

The Effects of Conjugated Linoleic Acid Supplementation during Resistance Training

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ABSTRACT

PINKOSKI, C., P. D. CHILIBECK, D. G. CANDOW, D. ESLIGER, J. B. EWASCHUK, M. FACCI, J. P. FARTHING, and G. A. ZELLO. The Effects of Conjugated Linoleic Acid Supplementation during Resistance Training. *Med. Sci. Sports Exerc.*, Vol. 38, No. 2, pp. 339–348, 2006. **Purpose:** We determined the effects of conjugated linoleic acid (CLA) supplementation during resistance training. **Methods:** Seventy-six subjects were randomized to receive CLA (5 g·d⁻¹) or placebo (PLA) for 7 wk while resistance training 3 d·wk⁻¹. Seventeen subjects crossed over to the opposite group for an additional 7 wk. Measurements at baseline, 7 wk, and 14 wk (for subjects in the crossover study) included body composition, muscle thickness of the elbow flexors and knee extensors, resting metabolic rate (RMR), bench and leg press strength, knee extension torque, and urinary markers of myofibrillar degradation (3-methylhistidine (3MH) and bone resorption (cross-linked N-telopeptides (Ntx)). **Results:** After 7 wk the CLA group had greater increases in lean tissue mass (LTM) (+1.4 vs +0.2 kg; $P < 0.05$), greater losses of fat mass (-0.8 vs +0.4 kg; $P < 0.05$), and a smaller increase in 3MH (-0.1 vs +1.3 $\mu\text{mol}\cdot\text{kg}^{-1}\text{LTM}\cdot\text{d}^{-1}$; $P < 0.05$) compared with PLA. Changes between groups were similar for all other measurements, except for a greater increase in bench press strength for males on CLA ($P < 0.05$). In the crossover study subjects had minimal changes in body composition, but smaller increases in 3MH (-1.2 vs +2.2 $\mu\text{mol}\cdot\text{kg}^{-1}\text{LTM}\cdot\text{d}^{-1}$; $P < 0.01$) and NTx (-4.8 vs +7.3 $\text{nmol}\cdot\text{kg}^{-1}\text{LTM}\cdot\text{d}^{-1}$; $P < 0.01$) while on CLA versus PLA. **Conclusions:** Supplementation with CLA during resistance training results in relatively small changes in body composition accompanied by a lessening of the catabolic effect of training on muscle protein. **Key Words:** STRENGTH, MUSCLE, FAT, BONE, RMR, EXERCISE

Conjugated linoleic acid (CLA) encompasses a group of 18-carbon polyunsaturated fatty acid isomers derived from the essential lipid, linoleic acid. Of the group of 28 possible isomers, cis-9, trans-11 (c9, t11) and trans-10, cis-12 (t10, c12) CLA are considered the two most biologically active (26). These and other CLA isomers are naturally occurring and found in foods such as beef, lamb, and dairy products.

Numerous studies have discovered an abundance of biological activities attributed to CLA. Of particular interest are the purported effects of CLA on body composition in animals and humans, and whether these changes are the results of alterations in metabolic rate and substrate utilization. A number of studies using animal models including rats, mice, pigs, and chickens have demonstrated that supplementing CLA reduces body fat (1,2,11,13,14,37) and increases lean body mass (2,14,28,31),

bone mineral mass (i.e., ash weight) (28), daily energy expenditure (24,32,37), and fatty acid oxidation (24,25).

CLA is currently marketed as an ergogenic aid purported to reduce body fat and increase lean mass; however, human studies have provided more equivocal results than animal studies concerning the effects of CLA supplementation on body composition (3,20,33,39). The largest study of humans to date ($N = 180$) showed that 1 yr of CLA supplementation reduced body fat mass in overweight subjects and increased lean tissue mass by small amounts (16). Only two studies have combined CLA supplementation with exercise training (20,33), and again the results were equivocal, with one study demonstrating a reduction in percent body fat with CLA (33) and the other showing no change in body composition (20). The type of exercise training in these studies was either not specified (33) or unsupervised (20). It was recently shown that supervised programs are more effective (22). The overall purpose of this study was to determine the effects of CLA supplementation during supervised resistance training on body composition and strength. Secondary analyses included measures of myofibrillar and bone degradation, RMR, and resting substrate utilization. Given the results of studies in animals using the active isoforms of CLA, we hypothesized that CLA, when combined with resistance training, would enhance increases in lean tissue mass and strength, reduce fat mass, increase RMR, increase fat oxidation, and reduce urinary markers of bone and muscle protein degradation, compared with resistance training alone.

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METHODS

Study Design

Healthy men and women were stratified according to gender and randomly assigned to the CLA or placebo-control group in a double-blind fashion. Subjects concurrently participated in a periodized total-body resistance-training program for a 7-wk period. The participants were measured before and shortly after completion of the study. Measurements included total body weight, percent body fat, lean body mass, fat mass, strength (leg and bench press), peak torque for knee extension, muscle thickness (elbow flexors and knee extensors), RMR, respiratory exchange ratio (RER), urinary 3-methylhistidine (as a marker of myofibrillar degradation), and N-telopeptide cross-links of Type I collagen (as a marker of bone resorption). The study was approved by our university's biomedical ethics review board for research in human subjects, and each subject signed a written informed consent for participation.

Following completion of this initial study, 17 subjects completed a further 7 wk of resistance training, crossing over to the opposite group (i.e., either CLA or placebo). The same dependent variables were assessed after the crossover, allowing a within-subjects comparison of CLA versus placebo.

Subjects, Randomization, and Supplementation

Eighty-five (43 female and 42 male) participants, ranging in age from 18 to 45 yr, originally volunteered for the study. Most participants had strength training experience and the majority had more than 2 yr of experience and had been training at least two times per week for a minimum of 3 months before involvement in the study. Participants were healthy, nonsmoking individuals, and did not have any known preexisting diseases as assessed by a physical activity readiness questionnaire (34) and initial interviews. Subjects were moderately physically active, as determined by questionnaire (17), and were asked not to change their diets or aerobic exercise regimes throughout the duration of the investigation.

An individual who was not involved in any other aspect of the study was responsible for randomizing the participants and coding the supplements to ensure that all participants and investigators remained blinded throughout the study. Investigators were blinded during the entry and analysis of data by coding the groups. The participants were randomly assigned to either receive CLA (Tonalin, Natural, Inc., Vernon Hills, IL, 5 g of CLA per day in seven 1-g capsules; see Table 1 for complete composition) or the placebo (7 g of sunflower oil per day) for 7 wk. Nine subjects (two females and two males in the CLA group, and one female and four males in the placebo group) withdrew during the course of the study, all because of lack of time, except for one male in the placebo group who withdrew because he started smoking and was concerned that it might affect the results, and one male each in the placebo and CLA groups who withdrew due to personal reasons. Characteristics for those who completed the study are presented in Table 2. Placebo and CLA capsules were identical in

appearance. Capsules were placed in sealed and labeled (participant's name) envelopes with a week's supply and delivered to the investigator to distribute. To monitor compliance to the supplementation, subjects were required to return empty envelopes. The decision to supplement 5 g of CLA per day was based on previous research demonstrating that higher doses may be necessary to effectively alter body composition in human subjects (3).

Resistance Training

The resistance training program involved 12 exercises chosen to include all major muscle groups. These exercises included leg press, leg (knee) extension, and leg (knee) flexion using Hammer Strength equipment (Life Fitness; Franklin Park, IL), and bench press, shoulder press, lateral pull down, biceps curl, back extension, and hip (extension, flexion, adduction, abduction) exercises using Lever equipment (Pulse Fitness Systems; Winnipeg, MB, Canada). Each exercise was performed three times per week consisting of 3–4 sets of 4–10 repetitions at approximately 75–90% of one-repetition maximum (1RM). Training sessions were supervised because previous research has demonstrated greater gains compared with unsupervised training (22). Each successive session was separated by at least one rest day to reduce the chance of injury and minimize fatigue. To ensure maximal gains the 7 wk of training were periodized on roughly a 2 1/3-wk cycle. The periodized program was therefore broken up into three blocks consisting of seven complete workouts each. Block 1 (sessions 1–7) consisted of 4 sets of 8–10 repetitions, with 1 min of rest between sets. Block 2 (sessions 8–14) consisted of 4 sets of 6–8 repetitions, with 1.5 min of rest between sets. Block 3 (sessions 15–21) consisted of 3 sets of 4–5 repetitions, with a 2-min rest between sets. For block 1, intensity for the bench press and leg press were initially set at 70% of the subject's 1RM, whereas other intensities for additional exercises were set at approximately the subject's 10RM (i.e., the maximal weight the subject could lift for 10 repetitions). For blocks 2 and 3, initial intensities were set at subjects' 8RM and 5RM, respectively. We chose this periodized program because we previously had found it effective for inducing muscle hypertrophy (10). Intensities were progressively increased in 2- to 5-kg increments when a subject was able to complete the maximum number of repetitions required in a given block with good form.

TABLE 1. Compositional list of conjugated linoleic acid oil supplement.

Carbons: Double Bond	Common Name	Percent Composition
14:0	Myristic acid	0.1%
16:0	Palmitic acid	6.9%
16:1	Palmitoleic acid	0.1%
18:0	Stearic acid	2.5%
18:1	Oleic acid	14.5%
18:2	Linoleic acid (c9,c12)	2.7%
18:2	c9,t11 CLA isomer	36.1%
18:2	t10,c12 CLA isomer	36.3%
20:0	Arachidic acid	0.3%
20:1	Eicosenoic acid	0.3%
24:0	Lignoceric acid	0.1%
24:1	Nervonic acid	0.1%

TABLE 2. Subject characteristics.

	Age (yr)	Mass (kg)	Height (cm)	Physical Activity Score (Arbitrary)
CLA				
Males (N = 19)	26.6 ± 5.7	84.9 ± 13.7	178 ± 9	73 ± 44
Females (N = 19)	23.8 ± 6.2	66.4 ± 13.2	167 ± 9	60 ± 35
Placebo				
Males (N = 17)	23.9 ± 4.1	80.0 ± 16.0	178 ± 8	73 ± 41
Females (N = 21)	26.4 ± 9.2	66.3 ± 12.4	165 ± 5	58 ± 32

All values are means ± SD. No differences between CLA and placebo groups. Physical activity score determined by questionnaire. Subjects were asked how many times over a week they perform activities that are classified as "strenuous," "moderate," or "mild." Frequency of "strenuous" activities was multiplied by 9, frequency of "moderate" activities was multiplied by 5, and frequency of "mild" activities was multiplied by 3. The total was then summed for the physical activity score.

Measurement of Dependent Variables

Cessation of any strength training for at least 3 d before testing was required of each participant. Two separate sessions of testing were necessary both before and after the 7-wk training protocol. During the first session the following were measured, in order: RMR and respiratory exchange ratio, body composition, muscle thickness of knee extensors and elbow flexors, and peak torque of knee extensors. In the second session maximal strength (bench press and leg press) was measured. There was a minimum of 2 d of rest between the first and second sessions to offset any fatigue or muscle soreness caused by peak torque measurements.

Body Composition

Body composition was determined using air displacement plethysmography (Bod Pod SIL, Life Measurement Inc., with software version 1.69; Concord, CA) for measurement of body volume, as previously described (12). Body density was calculated by dividing the individual's mass by body volume, corrected for estimated lung volume. Percent body fat was estimated by the equation derived by Siri (30). Body fat was determined as body mass multiplied by percent body fat, and lean tissue mass as the difference between body mass and body fat mass. Reproducibility for lean tissue and fat mass was determined on 29 subjects and measured on two occasions, 1 wk apart. The coefficients of variation for lean tissue and fat mass were 0.9 and 5.4%, respectively. The validity of our Bod Pod was checked in a separate experiment by measuring 12 subjects on the Bod Pod and by dual-energy x-ray absorptiometry (DXA; Hologic QDR 2000, Waltham, MA). Correlation coefficients between Bod Pod and DXA measurements were 0.98 ($P < 0.01$) for both lean tissue and fat mass. Standard deviations for lean tissue and fat mass measurement were also similar between the two measurement instruments (SD of 8.0 and 7.7 kg for fat mass measured by the Bod Pod and DXA, respectively, and SD of 6.5 and 5.5 kg for lean tissue mass measured by the Bod Pod and DXA, respectively).

Muscle Thickness

Muscle thickness for elbow flexors and knee extensors on the right limbs was assessed using B-Mode ultrasound (Aloka SSD-500, Tokyo, Japan), using methods we have described recently in detail (9). Reproducibility of measurements of muscle thickness was determined on two separate days for 10 subjects. The coefficients of variation for elbow flexors and knee extensor muscle thickness were 1.4 and 0.8%, respectively.

Muscular Strength

Bench press and leg press strength were assessed by the 1RM using a standard testing protocol (6,9). Reproducibility of bench press and leg press was assessed on 10 subjects on two separate occasions. The coefficients of variation for bench press and leg press were 3.6 and 3.0%, respectively.

Maximal Torque

Concentric knee extension torque was assessed using an isokinetic dynamometer set at a velocity of 60°s^{-1} (Biodex System 3, Biodex Medical Systems, Inc., Shirley, NY) using procedures we have previously described in detail (6,7). Reproducibility was determined by measuring eight subjects on two occasions. The coefficient of variation for knee extension torque was 2.4%.

Resting Metabolic Rate and Respiratory Exchange Ratio

Before measurement of RMR each participant was asked to refrain from all exercise for 48 h, omit any caffeine intake for 24 h, and fast for 12 h. Measurements were taken in the morning between 5:30 and 9:00 a.m., and the testing before and after the 7-wk intervention was completed at the same time of day for each subject. Upon arrival the participants were weighed and then rested in the supine position on a bed for 30 min. RMR and respiratory exchange ratio (RER) were assessed by open circuit indirect calorimetry using a ventilated hood connected to a metabolic cart (Sensor Medics Vmax29 Series, Anaheim, CA), with subjects supine, as previously described (4). Measurements continued until the "steady state" had been reached, defined as five consecutive readings (separated by at least 1 min) with exhaled ventilation, oxygen uptake, and carbon dioxide expired values within 5% of their previous values. Estimates of daily energy expenditure ($\text{kcal}\cdot\text{d}^{-1}$) were provided by the software (Vmax/Sensor Medics Vision Software Version 4.3) accompanying the metabolic cart based on oxygen consumption and RER.

Myofibrillar Protein Degradation and Bone Resorption

Myofibrillar protein degradation was assessed from 3-methylhistidine, and bone resorption was assessed from cross-linked N-telopeptides of Type I collagen,

from urine samples collected before and immediately following the 7 wk of training and supplementation. Urine collection was preceded by a 3-d meat-free diet. Meat consumption affects 3-methylhistidine levels, and at least 3 d of a meat-free diet are required to return urine concentrations of 3-methylhistidine to baseline levels (21). The urine collection procedure involved discarding the first urination upon waking in the morning and then collecting all samples for the following 24 h, including the first one upon waking up the next morning. Urine samples were brought to the researcher, where the individual's urine volume was recorded. Aliquots of each urine sample were drawn off from the 24-h collection and stored at -20°C until analyzed.

The concentration of 3-methylhistidine was determined by high-performance liquid chromatography (3 mm Chromsep ODS-2 column, Varian Inc, Mississauga Ontario, Canada; flow rate $1.0\text{ mL}\cdot\text{min}^{-1}$) and 2475 multiwavelength fluorescence detection (Waters, Mississauga Ontario, Canada) using the methods of Wassner et al. (35) with modification for sample volumes. Derivatization was completed by placing $200\ \mu\text{L}$ of diluted (10 times with 0.9% NaCl) urine samples or 3-methylhistidine standards (Pfaltz and Bauer, Waterbury, CT), 1 mL of borate buffer (0.25 M boric acid, adjusted to pH 9.5 with NaOH), 1 mL of fluorescamine reagent (acetonitrile containing 1 mg of fluorescamine per milliliter) in glass autosampler vials, which were then mixed and allowed to stand at room temperature for 5 min. Two hundred microliters of 70% perchloric acid was added to the vials, which were capped with teflon-lined seals and heated at 80°C for 1 h. After cooling to room temperature, samples were injected ($20\ \mu\text{L}$) with an autosampler (715 Ultra WISP autoinjector, Waters). The mobile phase was 23% acetonitrile and 77% 20 mM Na_2HPO_4 adjusted to pH 7.2 with NaOH. Peaks were monitored at 365 nm (excitation) and 460 nm (emission) and integrated with chromatography software (Millennium chromatography manager Millennium32, version 4, Waters). The intraassay coefficient of variation from duplicate samples was 5.1%. The daily amount of 3-methylhistidine excreted by each subject was determined by multiplying the concentration by the 24-h urine volume.

The concentration of cross-linked N-telopeptides of Type I collagen was determined using a competitive-inhibition enzyme-linked immunosorbent assay (ELISA) according to procedures from commercially available kits (Osteomark NTx test, Ostex International, Inc., Seattle, WA). Samples were analyzed in triplicate within a single assay. The intraassay coefficient of variation was 9.4%. The daily amount of cross-linked N-telopeptides of Type I collagen excreted by each subject was determined by multiplying the concentration by the 24-h urine volume.

Dietary Assessment

Energy and macronutrient intake were assessed with a 3-d food record before and after the 7-wk intervention. Subjects were instructed to complete the food records on

days separate from the 3-d meat-free period required before urine collection. A booklet was provided for each participant to record the amount and type of food and beverage consumed for two weekdays and one weekend day. Fuel Nutritional software 2.1a (Logiform International, Inc., Saint-Foy PQ, Canada) was used to estimate average daily energy and macronutrient consumption.

Compliance, Side Effects, and Blinding Questionnaire

Subjects were given a questionnaire at the end of the study to evaluate side effects and the success of blinding for the supplement. The supplements consumed were confirmed by asking the subjects to return supplement envelopes, whether empty or not. To evaluate the success of the blinding the subjects were asked to indicate whether they thought they were in the CLA group, the placebo group, or "did not know."

Crossover Study

After the initial study, 27 subjects volunteered to continue training for an additional 7 wk, crossing over to the opposite supplement group after a 2-wk washout period. This additional study was approved by our university's biomedical ethics review board for research in human subjects, and each subject signed a new informed consent for participation in the extension. Subjects were informed in this consent that they would remain blinded to their group assignment until the end of the extension study. Of these subjects, 10 withdrew from the study (two males and three females from each of the CLA and placebo groups). Eight of these subjects withdrew because of lack of time. Two subjects (one from the CLA group and one from the placebo group) withdrew because of gastrointestinal distress that they felt was related to the supplement. Of the 17 remaining subjects, eight received CLA for the first 7 wk and placebo for the final 7 wk (CLA placebo, four males and four females; age = 28.1 ± 2.3 yr, mass = 83.7 ± 6.3 kg, height = 176 ± 4 cm), and nine received placebo for the first 7 wk and CLA for the final 7 wk (placebo CLA, four males and five females; 32.4 ± 3.0 yr, 81.4 ± 6.0 kg, 172 ± 2 cm). Subjects and investigators remained blinded to group assignments until the completion of the study and locking of the database. All dependent variables measured at baseline and 7 wk were measured again after 14 wk. At the end of the second 7-wk period, subjects were again given the questionnaire on blinding and side effects.

Statistical Analyses

Baseline characteristics were assessed by a two-factor (supplement group \times gender) ANOVA. For the first 7-wk study, all dependent variables were assessed by a three-factor (supplement group \times gender \times time) ANOVA, with repeated measures on the last factor. To clarify presentation of the results, a two-factor ANOVA (supplement group \times gender) was used to evaluate change scores for the dependent variables. Change scores were determined by

subtracting baseline measurements from week 7 measurements. An LSD *post hoc* test was used to identify differences between means when interactions were found.

For the crossover study, a three-factor (gender \times supplement \times time) ANOVA, with repeated measures on the last two factors, was used for assessment of dependent variables. A two-factor (gender \times supplement) ANOVA, with repeated measures on the second factor, was used to compare the change scores between the 7-wk phase during which CLA was consumed versus the 7-wk phase during which placebo was consumed.

Statistics were analyzed using Statistica version 5 (Stat Soft Inc., Tulsa, OK). All results are expressed as mean \pm SD. Significance was accepted at $\alpha \leq 0.05$.

RESULTS

Study 1: 7-wk Comparison Between CLA and Placebo Groups

There were no differences in any of the dependent variables at baseline between the CLA and placebo groups. Males had greater lean tissue mass, elbow flexors muscle thickness, bench press and leg press strength, knee extension torque, and RMR, and lower percent fat compared with females at baseline (all $P < 0.01$). Males and females had similar fat mass, knee extensor muscle thickness, and respiratory exchange ratio at baseline.

Body Composition

There was a gender \times time interaction for body mass ($P < 0.05$). Males had a significant increase in body mass over time (82.5 ± 14.4 to 83.4 ± 15.0 kg; $P < 0.01$), whereas females had no change (65.9 ± 12.6 to 66.2 ± 12.6 kg). There were no differences between CLA and placebo groups for body mass over time. There were supplement group \times time interactions for lean tissue mass, fat mass, and percent fat (Table 3). The CLA group had a greater increase in lean tissue mass (Fig. 1A), and a greater decrease in fat mass (Fig. 1B) and percent fat (Fig. 1C) compared with the placebo group.

Muscle Thickness

There was a time main effect for muscle thickness of the knee extensors (4.79 ± 0.87 cm at baseline vs 4.95 ± 0.78 cm at 7 wk; $P < 0.01$) and elbow flexors (2.55 ± 0.70 cm at baseline vs 2.78 ± 0.70 cm at 7 wk; $P < 0.01$). There were no differences between CLA and placebo groups over time. There was a trend for gender \times time interactions for both knee extensors ($P = 0.06$) and elbow flexors ($P = 0.08$) muscle thickness, with a trend for males to have greater increases than females.

Strength

For leg press strength, there was a main effect for time (190 ± 79 kg at baseline vs 236 ± 78 kg at 7 wk; $P < 0.01$),

TABLE 3. Changes in body composition for study 1.

	CLA		Placebo		P Group \times Time
	Pre	Post	Pre	Post	
Lean tissue mass (kg)	55.5 \pm 12.1	56.8 \pm 13.0**	52.5 \pm 10.8	52.7 \pm 11.0	0.033
Fat mass (kg)	19.9 \pm 9.1	19.1 \pm 9.3*	19.4 \pm 9.9	19.8 \pm 10.3	0.028
Fat percentage	26.0 \pm 8.5	24.7 \pm 9.0*	26.3 \pm 9.8	26.5 \pm 10.0	0.041

All values are means \pm SD.

* Significant compared with pre value ($P < 0.05$).

** Significant compared with pre value ($P < 0.01$).

with no differences between supplement groups or genders over time. For bench press strength, there was a significant supplement group \times gender \times time interaction (Fig. 2; $P < 0.05$). All groups increased bench press strength over time ($P < 0.01$); however, the males in the CLA group had greater increases than all other groups ($P < 0.05$).

Knee Extension Torque

There was a time main effect for knee extension torque (206 ± 61 N·m at baseline vs 217 ± 61 N·m at 7 wk; $P < 0.01$) with no differences between supplement groups or genders over time.

Resting Metabolic Rate and Respiratory Exchange Ratio

There was a supplement group \times time interaction for absolute RMR (Fig. 3A; $P < 0.05$). The CLA group had no change over time, whereas the placebo group had a significant decrease in RMR ($P < 0.05$). When expressed relative to lean tissue mass, there was no longer a difference in RMR between groups over time (Fig. 3B; $P = 0.10$). There were no changes in respiratory exchange ratio over time in any groups (CLA: 0.74 ± 0.06 to 0.75 ± 0.06 ; placebo: 0.75 ± 0.06 to 0.74 ± 0.06).

3-methylhistidine and Cross-linked N-telopeptides of Type I Collagen

There was a supplement group \times time interaction for 3-methylhistidine (Fig. 4; $P < 0.05$). The CLA group had no change over time, whereas the placebo group had a significant increase in 3-methylhistidine ($P < 0.05$). There were no changes in cross-linked N-telopeptides of Type I collagen over time in either group (CLA: 12.1 ± 11.7 to 12.5 ± 14.8 nmol·kg⁻¹ lean tissue mass·d⁻¹; placebo: 9.4 ± 6.5 to 11.8 ± 6.0 nmol·kg⁻¹ lean tissue mass·d⁻¹).

Dietary Intake

There were no differences between supplement groups from baseline to 7 wk for intake of calories (CLA: 2169 ± 676 to 2220 ± 666 kcal·d⁻¹; placebo: 2403 ± 868 to 2236 ± 710 kcal·d⁻¹), carbohydrate (CLA: 270 ± 88 to 271 ± 94 g·d⁻¹; placebo: 306 ± 143 to 285 ± 105 g·d⁻¹), protein (CLA: 97 ± 39 to 98 ± 39 g·d⁻¹; placebo: 99 ± 50 to 94 ± 44 g·d⁻¹), or fat (CLA: 78 ± 33 to 79 ± 33 g·d⁻¹; placebo:

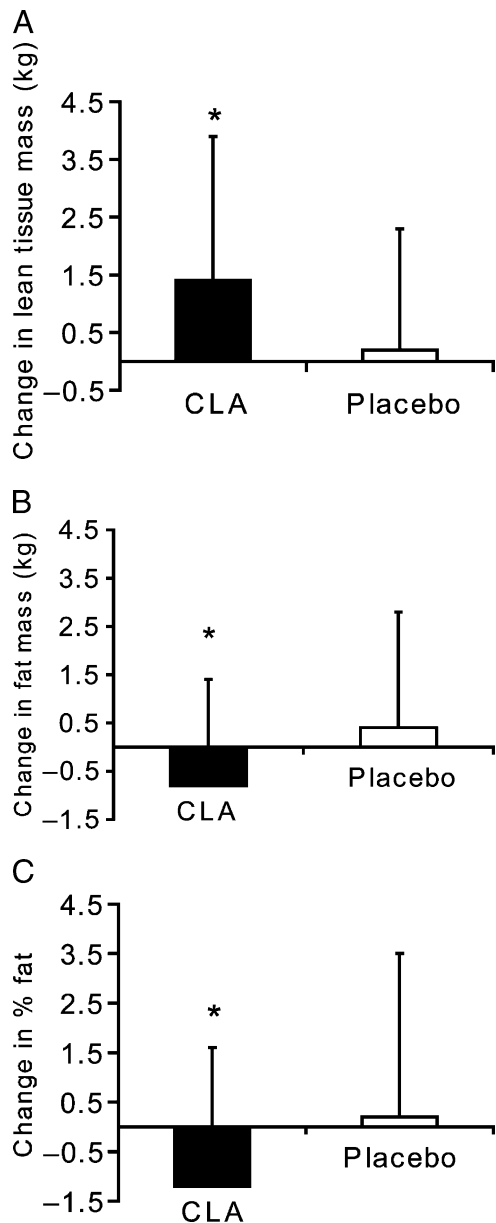


FIGURE 1—Changes in A) Lean tissue mass, B) Fat mass, and C) percent fat after 7 wk of supplementation and resistance training for CLA ($N = 37$) and placebo ($N = 38$) groups. Values are means \pm SD. * CLA group significantly different than placebo ($P < 0.05$).

85 ± 39 to 75 ± 33 $\text{g}\cdot\text{d}^{-1}$). Males had significantly greater intakes for calories ($P < 0.05$), protein ($P < 0.01$), and a trend for greater fat intake ($P = 0.055$) compared with females.

Compliance, Blinding, and Side Effects

From the counts of returned supplements, the CLA group consumed $99.1 \pm 2.5\%$ of their capsules, whereas the placebo group consumed $99.6 \pm 1.2\%$ of their capsules.

Eight subjects in the CLA group and seven subjects in the placebo group reported adverse events that the investigators considered related to the supplement. Two of the eight subjects in the CLA group and four of the seven subjects in the placebo group reported multiple adverse events. The adverse events reported from CLA included upset stomach/indigestion (six), heartburn/reflux

(two), and nausea (two). Adverse events reported from the placebo included upset stomach (five), diarrhea (two), loss of appetite (two), bloating (two), and constipation (one). The intensities of all adverse events were considered “mild” to “moderate” and were transient. There were no differences between CLA and placebo groups for number of adverse events. No serious adverse events occurred.

Study 2: Cross-over Study

There were no differences in any measures at baseline for the subjects in the CLA-placebo group versus the placebo-CLA group. Physical activity levels outside the training program were similar between the two phases (physical activity score (arbitrary units) = 52 ± 33 during CLA and 51 ± 25 during placebo), as were dietary intakes. Results for the crossover study are presented in Table 4. There were significant supplement \times time interactions for body mass, fat mass, percent fat, elbow flexors muscle thickness, 3-methylhistidine, and cross-linked N-telopeptides of Type I collagen ($P < 0.05$). Subjects increased body mass, fat mass, and percent fat while on placebo ($P < 0.05$) but not while on CLA. Subjects increased elbow flexor muscle thickness while on CLA ($P < 0.05$) but not while on placebo. Subjects increased 3-methylhistidine ($P < 0.01$) and cross-linked N-telopeptides of Type I collagen ($P < 0.01$) while on placebo, and decreased cross-linked N-telopeptides of Type I collagen while on CLA ($P < 0.05$). There were no differences for changes in lean tissue mass, knee extensor muscle thickness, strength, torque, RMR, or respiratory exchange ratio while on CLA versus placebo. From counts of returned supplements, subjects consumed 98.4% of capsules during the CLA phase and 99.8% of capsules during the placebo phase.

Our questionnaire on the success of the blinding indicated that after the CLA phase, six subjects correctly indicated they

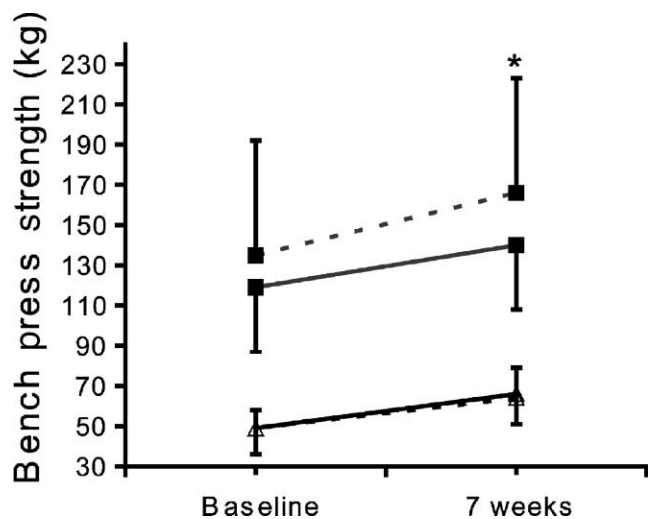


FIGURE 2—Bench press strength (1RM) at baseline and after 7 wk of supplementation and resistance training for CLA males ($N = 19$; squares, dashed line), placebo males ($N = 16$; squares, solid line), CLA females ($N = 19$; triangles, dashed line), and placebo females ($N = 20$; triangles, solid line). Values are means \pm SD. * The increase in bench press strength for males on CLA was greater than those of males on placebo and females on CLA or placebo ($P < 0.05$).

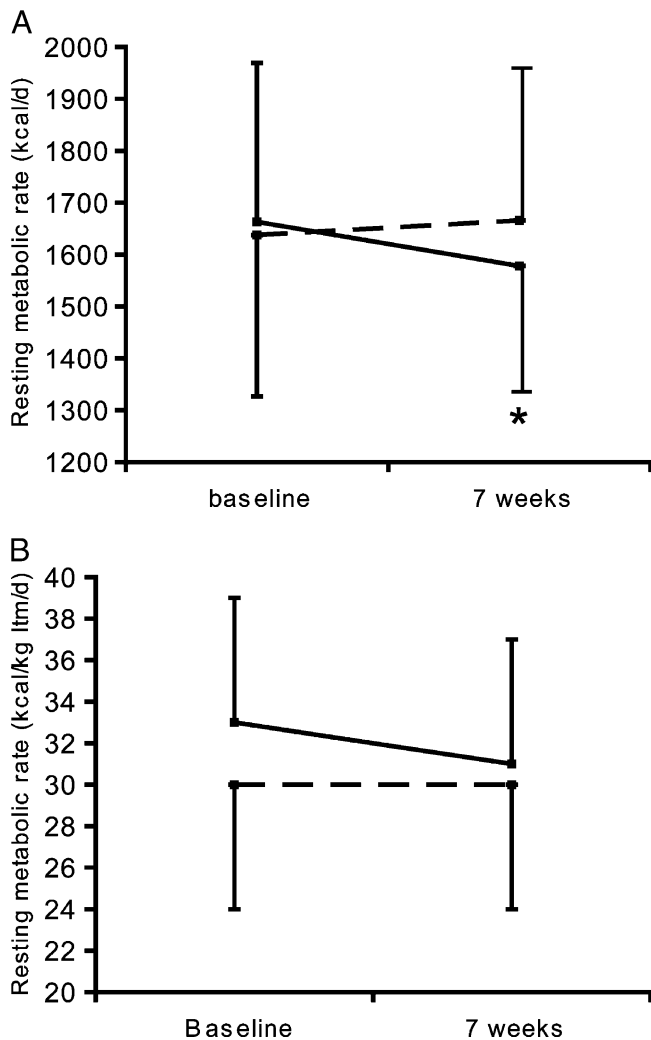


FIGURE 3—A) Absolute and B) relative (to lean tissue mass) RMR at baseline and after 7 wk of supplementation and resistance training for CLA ($N = 31$; dashed line) and placebo ($N = 32$; solid line) groups. Values are means \pm SD. * Placebo group significantly decreased absolute RMR from baseline to 7 wk ($P < 0.05$).

were taking CLA, six incorrectly indicated they were taking placebo, and five indicated they did not know which supplement they were taking. After the placebo phase, seven subjects correctly indicated they were taking placebo, five incorrectly indicated they were taking CLA, and five indicated they did not know which supplement they were taking.

Of the 17 subjects who completed the crossover study, four reported adverse events considered related to the supplement while taking CLA, and two reported adverse events while taking placebo. The two subjects reporting side effects during placebo reported multiple adverse events. Adverse events while taking CLA included upset stomach (three) and diarrhea (one). Adverse events while taking placebo included bloating (one), constipation (one), upset stomach (one), and diarrhea (one). Two additional subjects withdrew from the study during the second 7-wk period due to recurring upset stomach—one while taking CLA and one while taking placebo. All adverse events were considered mild to moderate in intensity and there were no differences between the CLA and placebo phase.

DISCUSSION

The main findings of our first 7-wk study were that CLA supplementation during resistance training significantly increased lean tissue mass and reduced fat mass. Although these results were statistically significant, the changes in the CLA group were small, and one could question their clinical significance. The small increase in lean tissue mass may be attributed to a lessened catabolic effect of training with CLA supplementation, as evidenced by an increased urinary 3-methylhistidine output in the placebo group with no change in the CLA group. The small increase in lean tissue mass with CLA was not sufficient for increasing muscular strength, with the exception of bench press strength in males. The contention that CLA affects body composition is not supported fully by our second study, in which a number of subjects crossed over to opposite treatment groups. There were supplement \times time interactions for fat mass and fat percentage, but this was due to significant increases during the placebo phase; there were no significant changes during the CLA phase of our crossover study. The crossover study did indicate that CLA may decrease bone resorption.

A longer duration of study may be necessary to see clinically relevant changes in body composition. In the largest ($N = 180$) and longest (1 yr) study so far on CLA in humans, Gaullier et al. (16) found larger reductions in body fat mass in overweight subjects on CLA compared with placebo (i.e., 1.7-kg reduction in fat mass for a CLA-free fatty acid group and 2.4-kg reduction for a CLA-triacylglycerol group, compared with a 0.2-kg increase for a placebo group). Similar to our study, they found minimal changes in lean tissue mass (i.e., 0.6–0.7 kg increases in their CLA groups compared with no change for their placebo group). The slightly greater increase in lean tissue mass (1.4 kg) in our study may be due to a primary effect of CLA on increasing training capacity.

Only two previous studies have evaluated the effects of CLA in conjunction with exercise training in humans. Thom

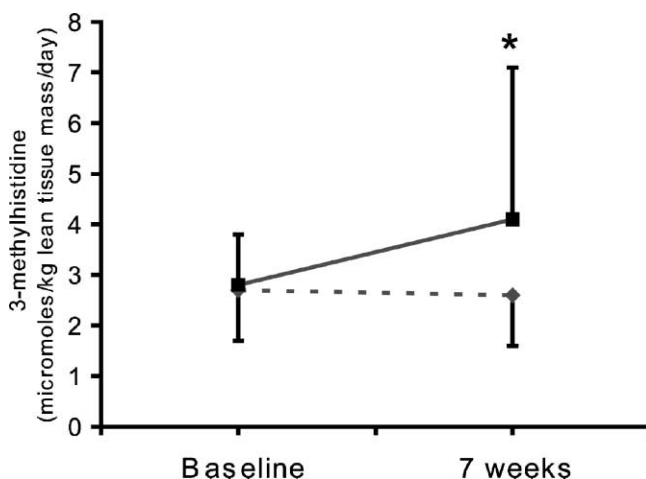


FIGURE 4—Urinary 3-methylhistidine at baseline and after 7 wk of supplementation and resistance training for CLA ($N = 26$; diamonds, dashed line) and placebo ($N = 25$; squares, solid line) groups. Values are means \pm SD. * Placebo group significantly increased 3-methylhistidine from baseline to 7 wk ($P < 0.05$).

TABLE 4. Changes in dependent variables during the crossover study.

	CLA		Placebo		P Supplement × Time
	Pre	Post	Pre	Post	
Body mass (kg, <i>N</i> = 17)	82.7 ± 17.2	83.0 ± 17.2	81.9 ± 17.2	83.2 ± 17.2*	0.025
Lean tissue mass (kg, <i>N</i> = 17)	56.1 ± 11.1	56.5 ± 11.1	56.5 ± 10.7	56.3 ± 11.1	NS
Fat mass (kg, <i>N</i> = 17)	26.6 ± 11.9	26.4 ± 11.9	25.4 ± 12.3	26.8 ± 9.0*	0.038
Fat percentage (<i>N</i> = 17)	31.3 ± 9.8	31.0 ± 9.8	29.9 ± 10.3	31.4 ± 9.4*	0.043
Elbow flexors muscle thickness (cm, <i>N</i> = 15)	2.8 ± 0.8	3.2 ± 0.8	2.9 ± 0.8	3.0 ± 0.8	0.030
Knee extensor muscle thickness (cm, <i>N</i> = 14)	5.2 ± 0.7	5.3 ± 0.7	5.2 ± 0.7	5.2 ± 1.1	NS
Bench press strength (kg, <i>N</i> = 14)	98 ± 67	115 ± 78*	98 ± 78	109 ± 63*	NS
Leg press strength (kg, <i>N</i> = 14)	216 ± 89	243 ± 89*	210 ± 81	250 ± 93*	NS
Knee extension torque (N·m, <i>N</i> = 17)	228 ± 62	239 ± 62*	226 ± 66	233 ± 62	NS
Resting metabolic rate (kcal·d ⁻¹ , <i>N</i> = 11)	1764 ± 350	1762 ± 380	1834 ± 327	1763 ± 323	NS
Respiratory exchange ratio (<i>N</i> = 11)	0.72 ± 0.07	0.75 ± 0.07	0.70 ± 0.07	0.76 ± 0.03	NS
3-MH (μmol·kg ⁻¹ lean tissue·d ⁻¹ , <i>N</i> = 13)	4.1 ± 2.2	2.9 ± 1.1	2.4 ± 0.7	4.6 ± 1.8*	0.010
Ntx (μmol·kg ⁻¹ lean tissue·d ⁻¹ , <i>N</i> = 13)	13.5 ± 6.1	8.7 ± 4.3*	6.1 ± 3.2	13.4 ± 6.1*	0.0015

All values are means ± SD. 3-MH, 3-methylhistidine; Ntx, N-telopeptides.

* Significant compared with pre value (*P* < 0.05).

et al. (33) had subjects (men and women) supplement 1.8 g·d⁻¹ of CLA in combination with regular (90 min, three times per week) strenuous exercise and reported a marked reduction in percent body fat compared with the placebo group. Furthermore, significant group differences (in percent body fat) were observed in as little as 4 wk and continued to conclusion (12 wk) of the study. The type of exercise in the study of Thom et al. (33) was not described as making it difficult to make more definitive judgments and comparisons. Kreider et al. (20) recruited experienced (>1 yr, 3 × wk⁻¹) resistance-trained men to supplement with CLA (6.0 g·d⁻¹) while performing their regular training program for a 4-wk period. Despite the higher dosage, CLA was found to have no effect on fat or lean mass. The studies of Thom et al. (33) and Kreider et al. (20) differed in their methods for assessing body composition, with the former using near infrared light and the latter using dual energy x-ray absorptiometry. Similar to the study by Thom et al. (33), the changes in body composition with CLA in the current study were fast compared with other studies of CLA (3,16). This may be due to the higher amount of CLA we used or because of the combination of exercise training along with the CLA supplementation. Our body composition findings are supported by a recent study that combined exercise training (treadmill running) and CLA in mice (2). Mice that were exercise trained and that received CLA increased lean tissue mass and reduced fat mass to a greater extent than mice that were only exercised, only received CLA, or received neither intervention.

Various mechanisms have been suggested for the physiological effects of CLA. Previous studies have suggested that CLA may attenuate skeletal muscle atrophy by modulating the immune response to stress (2,23). Miller et al. (23) found that feeding CLA to mice partially attenuated the catabolic response to an endotoxin injection. The end result of feeding CLA was a reduction of the overall loss of muscle mass in the injected mice. Bhattacharya et al. (2) found CLA to ameliorate the inflammatory effect of treadmill exercise training in mice by decreasing production of the proinflammatory cytokines interleukin-6 and tumor necrosis factor-α. This occurred in conjunction with an increase in lean tissue mass in their exercise-trained mice that received CLA. The amount of lean tissue mass accrued through resistance

training results from the balance between protein synthesis and degradation, both of which are stimulated by bouts of resistance training (7,29). Our results indicated that CLA may have lessened the catabolic effects of training, because 3-methylhistidine increased with placebo supplementation, but was unchanged with CLA supplementation (Fig. 4; Table 4).

Supplementation with CLA did not affect muscular performance measures (with the exception of bench press strength in males). The relative lack of improvement in strength with CLA supplementation is consistent with the relatively small increase (i.e., 1.4 kg) in lean tissue mass. Increases in strength with short durations of training, as in the 7 wk protocol of the current study, can be attributed to mainly neural changes or learning effects (8). Similar to the first part of the current study, there were no differences for strength improvements between the CLA and placebo, and no effect of CLA on lean tissue mass in our crossover study. The changes in strength and lean tissue mass with CLA in the current study are especially small when compared with other nutritional supplements such as creatine monohydrate (6).

Animal and cellular studies have suggested that CLA may reduce body fat mass by a number of mechanisms. These include 1) increased energy expenditure, possibly through an increase in uncoupling protein (37,38); 2) inhibition of lipoprotein lipase, therefore reducing the amount of fat that can be stored in adipocytes (27); 3) increased activity of carnitine palmityltransferase, therefore increasing capacity for fatty acid oxidation (27); 4) apoptosis or decreased differentiation of adipocytes, possibly through an inhibition of peroxisome proliferator activated receptor γ, a nuclear receptor involved in regulation of genes that regulate energy metabolism, cell apoptosis, proliferation, and differentiation (5,15); and 5) decreased serum leptin, a cytokine released by adipose tissue that plays a role in regulating energy balance through effects on food intake and energy expenditure (2). CLA has, to a lesser extent, been shown to reduce body fat in humans (3,18,33). Our results indicate that CLA had no effects on RMR, fat oxidation (i.e., respiratory exchange ratio), or dietary intake. We did find a group × time interaction for RMR; however, this was due to a significant decrease in RMR in the placebo group, with no significant change in the CLA group. The decrease in RMR in the placebo group could be

explained by seasonal changes or measurement error, as the day-to-day variation in this measurement is high. The majority of subjects had their baseline RMR measured in the month of May, when outdoor temperatures are still relatively cool in Saskatchewan, whereas their 7-wk measurements were during the month of July, when outdoor temperatures are quite hot. Although indoor temperature was constant during the study, cooler outdoor temperatures result in higher RMR than warmer outdoor temperatures, even if the RMR measurement is performed indoors at a constant temperature (19). Previous studies in humans are equivocal with regards to an effect of CLA on RMR, with one recent study showing CLA increased RMR (18), whereas another showed no effects of CLA (39).

One finding of interest in the current study was that a marker of bone resorption, urinary cross-linked N-telopeptides of Type I collagen, was decreased in subjects who participated in the crossover portion of our study while on CLA compared with placebo (Table 4). This result, however, was not supported in the first part of our study, where a larger group of individuals on CLA had similar changes in this marker of bone resorption compared with subjects on placebo. The differences between the two studies could be due to their designs—the crossover study involved a within-subjects comparison, whereas the first study involved a between-subjects comparison. The within-subjects comparison would result in less variability when comparing CLA with placebo. Research in animals indicates CLA may have a positive influence on bone mineral (28). Conjugated linoleic acid may inhibit the production of prostaglandin E (PGE₂), which when released in high amounts is a potent stimulator of bone resorption. Conjugated linoleic acid may inhibit PGE₂ synthesis by blocking the access of its precursor, arachidonic acid, to cyclooxygenase enzyme (36). Alternatively, CLA may modulate production of bone resorptive cytokines (36). Only two other studies in humans have evaluated the effects of CLA on bone. Kreider et al. (20) found a trend for an increase in whole-body bone mass ($P = 0.08$) with a moderate-to-large effect size during 28 d of CLA supplementation combined with strength training. Gaullier et al. (16) found that whole-body bone mineral mass was slightly reduced over 1 yr in one of their two CLA groups (the CLA-free fatty acid group, but not the CLA-triacylglycerol group). The reduction was small (i.e., 1.4%) and without significance between groups. Further

long-duration studies in humans are necessary to investigate the effects of CLA on sites in the body that are susceptible to bone loss and osteoporotic fracture (i.e., the proximal part of the femur and the lumbar spine).

One limitation of our crossover study was a lack of power for some of the measurements due to low subject numbers or high variability for some measurements. The body composition changes in the first study (i.e., increased lean tissue mass and decreased fat mass) were not supported by the crossover study. In the crossover study, subjects unexpectedly gained a significant amount of fat mass during the placebo phase, whereas fat mass was unchanged in the CLA phase (Table 4). This increase in fat mass may have been due to a seasonal effect, but this was not reflected in the diet records, which were not different between the two phases. The dietary records were quite variable and small differences may not have been detected. One additional limitation was that the dietary records were recorded 3 d after the supplement phases, and therefore may have been affected by the end of the supplement. Additionally, the washout period between the phases of the crossover study was only 2 wk; this may not have been adequate, and there may have been some carryover of effects between phases.

In summary, our initial study indicated CLA has small effects for decreasing fat mass and increasing lean tissue mass. Although these effects were statistically significant, the clinical significance is most likely quite small. The effect of CLA on fat and lean tissue mass was not supported in our crossover study, as subjects had no changes during the phase they took CLA. When deciding whether to use CLA as a dietary supplement, one would have to weigh these relatively small beneficial effects against the relatively high monetary costs of CLA.

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