18) and middle-aged adults (19) than in younger individuals. People with low intramuscular total creatine concentrations show an enhanced ability to increase intracellular creatine content following CrM supplementation (20). For example increases in PCr were greater in middle aged than young individuals (19). Consequently, older adults may benefit more from a combination of resistance exercise and CrM supplementation than that in young men (15, 17) and women (16). Given the relative safety of CrM supplementation (21), it may be an efficacious, safe, and less expensive alternative to pharmacological interventions in the treatment of age-related sarcopenia.

A few studies have examined the effects of CrM upon muscle function in older adults (19, 22, 23, 24, 25, 26). Two have examined the potential for CrM to enhance the gains in strength and fat-free mass following a resistance training program (22, 26) and reported conflicting results. One study found that CrM supplementation did not enhance the resistance training induced gains in strength (22). In contrast, a more recent study found that CrM supplementation and resistance exercise training resulted in significantly greater increases in lean body mass and strength in elderly men as compared to resistance training alone (26). A limitation to the interpretation of both aforementioned studies was the lack of any direct measurements of muscle fiber size or creatine content or any functional outcome measurements. A final issue of importance was that in the positive study, only males were studied (26), whereas in the negative study, both males and females were evaluated (25). Gender is an important factor to consider, for we have found that during acute CrM supplementation, males show a greater increase in fat-free mass (27) and only males showed a reduction in amino acid oxidation and protein breakdown following CrM supplementation (28).

We hypothesized that CrM supplementation would enhance the resistance exercise mediated increases in strength, functional capacity, muscle fiber area, and body composition in elderly men and women. In addition, we hypothesized that both the exercise training and the CrM supplementation would not result in significant side effects.

Methodology

Subjects.

Fifteen men (67.8 ± 4.0 y) and 15 women (69.3 ± 6.3 y) volunteered to participate in a 14-wk resistance training program. Each subject underwent a thorough screening including: a

telephone interview, a medical evaluation, and a 12-lead electrocardiogram before and after progressive cycle ergometry to 6 mets on a mechanically braked cycle ergometer (Monarch, Sweden). Exclusion criteria included: evidence of coronary heart disease; congestive heart disease; uncontrolled hypertension; chronic obstructive pulmonary disease; diabetes mellitus; renal failure; major orthopedic disability; and smoking. All the women were postmenopausal and were not taking hormone replacement therapy. The study was approved by the McMaster University Medical Ethics Committee.

Of the original 30 volunteers, 15 males and 13 females completed all aspects of the study. Two females in the creatine group dropped out during the training for personal reasons unrelated to the training or supplementation.

Nutritional Supplementation.

Prior to training, subjects were randomly assigned in a double-blind manner to either a creatine monohydrate (CrM) (Neotone, Avicena, Cambridge, MA; 5 g CrM + 2 g of dextrose per day x 14 weeks) or placebo (PL) (7 g dextrose per day x 14 weeks) group. The CrM group consisted of 8 men and 6 women, while the PL group consisted of 7 men and 7 women (Table 1). The flavor and appearance of the supplements were indistinguishable by the subjects and the investigators. Subjects were instructed to consume their supplement dissolved in juice and to return their empty sachets on a weekly basis to ensure compliance. A 3-day dietary record was completed prior to and after training (including the minimal contribution from the supplements). The diets were analyzed using a commercially available program (Nutritionist V, First Data Bank, San Bruno, CA), and the subjects maintained similar dietary patterns during the study.

Strength Training.

Training was conducted three times weekly on nonconsecutive days for 14 weeks. Each training session was preceded by a 5-minute warm-up and followed by stretching of the muscle groups involved in the resistance exercises. Twelve exercises were used to train the major muscle groups of the upper and lower body in a circuit set system using weight training machines (Universal Gym Equipment Inc., Cedar Rapids, Iowa). Subjects performed 10 repetitions of each arm exercise and 12 repetitions of the remaining exercises. Training progressed from one set of each exercise at 50% of the initial 1 repetition maximum (1 RM) strength to three sets at 80% of 1 RM over the training period. The 1 RM was re-evaluated every 2 weeks, and the training loads were adjusted accordingly.

Testing.

All testing procedures were conducted before and after 14 weeks of resistance training, with post testing at 48 h following the last exercise bout.

i. Dynamic strength testing

Before initial strength testing, two low-intensity training sessions were completed to habituate the subjects to equipment and proper techniques. Prior to and after training, the 1 RM was used to assess strength in four different exercises (upright chest press, leg press, arm flexion, and knee extension). The preliminary 1 RM values were used to calculate the initial training load of 50% of 1 RM. In addition, at the end of the training program, each subject performed as many repetitions as possible with the pre-training 1 RM to provide a measure of endurance.

ii. Isometric Strength

Handgrip, ankle dorsi-flexion, and knee extensor strength were measured using custom made isometric devices as previously described (29, 30). For each measurement, subjects performed three maximal 5-second voluntary contractions with 1-minute rest between each of

three attempts. The highest peak torque value of each of the attempts was recorded as the maximal isometric strength value.

iii. Functional testing

Three functional ability tests were performed before and after training. The 30-second chair stand test required subjects to rise up and sit down for 30 s with arms folded in front of their chest as quickly as possible on a firm, armless chair placed against a wall (31). The timed stair climb required subjects to walk as fast as possible up 14 stairs without the use of railings. The timed walk required subjects to walk a distance of 30 meters as fast as possible without the use of external aids. Participants completed each of the functional tests as part of an initial familiarization trial during the recruitment phase. The functional tasks were all measured by the same evaluator and were timed to the nearest 0.1 s using an electronic stopwatch.

iv. Body composition assessment

Body mass and height were measured to the nearest 0.1 kg and 0.5 cm, respectively, using a calibrated electronic scale (Healthometer Pro Series Electronic Scale, Bridgeview, IL). A total body dual-energy X-ray absorptiometry (DEXA) scan (Hologic QDR® 4500A; Waltham, MA) was used to determine body fat percentage (%BF), fat mass (FM), fat/bone free mass (FFM) and bone mineral content (BMC), using the Hologic software program (V.8.26a).

v. Muscle, Blood, and Urine Collection

Muscle biopsies (~100 mg) were obtained from the *vastus lateralis* muscle of the dominant leg under local anesthesia (1% lidocaine) using a modified Bergström biopsy needle. One section was mounted in embedding medium (OCT, Tissue-Tek, Torrance, CA), cut and stained with myosin ATPase at pH 4.3 and 10.1 for fiber type discrimination, as previously described (32). An average of 425 ± 102 (range 252-683) fibers were counted per biopsy for determination of muscle fiber type and mean fiber area.

A second piece of muscle (~10-30 mg) was frozen in liquid nitrogen and stored at -80°C for subsequent determination of creatine (Cr), phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations as previously described (33). Intra-assay CV for ATP, PCr, and Cr were 7.3%, 8.5%, and 8.4% respectively.

Blood was drawn from an antecubital vein into 10-ml non-treated tubes and allowed to clot (serum), and into 5-ml ethylenediaminetetraacetic acid (EDTA)-treated tubes (plasma). After centrifugation, serum and plasma were stored at -70°C for subsequent analysis of total testosterone (TT), insulin-like growth factor-1 (IGF-1), dehydroepiandrosterone sulfate (DHEAS), and osteocalcin (OC), using radio-immuno-assays (Coat-A Count®, Diagnostics Products, LA, CA; osteocalcin, Biomedical Technologies, Stoughton, MA, IGF-1, Alpco Diagnostics, Windham, NH). Creatine kinase activity (CK), gamma-glutamyl transferase activity (γ GT) and creatinine (Crn) were measured in serum (Kodak, Ektachem, Rochester, NY). Thereafter, a urine sample was collected for subsequent analysis of Crn and creatine (Cr) using a standard picric acid method.

Statistical Analysis.

Values are reported as mean \pm SD. All statistics were performed using a commercially available software program (V5.0, Statistica, Statsoft, Tulsa, OK). All variables were analyzed using a three-way, repeated measures analysis of variance: a 2 (condition: Cr vs. Pl) \times 2 (gender: male vs. female) \times 2 (time: pre- vs. post- training) design, with repeated measures on the last factor. A P level of < 0.05 was used to determine significance, and significant differences were further analyzed using Tukey's post hoc test.

Results

Subject characteristics and body composition.

The treatment groups were comparable in baseline age, height, weight, %BF and FFM. Men were taller, had greater FFM, TBM and lower %BF, and had higher daily energy (kcal/day) intake as compared to women ($P < 0.05$), with no between-group differences in energy intake or in the proportions of protein, fat, and carbohydrate. Nutritional intake was unchanged during the training period (Table 1 and 2).

There was a greater increase in TBM (1.2 ± 1.7 kg) and FFM (1.7 ± 1.2 kg) for CrM supplementation, as compared to PL (TBM: -0.2 ± 1.3 kg; FFM: 0.4 ± 0.5 kg) following exercise training (Table 2, Figure 1; $P < 0.05$). The %BF and fat mass did not change after the training for either group.

Muscle high-energy phosphates.

At baseline, muscle free Cr, PCr, and TCr were not different between groups, nor were there any gender differences. CrM supplementation increased muscle TCr by 27.0% (men: pre: 116.8 ± 14.5 mmol•kg⁻¹ vs. post: 159.3 ± 23.9 mmol•kg⁻¹; women: pre: 129.7 ± 25.4 mmol•kg⁻¹ vs. post: 151.7 ± 18.7 mmol•kg⁻¹) with no increases in the PL group (group x time interaction, $P < 0.01$). In addition, the increase in TCr was greater for men as compared to women (group x gender x time interaction, $P < 0.05$). CrM supplementation also increased muscle PCr in the men only ($P < 0.05$), and had no effect on free Cr or ATP concentrations (Table 3).

Isometric Strength.

At baseline, there were no between-group differences for any of the isometric strength measures. The men were stronger than the women in all three exercises ($P < 0.001$). The increase in knee extensor strength was greater for the CrM group (46.2 ± 22.5 %) than the placebo group (22.5 ± 14.4 %) for both genders (Figure 2; group x time interaction, $P < 0.05$). There was an increase in dorsiflexion in the CrM group only for the men (17.8 ± 11.6 % vs placebo 2.2 ± 5.6 %)(group x time x gender interaction, $P < 0.05$). There was no effect of training or supplementation on handgrip strength (Table 4).

Dynamic Strength.

Following training, 1 RM increased in all four exercises, with no differential increases between treatments ($P < 0.001$; Table 4). The men in the CrM group had a significantly higher arm flexion 1RM as compared to the men in the PL group ($P < 0.05$). There were no other between-group differences in baseline 1 RM for the remaining strength measures. The men had greater maximum voluntary muscle strength (1 RM) than the women on all four exercises tested ($P < 0.001$; Table 4). The male subjects improved their absolute 1 RM more than the females in both the arm flexion and seated chest press (gender x time, $P < 0.05$). The absolute endurance increased after training, such that subjects were able to lift their pre-training 1 RM an average of 31, 13, 13, and 12 times, in the leg press, knee extension, arm flexion, and chest press, respectively ($P < 0.001$).

Muscle histology.

Fiber type distribution was not altered by training for any group. Type I, type IIa and type IIx mean fiber areas were greater in men than women ($P < 0.05$). There was an increase in the mean fiber area for type I ($P < 0.05$) and type IIx ($P < 0.001$) fibers but not type IIa fibers following training. The mean fiber area increased more in the type IIx fibers than type I fibers with training; consequently, there was an increase in the type IIx:type I area ratio ($P < 0.05$). In addition, the men had a greater percentage area of type IIx fibers ($P < 0.05$) and smaller percentage area of type I fibers ($P < 0.05$) than the women (Table 5).

Functional Measures.

The number of chair stands that could be performed in 30 seconds after training increased by 3.5 repetitions for CrM (25 %) and 2.9 repetitions for PL (21 %) ($P < 0.001$ main effect for training). Training decreased the 30 m walk time by 1.7 s for the CrM group (10 %) and 1.5 s for the PL group (9 %) ($P < 0.001$ main effect for training). The time to climb 14 stairs was improved by an average of 1.0 s for the CrM group (15 %) and 1.5 s for PL (22 %) ($P < 0.001$ main effect for training). There were no significant main effects or interactions involving the treatment interventions in any of the functional measurements. Males were overall faster than females at the stair climb ($P < 0.05$).

Blood and Urine Analyses.

Plasma Crn concentration increased for the CrM group following training (men: 13 % increase; women: 22 % increase) ($P < 0.05$). Plasma CK activity was also higher in the CrM group after training as compared to the PL group ($P < 0.05$). The urine creatine:creatinine ratio was greater after training in the CrM supplemented group ($P < 0.05$). Neither CrM supplementation nor resistance training altered any of the measured hormone concentrations (Table 6).

Side Effects.

Subjects tolerated the supplementation protocol well with only 2 reports of gastrointestinal distress (one in each treatment group). In addition, there were no reports of muscular cramping or any other subjective symptoms during the study.

Discussion

This study demonstrated that 14 weeks of resistance training increased muscle strength, muscle fiber area, and performance on functional tasks in healthy, community dwelling older adults. CrM supplementation increased intramuscular total creatine, and enhanced the exercise induced gains in total body mass, fat-free mass, isometric knee extension in both men and women and isometric dorsi-flexion strength in men. Together, these results confirm that: (1) resistance training is an effective countermeasure to sarcopenia and strength loss, and (2) CrM supplementation combined with strength-training increased TBM and FFM and improved some of the isometric strength measures as compared to placebo. The ability of older adults to reverse these losses in strength and function through strength exercise training, and the ability of CrM supplementation to enhance these improvements may ultimately be reflected by an improved quality of life in older adults. The current results suggest that further investigation with a longer intervention duration and a larger sample size is justified to determine whether or not there are beneficial effects of CrM supplementation in strength trained older adults and whether these are maintained for a longer period of time.

In the present study there were increases in strength for all exercises which ranged from 26 to 60 %. These observations are consistent with data reported in the literature where older adults trained under similar conditions as in the present study (10, 34, 35, 36). Furthermore, no injuries were reported during training, confirming that high intensity resistance training is a safe and effective method for strength development in older adults.

The measurements of functional capacity used in the current study all showed improvements following the strength training program. The 11% improvement in walking speed was consistent with the findings reported by other investigators (35, 37). We also reported a significant improvement in the 30-second chair stand, a test that is thought to reflect lower body strength. Other studies evaluating the effect of resistance training on sit to stand performance

have reported equivocal results (12, 37, 38), likely attributable to methodological differences and the inherent variability in functional assessments (as compared to objective strength measurements). We also found reductions in the time to climb 14 stairs (24%), which confirmed a previous report showing an improvement in stair climbing performance in community dwelling older adults following 10 months of resistance training (39). Together, these results suggest that resistance exercise training improves muscular strength, which ultimately can enhance functional tasks that have relevance to activities of daily living. For example, lower body muscle weakness results in a deterioration of walking speed, and the ability to stair climb and rise from a chair (40, 41), and nursing home residents with a history of falls demonstrated significantly lower dynamic strength measurements of the knees and ankles when compared to non-fallers (42). Ultimately, the practicality of these observations comes from studies that demonstrated a correlation between declining strength and an increased prevalence of falls and fractures (43,44).

CrM supplementation during 4 months of resistance training enhanced the improvements in several measures of isometric strength. CrM supplementation further augmented knee extensor strength by 24% in both men and women and dorsiflexion strength by 18 % in the men following resistance training but did not affect maximal isometric handgrip strength. The disparity between our results for 1-RM strength and isometric strength may be explained by the increased sensitivity associated with our custom-built isometric devices. These devices allow us to isolate a muscle group and control for all extraneous movements, reducing the variability in strength measurement. The test-retest reliability for our custom-made strength devices is 0.5 – 2.0 %, which is less than that for conventional weight machines (5.0 %). Although these improvements were not realized in functional tasks, it is likely that these subtle changes would not have been detected due to the inherent variability using these type of tests and that either a greater sample size or a longer treatment period would have been required. Our results are consistent with those from a recent study where elderly males showed a greater increase in strength, muscle power and endurance following a strength training program while taking CrM as compared to a placebo (26). Furthermore, previous work has also demonstrated that CrM supplementation during resistance training resulted in enhanced muscle strength gains in healthy young men (15, 17) and women (16). In one other study in the elderly, there were no additional benefits of CrM supplementation on muscle strength following resistance exercise training (22). As in the present study, 1-RM strength was also not differentially affected by CrM, however, only isometric endurance was determined, not maximal isometric strength (22). Thus, in the latter study (22), it is possible that 8 weeks was not long enough to detect subtle improvements in strength, and/or maximal peak isometric strength (such as was determined in the current study) rather than isometric endurance may have been more appropriate to detect improvements in peak strength. Given that leg strength has been correlated to falls and fractures (42,43,44), any increase in knee extension strength (climbing stairs, getting up from a chair, etc.) could ultimately result in a reduction in morbidity and an improvement in functional capacity. While the potent effects of exercise on leg strength have been documented previously and in the current study, future studies will be required to determine whether the effect of CrM supplementation on isometric knee extension strength ultimately translates in to functional gains.

The effect of resistance exercise training on FFM in older adults has produced conflicting results. While some studies have reported significant increases in FFM following resistance training (12, 13, 45), others have reported no increase in FFM (9, 22). These equivocal results may be explained by exercise intensity and duration, as well as the precision of measurement. Studies reporting no change in FFM following resistance training have used skinfolds (22) and

hydrodensitometry (9) to estimate muscle mass. It is possible that these techniques are not sensitive enough to detect subtle changes in body composition following resistance training (46). In contrast, studies using more sensitive measures to determine body composition such as DEXA (12, 13, 47) and computerized tomography (11, 34, 43) have reported increases in FFM following resistance exercise training. In the present study, we did not detect any changes in FFM following resistance exercise training in the placebo group; however, the CrM group did show an increase in TBM and FFM, as determined by DEXA.

A limited number of studies have investigated the potential benefits of CrM supplementation on body composition in older adults (22, 24, 25, 26). Most of these studies have been carried out in men and have generated equivocal findings. Rawson and colleagues reported that 30 days of CrM supplementation, without exercise training, did not affect body composition as determined by hydrostatic weighing (25). In addition, 8 weeks of CrM supplementation, with or without resistance exercise training, did not alter anthropometrically determined body composition (22). Our findings are in agreement with a recent study which reported that strength training in conjunction with CrM supplementation induced greater increases in TBM and FFM as compared to strength training without supplementation (26). The increase in TBM and FFM following chronic CrM supplementation combined with resistance training is comparable with findings of other studies in young men (15, 17) and women (16). The underlying mechanism(s) explaining the increase in TBM and FFM remain to be elucidated. Several potential mechanism(s) have been identified including increased water retention (48, 49), a creatine-stimulated increase in myofibrillar mRNA and protein content (50), and/or a reduction in whole body amino acid oxidation and protein breakdown (28). Given that the increases in muscle fiber area were not differentially affected by CrM supplementation, it could be concluded that the increase in fat-free mass was not due to increases in myofibrillar protein content *per se*, however, it is important to note that a very small change in muscle fiber area in every muscle in the body could easily account for a one or two kilogram increase in fat-free mass and the change in fiber area may be below the limit of detection using routine histochemistry (see below).

Following resistance exercise training, significant increases in type I (11, 34, 36) and type II (10, 11, 34, 36) muscle fiber area have been consistently reported. More recently, type IIa and IIx muscle fiber areas have been reported to increase following resistance exercise in the elderly (51, 52). Similarly, we demonstrated a 13% and 31% increase in type I and type IIx muscle fiber area following resistance training in the current study, however we did not observe any effect of CrM supplementation on enhancing muscle fiber area increases. An earlier, longer-term study (1 y) found that creatine supplementation (1.5 g/d) resulted in a significant increase in type II muscle fiber diameter in patients with gyrate atrophy (53). Recently, CrM supplementation was shown to potentiate the increase in muscle fiber area for all three fiber-types following resistance training (17). Given the variability in the determination of muscle fiber area, further experiments will be needed to evaluate the potential link between CrM supplementation and muscle fiber area in the elderly.

Healthy older men and women (58-75 years) have significantly lower muscle PCr and TCr concentrations as compared to young healthy men and women (18, 19). The magnitude of the increase in muscle TCr and PCr concentration appears to be inversely proportional to the basal concentration. One study examined basal concentrations of muscle PCr and PCr re-synthesis rates in middle aged (58 ± 4.4 years) males and females before and after creatine supplementation using ^{31}P -MRS (19). This study (19) found that resting muscle PCr content was lower in the middle-aged subjects as compared to the younger subjects, and the middle-aged

subjects experienced a greater increase (30 %) in muscle PCr stores as compared to younger subjects (15 %) following CrM supplementation (19). Together this data suggests that older adults may be at a disadvantage in activities requiring rapid rates of energy turnover. The current study is the first to directly examine intramuscular TCr concentration following CrM supplementation using the muscle biopsy technique. As hypothesized, CrM supplementation increased muscle TCr in males and females by an average of 26 % which is in agreement with previous findings in young men (20, 53, 54). Traditionally, long term CrM supplementation at a lower dosage is often preceded by short term high dose loading (i.e., 20 g/d for 4-5 days). However, a novel finding of the present study was that the older subjects were able to increase and maintain TCr stores with the ingestion of only 5 g/d for 4 months with no initial loading period.

Most studies have not reported any adverse side effects resulting from short- (21, 55, 56) or longer- (57, 58) term CrM supplementation. We found that the CrM supplement was well tolerated by the subjects. Plasma Crn concentration and CK activity increased to a greater extent for the CrM supplemented group following training yet the levels remained within the normal limits for an older population. An increase in plasma creatinine would be expected based upon the fact that the elevated total muscle creatine (non-enzymatic degradation to creatinine) and the increased FFM, would both increase the rate of appearance of creatinine into the plasma. In addition, urine creatine, but not urine Crn, increased following CrM supplementation. We did not complete a full 24-hour urine collection in the current study, however previous findings have reported no changes in Crn clearance in response to CrM supplementation (27, 55, 56). Finally, the liver enzyme γ GT also did not change in response to CrM supplementation, which is in agreement with studies also showing no change in indices of hepatic dysfunction following CrM supplementation (58).

In summary, 14 weeks of resistance training resulted in improvements in muscle strength and functional task performance. In addition, there was a greater increase in FFM, TBM and isometric knee extension strength in those who supplemented with CrM. Although the mechanisms of these improvements remain to be elucidated, our results represent the first line of evidence suggesting that CrM supplementation may be beneficial to older adults who perform resistance exercise training.

Acknowledgements. This study was partially funded by Avicena Corporation (50 %) and partially funded by the Hamilton Health Sciences Corporation, Department of Rehabilitation (50 %). None of the authors have any financial or consulting links with Avicena Corporation. The creatine monohydrate was also provided by Avicena Corporation (Neotine[®]). Gianni Parise was a recipient of an NSERC, Canada, graduate fellowship at the time of data collection. The testing and exercise training equipment was purchased with support from the Canadian Foundation for Innovation Grant.

References.

1. Grimby G, Danneskiold-Samsøe B, Hvid K, Saltin B. Morphology and enzymatic capacity in arm and leg muscles in 78-81 year old men and women. *Acta Physiol Scand.* 1982;115:125-134.
2. Larsson L, Grimby G, Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. *J Appl Physiol.* 1979;46:R451-456.
3. Lexell J, Taylor CC, Sjöström M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year old men. *J Neurol Sci.* 1988;84:275-294.
4. Doherty TJ, Vandervoort AA, Taylor AW, Brown WF. Effects of motor unit losses on strength in older men and women. *J Appl Physiol.* 1993;74:868-874.
5. Birkenhager-Gillesse EG, Derksen J, Lagaay AM. Dehydroepiandrosterone sulfate (DHEAS) in the oldest old, aged 85 and over. *Ann N Y Acad Sci.* 1994;719:543-552.
6. Morley JE, Kaiser FE, Perry HM, et al. Longitudinal changes in testosterone, luteinizing hormone, and follicle-stimulating hormone in healthy older men. *Metabolism.* 1997;46:410-413.
7. Zadik Z, Chalew SA, McCarter RJ, Meistas M, Kowarski AA. The influence of age on the 24-hour integrated concentration of the growth hormone in normal individuals. *J Clin Endocrinol Metab.* 1985;60:513-516.
8. Schneider EL, Guralnik JM. The aging of America: impact on health care costs. *JAMA.* 1990;263:2335-2340.
9. Ades PA, Ballor DL, Ashikaga T, Utton JL, Nair KS. Weight training improves walking endurance in healthy elderly persons. *Ann Intern Med.* 1996;124:568-572.
10. Charette SL, McEvoy L, Pyka G, et al. Muscle hypertrophy response to resistance training in older women. *J Appl Physiol.* 1991;70:1912-1916.
11. Frontera WR, Meredith CN, O'Reilly KP, Knuttgen HG, Evans WJ. Strength conditioning in older men: skeletal muscle hypertrophy and improved function. *J Appl Physiol.* 1988;64:1038-1044.
12. Taaffe DR, Duret C, Wheeler S, Marcus R. Once-weekly resistance exercise improves muscle strength and neuromuscular performance in older adults. *J Am Geriatr Soc.* 1999;47:1208-1214.
13. Treuth MS, Ryan AS, Pratley RE, et al. Effects of strength training on total and regional body composition in older men. *J Appl Physiol.* 1994;77:614-620.
14. Fiatarone MA, O'Neill EF, Ryan ND, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med.* 1994;330:1769-1775.
15. Kreider RB, Ferreira M, Wilson M, et al. Effects of creatine supplementation on body composition, strength, and sprint performance. *Med Sci Sports Exerc.* 1998;30:73-82.
16. Vandenberghe K, Goris M, Van Hecke P, Van Leemputte M, Vangerven L, Hespel P. Long-term creatine intake is beneficial to muscle performance during resistance training. *J Appl Physiol.* 1997;83:2055-2063.
17. Volek JS, Duncan ND, Mazzetti SA, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med Sci Sports Exerc.* 1999;31:1147-1156.
18. Campbell WW, Barton ML, Cyr-Campbell D, et al. Effects of an omnivorous diet compared with a lactoovo-vegetarian diet on resistance-training-induced changes in body composition and skeletal muscle in older men. *Am J Clin Nutr.* 1999;70:1032-1039.
19. Smith SA, Montain SJ, Matott RP, Zientara GP, Jolesz FA, Fielding RA. Creatine supplementation and age influence muscle metabolism during exercise. *J Appl Physiol.* 1998;85:1349-1356.
20. Harris RC, Söderlund K, Hultman E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci.* 1992;83: 367-374.
21. Juhn MS, Tarnopolsky M. Potential side effects of oral creatine supplementation: a critical review. *Clin J Sport Med.* 1998;8:298-304.
22. Bermon S, Venembre P, Sachet C, Valour S, Dolisi C. Effects of creatine monohydrate ingestion in sedentary and weight-trained older adults. *Acta Physiol Scand.* 1998;164:147-155.
23. Jakobi JM, Rice CL, Curtin SV, Marsh GD. Neuromuscular properties and fatigue in older men following acute creatine supplementation. *Eur J Appl Physiol.* 2001;84:321-328.
24. Rawson ES, Clarkson PM. Acute creatine supplementation in older men. *Int J Sports Med.* 1999;20:1-5.
25. Rawson ES, Wehnert ML, Clarkson PM. Effects of 30 days of creatine ingestion in older men. *Eur J Appl Physiol.* 1999;80:139-144.
26. Chrusch MJ, Chilibeck PD, Chad KE, Davison KS, Burke DG. Creatine supplementation combined with resistance training in older men. *Med Sci Sports Exerc.* 2001;33:2111-2117.
27. Mihic S, MacDonald JR, McKenzie S, Tarnopolsky MA. Acute creatine loading

- increases fat-free mass, but does not affect blood pressure, plasma creatinine, or CK activity in men and women. *Med Sci Sports Exerc.* 2000;32:291-296.
28. Parise G, Mihic S, MacLennan D, Yarasheski KE, Tarnopolsky MA. Effects of acute creatine monohydrate supplementation on leucine kinetics and mixed-muscle protein synthesis. *J Appl Physiol.* 2001;91:1041-1047.
 29. Tarnopolsky MA, Roy BD, MacDonald JR. A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies. *Muscle Nerve.* 1997;20:1502-1509.
 30. Tarnopolsky MA, Martin J. Creatine monohydrate increases strength in patients with neuromuscular disease. *Neurology.* 1999;52:854-857.
 31. Rikli RE, Jones CJ. Development and validation of a functional fitness test for community-residing older adults. *J Aging Phys Act.* 1999;7:129-161.
 32. Carter SL, Rennie CD, Hamilton SJ, Tarnopolsky MA. Changes in skeletal muscle in males and females following endurance training. *Can J Physiol Pharmacol.* 2001;79:1-7.
 33. Tarnopolsky MA, Parise G. Direct measurement of high-energy phosphate compounds in patients with neuromuscular disease. *Muscle Nerve.* 1999;22:1228-1233.
 34. Brown AB, McCartney N, Sale DG. Positive adaptations to weight-lifting training in the elderly. *J Appl Physiol.* 1990;69:1725-1733.
 35. Hunter GR, Treuth MS, Weinsier RL, et al. The effects of strength conditioning on older women's ability to perform daily tasks. *J Am Geriatr Soc.* 1995;43:756-760.
 36. Pyka G, Lindenberger E, Charette S, Marcus R. Muscle strength and fiber adaptations to a year-long resistance training program in elderly men and women. *J Gerontol Med Sci.* 1994;49:M22-27.
 37. Schlicht J, Camaione DN, Owen SV. Effect of intense training on standing balance, walking speed, and sit-to-stand performance in older adults. *J Gerontol Med Sci.* 2001;56A:M281-286.
 38. Skelton DA, Young A, Greig CA, Malbut KE. Effects of resistance training on strength, power, and selected functional abilities of women aged 75 and older. *J Am Geriatr Soc.* 1995;43:1081-1087.
 39. Rooks DS, Kiel DP, Parsons C, Hayes WC. Self-paced resistance training and walking exercise in community-dwelling older adults: effects on neuromotor performance. *J Gerontol Med Sci.* 1997;52A:M161-168.
 40. Bassey EJ, Fiatarone MA, O'Neill EF, Kelly M, Evans WJ, Lipsitz LA. Leg extensor power and functional performance in very old men and women. *Clin Sci.* 1992;82:321-327.
 41. Fiatarone MA, Marks EC, Ryan ND, Meredith CN, Lipsitz LA, Evans WJ. High-intensity strength training in nonagenarians. *JAMA.* 1990;263:3029-3034.
 42. Whipple RH, Wolfson LI, Amerman PM. The relationship of knee and ankle weakness to falls in nursing home residents: an isokinetic study. *J Am Geriatr Soc.* 1987;35:13-20.
 43. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med.* 1995;332:556-561.
 44. Province MA, Hadley EC, Hornbrook MC, et al. The effects of exercise on falls in elderly patients. *JAMA.* 1995;273:1341-1347.
 45. Hunter GR, Wetzstein CJ, Fields DA, Brown A, Bamman MM. Resistance training increases total energy expenditure and free-living physical activity in older adults. *J Appl Physiol.* 2000;89:977-984.
 46. Lohman TG. Skinfolts and body density and their relation to body fatness: a review. *Hum Biol.* 1981;53:181-225.
 47. Nichols JF, Omizo DK, Peterson KK, Nelson KP. Efficacy of heavy-resistance training for active women over sixty: muscular strength, body composition, and program adherence. *J Am Geriatr Soc.* 1993;41:205-210.
 48. Hultman E, Söderlund K, Timmons JA, Cederblad G, Greenhaff PL. Muscle creatine loading in men. *J Appl Physiol.* 1996;81:232-237.
 49. Ziegenfuss TN, Lowery LM, Lemon PWR. Acute fluid changes in men during three days of creatine supplementation. *J Exerc Physiol.* 1998;1:1-14.
 50. Willoughby DS, Rosene J. Effects of oral creatine and resistance training on myosin heavy chain expression. *Med Sci Sports Exerc.* 2001;33:1674-81.
 51. Häkkinen K, Newton RU, Gordon SE, et al. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. *J Gerontol Biol Sci.* 1998;53A:B415-423.
 52. Hikida RS, Staron RS, Hagerman FC, et al. Effects of high-intensity resistance training on untrained older men: muscle fiber characteristics and nucleo-cytoplasmic relationships. *J Gerontol Biol Sci.* 2000;55A:B347-354.
 53. Sipilä I, Rapola J, Simell O, Vannas A. Supplementary creatine as a treatment for gyrate atrophy of the choroid and retina. *N Engl J Med.* 1981;304:867-870.

53. Balsom PD, Söderlund K, Sjödén B, Ekblom B. Skeletal muscle metabolism during short duration high-intensity exercise: influence of creatine supplementation. *Acta Physiol Scand.* 1995;154:303-310.
54. Greenhaff PL, Bodin K, Söderlund K, Hultman E. Effect of oral creatine supplementation on skeletal muscle phosphocreatine resynthesis. *Am J Physiol.* 1994;266:E725-730.
55. Poortmans JR, Auquier H, Renaut V, Durussel A, Saugy M, Brisson GR. Effect of short-term creatine supplementation on renal responses in men. *Eur J Appl Physiol.* 1997;76:566-567.
56. Robinson TM, Sewell DA, Casey A, Steenge G, Greenhaff PL. Dietary creatine supplementation does not affect some haematological indices, or indices of muscle damage and hepatic and renal function. *Br J Sports Med.* 2000;34:284-288.
57. Poortmans JR, Francaux M. Long-term oral creatine supplementation does not impair renal function in healthy athletes. *Med Sci Sports Exerc.* 1999;31:1108-1110.
58. Kamber M, Koster M, Kreis R, Walker G, Boesch C, Hoppeler H. Creatine supplementation—Part I: performance, clinical chemistry, and muscle volume. *Med Sci Sports Exerc.* 1999;31:1763-1769.

Tables.**Table 1. Subject characteristics and dietary analysis before and after training.**

	Creatine				Placebo			
	Men		Women		Men		Women	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Age, y	68.7 ± 4.8	same	70.8 ± 6.1	same	68.3 ± 3.2	same	69.9 ± 5.6	same
Height, cm	172.0 ± 6.3*	same	159.3 ± 6.9	same	168.1 ± 4.9*	same	160.4 ± 7.7	same
Weight, kg	84.1 ± 14.0 [†]	85.5 ± 13.5* [†]	65.4 ± 16.2	66.5 ± 16.8 [†]	76.6 ± 9.8*	76.2 ± 9.7*	66.2 ± 14.0	66.2 ± 13.7
kcal/d	2249 ± 488*	2333 ± 640*	1821 ± 457	1659 ± 341	2603 ± 587*	2327 ± 343*	1788 ± 507	1725 ± 484
% PRO	16 ± 2	17 ± 3	16 ± 3	17 ± 2	16 ± 3	17 ± 3	16 ± 4	18 ± 4
% CHO	54 ± 6	53 ± 6	50 ± 7	53 ± 5	49 ± 12	46 ± 9	46 ± 12	47 ± 10
% FAT	29 ± 5	28 ± 7	33 ± 5	29 ± 2	30 ± 6	32 ± 5	30 ± 5	31 ± 6
PRO (g/kg/d)	1.1 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.2	1.4 ± 0.4	1.3 ± 0.3	1.1 ± 0.4	1.2 ± 0.4

Values are means ± SD. * men significantly higher than women (P<0.05); † main effect for creatine to show a greater increase in total mass as a result of training as compared to placebo (P < 0.05).

Table 2. Body composition before and after training.

	Creatine				Placebo			
	Men		Women		Men		Women	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
TBM, kg*	84.1 ± 14.0	85.5 ± 13.5†	65.4 ± 16.2	66.5 ± 16.8†	76.6 ± 9.8	76.2 ± 9.7	66.2 ± 14.0	66.2 ± 13.7
FFM, kg*	56.0 ± 7.1	57.4 ± 7.4†	33.7 ± 3.7	35.7 ± 4.1 †	52.0 ± 5.3	52.0 ± 5.8	37.2 ± 1.8	37.8 ± 2.0
FM, kg	22.0 ± 5.4	22.3 ± 5.8	21.4 ± 9.7	17.3 ± 12.1	12.2 ± 2.9	12.1 ± 3.0	24.2 ± 11.2	23.9 ± 11.8
BF, %*	27.0 ± 3.9	26.8 ± 4.7	36.2 ± 11.8	34.2 ± 9.9	18.3 ± 2.1	18.1 ± 2.0	36.4 ± 11.2	35.5 ± 12.6

Values are mean ± SD. Pre = before training; Post = after training; TBM = total body mass; FFM = fat/bone-free mass; BF = body fat. * men had high TBM and FFM and lower BF % versus women (P<0.05). † CrM increases were greater than placebo (P<0.05). Due to a technical problem with the DEXA; Men, creatine (n=5); Women, creatine (n=5); Men, placebo (n=2); Women, placebo (n=4) for FFM, FM, and % BF. Men, creatine (n=8); Women, creatine (n=6); Men, placebo (n=7); Women, placebo (n=7) for TBM.

Table 3. Muscle metabolites before and after training.

	Creatine				Placebo			
	Men (N=7)		Women (N=6)		Men (N=7)		Women (N=6)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PCr	67.4 ± 19.7	88.0 ± 20.5*	83.1 ± 15.2	91.1 ± 30.3	89.2 ± 25.6	70.7 ± 19.3	74.8 ± 13.8	85.2 ± 12.8
Cr	49.4 ± 16.0	71.3 ± 10.9	46.6 ± 13.8	60.6 ± 23.3	51.6 ± 22.9	54.7 ± 21.3	63.6 ± 15.9	62.4 ± 25.4
TCr	116.8 ± 14.5	159.3 ± 23.9 [†]	129.7 ± 25.4	151.7 ± 18.7 [†]	140.8 ± 20.6	125.5 ± 25.4	138.5 ± 14.0	147.5 ± 20.3
ATP	18.1 ± 1.9	19.9 ± 6.1	17.0 ± 3.4	19.2 ± 3.9	20.3 ± 2.7	18.1 ± 3.9	18.9 ± 2.9	20.9 ± 2.6

Values are mean ± SD. PCr = phosphocreatine; Cr = free creatine; TCr = total creatine; ATP = adenosine triphosphate * Men increased PCr on CrM supplements after training (P<0.0). † TCr increased for both men and women after training only in the CrM group (P<0.01). All values are in mmol·kg⁻¹ dm. .

Table 4. Isometric and 1 RM measurements before and after training.

	Creatine				Placebo			
	Men*		Women		Men*		Women	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Isometric strength								
Knee extension (Nm)	153 ± 28	217 ± 36 [‡]	94 ± 38	126 ± 30 [‡]	156 ± 32	180 ± 29 [†]	89 ± 17	113 ± 25 [†]
Dorsi-flexion (Nm)	54 ± 14	62 ± 15 [§]	37 ± 4	40 ± 6	52 ± 8	52 ± 10	34 ± 9	39 ± 10
Grip (kg)	438 ± 63	469 ± 98	259 ± 67	259 ± 76	397 ± 56	406 ± 47	265 ± 31	267 ± 29
1 RM strength (lbs)								
Seated chest press	116 ± 26	146 ± 33 [†]	48 ± 11	63 ± 21 [†]	97 ± 20	119 ± 15 [†]	46 ± 9	59 ± 11 [†]
Arm Flexion	81 ± 22 [†]	112 ± 18 [†]	25 ± 7	40 ± 11 [†]	60 ± 16	85 ± 16 [†]	26 ± 8	40 ± 6 [†]
Leg press	194 ± 47	244 ± 55 [†]	111 ± 28	153 ± 54 [†]	166 ± 39	231 ± 33 [†]	105 ± 26	152 ± 30 [†]
Knee extension	119 ± 18	162 ± 24 [†]	71 ± 10	113 ± 22 [†]	107 ± 31	151 ± 32 [†]	73 ± 16	115 ± 30 [†]

Values are mean ± SD. * men were stronger than women on all strength measures (P<0.001). † indicates a training induced increase in strength (P<0.01). ‡ indicates a greater increase in strength for the CrM supplemented groups (P<0.05). § indicates a group x gender x time interaction with the men in the creatine group showing a significant increase after training (P<0.05). the males increased strength by a larger amount as compared to the females (P<0.05).

Table 5. Muscle fiber characteristics before and after training.

	Creatine				Placebo			
	Men		Women		Men		Women	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Mean Fiber Area (mm²)								
Type I*	4856 ± 1331	5477 ± 1677 [†]	3914 ± 857	4106 ± 1383 [†]	4690 ± 1056	5827 ± 1388 [†]	4326 ± 973	4558 ± 1459 [†]
Type IIa*	5055 ± 1018	6105 ± 2230	3230 ± 1029	3037 ± 1030	4226 ± 615	5268 ± 1742	3138 ± 639	3237 ± 817
Type IIx*	3900 ± 1240	4998 ± 1796 [†]	1808 ± 555	2542 ± 1204 [†]	2899 ± 418	4076 ± 1611 [†]	2250 ± 698	2633 ± 814 [†]
% Area								
Type I*	40.5 ± 14.8	39.0 ± 16.3	58.3 ± 14.8	55.2 ± 12.4	41.6 ± 7.0	46.5 ± 12.7	53.8 ± 19.1	56.2 ± 17.5
Type IIa	39.0 ± 12.9	34.8 ± 4.4	30.5 ± 11.3	36.8 ± 12.2	40.5 ± 7.9	30.2 ± 4.8	30.6 ± 14.5	27.4 ± 9.7
Type IIx*	20.5 ± 18.8	26.2 ± 16.7	11.3 ± 10.0	9.4 ± 6.5	17.8 ± 11.2	23.2 ± 12.2	15.7 ± 11.3	16.4 ± 12.4
Fiber Distribution (%)								
Type I	40.6 ± 11.8	39.1 ± 13.7	51.5 ± 19.8	48.0 ± 11.2	37.1 ± 8.3	41.4 ± 7.9	45.5 ± 19.3	48.2 ± 17.2
Type IIa	37.3 ± 13.1	32.5 ± 2.9	30.0 ± 6.8	40.8 ± 13.6	39.5 ± 10.0	30.4 ± 7.5	32.4 ± 15.0	29.7 ± 9.7
Type IIx	22.0 ± 15.5	28.4 ± 14.7	18.5 ± 14.0	11.1 ± 7.9	23.4 ± 13.4	28.1 ± 11.7	22.2 ± 13.7	22.1 ± 14.2

Values are mean ± SD. * men showed higher absolute size for all fiber types as compared to women; however, the % of the total area represented by type I fibers was higher for women and for type IIx fibers was higher for men (P<0.05); † type I and IIx fibers increased after training for both groups (P<0.05).

Table 6. Blood analysis before and after training.

	Creatine				Placebo			
	Men		Women		Men		Women	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Blood analysis								
TT (nmol/L)*	15.4 ± 5.4	16.6 ± 4.9	0.7 ± 0.4	0.6 ± 0.4	19.3 ± 12.6	22.6 ± 6.3	0.3 ± 0.2	0.4 ± 0.4
DHEAS (µmol/L)*	3.7 ± 2.0	3.0 ± 1.8	2.3 ± 1.3	1.8 ± 1.3	2.8 ± 1.5	2.8 ± 1.6	1.7 ± 1.1	1.7 ± 1.0
IGF-1 (nmol/L)	10.5 ± 3.0	10.9 ± 3.3	10.2 ± 3.1	8.3 ± 4.0	9.0 ± 4.0	8.0 ± 1.3	7.3 ± 2.7	7.1 ± 1.3
OC (ng/mL)	16.0 ± 4.5	16.1 ± 2.7	17.1 ± 5.1	18.0 ± 5.0	14.1 ± 2.1	14.1 ± 1.8	16.3 ± 3.6	16.6 ± 3.6
CK activity (U/L)	53.3 ± 31.5	107.4 ± 76.4 [†]	83.3 ± 85.0	112.3 ± 98.2 [†]	83.7 ± 50.0	81.9 ± 43.8	67.6 ± 40.9	47.0 ± 22.1
Crn (µmol/L)	111.4 ± 24.4	126.2 ± 33.4 [†]	95.4 ± 16.6	116.3 ± 16.3 [†]	107.9 ± 30.3	95.7 ± 21.3	100.5 ± 19.4	89.3 ± 29.4
GGT (U/L)	31.4 ± 23.6	28.4 ± 17.7	17.7 ± 2.4	17.8 ± 3.5	24.7 ± 8.5	23.3 ± 6.9	28.0 ± 16.1	28.1 ± 15.4
Urine Analysis								
Cr (mg/mL)	0.8 ± 0.6	2.2 ± 2.1 [†]	0.6 ± 0.4	3.9 ± 4.2 [†]	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.3	0.4 ± 0.4
Crn (mg/mL)	0.9 ± 0.4	1.0 ± 0.4	0.7 ± 0.4	0.9 ± 0.6	0.7 ± 0.6	0.8 ± 0.4	0.6 ± 0.5	0.6 ± 0.4
Cr:Crn Ratio	0.89 ± 0.73	2.13 ± 1.97 [†]	0.88 ± 0.38	3.46 ± 2.25 [†]	0.82 ± 0.75	0.53 ± 0.37	0.63 ± 0.25	0.43 ± 0.44

Results are mean ± SD. TT = total testosterone; DHEAS = dehydroepiandrosterone sulfate; IGF-1 = Insulin-like growth factor-1; OC = osteocalcin; CK = creatine kinase; Crn = creatinine; γGT = gamma glutamyl transferase; Cr = creatine. * men showed higher values than women (TT, P < 0.000001; DHEAS, P < 0.05). † CrM group increased more than the placebo group (P < 0.05).