Effect of Creatine Ingestion after Exercise on Muscle Thickness in Males and Females

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ABSTRACT

CHILIBECK, P. D., D. STRIDE, J. P. FARTHING, and D. G. BURKE. Effect of Creatine Ingestion after Exercise on Muscle Thickness in Males and Females. *Med. Sci. Sports Exerc.*, Vol. 36, No. 10, pp. 1781–1788, 2004. Muscles exercised before creatine (Cr) supplementation have a greater elevation of intramuscular Cr than nonexercised muscles. **Purpose**: To determine whether preferential increase of muscle thickness could be achieved by ingesting Cr immediately after exercise of specific muscles over 6 wk. Another purpose was to determine if the increase in lean tissue mass (LTM) with Cr supplementation is greater in males than females. **Methods:** Subjects randomly assigned to Cr (six males, five females, 0.2 g $Cr \cdot kg^{-1}$) and placebo (PL; five males, five females) performed single-limb training with one side of the body two times per week and with the opposite limbs two times per week. Cr was consumed after training of one side of the body and PL after training the opposite side. Subjects on PL always consumed PL after exercise. Elbow flexors and knee extensors muscle thickness, LTM, fat, and bone mass, and single-limb bench and leg press one-repetition maximum (1-RM) were assessed before and after 6 wk. **Results:** Within the Cr group, elbow flexors muscle thickness increased more in the limbs trained on days Cr was supplemented compared with limbs trained on days PL was supplemented ($P < 0.02$). All other measures changed to a similar extent between limbs. Males on Cr had the greatest increase in LTM ($P < 0.05$) with no difference between females on Cr and PL. Bench press 1-RM increased more in Cr than PL groups ($P < 0.01$). All other measures changed to a similar extent between groups. Males increased bone mass ($P < 0.01$) with no effect of Cr supplementation. **Conclusion:** Supplementing with Cr after training of the arms resulted in greater increase in muscle thickness of the arms. Males have a greater increase in LTM with Cr supplementation than females. **Key Words:** GENDER, BONE, LEAN TISSUE MASS, STRENGTH-TRAINING, SIDE-EFFECTS

Supplementation with creatine monohydrate during resistance training enhances muscle hypertrophy compared with resistance training alone (13,33). Uptake of creatine into skeletal muscle may stimulate transcription factors that control protein synthesis (18) or increase phosphocreatine stores (6,29), which may allow for greater amount of work performed during individual training sessions (7,13,34). This in turn may lead to an increased stimulation for muscle hypertrophy.

Several strategies are effective for increasing the amount of creatine transported into skeletal muscle including co-ingestion of creatine with carbohydrate, protein, and alpha-lipoic acid (6,29). All of these increase insulin release, which may aid in transport of creatine into skeletal muscle (29).

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An additional strategy for increasing uptake of creatine into muscle involves performance of exercise immediately before creatine supplementation (17,26). The increase in blood flow to active muscle or activation of the sarcolemmal sodium-potassium pump may result in enhanced delivery and uptake of creatine into skeletal muscle (26). If exercise enhances uptake of creatine into skeletal muscle, and creatine stimulates muscle hypertrophy, one could theoretically ingest creatine only after exercise of muscle groups for which greater hypertrophy is desired (i.e., weaker or previously injured muscle groups). The purpose of this study was to determine whether greater increases in muscle thickness could be achieved by ingesting creatine, compared with placebo, immediately after exercise of muscle groups over a period of 6-wk training. This study used a unique design where subjects performed single-limb (arm and leg) training with one side of the body twice per week, immediately after which creatine was ingested. On two other days of the week, exercise was performed with the opposite limbs, after which placebo was ingested. We hypothesized that the limbs trained on the days creatine was ingested would show greater increases in muscle thickness than the limbs trained on days placebo was ingested.

A secondary purpose of this study was to determine whether males and females differed in their response to

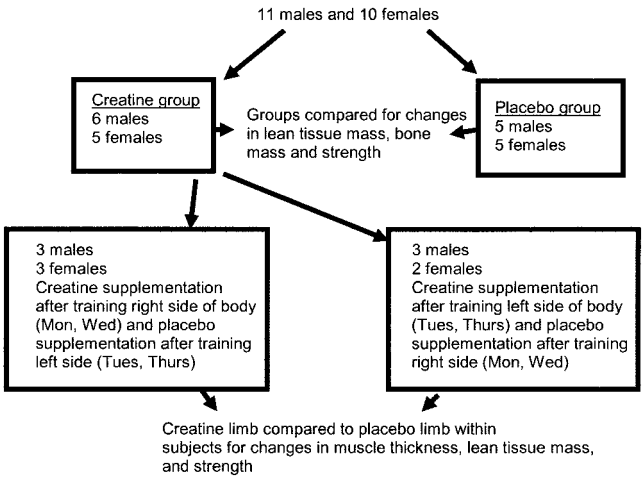

FIGURE 1—Flow diagram showing the study design.

creatine supplementation while engaged in resistance training. Studies involving male (4) and female (31) subjects engaged in resistance training indicate that creatine supplementation increases lean tissue mass compared with placebo. No study, however, has directly compared young male and female subjects supplementing with creatine and engaged in the same resistance training program. Acute creatine supplementation (5 d) without training results in a greater increase in lean tissue mass in males compared with females (22). Additionally, creatine supplementation has anti-catabolic actions in some proteins in men but not women (24). In the second part of the present experiment, we compared males and females supplemented with creatine to those supplemented with placebo for changes in lean tissue mass. We hypothesized that males would have a greater increase in lean tissue mass compared with females while supplementing with creatine during resistance training.

METHODS

Our study design is outlined in Figure 1 and described in detail below. After receiving approval from our institution's biomedical ethics review board for research in human subjects and obtaining written informed consent, 13 men and 11 women were randomized to receive creatine supplementation (seven males and six females; 0.2 g creatine·kg^{-1} body weight) or placebo (six males and five females; 0.2 g·kg^{-1} corn starch) and simultaneously engaged in a 6-wk resistance training program. This dose was consumed twice per week after training of only one side of the body (see design below and Fig. 1). The creatine was not consumed on nontraining days. The dose was only given twice per week because of the purpose of the study; that is, we wanted to assess the effect of creatine given only after training of specific muscle groups (i.e., one side of the body which was trained twice per week). The dose of 0.2 g·kg^{-1}·d^{-1} was chosen because it is equal to the dose we previously found to result in minimal excretion of creatine in the urine during the acute loading phase (5). None of the subjects had supplemented with creatine in the past month, none of the subjects were vegetarians, and all the females were eumenorrheic during the study. The creatine and cornstarch were mixed with 0.5 g·kg^{-1} of a maltodextrine/grape-flavored sucrose mixture to mask the taste of the creatine and placebo. One female and one male in the creatine group and one male in the placebo group dropped out of the study due to lack of time, leaving six males and five females in the creatine group (age = 24.6 ± 1.3 yr; mass = 77.2 ± 5.6 kg; height = 170 ± 3 cm; weight training experience = 5.5 ± 1.0 yr) and five males and five females in the placebo group (age = 29.6 ± 2.5 yr; mass = 74.6 ± 5.0 kg; height = 172 ± 3 cm; weight training experience = 5.8 ± 1.3 yr).

The resistance-training program involved unilateral exercises of the arms and legs. Bench press, elbow flexion, lat pull-down, shoulder press, and hip flexion, extension, abduction, and adduction were done on Lever weight-training equipment (Pulse Fitness Systems, Winnipeg, MB, Canada) and leg press, knee extension, and knee flexion on Hammer Strength training equipment (Life Fitness, Franklin Park, IL). Subjects trained the right side of the body 2 d·wk^{-1} (i.e., Monday and Wednesday) and the left side of the body on two alternating days per week (i.e., Tuesday and Thursday). To ensure that the nontraining side of the body was not substantially activated (i.e., by stabilizing isometric contractions), we measured electromyographic (EMG) activity over the active muscle group in the training limb and the contralateral muscle group in the nontraining limb during a training session in two subjects. We have previously described the EMG set-up in detail elsewhere (14). The nontraining side EMG mean absolute value, expressed as a percentage of the mean absolute value of the training side, averaged 3.2%, with a range from 1% (for bench press) to 4.6% (for shoulder press), indicating that the nontraining side was minimally activated during the unilateral training. Training was performed using a program where training volume (sets and repetitions) was progressively decreased and training intensity was progressively increased every 2 wk. During weeks 1 and 2, subjects performed four sets of 8–10 repetitions using 70–75% of one-repetition maximum (1-RM). During weeks 3 and 4, subjects performed four sets using a weight that could be lifted for six to eight repetitions. During weeks 5 and 6, subjects performed three sets using a weight that could be lifted for four to five repetitions. During each 2-wk block weight was progressively increased by increments of 1.1 kg once the upper target number of repetitions (i.e., 10 repetitions for weeks 1 and 2, eight repetitions for weeks 3 and 4, and five repetitions for weeks 5 and 6) could be performed with good form. We chose this program because we previously found it to be effective for inducing muscle hypertrophy (12). The length of the program was chosen because we have previously found that creatine supplementation produces increases in lean tissue mass within 6 wk (4,7).

To determine whether creatine supplementation immediately after exercise resulted in increased thickness only of the muscle groups engaged in exercise (hypothesis 1), the subjects in the creatine group were further randomized to receive creatine only after training of one side of the body

and placebo after training the other side of the body (Fig. 1). Six subjects (three males and three females) received creatine on the days they trained the right side of their body and five subjects (three males and two females) received creatine on days they trained the left side of their body. All subjects were right-handed. Subjects were instructed to consume the supplement immediately after their training session. Compliance was ensured by supervising the subjects while the supplement was consumed. Subjects and investigators were blinded to whether subjects were receiving creatine or placebo. This was achieved by having an individual that was not involved in training or data collection mix the supplements in plastic bags labeled with subjects' names. To assess the success of our blinding, subjects were given a questionnaire at the end of the study that asked which group they thought they were in (possible answers were "creatine," "placebo," or "don't know"). If they thought they were in the creatine group, they were then asked which days they thought they received creatine and which days they thought they received placebo. The questionnaire also included a list of potential side effects for subjects to identify. This side effects list was generated from a questionnaire we previously used to detect side effects during creatine supplementation (13).

Subjects were tested before and after training for muscle thickness (ultrasound) of the elbow flexors and knee extensors of both limbs, for whole-body and subregional lean tissue, fat, and bone mass by dual energy x-ray absorptiometry, and for unilateral bench press and leg press strength of both limbs. The elbow flexors and knee extensors were chosen as representative upper- and lower-body muscle groups because ultrasound measurements of these muscle groups are the most reproducible in our lab, and it is easy to achieve hypertrophy of these muscle groups with a relatively short duration of training (1,12,15).

Muscle thickness measures were performed on the day before any strength measures. Measurements were taken 3–5 d after the last training session to prevent any swelling in response to the last training session from contributing to the muscle thickness measurement. During this time, subjects were instructed not to participate in any other exercise sessions or intense activity. Muscle thickness was measured using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan). For the elbow flexors, a small mark was drawn on the lateral side of the arm to indicate exactly two-thirds of the distance down from the acromion process to the olecranon process. A tape measure was then wrapped around the arm at the two-thirds mark and used to mark another reference point on the bulk of the biceps, where the center of the ultrasound probe would be placed. Once the reference points were taken, each subject laid their arm flat down on a tabletop with their biceps facing upward and forearm supinated. For the knee extensors, the reference point for placement of the ultrasound probe was measured as two-thirds the distance from the greater trochanter to the lateral epicondyle and 3 cm lateral to the midline of the anterior thigh. Great care was taken using overhead transparency film and markings on the skin to ensure that identical sites were measured on each occasion.

A water-soluble transmission gel was applied to the measurement site and a 5-MHz ultrasound probe was placed on the scan site while not depressing the skin. Once the researcher was satisfied with the quality of the image produced, the image on the monitor was frozen. With the image frozen, a cursor was enabled in order to measure the thickness of the muscle groups (cm) at three sites: the proximal site, the mid site, and the distal site, as determined by the divisions (1 cm) on the monitor. The distal and proximal sites on the monitor were 6 cm apart with the mid site located equidistant between them. The mid site would correspond to where the reference mark was drawn on the muscle group.

The muscle thickness measure was extracted from the monitor image using a similar method as described by Abe et al. (1), with the distance from the subcutaneous adipose layer to the surface of the humerus bone taken as elbow flexor muscle thickness, and the distance from the subcutaneous adipose layer to the surface of the femur as knee extensor muscle thickness. Three muscle thickness measures were taken at each of the three sites (proximal, mid, and distal). The closest two values were then taken and averaged to achieve a final muscle thickness value for that site. If the two closest could not be determined by the previous three measures (i.e., high and low values were the same distance from the middle value), then a fourth measurement was performed. To arrive at an overall mean muscle thickness score, the muscle thicknesses at each site were averaged. Reproducibility of measurements of muscle thickness was determined on two separate days for 10 subjects (six males, four females). The coefficients of variation for elbow flexors and knee extensors muscle thickness were 1.4% and 0.8%, respectively.

Lean tissue, bone, and fat mass (excluding the head) were determined by dual energy x-ray absorptiometry on a Hologic-2000 densitometer in array mode and analyzed using system software version 7.01. Reproducibility was determined on 10 subjects on two separate occasions. The coefficients of variation for lean tissue, bone, and fat mass were 0.5%, 0.5%, and 3.0%, respectively. To confirm any changes in muscle thickness in the limbs we also assessed lean tissue mass in the individual upper and lower body limbs from the dual energy x-ray absorptiometry scans. We considered this a secondary measure to the muscle thickness measures because the reproducibility of lean tissue mass from single limbs is not as good as the muscle thickness measures (with coefficients of variation of 2.0–6.6%; 8).

Strength was assessed for unilateral leg press and bench press on each limb, in random order, by determining the 1-RM. A warm-up consisted of one set of eight repetitions with low weight. Weight was then progressively increased for each subsequent 1-RM attempt with a 2-min rest interval. The 1-RM was usually reached in four to six sets, including the warm-up set. Each test exercise for left and right unilateral leg press, and left and right unilateral bench press was separated by a 3-min rest interval. Reproducibility

of measurements of 1-RM was determined on two separate days for 10 subjects (6 males, 4 females). The coefficients of variation for unilateral leg press and unilateral bench press were 4.5% and 5.8%, respectively.

Statistical analyses. To determine whether there were differences between males or females or subjects assigned to creatine or placebo groups, baseline data was assessed by a two-factor (gender × supplement) ANOVA.

To test our first hypothesis (that the limbs trained on the days creatine was ingested would show greater increases in muscle size than the limbs trained on days placebo was ingested), muscle thickness, limb lean tissue mass, and unilateral strength changes were compared only within the group that received creatine. Absolute change scores for elbow flexor and knee extensor muscle thickness, arm and leg lean tissue mass, and unilateral leg press and bench press for each limb were determined by subtracting the baseline measurement from the measurement after 6 wk of training. Relative change scores were determined by dividing the absolute change score by the baseline score and multiplying by 100 to give a percentage. To evaluate differences between change scores of each limb (i.e., change in creatine limb vs change in placebo limb), we used a two-factor ANOVA, with one between-groups factor for gender and a within-group factor for limb (creatine vs placebo limb).

To test our second hypothesis (that males would have a greater increase in lean tissue mass while taking creatine compared with females), lean tissue mass and muscle thickness and strength scores (of limbs combined) were compared between male and female subjects in both the creatine and placebo groups. Secondary dependent variables of fat mass and bone mineral content (BMC) (mass) were also analyzed because fat mass has occasionally been shown to decrease with strength training (10) and bone mass occasionally increases with either strength training (11) or creatine supplementation (20). A three-factor ANOVA, with two between groups factors for gender (male vs females) and supplementation (placebo vs creatine), and one repeated factor for time (pre- vs posttraining) was used to assess dependent variables. To clarify the presentation of results, a two-factor ANOVA was used to compare absolute and relative change scores for the dependent variables with factors of gender (male vs females) and supplementation (placebo vs creatine). A Tukey's *post hoc* test was used to determine differences between means when significant interactions were found.

A chi-square analysis was used to determine whether side-effect frequencies were different between creatine and placebo groups. All results are expressed as means ± SEM. Significance was accepted at $\alpha \leq 0.05$.

RESULTS

Creatine group only: limbs exercised on days creatine was supplemented versus limbs exercised on days placebo was supplemented. The muscle thickness of the elbow flexors trained on days creatine was supplemented increased by 9.2 ± 2.0% (0.32 ± 0.07 cm), whereas the elbow flexors trained on days placebo was supplemented increased by 6.2 ± 1.8% (0.24 ± 0.07 cm). There was a trend for the difference between arms to be significant ($P = 0.071$). Upon inspection of the data, there was an outlier who had a muscle thickness in the creatine arm (5.3 cm) that was more than 2 SD higher at baseline compared with the mean of the rest of the group (3.5 cm, SD ±0.5 cm). Also, his change score for his creatine arm (−1.3%) was greater than 2 SD different than the mean change score for the rest of the group (+10.3%, SD ± 5.4%). After removal of this outlier, the increase in the creatine arm (10.3 ± 1.7%, 0.36 ± 0.06 cm) was greater than the increase in the placebo arm (6.3 ± 1.9%, 0.24 ± 0.07 cm) ($P < 0.02$; Fig. 2A). With the outlier included, the lean tissue mass, from regional analysis of the dual energy x-ray absorptiometry scans, increased more in the creatine

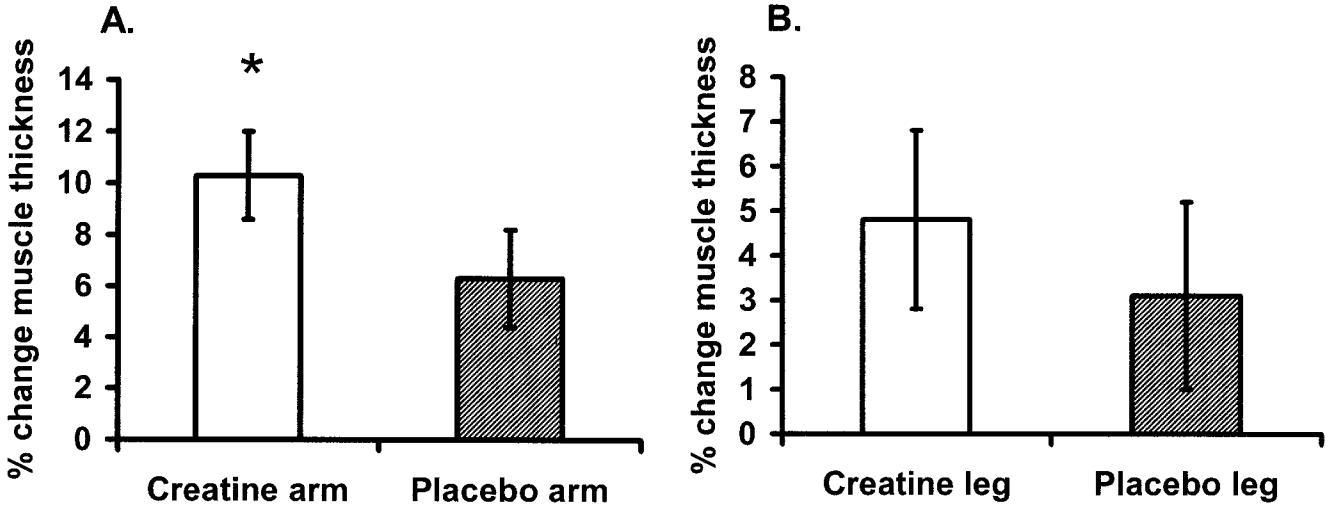

FIGURE 2—A. Relative changes in elbow flexors muscle thickness of arms trained on days creatine was supplemented vs arms trained on days placebo was supplemented. *Percent change in elbow flexors muscle thickness of the creatine arm was greater than the change in the placebo arm ($P < 0.02$) after removal of an outlier. B. Relative change in knee extensors muscle thickness of legs trained on days creatine was supplemented vs legs trained on days placebo was supplemented. Values are means ± SEM.

arm (12.8 ± 2.1%, 0.36 ± 0.07 kg) compared with the placebo arm (6.5 ± 1.5%, 0.20 ± 0.06 kg) ($P < 0.01$). For the absolute increase in muscle thickness of the arm flexors and lean tissue mass of the arms, there was a main effect for gender with males increasing more than females ($P < 0.05$) but no gender × limb interaction. For relative change, there was no difference between males and females.

The muscle thickness of the knee extensors trained on days creatine was supplemented increased by 4.8 ± 2.0% (0.22 ± 0.09 cm), which as not statistically different from the 3.1 ± 2.1% (0.14 ± 0.09 cm) increase in the knee extensors trained on days placebo was supplemented (Fig. 2B). Likewise, lean tissue mass from the legs region of the dual energy x-ray absorptiometry scan indicated no difference between increases in the creatine (6.2 ± 1.2%, 0.54 ± 0.11 kg) and placebo (6.2 ± 1.3%, 0.55 ± 0.14 kg) legs. For both absolute and relative increase in muscle thickness of the knee extensors and leg lean tissue mass, there was a main effect for gender with males increasing more than females ($P < 0.05$). Again there was no gender × limb interaction.

When elbow flexors and knee extensors muscle thicknesses were combined for each side of the body, the increase on the creatine side (7.1 ± 1.6%, 0.57 ± 0.11 cm) was greater than the increase on the placebo side (4.2 ± 1.6%, 0.36 ± 0.12 cm) ($P < 0.05$). However, the increase in lean tissue mass from combined arm and leg on the creatine side (7.8 ± 1.2%, 0.89 ± 0.16 kg) was not different from the placebo side (6.2 ± 1.2%, 0.75 ± 0.17 kg).

Bench press strength for the side of the body trained on days creatine was supplemented increased by 31 ± 6% (15 ± 1 kg), which was not different than the 33 ± 6% (15 ± 1 kg) increase in bench press strength for the side of the body trained on days placebo was supplemented. Leg press strength for the side of the body trained on days creatine was supplemented increased by 37 ± 8% (34 ± 6 kg), which was not different than the 28 ± 5% (29 ± 5 kg) increase in leg press strength for the side of the body trained on days placebo was supplemented.

Creatine and placebo groups: comparisons between males and females. There were no significant differences for baseline measurements between creatine and placebo groups. Males were taller compared with females (177 ± 2 vs 164 ± 2 cm; $P < 0.01$), had more lean tissue mass (54.8 ± 1.7 vs 38.6 ± 1.5 kg; $P < 0.01$), more bone mineral mass (2378 ± 88 vs 1717 ± 76 g; $P < 0.01$), greater elbow flexors muscle thickness (4.0 ± 0.1 vs 3.1 ± 0.1 cm), greater bench press 1-RM (151 ± 10 vs 61 ± 7 kg; $P < 0.01$), greater leg press 1-RM (153 ± 10 vs 72 ± 11 kg; $P < 0.01$), and there was a trend for males to be heavier (83.0 ± 5.8 vs 68.1 ± 3.4 kg; $P = 0.059$). Males and females were similar in age (26.2 ± 1.9 vs 27.9 ± 2.3 yr) and had similar fat mass (19.6 ± 4.4 vs 22.7 ± 2.5 kg), quadriceps muscle thickness (4.7 ± 0.2 vs 4.5 ± 0.2 cm), and weight training experience (5.2 ± 1.3 vs 6.0 ± 0.9 yr).

For body weight, there was a significant time main effect ($P < 0.01$), with groups collectively increasing by 1.8 ± 0.4 kg. Changes in body weight did not differ between creatine and placebo groups or males and females.

For lean tissue mass there was a significant supplement × sex × time interaction ($P < 0.02$). Males on creatine and males on placebo significantly increased lean tissue mass ($P < 0.01$), but neither females on creatine nor females on placebo increased lean tissue mass. Males on creatine had significantly greater absolute and relative (Fig. 3) increases in lean tissue mass compared with all other groups ($P < 0.01$). Changes for females on creatine were not different from females on placebo. There were no significant changes in fat mass for any groups over time.

For whole-body bone mineral mass, there was a significant sex × time interaction ($P < 0.02$). Males significantly increased bone mineral mass over time (by 39 ± 11 g; $P < 0.01$) whereas females had no increase (2 ± 7 g). Likewise, the relative increase for males was greater than the relative increase for females (1.8 ± 0.5% vs 0.1 ± 0.5%; $P < 0.05$). The greater increase in the males was due to a greater increase in the legs region (sex × time; $P < 0.01$). Males significantly increased bone mass of the legs (by 34 ± 7 g; $P < 0.01$) whereas females had no increase (10 ± 3 g). Likewise, the relative increase in the legs of males was greater than the increase for females (3.1 ± 0.7% vs 1.1 ± 0.5%; $P < 0.05$). There were no differences over time for bone mineral mass in any of the other regions (i.e., spine, pelvis, arms, and ribs).

For elbow flexors muscle thickness (arms combined), there was a sex × time ($P < 0.05$) interaction. Both males and females increased muscle thickness over the training program ($P < 0.05$), and males increased more than females (0.3 ± 0.1 vs 0.2 ± 0.1 cm). Relative changes between males and females were similar (8.5 ± 1.6 vs 5.4 ± 1.5%). There were no differences between creatine and placebo groups for absolute (0.3 ± 0.1 vs 0.2 ± 0.1 cm) or relative changes (7.7 ± 1.7 vs 6.3 ± 1.5%). For knee extensors' muscle thickness (legs combined), there was a time main effect ($P < 0.01$), with no differences in absolute increases between males and females (0.3 ± 0.1 vs 0.2 ± 0.1 cm) or subjects in creatine and placebo groups (0.2 ± 0.1 vs 0.3 ±

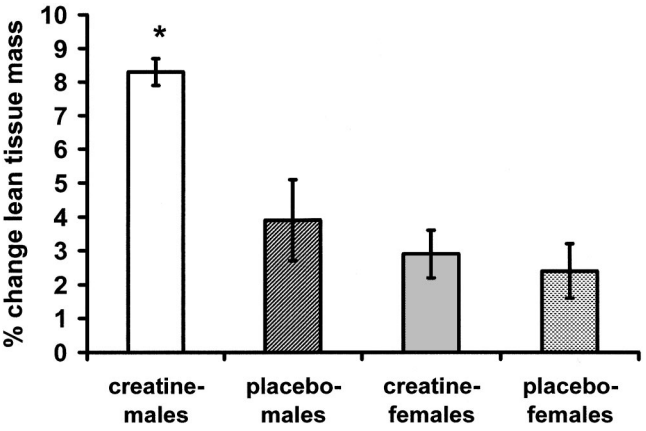

FIGURE 3— Relative change in lean tissue mass for males on creatine ($N = 6$), males on placebo ($N = 5$), females on creatine ($N = 5$), and females on placebo ($N = 5$). * Percent change in lean tissue of males on creatine was greater than all other groups ($P < 0.01$). Values are means ± SEM.

0.1 cm). Likewise, there were no differences in relative changes between males and females (6.3 ± 2.1 vs 3.3 ± 1.9%) or creatine and placebo groups (3.9 ± 1.7 vs 5.9 ± 2.4%).

For bench press strength (arms combined), there were supplement × time ($P < 0.01$) and sex × time ($P < 0.05$) interactions. All groups increased significantly over time ($P < 0.01$), with the creatine group having a greater increase in strength compared with the placebo group (29 ± 2 vs 17 ± 3 kg) and males having a greater increase in strength compared with females (27 ± 3 vs 19 ± 3 kg). There was a trend for the relative increase in strength to be greater for the creatine versus placebo group (32 ± 5 vs 21 ± 4%; $P = 0.052$), and females had a greater relative increase in strength compared with males (35 ± 6 vs 19 ± 2%; $P < 0.01$).

For leg press strength (legs combined), there was a sex × time ($P < 0.02$) interaction. Both males and females increased significantly over time ($P < 0.01$), with the increase in strength being greater for males compared with females (67 ± 8 vs 40 ± 6 kg). There was no difference in relative increases in strength between males and females (29 ± 4 vs 29 ± 5%). There were no differences between creatine and placebo groups for absolute (62 ± 9 vs 45 ± 7 kg) or relative changes in leg press strength (32 ± 4 vs 25 ± 3%).

From the questionnaire on the success of our blinding, of the 10 subjects in the placebo group, 3 correctly indicated they were in the placebo group, 5 incorrectly indicated they were in the creatine group, and 2 indicated they did not know which group they were in. Of 11 subjects in the creatine group, 6 correctly indicated they were in the creatine group, 3 incorrectly indicated they were in the placebo group, and 2 indicated they did not know which group they were in. Of the six subjects in the creatine group that correctly indicated they were in the creatine group, four were able to correctly identify the limbs trained on the days creatine was received. There were no significant differences for frequency of side effects between creatine and placebo groups.

DISCUSSION

The first major finding of this study is that individuals can increase thickness of upper (but not lower) limb musculature during a resistance training program by consuming creatine immediately after training sessions. Exercise before creatine supplementation enhances the uptake and storage of creatine in the specific muscles that are exercised. Previous studies showed that single-leg cycling followed by creatine supplementation resulted in a greater increase in creatine content of the exercised than the nonexercise leg (17,26). This greater creatine uptake has been attributed to an increased blood flow to the exercised limb or a greater activation of the sodium-potassium pump, where creatine is co-transported across the sarcolemma (26). Because creatine stimulates muscle hypertrophy (33), we predicted that muscles exercised immediately before creatine supplementation would show preferential increases in muscle thickness. In the current study, supplementation of creatine immediately after resistance training of one arm caused a greater increase in muscle thickness of the elbow flexors than when placebo was supplemented after training of the opposite contralateral arm (Fig. 2A). This greater increase in muscle thickness of the creatine arm was confirmed by a greater increase in lean tissue mass, measured by dual energy x-ray absorptiometry, compared with the placebo arm. There was a trend for the same result in the legs (knee extensors), but the difference between legs did not reach statistical significance (Fig. 2B). These results have practical implications for individuals that may want to preferentially increase size of muscle groups that may have been atrophied due to previous injury or disease (i.e., stroke). Indeed, creatine supplementation was recently found to increase muscle size to a greater extent than placebo during resistance training after limb immobilization by casting (18).

A limitation of our between-limb results is that the difference in elbow flexors muscle thickness for arms trained on days creatine was supplemented versus days placebo was supplemented was significant only after removal of an outlier. This outlier had an unusually large muscle thickness of his creatine arm at baseline and was the only subject to have a decrease in muscle thickness of this arm during the training program. His unusually high muscle thickness in this arm at baseline may have been due to measurement error, as his elbow flexor muscle thickness was actually greater than his knee extensor muscle thickness (all other subjects had substantially greater muscle thickness of the knee extensors compared with elbow flexors, as would be expected). If we included the outlier in our analysis we would have required a total of 24 subjects, approximately double than in the current study, to reach a level of statistical significance ($P = 0.05$) with a power of 80% (nQuery advisor release 3.0 study planning software, Statistical Solutions Ltd., Cork, Ireland). Our assumption that the outlier result may have been due to experimental error is supported by our finding that with the outlier included, the increase in lean tissue mass, assessed by dual energy x-ray absorptiometry, of the creatine arm was greater than the increase in the placebo arm.

Another limitation of the current study is the relatively short period of our training and supplementation (6 wk). Staron et al. (28) found little muscle fiber hypertrophy in men and women over 8 wk of resistance training when muscle fiber area was assessed at 2-wk intervals. The increase in muscle thickness during our protocol may be due to water retention with the creatine supplementation. Intracellular water has been shown to increase with creatine supplementation when measured by bioelectrical impedance or magnetic resonance spectroscopy (2,27), but others have not found an increase in intracellular water (relative to extracellular water) using either bioelectrical impedance (5,16) or deuterium oxide and sodium bromide dilution (25).

Unlike the muscle thickness measurements, there were no differences in strength after the training program for limbs trained on days creatine was supplemented compared with limbs trained on days placebo was supplemented. Changes in strength are due to changes in both muscle size and neural factors. During short-duration training, as in the current study, early increases in strength are more likely due to neural factors (9). In addition, when using a within-subjects

design to compare contralateral limbs, results may be affected by "cross-education," a neural phenomenon where training of one limb contributes to the increase in strength of the opposite contralateral limb (14).

The second major finding of this study was that creatine supplementation during resistance training increased lean tissue mass in males but not females (Fig. 3). Creatine supplementation has been shown to enhance the increase in lean tissue mass during resistance training in males (4) and females (31), but only one previous study has compared males and females on the same supplementation and resistance training program, but this was in a population of older adults (3). Ours is the first study to compare young male and female subjects, with similar training backgrounds for increases in lean tissue mass during resistance training and creatine supplementation. Our results are consistent with one previous study where acute loading with creatine (without exercise training) increased lean tissue mass more in males than females (22). The greater responsiveness in males may be due to a greater inhibition of muscle catabolism with creatine supplementation compared with females. Parise et al. (24) showed that nine days of creatine supplementation reduced leucine oxidation and plasma leucine rate of appearance in men but not women.

Contrary to our finding for lean tissue mass, creatine supplementation did not result in greater increases in muscle thickness in the creatine compared with the placebo group. We only assessed muscle thickness in two muscle groups; perhaps a small increase with creatine supplementation in mass of multiple muscle groups would result in an overall increase in whole-body lean tissue mass. When we summed the muscle thickness scores across the elbow flexors and knee extensors, the pattern of increase in total muscle thickness across groups mirrored the increases in lean tissue mass. There was a trend for the increase in muscle thickness in males on creatine to be greater than all other groups; however, this did not reach statistical significance ($P = 0.18$).

Creatine supplementation was more effective than placebo for increasing bench press strength. Unlike the increase in lean tissue mass, creatine was equally effective for increasing strength in both males and females. This is in agreement with one other study that found an equal enhancement of high-intensity exercise performance after creatine supplementation in males and females (30). Perhaps the increase in intramuscular creatine and phosphocreatine stores are effective for improving exercise performance in both genders but only effective for increasing lean tissue mass in males. Again, our study is limited in that we did not collect muscle biopsies and this remains a topic for future research. It is unclear why creatine supplementation enhanced bench press but not leg press strength, but this is in agreement with other studies from our lab (4). Increases in leg press strength may be more dependent on neural mechanisms than increases in bench press strength.

A limitation of our gender comparison was the low number of subjects used in the study and the fact we used relatively well-trained individuals. Untrained individuals may respond differently to supplementation as they would have a greater potential for improvement in lean tissue mass and muscular strength. Another limitation is the relatively low dose of creatine used in the current study. Because of the design of the study, with the main purpose of determining the responsiveness to supplementation only after exercise training, creatine was consumed only twice per week at a dose of 0.2 $g \cdot kg^{-1}$ body mass, which resulted in an intake of about 15.5 g for each of the 2 d. This amounts to approximately 4.5 $g \cdot d^{-1}$ if averaged over an entire week. This is different from the standard practice of including a "loading" phase where subjects consume ~20–25 $g \cdot d^{-1}$ for 5–7 d and a "maintenance" phase of ~5 $g \cdot d^{-1}$ thereafter (16,33). However, our lab (7) and others (3) have excluded the loading phase and used a continuous low dose (i.e., 5–7.7 g $Cr \cdot d^{-1}$) to increase lean tissue mass and muscular strength over placebo-supplemented groups.

We evaluated side effects of creatine supplementation with a questionnaire that was developed based on anecdotally reported side effects (19). We previously used this questionnaire to detect some side effects (i.e., cramping, muscle pulls, and strains) in an older group of males on creatine supplementation (13). In the current study, with a younger group and a smaller dose of creatine supplementation, these side effects were not evident. Others have demonstrated that higher doses of creatine in young subjects are also without side effects (32).

Similar to previous comparisons of males and females (23,28), we found males to have greater absolute increases in strength and muscle size (muscle thickness of the arms), whereas relative (%) increases were similar between the genders and for one measurement (bench press) greater in females. The only exception was that absolute increase in muscle thickness of the knee extensors was similar in males and females.

An interesting finding of the current study was that males increased BMC (mass) whereas females did not. This increase was restricted to the legs region. The increase in BMC of males was small (i.e., 1.8% for the whole body and 3.1% for the legs), and this is most likely due to the short duration of training. Our results are supported by one other study that found a greater increase in bone mineral during resistance training in older males compared with females (21). The greater responsiveness in males may be due to greater hormonal responsiveness with training (28).

In conclusion, creatine supplementation immediately after resistance training resulted in enhancement of muscle thickness of the elbow flexors, but not knee extensors, over a 6-wk training program. In addition, creatine was more effective for increasing lean tissue mass in males compared with females during resistance training. Future studies could evaluate whether creatine supplementation is beneficial for increasing muscle mass in limbs that are atrophied due to disease or disuse. Changes in hormone responsiveness and properties of muscle fibers (i.e., transcription factors, metabolite concentrations) should be assessed to determine reasons for the greater increases in lean tissue mass with creatine supplementation in males compared with females.

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REFERENCES

1. ABE, T., D. V. DEHOYOS, M. L. POLLOCK, and L. GARZARELLA. Time course for strength and muscle thickness changes following upper and lower body resistance training in men and women. *Eur. J. Appl. Physiol.* 81:174–180, 2000.
2. BRILLA, L. R., M. S. GIROUX, A. TAYLOR, and K. M KNUTZEN. Magnesium-creatine supplementation effects on body water. *Metabolism* 52:1136–1140, 2003.
3. BROSE, A., G. PARISE, and M. A. TARNOPOLSKY. Creatine supplementation enhances isometric strength and body composition improvements following strength exercise training in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 58:11–19, 2003.
4. BURKE, D. G., P. D. CHILIBECK, K. S. DAVISON, D. G. CANDOW, J. FARTHING, and T. SMITH-PALMER. The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength. *Int. J. Sport Nutr. Exerc. Metab.* 11:349–364, 2001.
5. 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.* 35:1946–1955, 2003.
6. BURKE, D. G., P. D. CHILIBECK, G. PARISE, M. A. TARNOPOLSKY, and D. G. CANDOW. Effect of α-lipoic acid combined with creatine monohydrate on human skeletal muscle creatine and phosphagen concentration. *Int. J. Sport Nutr. Exerc. Metab.* 13:297–305, 2003.
7. BURKE, D. G., S. SILVER, L. E. HOLT, T. SMITH PALMER, C. J. CULLIGAN, and P. D. CHILIBECK. The effect of continuous low dose creatine supplementation on force, power, and total work. *Int. J. Sport Nutr. Exerc. Metab.* 10:235–244, 2000.
8. CHILIBECK, P., A. CALDER, D. G. SALE, and C. WEBBER. Reproducibility of dual-energy x-ray absorptiometry. *Can. Assoc. Radiol. J.* 45:297–302, 1994.
9. CHILIBECK, P. D., A. W. CALDER, D. G. SALE, and C. E. WEBBER. A comparison of strength and muscle mass increases during resistance training in young women. *Eur. J. Appl. Physiol.* 77:170–175, 1998.
10. CHILIBECK, P. D., K. S. DAVISON, S. J. WHITING, Y. SUZUKI, C. L. JANZEN, and P. PELOSO. The effect of strength training combined with bisphosphonate (etidronate) therapy on bone mineral, lean tissue, and fat mass in postmenopausal women. *Can. J. Physiol. Pharmacol.* 80:941–950, 2002.
11. CHILIBECK, P. D., D. G. SALE, and C. E. WEBBER. Exercise and bone mineral density. *Sports Med.* 19:103–122, 1995.
12. CHILIBECK, P. D., D. G. SYROTUIK, and G. J. BELL. The effect of strength training on estimates of mitochondrial density and distribution throughout muscle fibres. *Eur. J. Appl. Physiol.* 80:604–609, 1999.
13. CHRUSCH, M. J., P. D. CHILIBECK, K. E. CHAD, K. S. DAVISON, and D. G. BURKE. Creatine supplementation combined with resistance training in older men. *Med. Sci. Sports Exerc.* 33:2111–2117, 2001.
14. FARTHING, J. P., and P. D. CHILIBECK. The effect of eccentric training at different velocities on cross-education. *Eur. J. Appl. Physiol.* 89:570–577, 2003.
15. FARTHING, J. P., and P. D. CHILIBECK. The effects of eccentric and concentric training at different velocities on muscle hypertrophy. *Eur. J. Appl. Physiol.* 89:578–586, 2003.
16. FRANCAUX, M., and J. R. POORTMANS. Effects of training and creatine supplement on muscle strength and body mass. *Eur. J. Appl. Physiol.* 80:165–168, 1999.
17. HARRIS, R. C., K. SODERLUNG, and E. HULTMAN. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin. Sci.* 83:367–374, 1992.
18. HESPEL, P., B. OP'T EIJNDE, M. VAN LEEMPUTTE, et al. Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and alters the expression of muscle myogenic factors in humans. *J. Physiol.* 536:625–633, 2001.
19. JUHN, M. S., and M. TARNOPOLSKY. Potential side effects of oral creatine supplementation: a critical review. *Clin. J. Sport Med.* 8:298–304, 1998.
20. LOUIS, M., J. LEBACQ, J. R. POORTMANS, et al. Beneficial effects of creatine supplementation in dystrophic patients. *Muscle Nerve* 27:604–610, 2003.
21. MADDALOZZO, G. F., and C. M. SNOW. High intensity resistance training: effects on bone in older men and women. *Calcif. Tissue Int.* 66:399–404, 2000.
22. MIHIC, S., J. R. MACDONALD, S. MCKENZIE, and M. A. TARNOPOLSKY. 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.* 32:291–296, 2000.
23. O'HAGAN, F. T., D. G. SALE, J. D. MACDOUGALL, and S. H. GARNER. Response to resistance training in young women and men. *Int. J. Sports Med.* 16:314–321, 1995.
24. PARISE, G., S. MIHIC, D. MACLENNAN, K. E. YARASHESKI, and M. A. TARNOPOLSKY. Effects of acute creatine monohydrate supplementation on leucine kinetics and mix-muscle protein synthesis. *J. Appl. Physiol.* 91:1041–1047.
25. POWERS, M. E., B. L. ARNOLD, A. L. WELTMAN, et al. Creatine supplementation increases total body water without altering fluid distribution. *J. Athl. Train.* 38:44–50, 2003.
26. ROBINSON, T. M., D. A. SEWELL, E. HULTMAN, and P. L. GREENHAFF. Role of submaximal exercise in promoting creatine and glycogen accumulation in human skeletal muscle. *J. Appl. Physiol.* 87:598–604, 1999.
27. SAAB, G., G. D. MARSH, M. A. CASSELMAN, and R. T. THOMPSON. Changes in human muscle transverse relaxation following short-term creatine supplementation. *Exp. Physiol.* 87:383–389, 2002.
28. STARON, R. S., D. L. KARAPONDO, W. J. KRAEMER, et al. Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. *J. Appl. Physiol.* 76:1247–1255, 1994.
29. STEENGE, G. R., E. J. SIMPSON, and P. L. GREENHAFF. Protein-and carbohydrate-induced augmentation of whole body creatine retention in humans. *J. Appl. Physiol.* 89:1165–1171, 2000.
30. TARNOPOLSKY, M. A., and D. P. MACLENNAN. Creatine monohydrate supplementation enhances high-intensity exercise performance in males and females. *Int. J. Sport Nutr. Exerc. Metab.* 10:452–463, 2000.
31. VANDENBERGHE, K., M. GORIS, P. VAN HECKE, M. VAN LEEMPUTTE, L. VANGERVEN, and P. HESPEL. Long-term creatine intake is beneficial to muscle performance during resistance training. *J. Appl. Physiol.* 83:2055–2063, 1997.
32. VOLEK, J. S., N. D. DUNCAN, S. A. MAZZETTI, M. PUTUKIAN, A. L. GOMEZ, and W. J. KRAEMER. No effect of heavy resistance training and creatine supplementation on blood lipids. *Int. J. Sport Nutr. Exerc. Metab.* 10:144–156, 2000.
33. VOLEK, J. S., N. D. DUNCAN, S. A. MAZZETTI, et al. Performance and muscle fiber adaptations to creatine supplementation and heavy resistance training. *Med. Sci. Sports Exerc.* 31:1147–1156, 1999.
34. VOLEK, J. S., W. J. KRAEMER, J. A. BUSH, et al. Creatine supplementation enhances muscular performance during high-intensity resistance exercise. *J. Am. Diet. Assoc.* 97:765–770, 1997.