Comparison of Creatine Ingestion and Resistance Training on Energy Expenditure and Limb Blood Flow

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This study determined the effects of 28 days of oral creatine ingestion (days 1 to 5 = 20g/d; [5 g 4 times daily]: days 6 to 28 = 10 g/d; [5 g twice daily]) alone and with resistance training (5 hours/week) on resting metabolic rate (RMR), body composition, muscular strength (1RM), and limb blood flow (LBF). Using a double-blind, placebo-controlled design, 30 healthy male volunteers (21 ± 3 years; 18 to 30 years) were randomly assigned to 1 of 3 groups; pure creatine monohydrate alone (Cr; n = 10), creatine plus resistance training (Cr-RT; n = 10), or placebo plus resistance training (P-RT; n = 10). Body composition (DEXA, Lunar DPX-IQ), body mass, bench, and leg press 1RM (isotonic), RMR (indirect calorimetry; ventilated hood), and forearm and calf LBF (venous occlusive plethysmography) were obtained on all 30 subjects on 3 occasions beginning at approximately 6:00 AM following an overnight fast and 24 hours removed from the last training session; baseline (day 0), and 7 days and 29 days following the interventions. No differences existed among groups at baseline for any of the variables measured. Following the 28-day interventions, body mass (Cr, 73.9 ± 11.5 *v* 75.6 ± 12.5 kg; Cr-RT, 78.8 ± 6.7 *v* 80.8 ± 6.8 kg; *P* < .01) and total body water (Cr, 40.4 ± 6.8 *v* 42.6 ± 7.2 L, 5.5%; Cr-RT, 40.6 ± 2.4 *v* 42.3 ± 2.2 L, 4.3%; *P* < .01) increased significantly in Cr and Cr-RT, but remained unchanged in P-RT, whereas, fat-free mass (FFM) increased significantly in Cr-RT (63 ± 2.8 *v* 64.7 ± 3.6 kg; *P* < .01) and showed a tendency to increase in Cr (58.1 ± 8.1 *v* 59 ± 8.8 kg; *P* = .07). Following the 28-day period, all groups significantly increased (*P* < .01) bench (Cr, 77.3 ± 4 *v* 83.2 ± 3.6 kg; Cr-RT, 76.8 ± 4.5 *v* 90.5 ± 4.5 kg; P-RT, 76.0 ± 3.4 *v* 85.5 ± 3.2 kg), and leg press (Cr, 205.5 ± 14.5 *v* 238.6 ± 13.2 kg; Cr-RT, 167.7 ± 13.2 *v* 238.6 ± 17.3 kg; P-RT, 200.5 ± 9.5 *v* 255 ± 13.2 kg) 1RM muscular strength. However, Cr-RT improved significantly more (*P* < .05) on the leg press 1RM than Cr and P-RT and the bench press 1RM than Cr (*P* < .01). Calf (30%) and forearm (38%) LBF increased significantly (*P* < .05) in the Cr-RT, but remained unchanged in the Cr and P-RT groups following the supplementation period. RMR expressed on an absolute basis was increased in the Cr (1,860.1 ± 164.9 *v* 1,907 ± 173.4 kcal/d, 2.5%; *P* < .05) and Cr-RT (1,971.4 ± 171.8 *v* 2,085.7 ± 183.6 kcal/d, 5%; *P* < .05), but remained unchanged from baseline in P-RT. Total cholesterol decreased significantly in Cr-RT (-9.9%; 172 ± 27 *v* 155 ± 26 mg/dL; *P* < .01) compared with Cr (174 ± 46 *v* 178 ± 43 mg/dL) and P-RT (162 ± 32 *v* 161 ± 36 mg/dL) following the 28-day intervention. These findings suggest that the addition of creatine supplementation to resistance training significantly increases total and fat-free body mass, muscular strength, peripheral blood flow, and resting energy expenditure and improves blood cholesterol.

MOST OF THE RESEARCH examining the effects of oral creatine ingestion as an ergogenic aid has shown an improvement in physical performance and body composition measures. These investigations have reported an elevated skeletal muscle creatine and phosphocreatine content following both short-term (5 to 7 days)[1-3] and longer-term (10 to 12 weeks)[4,5] oral creatine ingestion. Interestingly, the cellular elevation in creatine content following creatine ingestion coincides with enhanced physical performance and body composition changes,[4-6] although this finding is not universal.[7] Studies examining the short-term effects of oral creatine ingestion alone on physical performance and body composition measures show an improvement in intermittent high-intensity activities, including 1-repetition muscular strength[6,8,9-11], and increased body mass.[11,12]

A relatively small number of investigations have examined the longer-term (≥ 28 days) effects of creatine ingestion in combination with resistance training on measures of physical performance and body composition. These studies have shown an increase in muscular strength and fat-free mass (FFM),[4,5,13-15] as well as muscle fiber hypertrophy.[5,16] There is little information directly comparing the relative effectiveness of longer-term (≥ 28 days) creatine ingestion alone compared with creatine in combination with resistance training and resistance training alone on muscular strength and body composition. It is presently unknown what effect creatine ingestion (28 days) and resistance training may have on resting metabolic rate (RMR) and peripheral limb blood flow (LBF). Recent studies from our laboratory and others have demonstrated an elevated resting metabolic rate[17] and limb blood flow in the exercise-trained state.[17,18] Of further interest are the research findings showing an enhancement of the exercise-trained state in subjects consuming creatine as demonstrated by greater improvements in muscular strength,[11] FFM,[19] and skeletal muscle hypertrophy[5] compared with placebo ingestion. Thus, we hypothesize that creatine ingestion may be an additional contributing factor to the increase in RMR and LBF independent of exercise training status and adaptation. A plausible physiologic explanation of creatine's role in manifesting these changes in RMR and LBF may be related to the simultaneous increase in FFM and total body water (TBW),[20,21] both

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of which may lead to alterations in hemodynamic variables that augment blood flow parameters and resting energy expenditure.

Of further interest, is the recent finding that creatine ingestion alone may modulate lipid metabolism, as evidenced by a lowering of total cholesterol following 4 and 8 weeks of supplementation,[22] although others have shown no effect following 12 weeks.[23] Therefore, the purposes of this study were to systematically determine whether creatine ingestion (28 days) increases muscular strength and FFM independent of strength training and to determine the effects of creatine ingestion on RMR, peripheral blood flow, and blood lipids alone and in combination with resistance training.

MATERIALS AND METHODS

Subjects

Thirty healthy active, but resistance-untrained, males (21 ±3 years) volunteered to participate in the study after being informed of the experimental procedures and signing informed consent statements in adherence with the Human Subjects guidelines of Skidmore College. Only individuals who had not engaged in a structured resistance exercise training program (resistance training less than 1 time/week) and had not ingested any nutritional supplements containing creatine monohydrate for 6 months prior to the study were eligible for participation. All subjects met the following criteria for participation in the study: nonsmoker, no history of cardiovascular disease, no medication that could influence cardiovascular or metabolic function, no history of diabetes mellitus, renal failure, or liver disease, normal blood pressure (<140/90 mm Hg), nonobese (body mass index [BMI] <30), and no orthopedic problems that would impede performing the exercises.

Experimental Design and Procedures

In a double-blind, randomized fashion, subjects were assigned to 1 of 3 groups: creatine only (Cr, days 1 to 5 = 20 g/d; days 6 to 28 = 10 g/d); creatine plus resistance training (Cr-RT, identical protocol as Cr), and resistance training only (P-RT) (placebo administered in similar manner). Physiologic parameters were measured on 3 separate occasions pre, following 7 days, and 28 days of supplementation. Subject physical characteristics of the Cr, Cr-RT, and P-RT groups, respectively, were (mean ± SD): age, 24 ± 3, 20 ± 3, and 20 ± 1 years; height, 179 ± 9, 183 ± 4 and 179 ± 7 cm; body mass, 74.0 ± 11.4, 78.9 ± 6.7, and 78.8 ± 11.3 kg.

Supplementation. Both the creatine monohydrate and placebo (dextrose) supplements were prepared in powder form with similar texture, taste, and appearance and were independently packaged in generic foil packets for double-blind administration. The Cr and Cr-RT groups ingested 5 g of creatine monohydrate dissolved in a flavored dextrose drink mix, 4 times daily (20 g/d) for 5 days followed by 10 g/day (5 g twice daily) for 23 days. The P-RT group ingested the placebo drink 4 times daily for 5 days and twice daily for 23 days. Supplement packets were administered in blindly coded large envelopes containing a 2-week supply of the supplements. Although no objective measure of compliance in taking the supplements was performed, verification of subject compliance was attempted by having subjects return all empty packets on a regular basis to a research assistant. Subjects were informed that in order to receive the incentive for participating in the study, all empty packets must be returned.

Body mass and height. Total body mass (TBM) was determined following an overnight fast and urine void on the morning of each test session with subjects clothed in shorts and tee shirt using a calibrated balance beam scale with a precision of ±0.1 kg. Height was assessed to the nearest 0.10 cm using a sliding vertical scale stadiometer.

Blood chemistry. Morning fasted blood was obtained via a finger stick and subsequently analyzed for triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) concentrations, using the Cholestech LDX blood analysis system (Hayward, CA). Low-density lipoprotein cholesterol (LDL-C) was calculated using standard equations. The intra- and interassay variances were less than 5%.

Three-day food diary. Energy, macronutrient, and vitamin consumption were determined from a 3-day food diary during the presupplementation period, and subjects were provided a copy of their food diary and instructed to maintain similar compositions and intakes during the 28-day supplementation period. Briefly, each subject was asked to weigh and record all foods and beverages ingested for 2 weekdays and 1 weekend day. Particular emphasis was placed on the importance of maintaining typical eating habits and describing foods and method of preparation in accurate detail. The Nutritionist IV for Windows computer program (N-Squared Computing, First DataBank Division, The Hearst Corporation; 4.0version, San Bruno, CA) was used for analysis.

One-repetition maximum muscular strength of chest and legs (1RM). One-repetition maximum (1RM) bench press and leg press were performed twice (baseline day 0 and postday 28) on all subjects. Prior to 1-RM strength testing, trained personnel supervised a 2-week familiarization period. After a 5- to 10-minute warm-up, subjects performed 1RM leg and bench press using standard methods. An attempt in the leg press and bench press was considered successful when completed through a full range of motion without deviating from proper technique and form.

Body composition and total body water (TBW). FFM and fat mass (FM) were determined by dual energy x-ray absorptiometry (DEXA, model DPX-IQ; Lunar, Madison, WI). Percent body fat was calculated by dividing the amount of measured FM by total scanned mass (sum of fat-free soft tissue mass, bone mass, and FM). Quality control (QC) and assurance (QA) calibration tests were performed on a regular basis before each testing session. QC tests used a spine phantom that was scanned in the various modes. Subjects were positioned for scans according to standardized procedures during the initial scan.

Whole body resistance was measured with subjects lying supine on a nonconductive table in a thermal-neutral environment (22°C) with arms and legs slightly abducted. Subjects were fasted (12 hours) and refrained from alcohol, caffeine, and strenuous exercise for 24 hours. All bioimpedance measurements followed standardized procedures[20,21] and were obtained immediately following the 30-minute RMR test using a multifrequency bioelectrical impedance analyzer (Xitron 4000 Bioimpedance Analyzer; Xitron Technologies, San Diego, CA) during a logarithmic sweep of frequencies ranging from 1 to 500 kHz. TBW was calculated using the equations of Deurenberg et al.[24] Values for TBW are expressed in liters.

RMR. RMR was measured with a computerized open-circuit indirect calorimeter (Sensormedics Metabolic Cart model 2900, Yorba Linda, CA) for 30 to 45 minutes following an overnight stay in the Human Performance Laboratory. Briefly, subjects were supine and a plexiglass hood was placed over their head and attached to the metabolic cart. Energy expenditure (kcal/min) was calculated from the equation of Weir.[25] The test-retest intraclass correlation for RMR in our laboratory is greater than 0.90.[26]

LBF. Procedures for the measurement of LBF to the forearm and calf have been described previously.[17] All blood flow measurements were obtained with a Hokanson EC-5R plethysmograph (Bellevue, WA). Mercury-filled strain gauges were placed around the maximum circumference of the calf and forearm. A 10-cm venous occlusion cuff was placed around the upper arm and a 12-cm venous occlusion cuff was placed around the upper thigh. The occlusion cuff was inflated to approximately 60 mm Hg for 5 to 8 seconds to impede outflow to the

distal part of the limb using a rapid cuff inflator (forearm model no. SC12; calf no. CC22), which achieved inflation in less than 0.5 seconds. The changes in limb circumference were recorded on the EC-5R graph recorder. Blood flow was calculated as $mL.100mL^{-1}.min^{-1}$. The intraclass correlation and coefficient of variation (CV) for forearm and leg blood flow using test-retest in 15 volunteers reached 0.79% and 5.5% and 0.97% and 4.0%, respectively, in our laboratory.[27]

Resistance exercise training intervention. Subjects in the Cr-RT and P-RT began their resistance training the same day they started supplementation, whereas Cr subjects engaged in no resistance training during the 28-day period. Resistance training sessions consisted of heavy resistance workouts using a combination of free weights and machines (Titan Exercise Equipment, Carrollton, TX) and were supervised by trained personnel. Following a 2-week familiarization period in the weight room, subjects performed 1RM tests of the following 10 exercises to determine initial strength levels: leg press, leg curl, leg extension, calf raises, bench press, lat-pull down, military press, arm curl, arm extension, and abdominal crunch. Subjects assigned to the resistance lifting groups (Cr-RT, P-RT) lifted 3 days/week, performing 2 sets of 10 repetitions at 70% of their 1RM. An additional third set was performed until failure. Resistance on a given exercise was increased by 5% upon successful completion of 10 repetitions on the third set. Subjects were instructed not to engage in any other forms of intense physical training outside of the exercises performed in this investigation so that the training response would be optimized.

Statistical analysis. Resting metabolic rate, blood flow, hematologic, and body composition data were analyzed by a 3×3 repeated measures analysis of variance (ANOVA) to test for the effects of creatine and placebo ingestion among the 3 groups during the 3 (day 0, 7, and 28) test days (SPSS for Windows Version 10.1 software, Chicago, IL). 1RM data was analyzed using a 2-way analysis of variance with repeated measures. Delta scores (pre and postvalues) were calculated on selected variables and analyzed by a 1-way ANOVA. Data are presented as mean ± SD. Tukey's post hoc comparisons were performed to locate mean differences whenever an interaction was found. Statistical power ranged from 0.70 to 0.90 at a P value equal to .05. Data were considered significant when the probability of error was .05 or less.

RESULTS

Table 1 shows the ANOVA marginal main effects mean for group and time for body composition, RMR, and blood flow parameters. The only main effect for groups was RMR, in which the P-RT group was significantly greater than the Cr and Cr-RT groups. For the main effects due to time, statistical differences were found for BM, FFM, FM, and RMR denoting that day 28 values were greater than day 0 values.

Table 2 shows the ANOVA group × time interaction means for body composition, RMR, and blood flow parameters. Body mass increased at day 7 for Cr-RT and at day 28 for Cr and Cr-RT ($P < .01$). FFM increased significantly from baseline from day 7 and 28 only in the Cr-RT group. FM and percent body fat remained unchanged among the groups throughout the intervention period. TBW changes expressed as liters increased over baseline values at day 28 for Cr and Cr-RT ($P < .01$). Calf blood flow and fetal blood flow rates increased significantly at day 7 only in the Cr-RT and remained elevated at day 28. RMR was elevated at day 28 over day 0 values in the Cr and Cr-RT groups.

1RM for the bench and leg press was obtained only at baseline (day 0) and posttest (day 28) days. Thus, only comparisons among groups for these 2 days are presented. Following the 28-day treatment period, all groups significantly increased ($P < .01$) bench press (Cr, 77.3 ± 4 *v* 83.2 ± 3.6 kg, 7.6%; Cr-RT, 76.8 ± 4.5 *v* 90.5 ± 4.5 kg, 17.8%; P-RT, 76.0 ± 3.4 *v* 85.5 ± 3.2 kg, 12.5%) and leg press (Cr, 205.5 ± 14.5 *v* 239.0 ± 13.2 kg, 16.3%; Cr-RT, 168.0 ± 13.2 *v* 238.6 ± 17.3 kg, 42%; P-RT, 200.5 ± 9.5 *v* 255.0 ± 13.2 kg, 27%) strength, although Cr-RT improved significantly more on the leg press than Cr (44% *v* 18%, $P < .01$) and P-RT (44% *v* 27%, $P < .05$) and significantly more on bench press than Cr (18.4% *v* 8%, $P < .01$). It is important to note that even after correcting for body mass changes following the intervention (analysis of covariance [ANCOVA]), leg press strength, but not bench press strength, remained significantly greater in Cr-RT than the other 2 groups (data not shown).

Lipid profile analysis showed that total cholesterol decreased significantly in Cr-RT (-9.9%; 172 ± 27 *v* 155 ± 26 mg/dL; $P < .01$) compared with Cr (174 ± 46 *v* 178 ± 43 mg/dL) and P-RT (162 ± 32 *v* 161 ± 36 mg/dL). There were no significant differences in the other hematologic variables, either within or between groups following the treatment period. In addition, no significant differences were observed at baseline among groups for total energy (Cr, 2,430 ± 727; Cr-RT, 2,977 ± 731; P-RT,

Table 1. ANOVA Marginal Main Effect Means for Group and Time for Body Composition, Energy Expenditure, and Blood Flow Parameters

	Marginal Main Effect Means for Groups			Marginal Main Effect Means for Time		
	Cr	Cr-RT	P-RT	Day 0	Day 7	Day 28
BM (kg)	74.8 ± 3.2[a]	80.5 ± 3.2[a]	78.8 ± 3.2[a]	77.2 ± 1.8[a]	78.4 ± 1.8[a,b]	78.5 ± 1.9[b]
FFM (kg)	58.7 ± 2.1[a]	64.1 ± 2.1[a]	62.7 ± 2.1[a]	61.1 ± 1.1[a]	62.2 ± 1.2[b]	62.1 ± 1.2[b]
FM (kg)	12.7 ± 1.7[a]	12.9 ± 1.7[a]	12.9 ± 1.7[a]	12.8 ± 1.0[a]	12.6 ± 1.0[a]	13.1 ± 1.0[b]
% Fat	16.9 ± 1.6[a]	16.2 ± 1.6[a]	16.1 ± 1.6[a]	16.5 ± 0.9[a]	16.1 ± 1.0[a]	16.6 ± 0.9[a]
TBW (L)	40.0 ± 2.0[a]	41.6 ± 1.8[a]	40.5 ± 1.7[a]	40.2 ± 1.0[a]	40.5 ± 1.2[a]	41.4 ± 1.1[a]
Calf blood flow ($mL \cdot 100\ mL^{-1} \cdot min^{-1}$)	2.74 ± 0.23[a]	2.67 ± 0.24[a]	2.97 ± 0.24[a]	2.70 ± 0.15[a]	2.82 ± 0.16[a]	2.96 ± 0.19[a]
Forearm blood flow ($mL \cdot 100\ mL^{-1} \cdot min^{-1}$)	2.99 ± 0.38[a]	3.68 ± 0.38[a]	3.51 ± 0.38[a]	3.12 ± 0.29[a]	3.58 ± 0.27[a]	3.48 ± 0.26[a]
RMR, kcal/d	1,835.7 ± 75.3[a]	2,028.6 ± 70.4[a,b]	2,054.2 ± 66.4[b]	1,958.1 ± 38.2[a]	1,946.2 ± 60.7[a]	2,014 ± 37.7[b]

NOTE. Different letters denote statistical difference within main effect, whereas similar letters denote statistical similarity within main effect ($P \leq .05$). Values are mean ± SD.

Abbreviations: Cr, creatine only; Cr-RT, creatine and resistance training; P-RT, placebo and resistance training.

Table 2. ANOVA Interaction Means for Body Composition, Energy Expenditure, and Blood Flow Parameters

	Cr (n = 10)			Cr-RT (n = 10)			P-RT (n = 10)		
	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28	Day 0	Day 7	Day 28
TBM (kg)	73.9 ± 11.5[a]	74.9 ± 11.8[a]	75.6 ± 12.5[b]	78.9 ± 6.7[a]	81.7 ± 5.5[b]	80.8 ± 6.8[b]	78.8 ± 11.3[a]	78.7 ± 10.9[a]	79.0 ± 11.7[a]
FFM (kg)	58.1 ± 8.1[a]	59.0 ± 8.8[a]	59.0 ± 8.8[a]	63.0 ± 2.8[a]	64.5 ± 3.4[b]	64.7 ± 3.6[b]	62.3 ± 6.5[a]	62.2 ± 6.6[a]	62.5 ± 6.5[a]
FM (kg)	12.6 ± 3.8[a]	12.5 ± 3.8[a]	13.1 ± 3.9[a]	12.8 ± 4.7[a]	12.7 ± 4.4[a]	13.1 ± 4.4[a]	12.9 ± 7.0[a]	12.6 ± 8.0[a]	13.1 ± 7.6[a]
% Fat	16.9 ± 3.5[a]	16.6 ± 3.7[a]	17.2 ± 3.5[a]	16.4 ± 4.9[a]	16.0 ± 4.6[a]	16.4 ± 4.6[a]	16.3 ± 6.4[a]	15.8 ± 7.4[a]	16.4 ± 6.8[a]
TBW (L)	40.4 ± 6.9[a]	40.0 ± 7.3[a]	42.6 ± 7.3[b]	40.6 ± 2.4[a]	41.7 ± 3.8[a,b]	42.3 ± 2.2[b]	40.8 ± 5.2[a]	39.9 ± 6.7[a]	40.9 ± 5.3[a]
CBF (mL · 100 mL^{-1} · min^{-1})	2.98 ± 1.0[a]	2.58 ± 1.0[a]	2.65 ± 1.2[a]	2.22 ± 0.3[a]	2.91 ± 0.9[b]	2.87 ± 0.8[b]	2.91 ± 0.9[a]	2.95 ± 0.6[a]	3.04 ± 0.90[a]
FBF (mL · 100 mL^{-1} · min^{-1})	2.98 ± 1.3[a]	3.24 ± 1.2[a]	2.75 ± 0.7[a]	2.90 ± 1.1[a]	4.1 ± 1.5[b]	4.0 ± 1.6[b]	3.5 ± 2.0[a]	3.4 ± 1.5[a]	3.7 ± 1.6[a]
RMR	1,860 ± 165[a]	1,740 ± 477[a]	1,907 ± 173[b]	1,971 ± 172[a]	2,029 ± 175[a]	2,086 ± 184[b]	2,043 ± 211[a]	2,070 ± 180[a]	2,050 ± 192[a]

NOTE. Different letters denote statistical difference within main effect, whereas similar letters denote statistical similarity within main effect ($P \leq .05$). Values are mean ± SD.

Abbreviations: Cr, creatine only; Cr-RT, creatine and resistance training; P-RT, placebo and resistance training.

2,822 ± 828 kcal/d), carbohydrate (Cr, 304 ± 105; Cr-RT, 421 ± 122; P-RT, 377 ± 11,436 g/d), fat (Cr, 85 ± 30; Cr-RT, 87 ± 33; P-RT, 93 ± 37 g/d), and protein (Cr, 103 ± 33; Cr-RT, 129 ± 44; P-RT, 124 ± 37 g/d) intake.

DISCUSSION

The major findings of this study were that following the 28-day treatment period (1) FFM increased significantly only in the Cr-RT subjects (1.7 kg); (2) TBW increased significantly in the Cr (2.2 L) and Cr-RT (1.7 L) groups; (3) LBF of the calf (30%) and forearm (38%) increased significantly only in the Cr-RT group; (4) absolute RMR increased 2.5% (66 kcal/d) and 5% (65 kcal/d) in the Cr and Cr-RT groups; (5) 1RM of the bench and leg press increased significantly in all 3 groups, although the increases in strength were greatest in the Cr-RT subjects; and (6) total cholesterol decreased approximately 10% in the Cr-RT group only.

Increases in TBM and TBW were observed in the Cr (1.7 kg; 2.2 L) and Cr-RT (2.0 kg; 1.7 L) groups. Furthermore, FFM determined by DEXA, increased significantly in the Cr-RT group. These findings are consistent with most, but not all,[28,29] previous reports that creatine supplementation over 5 to 44 days increases TBM by approximately 0.6 to 3.8 kg,[4,5,13-15,19,30-32] stressing that the former investigations[28,29] were conducted in elderly subjects. It has been suggested that this increase in body mass may be due to a creatine-stimulated increase in body water,[21,33] particularly the intracellular volume (ICV), and/or protein synthesis.[5,16,34,35] Indeed, previous research investigating the effects of acute (3 to 7 days) creatine supplementation reported body mass increases of approximately 0.9 to 1.7 kg, accompanied by significant increases in TBW (2%) and ICV (3%).[21] However, studies of longer duration of creatine ingestion (7 to 140 days) have reported gains in TBM that could not be fully accounted for by increases in TBW.[15,31] The increases in TBW reported in the present study are in general agreement with others[15,21,31] showing that an increase in the absolute amount of TBW contributes to the increase in TBM and FFM following medium term creatine ingestion, although this is not a universal finding.[31] In fact, Vandenberghe et al[4] found that gains in FFM following 10 weeks of creatine supplementation were largely retained 4 weeks after cessation of supplementation, despite muscle phosphocreatine levels returning to baseline suggesting that increases in TBM and FFM observed following Cr supplementation also may be due, at least in part, to dry matter growth. However, our findings suggest that the acute increase in TBM and FFM are mainly due to increases in TBW and ICV.

At present, there have been no longitudinal training studies to examine the time course of changes in LBF with either endurance or resistance exercise training, and thus only cross-sectional (trained *v* control subjects) data are available for comparison. Previous research supports a greater basal LBF in endurance-trained compared with untrained individuals that may be the result of increased endothelial cell nitric oxide synthesis and cardiac output (primarily via an increased stroke volume and decreased vascular resistance).[18] The present study is also the first to show an increase in LBF to the calf (≈ 32%) and forearm (≈ 40%) following an intermediate-term (28-day) intervention of creatine ingestion and resistance training. Thus, our findings confirm an enhanced LBF in trained individuals and extend these findings by using an exercise training/nutritional intervention study design to directly assess the changes in blood flow over time. It is interesting to note that the enhanced blood flow occurred only in the group supplementing with creatine and resistance training simultaneously, suggesting a synergistic effect of both interventions that appears to occur early in the process. Moreover, in the present study, the increase in LBF occurred only in the Cr-RT group. The mechanism responsible for increased peripheral limb blood flow is currently unknown, but may be related to the increased in TBW impacting plasma volume, venous return, and cardiac output.

The role of resistance-exercise training in influencing RMR remains unclear due to the lack of longitudinal investigations that have been performed. In the present study, RMR increased 2.5% (47 kcal/d) and 5% (114 kcal/d) in the Cr and Cr-RT groups, respectively. Concomitant with the increase in RMR in these 2 groups of subjects was the increase in TBW, body mass, as well as FFM (although only significant in Cr-RT) that occurred following the 28-day period. Although weight gain has been a commonly observed finding following creatine ingestion, prior to this study, there was no documentation as to what specific effect this increased body mass had on resting

energy expenditure. In the present study, significant increases in TBW fully accounted for increases in body mass in the Cr and Cr-RT subjects. Based on the finding that the increase in total body mass and FFM is explained predominantly by increases in TBW, the increase in RMR may be independent of changes in lean tissue.

To date, there is a paucity of studies directly comparing the effects of creatine supplementation alone to creatine combined with resistance training and/or resistance training alone on muscle strength. The present study provides new information regarding the effects of creatine ingestion by systematically comparing the effects of creatine supplementation alone versus Cr combined with RT and RT alone on muscular strength, as measured by 1RM bench and leg press. Moreover, our findings are strengthened as a result of our study design (double-blind, placebo-controlled), random assignment of a larger number of subjects ($n = 30$) to 1 of 3 groups: Cr, Cr-RT, and P-RT], and statistical control of changes in body mass and composition (ANCOVA) that potentially influence our dependent variables (RMR, muscle strength). The present study found that strength significantly increased in all groups, although Cr-RT improved significantly more on the leg press than Cr (44% *v* 18%, $P < .01$) and P-RT (44% *v* 27%, $P < .05$) and significantly more on bench press than Cr (18.4% *v* 8%, $P < .01$). These findings are novel because they are the first to directly demonstrate that Cr in combination with a well-controlled RT program elicits greater increases in maximal strength than Cr or RT alone. Moreover, these findings are significant to elucidate the independent contributions of the effects of creatine and resistance training alone and the possible synergistic effects of both on muscular strength.

Our results extend previous findings showing that short (3 to 7 days) to intermediate term (8 to 30 days) creatine supplementation, independent of RT, improves muscle strength[4,5,14,36,37] by directly comparing the independent effects of Cr and RT with the synergistic effect of both combined. The compelling evidence supporting an increase in muscular strength following creatine ingestion[5,11,14] suggests that an increase in both resting total creatine concentrations (15%) and rate of phosphocreatine resynthesis (42%) between exercise bouts observed in subjects supplementing with creatine is responsible.[1] Specifically, an increase in pre-exercise total creatine concentrations and enhanced PCr resynthesis would allow for greater PCr concentrations during subsequent exercise bouts, thus enhancing muscular performance and work output by sustaining the required rate of adenine diphosphate (ADP) rephosphorylation for a longer time period during contraction and permit an increase in training volume. Although the present study did not directly measure Cr levels in the body, we corroborate these findings of increased muscular strength following Cr ingestion. In addition, we extend these previous findings by demonstrating that subjects supplementing with Cr while resistance training improved muscular strength more than Cr or RT alone, independent of changes in body mass. Thus, increases in TCr,[5] intramuscular PCr stores,[1] and intracellular fluid volume[35] following creatine ingestion are the likely mechanisms for the increased muscle strength in the Cr and Cr-RT groups in our study. Because creatine is an osmotically active substance, greater water influx into the cell occurs, increasing the cellular hydration state, which in turn, serves as an anabolic proliferative signal leading to protein accretion. Studies proposing creatine's ability to serve as a chemical signal for increased contractile protein synthesis[34] and promote hypertrophy of type II muscle fiber diameter[16] reinforce its muscle strengthening potential.

At present, there is limited and conflicting information available regarding the cholesterol lowering effect of creatine ingestion.[15,22,23] Investigators have observed a 6% decline in total cholesterol following 8 weeks of creatine supplementation alone in subjects with elevated total cholesterol concentrations (>200 mg/d)[22] and an increase of 13% in HDL-cholesterol and a decrease of 13% in very–low-density lipoprotein (VLDL)-cholesterol in collegiate football players after 28 days of resistance/agility training.[15] Still others have shown no effect of 12 weeks of creatine ingestion on total cholesterol concentrations in resistance-trained subjects.[23] Our finding of an approximately 10% reduction in blood cholesterol may be due to the synergistic effect of creatine and resistance training combined, as only those subjects resistance training and supplementing with creatine (20 g/d, days 0 to 5; 10 g/d, days 6 to 28) experienced a decrease in total cholesterol levels over the 4-week period. The study,[23] which showed no decrease in cholesterol concentrations over the 12-week period, may have been the result of a lower dosage of creatine administration (5 g/d). Thus, it appears that higher creatine dosages (>10 g/d) may favorably reduce total cholesterol in individuals with elevated (200 mg/dL) cholesterol levels, and in normal subjects (<200 mg/dL) higher creatine intakes (>10 g/d) combined with resistance exercise may be needed.

In summary, 28 days of creatine supplementation resulted in significant increases in body mass, body water, muscular strength, and RMR. The combination of creatine ingestion and resistance training appear to enhance these effects while decreasing total cholesterol and significantly increasing FFM and peripheral blood flow to the calf and forearm. Our results showing an increased body mass, FFM, RMR, and LBF following combined creatine ingestion and resistance training appear to be primarily influenced by the concomitant increase in TBW. These findings provide new insights into the metabolic and hemodynamic effects of creatine ingestion combined with resistance training.

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