

Effect of Creatine and Weight Training on Muscle Creatine and Performance in Vegetarians

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ABSTRACT

BURKE, D. G., P. D. CHILIBECK, G. PARISE, D. G. CANDOW, D. MAHONEY, and M. TARNOPOLSKY. Effect of Creatine and Weight Training on Muscle Creatine and Performance in Vegetarians. *Med. Sci. Sports Exerc.*, Vol. 35, No. 11, pp. 1946–1955, 2003. **Purpose:** To compare the change in muscle creatine, fiber morphology, body composition, hydration status, and exercise performance between vegetarians and nonvegetarians with 8 wk of creatine supplementation and resistance training. **Methods:** Eighteen VG and 24 NV subjects (19–55 yr) were randomly assigned (double blind) to four groups: VG + creatine (VGCr, $N = 10$), VG + placebo (VGPl, $N = 8$), NV + creatine (NVCr, $N = 12$), and NV + placebo (NVPl, $N = 12$). Before and at the end of the study, muscle biopsies were taken from the vastus lateralis m, body composition was assessed by DXA, and strength was assessed using 1-RM bench press and leg press. Subjects participated in the same 8-wk resistance-training program. Creatine dosage was based on lean tissue mass ($0.25 \text{ g}\cdot\text{kg}^{-1} \text{ LTM}\cdot\text{d}^{-1} \times 7 \text{ d}$; $0.0625 \text{ g}\cdot\text{kg}^{-1} \text{ LTM}\cdot\text{d}^{-1} \times 49 \text{ d}$). **Results:** Biopsy samples indicated that total creatine (TCr = free Cr + PCr) was significantly lower in VG compared with NV at baseline (VG = $117 \text{ mmol}\cdot\text{kg}^{-1}$; NV = $130 \text{ mmol}\cdot\text{kg}^{-1}$; $P < 0.05$). For Cr subjects, there was a greater increase in PCr, TCr, bench-press strength, isokinetic work, Type II fiber area, and whole-body lean tissue compared with subjects on placebo ($P < 0.05$). Vegetarians who took Cr had a greater increase in TCr, PCr, lean tissue, and total work performance than nonvegetarians who took Cr ($P < 0.05$). The change in muscle TCr was significantly correlated with initial muscle TCr, and the change in lean tissue mass and exercise performance. These findings confirm an ergogenic effect of Cr during resistance training and suggest that subjects with initially low levels of intramuscular Cr (vegetarians) are more responsive to supplementation. **Key Words:** LEAN TISSUE MASS, DUAL-ENERGY X-RAY ABSORPTIOMETRY, MUSCLE FIBER AREA, BIOELECTRICAL IMPEDANCE

Ingestion of creatine monohydrate (CM) has been shown to enhance adaptations to resistance training by augmenting changes in lean tissue mass, muscle fiber area, strength, and resistance to fatigue (4,17,21,30–32). These improved exercise adaptations with creatine supplementation may be due to an increased anaerobic work capacity resulting from an increased rate of phosphocreatine resynthesis (14) or buffered energy depletion during exercise bouts (30), allowing one to train at higher volumes (6,29,32).

Creatine supplementation has been found to result in significant increases in muscle creatine (Cr), phosphocreatine (PCr), and total creatine (TCr = free Cr + PCr)

concentrations (13,16). However, large interindividual differences in baseline resting TCr content and responsiveness to CM supplementation are evident (24,31). Because some individuals do not experience any significant change in cellular creatine or phosphocreatine or improved exercise performance with short-term creatine ingestion, it has been suggested that there is a range of individual responsiveness to creatine supplementation (14,18). One feature common to most studies that directly measure intramuscular creatine and phosphocreatine is that those subjects with the lowest initial total creatine experience the greatest increase after creatine supplementation (5,14,16,18). After 5–7 d of creatine loading, most individuals experience an increase of 20–25 $\text{mmol}\cdot\text{kg}^{-1}$ dry mass (dm) in total creatine of which about 30% is in the form of phosphocreatine (16). A few vegetarian subjects included within mainly omnivorous study populations have demonstrated the greatest increase in muscle total creatine concentration after acute loading (14,16), and a recent study demonstrated that improvements in anaerobic exercise performance were greater for vegetarians than nonvegetarians who supplemented with creatine (26). Lukaszuk et al. (23) demonstrated that 3 wk of a lacto-ovo-vegetarian diet reduced muscle creatine content in omnivorous subjects, which when followed up with creatine

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Submitted for publication February 2003.

Accepted for publication June 2003.

0195-9131/03/3511-1946

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DOI: 10.1249/01.MSS.0000093614.17517.79

TABLE 1. Subject characteristics for those vegetarian and nonvegetarian subjects who participated in 8 wk of resistance training and supplemented with either creatine or placebo.

Group	N = M/F	Age	RT Exp	Height (cm)	Weight (kg)	%Fat
VGCr	5/5	31 ± 2.2	1.8 ± 0.4	169.9 ± 2.8	67.5 ± 2.5	19.5 ± 2.6
VGPI	3/5	34 ± 4.2	2.0 ± 0.6	168.1 ± 2.9	66.7 ± 4.3	20.7 ± 2.2
NVGr	7/5	33 ± 2.6	1.4 ± 0.3	170.6 ± 2.5	69.6 ± 3.9	21.1 ± 2.0
NVPI	5/7	32 ± 2.4	1.9 ± 0.5	172.3 ± 2.6	71.7 ± 3.1	22.9 ± 2.6

VGCr, vegetarian creatine; VGPI, vegetarian placebo; NVGr, nonvegetarian creatine; NVPI, nonvegetarian placebo; vegetarian, lacto-ovo or vegan; N = M/F, number of male/female; RT Exp, years of resistance training experience; N = 42 subjects; values are mean ± standard error.

supplementation resulted in greater, though nonsignificant, increases in total creatine as compared with placebo.

The purpose of this study was to compare the change in muscle TCr content, fiber morphology, body composition, hydration status, and exercise performance between vegetarians and nonvegetarians with 8 wk of creatine supplementation and resistance training. It was hypothesized that vegetarians would have lower baseline intramuscular creatine and phosphocreatine concentrations compared with nonvegetarians. As well, it was hypothesized that the vegetarian subjects would experience the greatest change in total creatine, muscle fiber area, body composition, and exercise performance during creatine supplementation as compared with placebo.

METHODS

Subjects and study design. Forty-nine subjects (19 vegetarian, 30 nonvegetarian) volunteered for the study and had a muscle biopsy taken from the right vastus lateralis muscle. Forty-two of the original subjects (18 vegetarians, 24 nonvegetarians) agreed to take part in an 8-wk resistance-training and CM supplementation study and were randomly assigned (double-blind) to receive creatine or placebo in stratified blocks based on whether they were vegetarian (3 vegan, 15 lacto-ovo) or nonvegetarian and gender. Subjects were recreational athletes, and all participated in a minimum of 20–30 min of exercise (walking, jogging, swimming, cycling, and/or weight lifting) 3–5 × wk⁻¹. All subjects had some resistance training experience (> 1 yr but < 5 yr), but no subject performed only weight-lifting exercise as his or her only activity. No subject had supplemented with creatine within the previous 6 months, which is longer than the time it takes muscle creatine levels to return to baseline after creatine supplementation (11). Subjects were self-described as vegetarian or nonvegetarian; however, 3-d food records were completed and used for confirmation. Subjects were considered vegetarian whether they were lacto-ovo or vegan and were required to be vegetarian for a minimum of 3 yr. All subjects were measured at the beginning and end of the training and supplementation period for muscle fiber morphology and metabolite concentrations, body composition, hydration status (Bioelectrical Impedance Analysis), and exercise performance. This study was approved by the university's ethics committee for biomedical research involving human subjects, and written informed consent was obtained from each subject. Subject characteristics (mean ± SE) are presented in Table 1.

Supplementation. The supplementation protocol for this study was based on the results obtained from a preliminary study that indicated a creatine dose of 0.25 g·kg⁻¹ of lean tissue mass resulted in minimal excretion of creatine in the urine during the acute loading phase (unpublished findings). Because there is about a 4:1 ratio between the amount typically consumed during loading compared with maintenance (14,18,30), an individualized maintenance dose of 0.0625 g·kg⁻¹ lean tissue mass was selected. Therefore, subjects in the creatine group consumed 0.25 g of creatine per kg lean tissue mass (LTM) per day of creatine (0.0625 g·kg⁻¹ LTM, 4 × d⁻¹) for 7 d as a loading phase. This was followed by 0.0625 g of creatine per kg LTM per day (0.0625 g·kg⁻¹ LTM, 1 × d⁻¹) for an additional 49 d. The average absolute daily dose of creatine for subjects during loading and maintenance was 16.8 ± 0.7 g·d⁻¹ and 4.2 ± 0.2 g·d⁻¹, respectively. Subjects in the placebo group consumed the same amount of supplement as the creatine group; however, the supplement only included maltodextrin. Subjects mixed their supplement with ~300 mL of a fruit drink each time it was consumed. Fruit juice was used because it has been found that carbohydrate increases creatine uptake into skeletal muscle (13). The creatine and placebo supplements were identical in taste, texture, and appearance. All supplementation was double blind, in that an individual who was not involved in any other aspect of the study prepared the creatine and placebo supplements and did not reveal the code for subjects on creatine and placebo until the completion of the study. Subjects were given enough of their respective supplement to last for 5 d, after which they had to return their empty packages to receive an additional 5-d supply.

Body weight, body composition, and hydration status. Body weight was measured before the study and again at the end of the study on a scale accurate to the nearest 0.1 kg (Toledo Scales, Toledo, OH). Subjects were weighed in a T-shirt, shorts, and socks and at the same approximate time of day. The scale was calibrated at the beginning and end of the study to ensure accuracy in body weight measurements. Before the study and again at the end, subjects were measured for body composition using dual energy x-ray absorptiometry (DXA) to determine lean tissue mass and percent body fat. Whole body scans were performed on a Hologic QDR-2000 (Hologic, Waltham, MA) in array mode. All scans and regional assessments were made by the same technician using system software version 7.1. The coefficient of variation for the DXA measurement of lean

tissue mass using this unit was determined by the measurements of 12 subjects twice and found to be 0.54%.

For hydration status, total body water, extracellular water, and by deduction, intracellular water content (total body water minus extracellular water) was measured before the study and again at the end of the study using a dual frequency (5 kHz and 200 kHz) bioelectric impedance analyzer (BodyStat, Tampa, FL). Two surface electrodes were placed on the right foot and the right hand, and connected through four separate 3-m leads to the base of the impedance unit. This unit was calibrated at the beginning of each testing period. Coefficients of variation using this device were determined by measuring 10 subjects twice in one day and found to be 0.22% for total body water and 0.25% for extracellular water.

Muscle biopsy and metabolite assays. Percutaneous needle biopsies were obtained from the distal third of the vastus lateralis muscle using a 5-mm Stille needle (Mircrins, New York, NY) under local anesthetic with 1% lidocaine (Smith-Kline Beecham, Toronto, ON) and with suction applied via a 60-cc syringe (10). Pre- and postbiopsies were taken from the lateral portion of the same leg, with the first sample taken 15 cm proximal to the knee joint and the second sample 3–5 cm proximal to the first incision (15). The sample was obtained when the needle was inserted approximately 1 cm below the fascial resistance to control for possible variations in fiber type distributions from superficial to deep (28). The same physician performed all biopsies. Immediately after the muscle sample was excised, it was mounted onto cork, embedded with optimum cutting temperature (OCT) medium, and exposed to the room air for 60 s. The mounted sample was then submerged for 30–40 s in isopentane cooled by liquid nitrogen. Samples were then quickly wrapped (~1–3 s) in aluminum foil and stored in liquid nitrogen until being moved to -80°C refrigeration.

In lots of four at a time, samples were removed from ultra-low refrigeration and placed inside a microtome cutting chamber and warmed to -20°C for about 5 min. Four, 7- μm cross-section cuts were taken from each mounted muscle sample and prepared for their respective staining treatment (described in detail later). The remaining tissue was immediately dissected free of the cork and OCT, placed into plastic vials, and frozen in liquid nitrogen. These samples were then lyophilized overnight and stored at -80°C until subsequent analysis.

Freeze-dried muscle samples were powdered in a controlled environment, with 15–30% relative humidity to prevent rehydration of the muscle sample. Powdering was done manually using 7-cm curved tissue forceps, and all connective tissue and fat was dissected away from the powdered muscle. After powdering, 5–10 mg of tissue was weighed and placed into a 2-mL vial (VWR Canlab, Edmonton, AB) for extraction with perchloric acid.

Muscle metabolites were extracted with 0.5-M perchloric acid containing 1 mM of EDTA at a ratio of 800 μL to every 10 mg of powder for 15 min on ice, while periodically vortexing. Samples were then centrifuged in a precooled centrifuge (4°C) for 5 min at 15,000 rpm. The supernatant

was weighed into another 2-mL vial and neutralized with 2.2-M KHCO_3 added at a volume equal to one-fifth the mass of the extract supernatant. The subsequent metabolite assays were performed using methods previously described (15,25). Neutralized extracts were prepared for spectrophotometric determination of ATP, phosphocreatine, and free creatine using a Hitachi F2500 spectrofluorometer (Chromabec, Montreal, PQ) at an excitation wavelength of 340 nm and an emission wavelength of 445 nm. Coefficients of variation for the ATP, phosphocreatine, and free creatine assays using this machine were determined using the repeated measurement of 100 samples and found to be 2.05%, 3.78%, and 3.08%, respectively.

Histochemical staining and image analysis. Serial muscle tissue cross-sections from the same individual pre- and posttraining were placed together in the same preincubation medium and stained for myosin ATPase at pH 4.2, 4.6, and pH 9.4 (2). After staining, sections were mounted and the area positively stained was analyzed using Image Pro Plus Version 4.0 software (Media Cybernetics, Silver Spring, MD). First, each slide was viewed under $200\times$ magnification (Olympus BX60, Tokyo, Japan). Then, three to four pictures were taken per slide (Spot Diagnostic Instruments Inc, Sterling Heights, MI) and immediately saved as a JPEG file into a Dell Dimension XPS R450 (Dell Computer Company, Austin, TX). Approximately 100–150 muscle fibers were used for determination of mean fiber area.

Urine collection. Twenty-four-hour urine was collected in 4-L plastic containers (Fisher Scientific, Edmonton, AB) five times during the study: one day before supplementation, the first day of supplementation, the third day of supplementation, the fifth day of supplementation, and after the last day of supplementation. Total 24-h urine output volume was recorded, and then an aliquot was removed and stored at -20°C until the end of the study, when all samples were analyzed together.

Creatine and creatinine were determined using the Jaffe method (19), which results in the formation of an orange-red alkaline creatinine picric acid complex that can be measured for optical density spectrophotometrically. The following procedure as adapted from Jaffe (19) was performed for each urine sample: 100 μL of urine was mixed with 600 μL of water, 100 μL of 10% sodium tungstate, and 200 μL of 0.67-N sulfuric acid. This mixture was vortexed for 10 s and centrifuged for 10 min. After centrifugation, a 300- μL aliquot was pipetted off and added to 2700 mL of water. In an ice bath, 1 mL of 0.04-M picric acid followed by 1 mL of 0.75-N sodium hydroxide was added to this solution. This solution was then vortexed for 10 s and placed in a 20°C water bath for 20 min. Samples were read in a Hitachi U2010 UV-vis spectrophotometer (Chromabec) at 520 nm immediately upon removal from the water bath, which provided a measure of creatinine concentration. To determine urinary creatine, the same steps were followed up to and including the addition of picric acid; however, after the acid was added, the solution was then heated at 100°C for 1 h. After heating, the sample was removed and cooled in ice for 5 min. Then 1 mL of 0.75-N sodium hydroxide was added.

The solution was then vortexed for 10 s and placed into a 20°C water bath for 20 min. The sample was again read in the same spectrophotometer at the same setting immediately upon removal from the water bath. Heating in acid for 1 h converted all creatine to creatinine, and the second optical density measure subtracted from the first optical density measure of creatinine for the same sample gave the amount of creatine present. Samples were prepared in duplicate for both creatinine and creatine measurements. The coefficient of variation between duplicate samples for creatinine (and indirectly creatine) determination using this machine was 6.82%. Because the purpose of the urine collection was to monitor changes in creatine and creatinine excretion associated with creatine supplementation, only the urine from those subjects who supplemented with creatine were analyzed.

Muscle performance measures. Muscular strength and total work performed during 50 isokinetic knee extension and flexion repetitions at $180^{\circ}\cdot\text{s}^{-1}$ were measured at the beginning of the study and again after 8 wk of supplementation and training. Strength was assessed by 1-RM for bench press and leg press. Before the leg press, a warm-up consisted of the modified hurdler's stretch held twice on each leg for 20 s followed by 10 repetitions of leg press (Hammer Strength, Life Fitness, Franklin Park, IL) using a weight determined by each subject as an appropriate warm-up weight. Before the bench press 1-RM test, a warm-up consisted of 20 push-ups; two static stretches of the chest musculature against a wall, held for 8 s each; and 10 repetitions with a comfortable starting weight as determined by each subject. For bench press, subjects were positioned on the bench (Pulse Fitness Systems, Winnipeg, MB) with both feet flat on the floor and the handles of the press at the level of their nipples. Subjects were not allowed to lift their buttocks off the bench or arch their backs during a lift.

After warm-up, for both leg press and bench press testing, subjects selected a weight they felt they could complete three repetitions with. At this weight, they only performed one repetition. Subjects then selected a weight they felt would be their 1-RM and attempted one repetition with this weight. After successful attempts, weight was increased by 2–5 kg for subsequent 1-RM attempts. The 1-RM was usually reached in less than six sets, including the warm-up set. There was 3-min rest between attempts, and two assistants changed the weight between attempts. Reproducibility of the bench press and leg press was determined on two separate days in 10 subjects. The coefficients of variation for bench press and leg press were 3.6% and 3.0%, respectively.

Total work performed during 50 repetitions was measured in the right knee extensors and flexors, using an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley, NY). The dynamometer was set in the concentric mode for knee extension/flexion, at an angular velocity of $180^{\circ}\cdot\text{s}^{-1}$ (20). Range of motion consisted of movement from 90° to 170° of knee flexion (internal angle). Subjects sat against a back support, producing an angle of 85° of hip flexion. Stabilizing belts were placed over the lap, across the chest, and across the distal one-third of the tested leg thigh. The rotational axis of the dynamometer was positioned to be coaxial with the knee axis (lateral condyle)

during testing. Subjects were given two practice trials of 10 repetitions where they were told to perform knee extension and flexion as fast and hard as possible. After the practice trials and 10-min rest, subjects performed the test trial, which consisted of 50 repetitions of knee extension and knee flexion at maximal effort. Torque on the machine attachment was corrected for the effects of gravity on the lower leg and the dynamometer's resistance pad. The torque output on the dynamometer was checked with a calibration weight on a weekly basis throughout the study duration. Reproducibility of the total work measurements was determined on two separate days in 12 subjects. The coefficients of variation for knee extension and flexion total work were 4.93% and 8.31%, respectively.

The order of tests was the same each time: muscle biopsy, then 24-h later leg press, bench press, and isokinetic knee extension/flexion, with at least 15 min of rest between each test.

Exercise program. All subjects followed the same high volume, heavy load ($>70\%$ 1-RM), resistance-training program for 8 wk (1). This program was designed to increase lean tissue mass and strength and had been previously found to yield these results (3). In this program, subjects self-select a weight that permits completion of the required number of sets and repetitions for each exercise and to reach failure by the last repetition each time. This typically takes a subject two to three sessions to find the appropriate weight to use for each exercise but allows for individual progression throughout the program. The program involved a 3-d split routine using whole body musculature. Day 1 was for chest and tricep musculature and included the following exercises in order: bench press, incline bench press, flat bench dumbbell flies, incline dumbbell flies, cable tricep extensions, rope reverse tricep extensions, and french curls. Day 2 was for back and bicep musculature and included the following exercises in order: chin-ups, low row, lat pull-downs, alternate dumbbell row, standing EZ-curls, preacher curls, and alternate dumbbell curls. Day 3 was for leg, shoulder, and abdominal musculature and included the following exercises in order: leg press, leg extensions, hamstring curls, standing calve raises, military dumbbell press, upright row, shrugs, deltoid flies, and abdominal crunches. Day 4 was rest and recovery. These four days were considered one cycle, and the cycle was repeated continuously throughout the duration of the study. The program was broken into seven blocks of two cycles or 8 d, for a total of 56 d. Block 1 consisted of three sets of 10–12 repetitions, with 1-min rest between sets. Block 2 consisted of three sets of 8–10 repetitions, with 1.5-min rest between sets. Block 3 consisted of four sets of six to eight repetitions, with 2-min rest between sets. Block 4 consisted of five sets of four to six repetitions, with 3-min rest between sets. Block 5 consisted of four sets of six to eight repetitions, with 2-min rest between sets. Block 6 consisted of three sets of 8–10 repetitions, with 1.5-min rest between sets. Block 7 consisted of three sets of 10–12 repetitions, with 1-min rest between sets (3). Subjects trained with at least one partner and adjusted their exercise load accordingly to permit completion of the

desired repetitions. A personal trainer supervised and assisted with all training sessions.

Training logs detailing the weight used and numbers of sets and repetitions performed for each exercise were completed for every workout. Training volume was calculated ($\text{kg} \times \text{reps}$) for the entire 8 wk of training and compared between groups and supplements. As well, the training volume during the second week (three sets of 8–10 reps) was compared with week 7 (three sets of 8–10 reps) for pre- to postcomparisons between groups, supplement, and time. Week 2 and week 7 were compared because the sets and reps were the same and this exercise cycle represented the beginning (week 2) and end (week 7) of the study.

Dietary intake. Subjects were asked not to alter their diet (increase or decrease caloric consumption) for the duration of the study. Three-day food diaries were collected from each subject at the beginning and end of the study. Subjects were given instruction about proper portion sizes and how to accurately record all food or beverages consumed during the 3-d recording in the food diary. Fuel Nutrition Software 2.1a (LogiForm International Inc., Saint-Foy, PQ) was used to analyze the food records for total calories and the amount of energy from each of carbohydrate, protein, and fat. This information was collected to determine whether diet might be a confounding factor in the study and to confirm vegetarian status.

Statistical analysis. There were two separate analysis performed in this study. The first analysis involved the assessment of baseline measurements of muscle concentrations of creatine, phosphocreatine, total creatine, and adenosine triphosphate in the muscle biopsies from the initial subject pool, consisting of 19 vegetarian and 30 nonvegetarians. The second analysis involved assessment of changes in body composition, exercise performance, muscle metabolite concentration, and muscle fiber area in the subject population ($N = 42$; 24 NV; 18 VG) that participated in 8 wk of resistance training and supplementation with either creatine or placebo.

In the first analysis, muscle concentrations of creatine, phosphocreatine, total creatine, and adenosine triphosphate were analyzed by two-way analysis of variance (ANOVA) for differences between group (vegetarian vs nonvegetarian) and gender (male vs female). Whenever significance was evident, Tukey *post hoc* tests were performed to compare means. A P value of 0.05 was considered significant.

In the second analysis, results were analyzed using a group (vegetarian vs nonvegetarian) \times supplement (creatine vs placebo) \times time (pre- vs posttraining) ANOVA with repeated measures on the factor of time. Whenever significance was evident, Tukey *post hoc* tests were performed to compare means. A P value of 0.05 was considered significant. All results are presented as means \pm standard error. As part of the second analysis, Pearson correlations were calculated for the change in muscle creatine and initial muscle creatine, the change in muscle creatine and the change in lean tissue mass, and the change in muscle creatine and the change in exercise performance variables. A P value of 0.05 was considered significant.

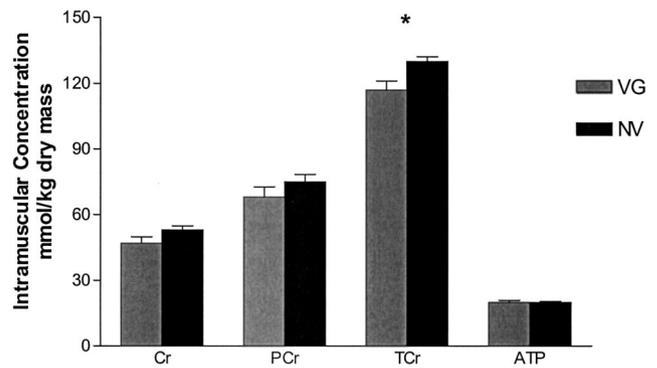


FIGURE 1—Baseline creatine (Cr), phosphocreatine (PCr), total creatine (TCr), and adenosine triphosphate (ATP) concentration for vegetarian (VG) and nonvegetarian (NV) subjects. Concentration is expressed as millimoles per kilogram dry mass. Values are mean \pm standard error for $N = 49$ subjects. * Indicates significant group difference ($P < 0.05$).

RESULTS

Baseline Measurements

The first purpose of this study was to determine whether a habitual vegetarian diet resulted in lower creatine, phosphagen, and total creatine concentrations. Because males and females were included in this study, gender was entered as a factor and assessed for baseline differences in the same above-mentioned variables. The results demonstrated that there was no significant difference between vegetarians and nonvegetarians or males and females for free creatine concentration. There were no significant differences between vegetarians and nonvegetarians or males and females for phosphocreatine or adenosine triphosphate concentrations. However, there was a significant difference between vegetarians and nonvegetarians for total creatine concentration ($P < 0.05$; Fig. 1), with the nonvegetarians demonstrating higher total creatine concentration. This difference was expected and was the basis for blocking subjects accordingly for the 8-wk training study.

Although males and females did not differ in muscle metabolite concentrations, there were expected differences between genders (16,24). In those subjects who participated in the training and supplementation study, males exhibited significantly higher values than females on all body composition variables except percentage fat, hydration measures, and muscular performance variables. This also was the basis of blocking subjects according to sex, which resulted in the random assignment of subjects to groups based on their gender. Numerous published reports indicate that there is no difference between how males and females respond to resistance training (7,22,28). Therefore, gender was not included as a factor in the statistical analysis for the subsequent training and supplementation study.

Training Study

Body composition and hydration status. There were no differences between groups at baseline for scale body weight or lean tissue mass. For body mass, there was

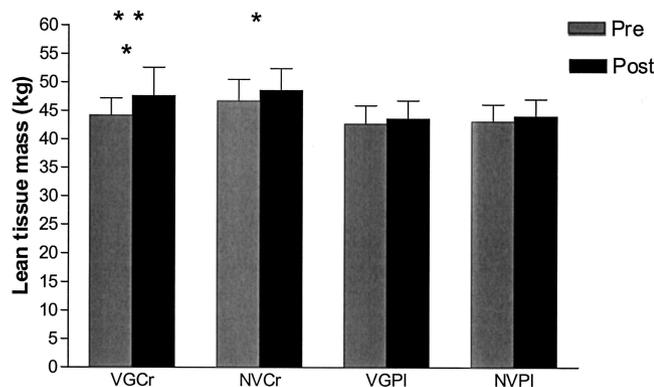


FIGURE 2—Graph of lean tissue mass (kg) at baseline (BL) and posttraining (Post) for subjects supplementing with creatine and placebo. VGCr, vegetarians on creatine; VGPI, vegetarians on placebo; NVCr, nonvegetarians on creatine; NVPI, nonvegetarians on placebo. Values are means expressed in kilograms (kg) \pm standard error for $N = 42$ subjects. * Indicates significant pre- to postchange ($P < 0.05$). ** Indicates significant group \times supplement \times time interaction ($P < 0.05$).

a supplement \times time interaction ($P < 0.05$), with those subjects supplementing with creatine demonstrating a greater increase from pre- to posttraining than those on placebo. For lean tissue mass there was a group \times supplement \times time interaction ($P < 0.05$). Vegetarians on creatine experienced an increase of 2.4 kg in lean tissue mass compared with an increase of 1.9 kg for nonvegetarians on creatine (significant interaction, $P < 0.05$; Fig. 2). Those subjects supplementing with creatine (vegetarians and nonvegetarians) demonstrated a greater increase in lean tissue mass as compared with those groups on placebo ($P < 0.05$). The change in lean tissue mass was significantly correlated with the change in muscle total creatine ($r = 0.61$, $P < 0.05$).

Total body water, extracellular water, and intracellular water all increased significantly (time effect) from baseline to posttraining and supplementation, and there were no differences between groups or supplements. The increase in total body water, extracellular water, and intracellular water was 35.4 ± 1.2 L to 37.0 ± 1.2 L, 17.2 ± 0.5 L to 17.9 ± 0.5 L, and 18.2 ± 0.7 L to 19.1 ± 0.8 L, respectively ($P < 0.05$).

Muscle metabolites. There were no group, supplement, or time main effects or interactions for free creatine content. There was a significant group \times supplement \times time interaction for phosphocreatine concentration ($P < 0.05$; Fig. 3) and total creatine concentration ($P < 0.05$; Fig. 4). *Post hoc* tests revealed that vegetarians on creatine experienced a significantly greater gain in phosphocreatine and total creatine concentration from pre to post than all other groups ($P < 0.05$). Nonvegetarians on creatine experienced a significant increase in phosphocreatine and total creatine from pre- to posttest ($P < 0.05$). There was a significant negative correlation between the change in muscle total creatine and initial muscle total creatine for those subjects supplementing with creatine ($r = -0.77$, $P < 0.05$). There were no changes in creatine, phosphocreatine, or total creatine for those subjects supplementing with placebo, and there was no effect of supplementation or training on adenosine triphosphate concentration.

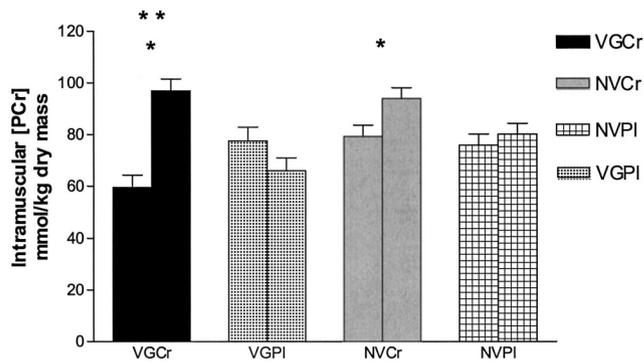


FIGURE 3—Graph of baseline (BL, first bar on left in each column) and posttraining (Post) measurement of phosphocreatine (PCr) concentration for those subjects who participated in 8 wk of weight training and supplementation. VGCr, vegetarians on creatine; VGPI, vegetarians on placebo; NVCr, nonvegetarians on creatine; NVPI, nonvegetarians on placebo. Values are mean \pm standard error for $N = 42$ subjects. * Indicates significant pre- to postchange ($P < 0.05$). ** Indicates significant group \times supplement \times time interaction ($P < 0.05$).

Muscle fiber area. There were inconsistencies with differentiation of fiber types at preincubation of pH 4.6. Therefore, to ensure accurate calculation of muscle fiber area, results from only those slides at pH 4.2 and 9.4 were reported, which permitted determination of Type I and II fiber area pre- and posttesting (2). There were no differences at baseline between groups for Type I or II muscle fiber area. There were no significant main effects or interactions for Type I muscle fiber area. There was a significant supplement \times time interaction for Type II fiber area ($P < 0.05$). Creatine supplementation resulted in a 28% increase in Type II fiber area, which was greater as compared with 9% for placebo supplementation (Fig. 5). Fiber type area and percentages for each group pre- and posttraining are listed in Table 3.

Urine. There were significant group differences in urine creatine output at baseline, with nonvegetarians excreting greater 24-h urinary creatine than vegetarians ($P < 0.05$). Urinary creatine output on day 3 and day 5 of creatine supplementation significantly differed from baseline, and

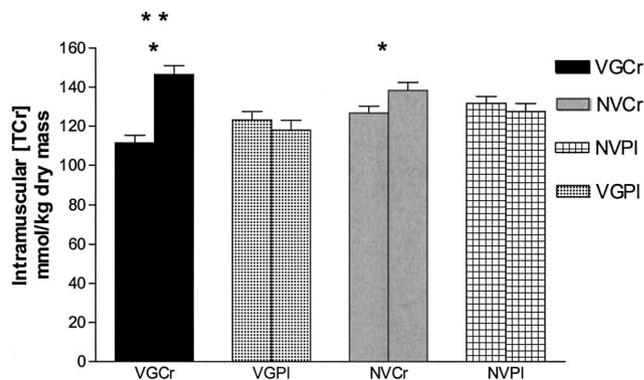


FIGURE 4—Graph of baseline (BL, first bar on left in each column) and posttraining (Post) measurement of total creatine (TCr) concentration. VGCr, vegetarians on creatine; VGPI, vegetarians on placebo; NVCr, nonvegetarians on creatine; NVPI, nonvegetarians on placebo. Values are mean \pm standard error for $N = 42$ subjects. * Indicates significant pre- to postchange ($P < 0.05$). ** Indicates significant group \times supplement \times time interaction ($P < 0.05$).

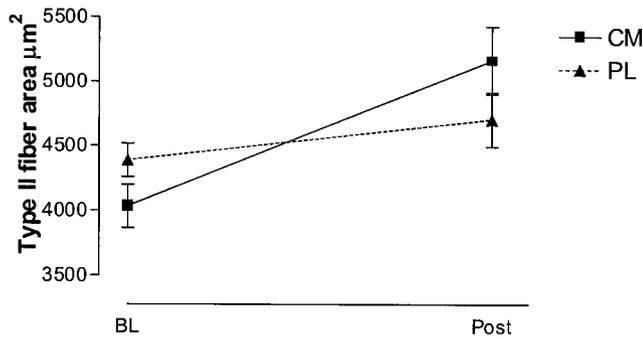


FIGURE 5—Graph of Type II muscle fiber area (μm^2) for creatine supplementing (CM) and placebo supplementing (PL) subjects both at baseline (BL) and posttraining (Post). Values are mean \pm standard error for $N = 42$ subjects. Plot demonstrates significant supplement \times time interaction ($P < 0.05$).

urinary creatine output on day 5 significantly differed from day 3 ($P < 0.05$). Creatinine output remained unchanged throughout the loading phase of supplementation but was significantly greater at posttest compared with pretest values (time main effect, $P < 0.05$). After supplementation ended, urinary creatine was not different from baseline. Urine creatine output is presented in Fig. 6.

Muscle performance measures. There were no differences in 1-RM bench press and leg press between groups at baseline. There was a supplement \times time interaction for 1-RM bench press ($P < 0.05$). Those subjects who supplemented with creatine demonstrated an increase in 1-RM bench press from 85.1 ± 8.5 kg to 101.0 ± 9.5 kg, which was greater than the placebo subjects who increased 1-RM bench press from 76.4 ± 9.0 kg to 85.1 ± 10.1 kg. Leg press increased as a result of training ($P < 0.05$) with no differences between groups. The mean increase in leg press was 22.1 ± 10 kg. There was a significant positive correlation between the change in muscle total creatine and change in bench press ($r = 0.62$, $P < 0.05$) and leg press ($r = 0.52$, $P < 0.05$) for those subjects supplementing with creatine.

The total work performed during 50 repetitions at 180°s^{-1} of knee flexion and extension was used as a measure of muscular endurance. There was a significant group \times supplement \times time interaction ($P < 0.05$) for total work. *Post hoc* tests indicated that the comparison of pre to post for vegetarians supplementing with creatine was significantly greater than all other groups ($P < 0.05$; Fig. 7). The change in total work performed was significantly correlated with the change in muscle total creatine for those subjects supplementing with creatine ($r = 0.59$, $P < 0.05$).

TABLE 2. Total calories ($\text{kcal}\cdot\text{d}^{-1}$) and macronutrient ($\text{g}\cdot\text{d}^{-1}$) content of vegetarian and nonvegetarian subjects at pre- and postexercise/supplement intervention.

Energy	Vegetarian		Nonvegetarian	
	Pre	Post	Pre	Post
Calories ($\text{kcal}\cdot\text{d}^{-1}$)	2159 (71)*	2213 (78)*	2638 (67)*	2629 (61)*
Carbohydrate ($\text{g}\cdot\text{d}^{-1}$)	332 (11)	330 (12)	349 (12)	348 (11)
Protein ($\text{g}\cdot\text{d}^{-1}$)	78 (2)*	80 (2)*	139 (2)*	138 (3)*
Fat ($\text{g}\cdot\text{d}^{-1}$)	59 (3)	61 (4)	67 (3)	66 (3)

All values are mean \pm (standard error); data are based on the average for 1 d from 3-d food records.

* Significant difference ($P < 0.05$) between groups (vegetarians and nonvegetarians).

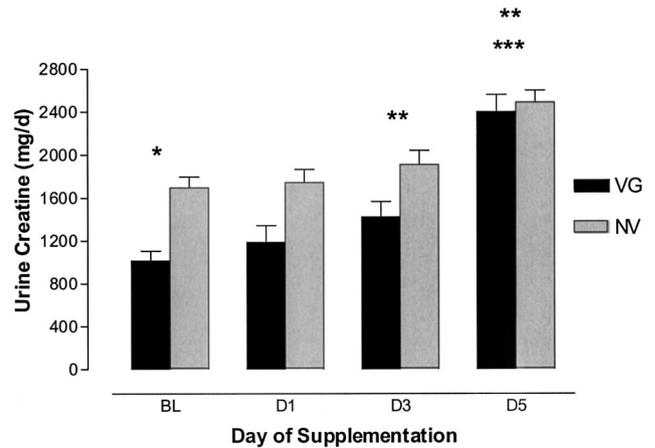


FIGURE 6—Graph of urine creatine output ($\text{mg}\cdot\text{d}^{-1}$) for baseline (BL), day 1 (D1), day 3 (D3), and day 5 (D5) of loading ($0.25 \text{ g}\cdot\text{kg}^{-1} \text{ LTM}\cdot\text{d}^{-1} \times 7 \text{ d}$) for vegetarian (VG) and nonvegetarian (NV) subjects supplementing with creatine. Values are mean \pm standard error for $N = 42$ subjects. * Indicates significant group main effect ($P < 0.05$). ** Indicates significant difference from baseline ($P < 0.05$). *** Indicates significant difference from previous measurement day ($P < 0.05$).

Training volume. There was a significant difference in total training volume between creatine and placebo supplementation ($P < 0.05$), and a significant supplement \times time interaction for training volume from week 2 and week 7 ($P < 0.05$). Creatine supplementation resulted in training volumes of $56,702 \text{ kg} \times \text{reps}$ and $65,198 \text{ kg} \times \text{reps}$ for weeks 2 and 7 (three sets, 8–10 reps), whereas placebo subjects had training volumes of $46,477 \text{ kg} \times \text{reps}$ and $50,105 \text{ kg} \times \text{reps}$ for weeks 2 and 7 (Fig. 8).

Diet. There was a significant difference between vegetarians and nonvegetarians for total calories and calories as protein before and at the end of the study ($P < 0.05$), with the nonvegetarians consuming higher total calories and calories as protein. The amount of total calories and calories as protein did not change for any group from pre- to posttest. Total calories and macronutrient (protein, carbohydrate, and fat) content of the vegetarian and nonvegetarian subjects are listed in Table 2.

DISCUSSION

This is the first study to demonstrate that habitual vegetarians (lacto-ovo or vegan) have lower resting intramuscular concentrations of total creatine as compared with omnivorous peers. As well, the results of this study are in agreement with previous work indicating that baseline muscle creatine concentrations affect the increase in muscle total creatine resulting from creatine monohydrate supplementation. The vegetarians supplementing with creatine in this study exhibited a greater increase in muscle concentrations of phosphocreatine and total creatine, and also a greater increase in lean tissue mass and total work output. These changes were also significantly correlated with the changes in muscle total creatine concentration.

Delanghe et al. (8) and Shomrat et al. (26) found that vegetarians had lower plasma creatine than nonvegetarian

TABLE 3. Muscle fiber cross-sectional areas and percentages.

Fiber Type	Area (μm^2)				Percentage			
	VGCr	VGPI	NVCr	NVPI	VGCr	VGPI	NVCr	NVPI
Type I								
Pre	3230 \pm 477	3291 \pm 229	3121 \pm 284	3470 \pm 394	46 \pm 3	44 \pm 2	42 \pm 4	43 \pm 4
Post	3944 \pm 201	3622 \pm 189	3674 \pm 229	3714 \pm 246	44 \pm 3	44 \pm 3	41 \pm 3	43 \pm 3
Δ (post-pre)	715 \pm 266	331 \pm 223	553 \pm 277	244 \pm 237	4 \pm 2	1 \pm 2	1 \pm 3	0 \pm 3
Type II								
Pre	3792 \pm 358	4189 \pm 381	4310 \pm 299	4601 \pm 511	54 \pm 2	56 \pm 4	58 \pm 3	57 \pm 3
Post	5019 \pm 349	4611 \pm 199	5287 \pm 473	4924 \pm 431	56 \pm 3	56 \pm 4	59 \pm 3	57 \pm 4
Δ (post-pre)	1227 \pm 255	422 \pm 311	977 \pm 263	323 \pm 455	2 \pm 2	0 \pm 3	1 \pm 3	0 \pm 4

VGCr, vegetarian creatine; VGPI, vegetarian placebo; NVCr, nonvegetarian creatine; NVPI, nonvegetarian placebo; vegetarian, lacto-ovo or vegan; values are mean \pm standard error.

peers, and Lukaszuk et al. (23) demonstrated that a 3-wk lacto-ovo-vegetarian diet could reduce muscle creatine in omnivorous men. The average plasma creatine concentration is about 30–70 μM for omnivorous adult males, and the vegetarians in the Delanghe et al. (8) and Shomrat et al. (26) studies had plasma creatine of 9 μM and 11 μM , respectively. The urinary analysis in our study supports these results in that vegetarians excreted significantly less urinary creatine than nonvegetarians, which would be expected of a diet that lacks animal meats, and therefore, little ingested creatine. In the only study to date involving creatine supplementation in a group of vegetarians, Shomrat et al. (26) gave seven vegetarians 21 g of creatine per day for 6 d and measured anaerobic exercise performance. Vegetarians experienced the same amount of weight gain as nonvegetarians, but they had significantly better fatigue resistance on the second of three Wingate anaerobic tests. In the present study, muscle biopsies provided direct evidence that muscle total creatine stores were lower in vegetarians than nonvegetarians, and after creatine supplementation and resistance training, these vegetarians experienced greater increases in TCr, PCr, lean tissue mass, and total work performance compared with nonvegetarians. These findings are significant in that they are the first to demonstrate that individuals with low initial intramuscular TCr have heightened response to creatine supplementation during resistance training. Previous studies have demonstrated that individual vegetarian

subjects respond to creatine supplementation with a greater increase in TCr than usual (16). Also, those with the greatest changes in TCr with creatine supplementation demonstrate greater increases in work output during fatiguing exercise tasks (5) and greater rates of PCr resynthesis after fatiguing exercise (14). An increase in ability to perform work and an increase in rate of PCr resynthesis would improve one's ability to perform work during individual training sessions, and this should result in a greater stimulus for muscle hypertrophy. This is supported by the results of the current study where vegetarians supplementing with creatine had greater increases in ability to perform work and a greater increase in lean tissue mass compared with nonvegetarians.

The greater gain in lean tissue mass experienced by the subjects supplementing with creatine in the present study is in agreement with previous creatine and weight training reports (4,21). In the present study, subjects supplementing with creatine had a 4% increase in lean tissue mass, which was greater than the 2% increase in the placebo group. Kreider et al. (21) used doses of 15.75 g·d⁻¹ for 4 wk and demonstrated increases in DXA determined lean tissue mass of 3.28% in subjects on creatine compared with 2% for placebo. Stout et al. (29) and Burke et al. (4) found an increase of 2.3–4.6% in lean tissue mass after 3–8 wk of resistance training and creatine supplementation. The increase in lean tissue mass demonstrated by those subjects supplementing with creatine in the present study parallels

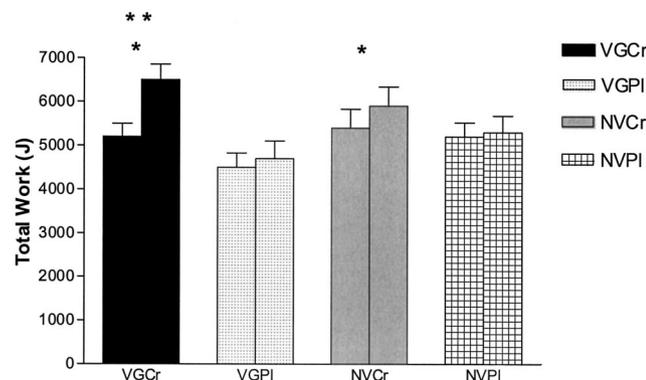


FIGURE 7—Graph of total work for knee flexion and extension (50 reps at 180°·s⁻¹) at baseline (BL) and posttraining (Post). VGCr, vegetarians on creatine; VGPI, vegetarians on placebo; NVCr, nonvegetarians on creatine; NVPI, nonvegetarians on placebo. Values are mean \pm standard error for $N = 42$ subjects. * Indicates significant pre-to postchange ($P < 0.05$). ** Indicates significant group \times supplement \times time interaction ($P < 0.05$).

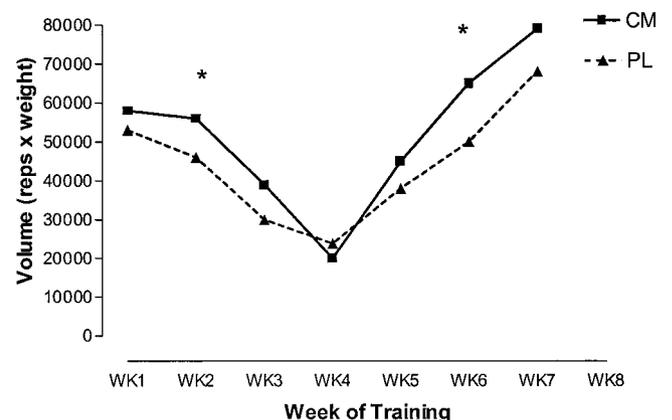


FIGURE 8—Graph of weekly training volume for all subjects supplementing with creatine (CM) and placebo (PL). Values are mean training volume (reps \times weight) for $N = 42$ subjects. Standard error bars were excluded for purposes of clarity. * Indicates significant difference between supplements ($P < 0.05$).

the increase in other variables such as muscle phosphocreatine and total work output resulting from creatine supplementation. Increased muscle phosphocreatine concentration resulting from creatine supplementation enhances phosphocreatine recovery, and this has been suggested as a reason for augmented increases in work performance during resistance training with subsequent increases in lean tissue mass (14,30). Our finding of an increase in self-selected training volume in subjects supplementing with creatine in the current and one previous study (6) supports this contention.

Extracellular, intracellular, and total body water increased in all subjects from pre- to posttraining, and there were no differences between groups or supplements regarding the changes that occurred. Francaux and Poortmans (12) reported a significant increase in intracellular water after 42 d of strength training and creatine supplementation. These authors used the same method to detect hydration status (BIA) and a similar program of exercise and supplementation as the present study. Compared with the current study, the increase in intracellular water was almost the same (~1 L) for creatine supplementing subjects, but like the present study, the percentage of intracellular water to body weight did not differ substantially. This would indicate that the increase in intracellular water paralleled an increase in muscle dry matter and occurred proportionately due to osmoregulatory changes. Hultman et al. (18) measured 24-h urine output volume and suggested that a short-term loading phase resulted in water retention similar in magnitude to that of Francaux and Poortmans (12). These results are limited, however, because the authors did not measure fluid consumption. In the present study, hydration status was measured by bioelectrical impedance (BIA) and body composition was assessed by DXA. The results of the BIA indicate that there was a significant increase in body water content (TBW, ECW, and ICW) from pre to post but that there were no significant differences between groups or supplements. DXA measures indicated that the subjects supplementing with creatine demonstrated a greater change in lean tissue mass than subjects on placebo. These combined results of body compartment fluid and composition would suggest that there was an increase in dry muscle mass due to creatine supplementation and resistance training that was superior to resistance training and placebo supplementation.

Assessment of muscle fiber morphology indicated that creatine supplementation resulted in greater increases in Type II fiber area compared with placebo supplementation (28% vs 9%). One year of creatine supplementation (1.5 g·d⁻¹) was found to increase Type II fiber area by 34% in a group of patients with gyrate atrophy (27). Volek et al. (32) reported that 12 wk of creatine supplementation and resistance training resulted in ~35% in Type II and Type I fiber area compared with ~10% for placebo. Likewise, Hespel et al. (17) found significant increases in fiber area of all fiber types during creatine supplementation combined with 2 wk of immobilization and 10 wk of strength training. From the results of the current study and those cited above, it is difficult to determine whether certain fiber types are

more responsive to creatine supplementation than others. The preferential hypertrophy of Type II muscle fibers in the current study may be related to the training stimulus (32) in addition to the Cr supplementation.

Creatine supplementation resulted in a 19% increase in 1-RM bench press compared with an 11% increase for placebo supplementation, which is similar to the results reported by Stout et al. (29) and Volek et al. (32). After 8 wk of resistance training and creatine supplementation (21 g·d⁻¹), Stout et al. (29) reported an increase of 13% for 1-RM bench press. Volek et al. (32) found a 24% increase in 1-RM bench press after 12 wk of creatine supplementation and weight training, which compared with a 16% increase for 1-RM bench press for placebo subjects. Improved strength after creatine supplementation and resistance training has been thought to be due to the ability to increase training volume at a greater rate (6,9). In the present study, creatine subjects had a greater increase in training volume (kg × reps) compared to placebo subjects. Earnest et al. (9) reported similar improved training volume for bench press associated with creatine supplementation. Volek et al. (32) also reported significantly greater training volume associated with creatine supplementation, which corresponded to statistically greater improvements in 1-RM bench press as compared with placebo supplementation. There was a significant increase in 1-RM leg press with training, but no differences between groups. The exercise program in the present study only included two leg exercises, emphasizing the knee extensors. The minimal focus on leg exercises may partly explain the lack of significant difference between creatine and placebo subjects for the leg press. Subsequent study with a greater emphasis on lower-limb exercises might elicit a significant difference similar to that for bench press 1-RM.

In summary, vegetarians have lower basal total creatine and urinary excreted creatine than nonvegetarian peers and that creatine supplementation combined with resistance training yields greater changes in muscle phosphocreatine, total creatine, Type II fiber area, lean tissue mass, and total work performed as compared with placebo supplementation. Furthermore, the increase in muscle concentrations of phosphocreatine and total creatine, lean tissue mass, and total work performed were greater in vegetarians supplementing with creatine compared with nonvegetarians supplementing with creatine. Future studies are needed to determine whether short-term dietary manipulation (vegetarian) can affect muscle concentrations of creatine and also whether there is supercompensation in creatine transport once it is reintroduced into the diet. The work of Lukaszuk et al. (23) indicated a trend toward this with a short duration (5 d) of supplementation. As well, additional work is necessary to further understand the mechanisms that regulate muscle concentrations of total creatine to determine why there is such individual variation in the magnitude of muscle creatine accumulation during loading.

This study was funded by a grant from MuscleTech Research and Development Inc., Mississauga, Ontario, Canada. The results of the present study do not constitute endorsement of the product by the authors or ACSM.

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