Conjugated Linoleic Acid Combined With Creatine Monohydrate and Whey Protein Supplementation During Strength Training

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Purpose: The authors examined the combined effects of conjugated linoleic acid (CLA), creatine (C), and whey protein (P) supplementation during strength training. ***Methods:*** Sixty-nine participants (52 men, 17 women; $M \pm SD$ age 22.5 ± 2.5 yr) were randomly assigned (double-blind) to 1 of 3 groups: CCP (6 g/d CLA + 9 g/d C + 36 g/d P; $n = 22$), CP (C + P + placebo oil; $n = 25$), or P (P + placebo oil; $n = 22$) during 5 wk of strength training (4–5 sets, 6–12 repetitions, 6 d/wk). Measurements were taken for body composition (air-displacement plethysmography), muscle thickness (ultrasound) of the flexors and extensors of the elbow and knee, 1-repetition-maximum (1-RM) strength (leg press and bench press), urinary markers of bone resorption (N-telopeptides, NTx), myofibrillar protein catabolism (3-methylhistidine; 3-MH), oxidative stress (8-isoprostanes), and kidney function (microalbumin) before and after training. ***Results:*** Contrast analyses indicated that the CCP group had a greater increase in bench-press (16.2% ± 11.3% vs. 9.7% ± 17.0%; $p < .05$) and leg-press (13.1% ± 9.9% vs. 7.7% ± 14.2%; $p < .05$) strength and lean-tissue mass (2.4% ± 2.8% vs. 1.3% ± 4.1%; $p < .05$) than the other groups combined. All groups increased muscle thickness over time ($p < .05$). The relative change in 3-MH (CCP –4.7% ± 70.2%, CP –0.4% ± 81.4%, P 20.3% ± 75.2%) was less in the groups receiving creatine ($p < .05$), with the difference for NTx also close to significance ($p = .055$; CCP –3.4% ± 66.6%, CP –3.9% ± 64.9%, P 26.0% ± 63.8%). There were no changes in oxidative stress or kidney function. ***Conclusion:*** Combining C, CLA, and P was beneficial for increasing strength and lean-tissue mass during heavy resistance training.

Keywords: exercise, nutraceutical, 3-methylhistidine, bone, oxidative stress, muscle

Exercising individuals often add nutritional supplements to their diet to accelerate the increase in muscle mass and strength from heavy resistance-exercise

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training. The effectiveness of most dietary supplements for improving body composition and exercise performance is small, however. Three dietary supplements consumed by exercising individuals include creatine, conjugated linoleic acid (CLA), and protein. Creatine, a nitrogen-containing compound involved in high-energy phosphate metabolism, has been shown to increase muscle mass and strength when combined with heavy resistance-exercise training (Burke et al., 2003; Burke et al., 2000; Chilibeck, Stride, Farthing & Burke, 2004; Chrusch, Chilibeck, Chad, Davison, & Burke, 2001; Kreider et al., 1998).

CLA encompasses a group of 18-carbon polyunsaturated fatty-acid isomers derived from the essential fatty acid linoleic acid. In animal models supplementation with CLA increases lean body mass (Dugan, Aalhus, Jeremiah, Kramer, & Schaefer, 1999; Dugan, Aalhus, Schaefer, & Kramer, 1997; Park et al., 1997) and reduces fat mass (Azain, Hausman, Sisk, Flatt, & Jewell, 2000; DeLany, Blohm, Truett, Scimeca, & West, 1999; Du & Ahn, 2002; Park et al.), possibly through an increase in fatty-acid oxidation (Nagao et al., 2003; Ohnuki, Haramizu, Ishihara, & Fushiki, 2001; West et al., 1998). We have recently shown that CLA supplementation (5 g/day) combined with strength training for 7 weeks significantly increased muscle mass and strength and reduced fat mass over strength training alone (Pinkoski et al., 2006).

Strength training results in significant muscle protein turnover (Rennie & Tipton, 2000; Tipton et al., 2001), and the rate of muscle protein synthesis after exercise is elevated with oral consumption of indispensible amino acids (Tipton, Ferrando, Phillips, Doyle, & Wolfe, 1999; Tipton et al., 2001). The increase in contractile protein that results from heavy resistance training suggests that protein intake might have to exceed basal levels to supply additional amino acids for muscle hypertrophy (Tarnopolsky et al., 1992). Supplementation with whey protein or milk, which contain these indispensible amino acids, combined with strength training significantly increases muscle mass and strength over strength training alone in young healthy men (Burke et al., 2001; Candow, Burke, Smith-Palmer, & Burke, 2006; Hartman et al., 2007).

The purpose of this study was to determine the effects of combining CLA, creatine, and whey protein on muscle mass and strength during a strength-training program. We hypothesized that the combination of CLA, creatine, and protein would be superior to creatine and protein combined or protein by itself.

A secondary purpose of the study was to determine whether the combination of CLA, creatine, and protein affected markers of muscle protein and bone catabolism, oxidative stress, and kidney function. CLA and creatine both exhibit anti-catabolic properties by reducing indicators of myofibrillar protein catabolism (Parise, Mihic, MacLennan, Yarasheski, & Tarnopolsky, 2001; Pinkoski et al., 2006) and bone resorption (Louis et al., 2003; Pinkoski et al.; Tarnopolsky et al., 2004). Some (Basu, Riserus, Turpeinen, & Vessby, 2000) but not all (Tarnopolsky et al., 2007) studies indicate that CLA might increase oxidative stress, whereas creatine might have the opposite effect (Rodriguez et al., 2007). There has also been some concern that creatine might affect kidney function (Thorsteinsdottir, Grande, & Garovic, 2006), but this effect is not consistently shown (Pline & Smith, 2005). We hypothesized that the combination of CLA, creatine, and protein would decrease myofibrillar protein catabolism and bone resorption more

than creatine and protein combined or protein by itself. We also hypothesized that creatine and CLA supplementation would have minimal effect on markers of oxidative stress and kidney function.

Methods

Experimental Protocol

Male ($n = 52$) and female ($n = 17$) participants (18–30 years old) who had already been performing regular strength training for at least 12 months before the study were recruited from a healthy population. Participants had no history of nutritional supplementation for at least 12 weeks before the start of the study. Exclusion criteria included having any disease or condition that prevented participation in high-volume strength training. The Physical Activity Readiness Questionnaire (PAR-Q) was used to identify individuals who required medical clearance before exercising (Thomas, Reading, & Shephard, 1992). Participants were informed of the risks and purposes of the study before their written consent was obtained. The study was approved by the Biomedical Research Ethics Board for Research in Human Subjects at the University of Saskatchewan.

We used a double-blind repeated-measures design, in which every participant underwent strength training and supplementation. A research assistant who was not involved in any other aspect of the study was responsible for randomizing participants (after they were stratified by gender) and coding the supplements to ensure that all participants and investigators remained blinded throughout the study. Investigators were blinded during entry and analysis of data by group coding.

The dependent variables measured before and after strength training and supplementation included lean-tissue mass, fat mass, muscle thickness of the elbow and knee flexors and extensors, leg-press and bench-press 1-repetition maximum (1-RM) strength, and urinary excretion of cross-linked N-telopeptides of Type I collagen (NTx), 3-methylhistidine, 8-isoprostanes, and microalbumin as indicators of bone and muscle protein catabolism, oxidative stress, and kidney function, respectively. In addition, participants completed dietary records for 3 days during the first and last weeks of strength training and supplementation to assess nutrient differences between groups. Participants were instructed not to change their diet during the study.

After being stratified by gender, participants were randomly assigned to one of three groups: CCP (6 g/day CLA, 9 g/day creatine, 36 g/day whey protein; $n = 22$: 16 men, 6 women), CP (9 g/day creatine, 36 g/day whey protein, 6 g/day placebo sunflower oil; $n = 25$: 19 men, 6 women), or P (45 g/day whey protein, 6 g/day placebo; $n = 22$: 17 men, 5 women) during 5 weeks of strength training. This training duration was chosen because 3–6 weeks is adequate for increasing strength and lean-tissue mass with creatine or whey protein supplementation (Burke et al., 2001; Burke et al., 2000; Kreider et al., 1998). All supplements were in powder form and supplied by Iovate Health Sciences (Mississauga, ON, Canada). The CLA was composed of approximately 36% each of the *cis*-9, *trans*-11, and *trans*-10, *cis*-12 isoforms, of which the *trans*-10, *cis*-12 isoform is

considered the most biologically active for affecting body composition (see Pinkoski et al., 2006 for a complete list of isoforms in the CLA supplement). We initially recruited 77 participants, of whom 8 dropped out during the study because of time constraints and unwillingness to comply with the study protocol. The supplement was divided into three equal servings each day. Participants were instructed to consume the first serving immediately before their training session and the second serving immediately afterward because it has been shown that the timing of nutritional supplementation might be important for creating an anabolic environment for muscle growth, with ingestion immediately before (Tipton et al., 2001) and immediately after strength training (Esmarck et al., 2001) appearing optimal. Participants were instructed to consume the final serving shortly before going to bed to help promote net muscle protein balance (Tipton et al., 1999). Participants were instructed to mix and consume the supplements in cold water to offset any potential water losses that might occur from additional dietary protein (Lemon, 1998).

The CLA dose of 6 g/day was chosen because we have recently shown that this approximate dose was effective for increasing muscle mass and strength and reducing fat mass, myofibrillar protein degradation, and bone resorption (Pinkoski et al., 2006). The creatine dose of 9 g/day was chosen because we have shown it to be effective when combined with protein (Burke et al., 2001). The whey protein dose of 36 g/day was chosen because it is the approximate amount shown to increase muscle mass during strength training (Burke et al., 2001). The placebo group (protein + sunflower oil placebo) received a slightly higher amount of protein (i.e., 45 g/day) to match the amount of nitrogen consumed by the other groups. Supplements were isoenergetic, isonitrogenous, and similar in taste, texture, and appearance. They contained approximately 1,030 kJ (246 kcal) of energy. Compliance with the supplementation protocol was monitored by verbal communication with participants and by having them return empty and unused supplement containers.

Strength Training

All participants followed the same high-volume, heavy-load, periodized, free-weight strength-training program for 5 weeks. We have previously used this program successfully to increase lean-tissue mass and strength in young adults (Burke et al., 2001; Candow, Chilibeck, Burke, Davison, & Smith-Palmer, 2001). Briefly, strength training started on the first day of supplementation and consisted of a 4-day split routine involving whole-body musculature: Day 1 involved chest and triceps muscles; Day 2 involved back and biceps muscles; Day 3 involved leg, shoulder, and abdominal muscles; and Day 4 was a rest day. This 4-day cycle was repeated continuously throughout the duration of the study. The periodized program was broken into three blocks of three cycles for 12 days, for a total of 36 days. Block 1 consisted of four sets of 10–12 repetitions, with 1-min rests between sets. Block 2 (Days 13–24) consisted of four sets of 6–8 repetitions, with 1.5-min rests between sets. Block 3 (Days 25–36) consisted of three sets of 4–5 repetitions, with 2-min rests between sets. Participants were instructed to keep training logs detailing the weight used and numbers of sets and repetitions performed for each exercise completed for every workout and were directed to increase their

resistance once the target number of repetitions was achieved with good form. Instructors were present to monitor correct form.

Data Collection

Detailed procedures for lean-tissue mass (Pinkoski et al., 2006), muscle thickness (Candow & Chilibeck, 2005), muscle strength (Chrusch et al., 2001), myofibrillar protein degradation and bone resorption (Pinkoski et al.), and diet (Candow et al., 2006) have previously been described. Briefly, lean-tissue and fat mass were assessed using air-displacement plethysmography (BOD POD, Life Measurement, Inc., Concord, CA). Our laboratory has demonstrated a coefficient of variation of 0.9% for fat-free mass and 3% for fat mass in young recreationally active adults.

Muscle thickness of the flexor and extensor muscles surrounding the elbow and knee were measured using B-mode ultrasound (Aloka SSD-500, Tokyo, Japan; Candow & Chilibeck, 2005). The coefficients of variation for muscle-thickness measurements in our laboratory are elbow flexors 2.5%, elbow extensors 2.2%, knee flexors 1.2%, and knee extensors 1.5%.

Leg-press and bench-press strength were assessed with 1-RM (Chrusch et al., 2001) on Hammer Strength (Life Fitness, Franklin Park, IL) and Lever (Pulse Fitness Systems, Winnipeg, Manitoba, Canada) plate-loaded machines. These two exercises were chosen as an index of muscle strength because they involve the major muscle groups in the lower and upper body. The coefficients of variation for leg-press and bench-press strength measures in our laboratory are 3.8% and 3.1%, respectively.

To measure urinary indicators of myofibrillar protein catabolism (3-methylhistidine), bone resorption (N-telopeptides), oxidative stress (8-isoprostanes), and kidney function (microalbumin), a 24-hr urine sample was obtained both before and immediately after the 5-week strength-training and supplementation protocol. Urine was collected after a 3-day meat-free diet. A meat-free diet was implemented to reduce 3-methylhistidine production from the breakdown of meat consumed in the diet (Hickson & Hinkelmann, 1985). Meat consumption affects 3-methylhistidine levels, and 3 days of a meat-free diet are required to return 3-methylhistidine levels to baseline (Lukaski, Mendez, Buskirk, & Cohn, 1981). The concentration of 3-methylhistidine was determined by high-performance liquid chromatography (3 mm Chromsep ODS-2 column, Varian Inc., Mississauga, ON, Canada; flow rate 1.0 mL/min) and 2475 multi-wavelength fluorescence detection (Waters, Mississauga, ON, Canada) using the methods of Wassner, Schlitzer, and Li (1980) with modification for sample volumes. The concentration of 3-methylhistidine in urine samples was corrected for creatinine content and multiplied by the 24-hr urine volume to produce a value for daily 3-methylhistidine excretion. The intra-assay coefficient of variation was 5.1%.

The concentration of NTx was determined by using a competitive-inhibition enzyme-linked immunosorbent assay (ELISA; Osteomark NTx Urine, Wampole Laboratories, Princeton, NJ). The daily amount of NTx excreted by each participant was determined by multiplying the concentration of NTx expressed in bone-collagen equivalents by their 24-hr urine volume. This amount of NTx was then

expressed relative to the daily amount of excreted creatinine (nmol of bone-collagen equivalents per mmol of creatinine). Samples were analyzed in duplicate within a single assay. The intra-assay coefficient of variation was 9%.

Urinary 8-isoprostanes were analyzed by ELISA (Neogen Corp., Lexington, KY). The concentration was multiplied by the 24-hr urine volume and expressed relative to daily creatinine excretion (i.e., ng/g creatinine). The intra-assay coefficient of variation was 12%.

Creatinine in urine was assessed by a commercially available colorimetric detection kit (Cayman Chemical Co., Ann Arbor, MI). The concentration was multiplied by 24-hr urine volume to determine the amount excreted over 24 hr. The intra-assay coefficient of variation was 1.8%. For each measure, samples from each participant (i.e., both at baseline and after 5 weeks) were assayed in duplicate within the same microplate or HPLC run to reduce variation.

Urinary microalbumin was determined with immunochromatographic lateral-flow membrane test strips containing specific monoclonal antibody against human albumin (Genzyme Diagnostics, Charlottetown, PEI, Canada). This semiquantitative test gives one of three results: microalbumin less than, equal to, or greater than 18 mg/L. A result greater than 18 mg/L indicates compromised kidney function. Duplicate tests resulted in a coefficient of variation of 0%.

Dietary intake was recorded for 3 days during the first and final weeks of strength training and supplementation to assess whether there were any differences in total energy and macronutrient composition between the groups. Participants used a 3-day food booklet to record what they ate for 2 weekdays and 1 weekend day. Nutribase Network Edition software (Version 6.21, Cybersoft Inc., Phoenix, AZ) was used to analyze the 3-day food records.

Statistical Analyses

A 3 (group: CCP vs. CP vs. P) $\times$ 2 (sex: male vs. female) $\times$ 2 (time: pre- and posttest periods) ANOVA with repeated measures on the third factor was used to assess changes in lean-tissue mass, fat mass, muscle thickness, strength, 3-methylhistidine, NTx, 8-isoprostanes, and diet (energy and macronutrient intake) over time. When interactions or main effects were found, a least-significant-difference post hoc test was used to determine differences between means. To simplify presentation of results we calculated relative (percent) change scores as (final measurement – baseline measurement)/baseline measurement $\times$ 100. A two-way ANOVA (Group $\times$ Sex) was used to analyze baseline measurements between groups, as well as differences in dependent variables for percent change scores. As a follow-up to these statistical analyses, contrast analyses were used to determine differences between groups consuming creatine (i.e., CCP and CP combined) and the protein-only group and between the group consuming CLA and the other two groups (i.e., CP and P combined). A contrast analysis allows testing of predicted specific differences in particular parts of a complex ANOVA design. Statistical tests for body-composition variables, strength, 3-methylhistidine, and NTx were one-tailed tests because the hypotheses concerning these variables were a priori. All results are expressed as $M \pm SD$. Statistical analyses were carried out using Statistica version 7 (Statsoft, Tulsa, OK). Significance was set at $p \leq .05$.

Results

Baseline characteristics for all groups are presented in Table 1. There were no differences between the CCP, CP, or P groups at baseline in any of the dependent variables. At baseline men had greater lean-tissue mass, bench- and leg-press strength, elbow-flexor muscle thickness, and total body mass; women had greater percentage body fat (all $p < .01$). Men and women were similar in elbow-extensor, knee-flexor, and knee-extensor muscle thickness, as well as 3-methylhistidine and NTx. Compliance with the supplementation, as assessed by returned supplement containers, was >98% for each group.

With the exception of muscle thickness, all measures changed to a similar extent in men and women, so strength, body composition, and markers of bone and muscle catabolism were analyzed with both sexes combined by a two-factor (Group × Time) ANOVA to increase statistical power.

Table 1   Baseline Characteristics of Protein + Placebo (P), Creatine + Protein + Placebo (CP), and Conjugated Linoleic Acid + Creatine + Protein (CCP) groups (N = 69), $M \pm SD$

	P, n = 17 men, 5 women	CP, n = 19 men, 6 women	CCP, n = 16 men, 6 women	p for group differences
Age (years)				.337
men	23.1 ± 2.3	22.6 ± 2.4	22.4 ± 2.4	
women	23.4 ± 2.4	21.8 ± 2.6	22.0 ± 2.8	
Height (cm)				.986
men	179 ± 7	181 ± 7	180 ± 7	
women	164 ± 5	160 ± 5	166 ± 6	
Body mass (kg)				.866
men*	83.3 ± 12.4	81.7 ± 10.5	80.3 ± 10.0	
women	60.5 ± 5.8	59.0 ± 5.3	65.3 ± 9.5	
FFM (kg)				.962
men*	65.0 ± 8.7	66.0 ± 8.7	64.6 ± 3.8	
women	45.3 ± 2.6	42.5 ± 3.5	46.3 ± 8.5	
Fat mass (kg)				.288
men	16.4 ± 7.7	13.3 ± 5.1	13.0 ± 6.3	
women	15.2 ± 5.0	13.5 ± 3.8	17.2 ± 6.5	
% Fat				.392
men*	19.1 ± 7.5	16.0 ± 5.3	15.7 ± 6.2	
women	24.7 ± 5.9	22.7 ± 4.8	25.9 ± 6.5	

Note. FFM = fat-free mass.

*Gender difference ($p < .05$).

Strength

There were time main effects for bench-press (123 ± 50 kg at baseline vs. 136 ± 50 kg at 5 weeks; $p < .01$) and leg-press strength (217 ± 66 kg at baseline vs. 235 ± 75 kg at 5 weeks; $p < .01$). In the contrast analyses bench-press and leg-press strength increased more in the CCP group than in the CP + P groups combined ($p < .05$; Figure 1).
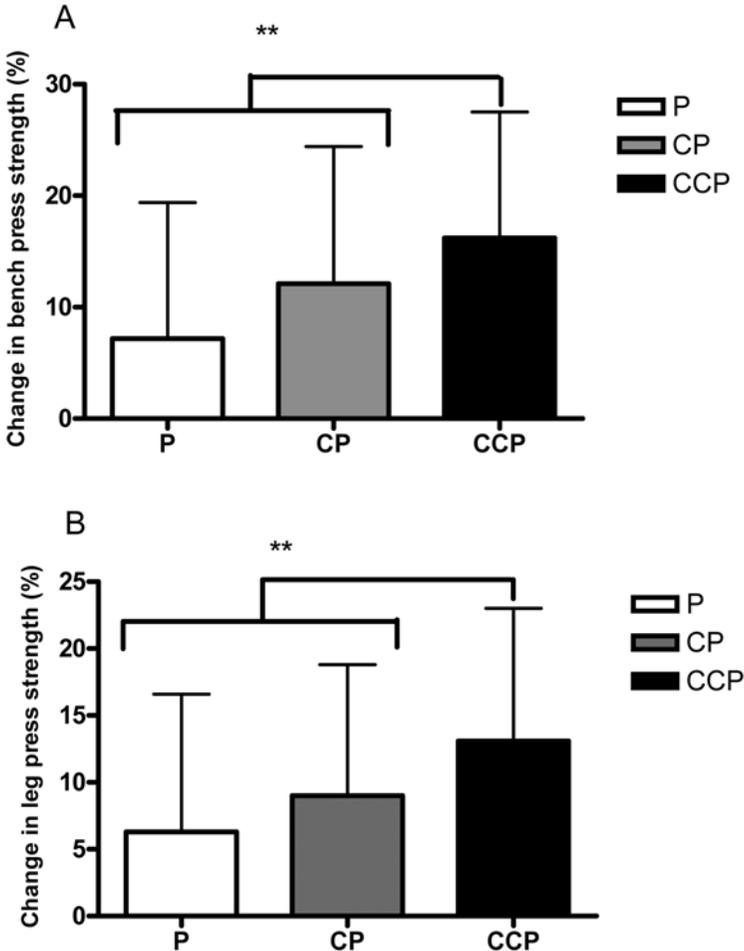

Figure 1 — (A) Change in bench-press strength after 5 weeks of supplementation and strength training for protein (P; $n = 22$), creatine + protein (CP; $n = 24$), and creatine + CLA + protein (CCP; $n = 22$) groups, $M \pm SD$. **CCP significantly greater than CP and P groups combined in the contrast analysis ($p < .05$). (B) Change in leg-press strength for P ($n = 22$), CP ($n = 25$), and CCP (N = 21) groups, $M \pm SD$. **CCP significantly greater than CP and P groups combined in the contrast analysis ($p < .05$).

Body Composition

There was a time main effect for lean-tissue mass (60.0 ± 11.6 kg at baseline vs. 61.1 ± 11.6 kg at 5 weeks; $p < .01$) and total body mass (76.8 ± 13.3 kg at baseline vs. 78.1 ± 13.3 kg at 5 weeks; $p < .01$). There were no changes in fat mass or percentage body fat over time. In the contrast analysis the CCP group increased lean-tissue mass more than the CP and P groups combined (Figure 2; $p < .05$). Contrast analysis also indicated that the two groups consuming creatine (CCP + CP) increased lean-tissue mass significantly more than the P group ($p < .05$).

Muscle Thickness

Muscle-thickness results are presented in Table 2. There were time main effects for all muscle-thickness measurements, with all measures increasing over time across groups ($p < .05$). There were no differences between men and women over time (i.e., no Sex $\times$ Time interactions). There was a Group $\times$ Sex interaction for the change in elbow-flexor muscle thickness ($p < .05$). The interaction occurred because of an unexpected greater percentage increase in elbow-flexor thickness in the female CP ($26.1 \pm 13.9\%$) than the female CCP group ($-2.1\% \pm 14.2\%$).

Urinary Analyses

There were no changes in 3-methylhistidine (CCP 25.8 ± 18.0–23.7 ± 15.8, CP 27.1 ± 17.5–24.1 ± 15.2, and P 36.2 ± 18.0–34.4 ± 15.5 μmol/mmol creatinine), NTx (CCP 140.3 ± 110.3–120.9 ± 61.2, CP 153.9 ± 107.2–123.8 ± 59.3, and P 133.5 ± 105.4–130.0 ± 58.8 nmol/mmol creatinine), and 8-isoprostanes (values when expressed relative to creatinine were CCP 418 ± 287–$708 \pm 1,284$, CP 521 ± 429–427 ± 317, and P 479 ± 305–661 ± 411 ng/g creatinine; 24-hr values were CCP 484 ± 316–477 ± 272, CP 493 ± 239–475 ± 216, and P 372 ± 179–436 ± 192 ng) over time in any group. In contrast analyses the percentage change scores for 3-methylhistidine of the CCP ($-4.7\% \pm 70.2\%$) and CP ($-0.4\% \pm 81.4\%$) groups combined were lower than the P group ($20.3\% \pm 75.2\%$; $p < .05$). The difference
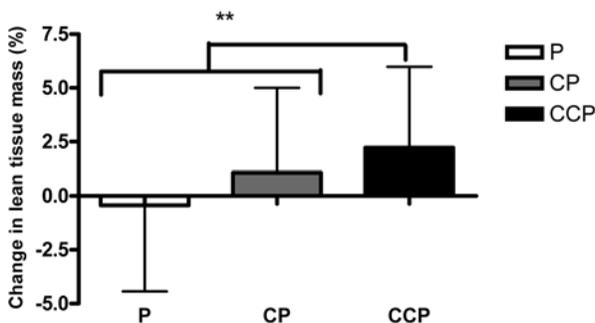

Figure 2 — Change in lean-tissue mass after 5 weeks of supplementation and strength training for protein (P; $n = 22$), creatine + protein (CP; $n = 24$), and creatine + CLA + protein (CCP; $n = 22$) groups, $M \pm SD$. **CCP significantly greater than CP and P groups combined in the contrast analysis ($p < .05$).

Table 2 Muscle-Thickness Percentage Changes Over 5 Weeks of Supplementation and Strength Training, $M \pm SD$

Muscles	P		CP		CCP	
	Women (n = 5)	Men (n = 17)	Women (n = 6)	Men (n = 19)	Women (n = 6)	Men (n = 16)
Elbow flexors*	17.0 ± 13.9	8.5 ± 13.9	26.1 ± 14.2†	13.0 ± 14.1	−2.1 ± 13.9†	10.4 ± 14.0
Elbow extensors*	21.7 ± 12.3	12.6 ± 12.7	15.4 ± 12.5	9.8 ± 12.8	16.6 ± 12.5	21.8 ± 12.8
Knee flexors*	7.8 ± 7.3	3.1 ± 7.4	2.9 ± 7.2	6.0 ± 7.5	6.0 ± 7.2	2.0 ± 7.2
Knee extensors*	2.8 ± 15.6	−0.3 ± 16.0	5.0 ± 14.3	4.7 ± 15.8	0.8 ± 15.6	13.0 ± 16.0

Note. P = protein and placebo group; CP = creatine, protein, and placebo group; CCP = conjugated linoleic acid, creatine, and protein group.

*Significant time main effect ($p < .05$). †Significant Group × Sex interaction; female CP > female CCP ($p < .05$).

between the CCP (–3.4% ± 66.6%) and CP (–3.9% ± 64.9%) groups combined compared with the P (26.0% ± 63.8%) group for the percentage change scores in NTx was close to significant ($p = .055$). There were no differences between groups in contrast analyses for percentage change in 8-isoprostanes (CCP +56.9% ± 204.8%, CP +11.3% ± 121.0%, and P +114.8% ± 190.9%). One man in the P group and 1 woman in the CCP group had microalbumin levels equal to 18 mg/L at baseline. All the other measures before and after training and supplementation were less than 18 mg/L.

Dietary Intake

No differences were found between supplement groups from baseline to 5 weeks for intake of total energy and macronutrients (see Table 3). This analysis did not include the macronutrients from the supplements.

Discussion

The most important results of this study were that bench-press and leg-press strength and lean-tissue mass increased more in the group consuming all three supplements (CLA, creatine, and protein) than in the other groups combined (i.e., consuming creatine and protein or only protein). Our contrast analyses also indicated that the two groups receiving creatine increased lean-tissue mass more than the group receiving protein alone, which supports our previous findings (Burke et al., 2001). These results support our primary hypothesis and indicate that CLA combined with creatine and protein might be additive for improving muscle strength and lean-tissue mass in strength-training individuals. Contrast analyses indicated that the two groups consuming creatine decreased 3-methylhistidine compared with the group receiving only protein ($p < .05$), with a similar difference for NTx, which approached significance ($p = .055$). These results partially support our secondary hypothesis that CLA and creatine would be effective for reducing bone resorption or muscle protein catabolism in young adults engaged in strength training.

Our previous research showed a greater increase in bench-press strength in males given CLA than in males on placebo, but there was no effect in females and no effect on leg-press strength (Pinkoski et al., 2006). One other study also found no effect of CLA on strength (Kreider, Ferreira, Greenwood, Wilson, & Almada, 2002); however, it did indicate a trend for overall strength to increase in the group supplemented with CLA versus placebo. Research in older adults completing a 6-month strength-training program (2 days/week) indicated that a combination of creatine and CLA increased isokinetic strength more than placebo and that women receiving the combination supplement improved knee-extension 1-RM more than the other groups (Tarnopolsky et al., 2007). In that study the authors suggested that the results were likely a result of the creatine supplementation as opposed to the CLA supplementation. In our study, contrast analyses indicated that bench-press and leg-press strength increased more in the CCP group than in the CP and P groups combined, which suggests that CLA supplementation during strength training might have more of an effect than creatine and protein on measures of strength.

Table 3 Estimated Daily Intake of Total Energy and Macronutrients for Participants at Baseline and After 5 Weeks of Supplementation and Strength Training ($N = 51$), $M \pm SD$

	P		CP		CCP	
	Baseline	5 weeks	Baseline	5 weeks	Baseline	5 weeks
Kilocalories	2,297 ± 903	2,365 ± 760	2,327 ± 929	2,456 ± 782	2,921 ± 968	2,426 ± 815
Kilojoules	9,615 ± 3,780	9,900 ± 3,183	9,741 ± 3,892	10,281 ± 3,275	12,227 ± 4,050	10,155 ± 3,411
Protein (g)	102 ± 42	106 ± 46	99 ± 46	115 ± 46	118 ± 45	115 ± 50
Carbohydrate (g)	280 ± 134	282 ± 92	314 ± 138	295 ± 97	362 ± 144	296 ± 99
Fat (g)	81 ± 42	90 ± 34	76 ± 41	88 ± 37	106 ± 45	86 ± 36

Note. P = protein and placebo group; CP = creatine, protein, and placebo group; CCP = conjugated linoleic acid, creatine, and protein group. Values do not include kilocalories or macronutrients from the supplements.

The results of the current study are in agreement with our previous research with CLA (Pinkoski et al., 2006), in which a group given CLA had greater increases in lean-tissue mass than a group given placebo during strength training. Our current results indicated that CLA supplementation with creatine and protein is more beneficial for increasing lean-tissue mass than the combined groups consuming creatine and protein and protein alone. Previous work in humans indicates that CLA supplementation has no effect on lean-tissue mass in young body builders during training (Kreider et al., 2002) and that a combination of creatine and CLA increased fat-free mass to a greater extent than placebo in older adults completing a strength-training program (Tarnopolsky et al., 2007). Supplementation with CLA, without exercise training, also increases lean-tissue mass in obese or overweight individuals (Gaullier et al., 2007; Steck et al., 2007) but not in normal-weight individuals (Nazare et al., 2007). Earlier, we found that CLA supplementation was ineffective for increasing muscle thickness more than placebo (Pinkoski et al.), which was confirmed with this study. The only difference between groups in the current study was that women on creatine and protein had a greater relative increase in elbow-flexor muscle thickness than women taking creatine, protein, and CLA (Table 2). Results for individual measures of muscle thickness differ from results for lean-tissue mass because whole-body lean tissue represents overall muscle mass, whereas our muscle-thickness measures only assessed four muscle groups. One mechanism whereby CLA might favorably influence skeletal muscle is a down-regulation of proinflammatory cytokines. The proinflammatory cytokine tumor necrosis factor-α, a known catabolic signaling agent, decreases with CLA supplementation (Song et al., 2005). Higher concentrations of tumor necrosis factor-α can signal apoptosis and result in loss of skeletal muscle (Pistilli, Jackson, & Alway, 2006). Other research in young and older strength-training individuals, however, showed no effect of CLA supplementation on other indicators of inflammation, such as interleukin-6 or C-reactive protein (Kreider et al., 2002; Tarnopolsky et al., 2007).

Fat mass was unaffected by CLA supplementation in this study. Our previous research indicated that CLA supplementation for 7 weeks decreased fat mass more than placebo (Pinkoski et al., 2006), and animal research has revealed similar results (Park et al., 1997). In addition, Tarnopolsky et al. (2007) found a reduction in fat mass of 1.9 kg in older adults supplementing with a mixture of creatine and CLA completing a 6-month strength-training program. They postulated that this reduction was likely caused by the CLA component of the supplement because creatine has consistently shown no effect on fat mass. CLA supplementation without exercise prescription resulted in no change in fat mass in healthy normal-weight men supplementing with CLA (Nazare et al., 2007), while overweight or obese individuals decreased fat mass (Gaullier et al., 2007). One suggested mechanism whereby CLA might exert its effect on fat mass is an increase in energy expenditure and fat oxidation via up-regulation of uncoupling proteins (Bhattacharya, Banu, Rahman, Causey, & Fernandes, 2006). Research on small animals is consistent with this fat-lowering effect of CLA; however, these same findings do not always translate well to human populations (Navarro et al., 2006). Further research on the clinical outcomes of CLA supplementation on body composition in a variety of populations is warranted.

Previous studies have indicated that creatine or CLA supplementation might decrease muscle protein catabolism (Parise et al., 2001; Pinkoski et al., 2006; Tarnopolsky et al., 2004). Our contrast analyses support these results in that the relative changes in 3-methylhistidine were lower in groups receiving creatine than in the protein-only group. Prior clinical research indicates that CLA supplementation has no effect on bone mineral during strength training in young (Kreider et al., 2002) or old (Tarnopolsky et al., 2007) healthy adults; however, Kreider et al. (2002) indicated a trend for total bone mass to increase (1.1%) in their CLA-supplemented group. Earlier we found a beneficial effect of CLA for decreasing bone resorption in a small crossover study (Pinkoski et al.). The current results indicate that bone resorption was unaffected by CLA supplementation; however, the relative decrease in NTx in the groups receiving creatine and/or CLA approached statistical significance ($p = .055$) compared with the protein-only group. Previous research from our laboratory indicated that creatine increased arm bone-mineral content in older men (Chilibeck, Chrusch, Chad, Davison, & Burke, 2005), and this was confirmed recently in an animal model (Antolic et al., 2007). Osteoblasts use phosphocreatine as an energy source and consequently secrete osteoprotegrin, which inhibits osteoclast activity, so creatine supplementation might effectively increase phosphocreatine, which would aid in bone formation and decrease bone resorption (Gerber, ap Gwynn, Alini, & Wallimann, 2005). Despite this potential, a recent 6-month clinical trial found no benefit of creatine (combined with CLA) on bone-mineral density (Tarnopolsky et al., 2007). Further research is necessary to evaluate longer term effects of creatine or CLA on bone.

There is some concern that CLA might increase oxidative damage to lipid membranes (Basu et al., 2000), and there have been case reports of kidney failure in people taking creatine supplements (Thorsteinsdottir et al., 2006). We did not observe increased oxidative stress as measured by 8-isoprostanes in our study, and there were no indications of kidney damage as assessed by urinary microalbumin. Tarnopolsky et al. (2007) assessed oxidative stress and kidney function in older adults consuming CLA and creatine for 6 months. There were no effects on kidney function and only an increase in 8-isoprostanes in women, but not men, taking CLA and creatine. There were no increases in a specific marker of DNA oxidative stress, and the researchers concluded that the biological significance of the increase in 8-isoprostanes was unclear. Longer term studies with more participants would be necessary to adequately determine the safety profile of dietary supplements combining CLA and creatine.

There are several limitations with our study. Although mean intake of macronutrients did not change in any group over the course of the study (Table 3), the effect of adding a supplement containing 1,030 KJ (246 kcal) might affect individual participants' regular intakes. Despite the increased energy intake and unchanged diet independent of the supplement, there were no significant increases in fat mass for any group. Although our participants were engaged in training before the study, they might have expended a greater amount of energy during our strength program because it was very high in volume, and this might have offset the increased energy intake. Another limitation from our dietary data is the 500-kcal/day decrease in energy intake in the CCP group. Although this is not statistically significant, this decrease might have negatively affected the results for this group. Another limitation is that we gave absolute doses of supplement to partici-

pants instead of scaling to body size. We felt that this increased external validity because most nutritional supplements bought off the shelf instruct users to consume absolute doses. A given absolute dose would most likely have a greater effect on a smaller individual; therefore, our approach might decrease the internal validity of the study.

The increase in lean-tissue mass was only significant when comparing the group that received CLA with the combined groups not receiving CLA (i.e., CCP compared with CP and P combined). The difference between individual groups (i.e., CCP vs. P) was not significant, and our study most likely lacked sufficient power to detect these differences. Based on the relative changes between the groups (Figure 2), for a power of 80% we would require 42 participants per group (almost twice our number) to achieve significance at $\alpha = .05$ in a one-tailed test. There were no differences between supplement groups for changes in muscle thickness. There was much variability in the percentage change scores between groups (Table 2), and the variability inherent in this measurement most likely contributed to this result. The effect of CLA on body composition was much smaller in our study (and other human studies) than in animal studies, most likely because the CLA doses used in human studies are only a fraction of those used in animal studies. Although compliance with our supplementation was high (>98%), participants were only instructed to consume supplement before and after workouts, and there was no check on the timing of consumption. Future studies should monitor compliance with respect to both amount and timing of consumption.

In summary, our results indicate that the addition of CLA to creatine and protein resulted in enhanced strength improvements and lean-tissue mass with high-volume strength training in well-trained young adults. Creatine combined with protein was more effective than protein alone for increasing lean-tissue mass. There were no indications of altered oxidative stress or altered kidney function with CLA and/or creatine supplementation.

Acknowledgments

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