Conjugated linoleic acid is related to bone mineral density but does not affect parathyroid hormone in men

Jason R. DeGuire, Nour Makarem, Catherine A. Vanstone, Suzanne Morin, Gustavo Duque, Hope A. Weiler

School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec, Canada
Metabolic Bone Centre, McGill University Health Centre, Montreal, Quebec, Canada
Ageing Bone Research Program, Sydney Medical School Nepean, University of Sydney, Sydney, New South Wales, Australia

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The relationships between conjugated linoleic acid (CLA) status, bone, body composition, and the effect of CLA on calcitropic hormones are unclear. A cross-sectional study was designed to examine the association between c9, t11 CLA status in erythrocyte membranes (RBC) and body composition. This preceded a dose-response trial investigating if c9, t11 CLA affected parathyroid hormone (PTH). It was hypothesized that (1) higher c9, t11 CLA status in RBC will be associated with a lower fat and higher bone mass and that (2) PTH will be reduced by 30% after supplementation of c9, t11 CLA. Fifty-four men (age, 19-53 years) were included in the cross-sectional analysis, of which 31 were studied in the dose-response trial and randomized to 1 of 3 groups: placebo (n = 10), 1.5 g/d (n = 11), or 3.0 g/d (n = 10) of c9, t11 CLA for 16 weeks. Men with RBC c9, t11 CLA status above the median had higher whole body bone mineral density (BMD) (1.359 ± 0.024 vs 1.287 ± 0.023 g/cm²; P = .04) and whole body lean mass percentage (78.8% ± 0.9% vs 75.3% ± 1.0%; P = .01), whereas body mass index (24.8 ± 0.5 kg/m² vs 27.3 ± 0.9 kg/m²; P = .01) and whole body fat mass percentage (17.3% ± 0.9% vs 21.3% ± 1.1%; P = .007) were lower. In regression analysis, RBC c9, t11 CLA status accounted for a significant proportion (r² = 0.10) of the variation in whole body BMD (P = .03). There were no time or treatment differences among any bone or biomarkers of bone metabolism including PTH. These findings indicate that RBC c9, t11 CLA status, a reflection of long-term (~4 months) dietary CLA intake, positively relates to BMD. However, c9, t11 CLA supplementation does not appear to affect PTH in healthy men.

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1. Introduction
Conjugated linoleic acid (CLA), specifically the cis-9, trans-11 (c9, t11) and trans-10, cis-12 (t10, c12) CLA isomers, is a commercially available nutraceutical marketed to promote weight loss or improve body composition. In addition to its positive effects on adipose [1] and lean mass [2], CLA supplementation increases whole body ash in mice [3,4]. In

Abbreviations: 1.5CLA, 1.5 g/d c9, t11 CLA; 25(OH)D, 25-hydroxyvitamin D; 3.0CLA, 3.0 g/d c9, t11 CLA; BAP, bone-specific alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; c9, t11, cis-9-trans-11 CLA isomer; CLA, conjugated linoleic acid; FFA, free fatty acid; FFQ, food frequency questionnaire; HDL, high-density lipoprotein cholesterol; HR, heart rate; iCa, ionized calcium; LDL, low-density lipoprotein cholesterol; OC, osteocalcin; PO₄, phosphate; PTH, parathyroid hormone; RBC, erythrocytes; t10, c12, trans-10, cis-12 CLA isomer; TG, triacylglycerol; WBF, whole body fat percentage; WBL, whole body lean percentage.

Corresponding author. School of Dietetics and Human Nutrition, McGill University, Montreal, Quebec, Canada H9X 3V9. Tel.: +1 514 398 7905; fax: +1 514 398 7739.
E-mail address: hope.weiler@mcgill.ca (H.A. Weiler).

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growing mice and chicks, CLA results in greater amounts of trabecular and cortical bone [5–7]. There are few studies that have examined the relationship between dietary CLA and bone in humans. A cross-sectional analysis in postmenopausal women suggested that dietary CLA was positively related to bone mineral density (BMD) of the hip, specifically Ward triangle [8]. However, 2 randomized controlled trials in adults and children show mixed results. In middle-aged overweight (body mass index [BMI], 25-30 kg/m²) men and women supplemented for 24 months with either 50:50 mix (c9, t11 and t10, c12) CLA in free fatty acid (FFA) form or triacylglycerol (TG) form, a significant positive change in whole body bone mineral content (BMC) from 12 to 24 months was found in the FFA group compared with the TG and placebo groups [9]. However, no differences in BMC and BMD were observed in children (age, 6-10 years), after a 7-month trial of 50:50 mix (c9, t11 and t10, c12) CLA in TG form or a placebo [10]. These studies suggest that supplemental CLA in FFA form may be associated with bone measurements in humans.

There is evidence that parathyroid hormone (PTH) is responsive to CLA in humans and animals, and this might explain the various responses in bone. In primigravidas with a family history of preeclampsia supplemented with calcium and 50:50 mix (c9, t11 and t10, c12) CLA from weeks 18 to 22 of pregnancy until delivery, PTH is reduced by 8.0%, but this change was not significant [11]. Furthermore, the c9, t11 CLA isomer decreased PTH by 40% in both healthy [12] and polycystic kidney disease [13] male rats. In line with average values for PTH being in the reference range for each rat model, no changes in BMC or BMD were observed [14, 15]. Chronic high PTH concentrations are known to promote bone loss, whereas intermittent or pulsatile PTH administration increases bone formation and BMD and reduce osteoporosis-related fractures [16]. Enhanced BMD in human trials of calcium supplementation is ascribed to reduced PTH levels [14, 17]. Therefore, to clarify the relationship between dietary CLA and BMD, it is essential to assess PTH response after CLA supplementation. The study was conducted in men because c9, t11 CLA reduced PTH in male but not female rats [12]. The objectives of this study were to (1) investigate if basal c9, t11 CLA status in erythrocytes (RBC), as assessed by gas chromatography analysis, is associated with enhanced body composition and bone mass (assessed by dual-energy x-ray absorptiometry) and (2) to determine the response of bioactive and intact PTH concentrations, measured by chemiluminescence, to supplemental c9, t11 CLA. It was hypothesized that higher c9, t11 CLA status in RBC at baseline, which reflects long-term (~4 months) CLA intake, will be associated with a lower fat and higher bone mass and that after a dose-response trial, PTH will be reduced by 30% in the highest c9, t11 CLA supplemental dose vs the placebo group.

2. Methods and materials

2.1. Participants

Fifty-four community-dwelling adult men from the greater Montreal area were studied for the cross-sectional analysis and screened for entrance criteria for the dose-response study. All study visits took place at the Mary Emily Clinical Nutrition Research Unit, School of Dietetics and Human Nutrition. Participants all gave written consent before inclusion into the study. Participants were excluded from the dose-response trial (but not for the cross-sectional analysis) if they were taking prescribed medication, had allergies to nuts or

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Fig. 1 – Consort diagram. One participant dropped out and was classified as intent to treat.
of this study received a letter of no objection by Health
fier: NCT00608400) and the supplements used for the purpose
McGill University (IRB A01-M11-09B; ClinicalTrials.gov identi-
This study was approved by the Research Ethics Boards of
by the study physician. Of the 54 men screened, 31 met the
4 BMD z-score greater than
4.5, and general health clearance by the study physician. Of
ethnicity [15]), plasma 25-hydroxyvitamin D (25[OH]D) con-
predictions of percentage body fat by BMI, age, sex, and
1.0, and general health clearance by the study physician. Of
and/or a percentage body fat less than 23% (based on
of percentage body fat by BMI, age, sex, and
effect [15]), plasma 25-hydroxyvitamin D (25[OH]D) concen-
tion greater than 60 nmol/L, lumbar spine vertebrae 1 to
4 BMD z-score greater than −1.0, and general health clearance
by the study physician. Of the 54 men screened, 31 met the
criteria and were included in the dose-response study (Fig. 1). This study was approved by the Research Ethics Boards of McGill University (IRB A01-M11-09B; ClinicalTrials.gov identifier: NCT00608400) and the supplements used for the purpose of this study received a letter of no objection by Health Canada’s Natural Health Products Directorate.

2.2. Dose-response trial design
This study was a double-blind, placebo-controlled randomized clinical trial phase II. The participants were stratified by age (<37 or >38 years) and then randomized to 1 of 3 groups: (1) 4.2 g olive oil (placebo, n = 10), (2) 2.1 g olive oil and 2.1 g (1.5 g c9, t11 CLA [1.5CLA], n = 11), or (3) 4.2 g (3.0 g c9, t11 CLA [3.0CLA], n = 10), as shown in Fig. 1. The incomplete randomization block was started before the decision to stop recruiting, resulting in having 11 participants in the 1.5CLA group only. Olive oil was selected as the placebo because the small amount used has no significant biological activity on PTH, bone, or body composition [1,9,18]. The fatty acid composition of the CLA (in kind, Lipid Nutrition; Loders Croklaan BV, Wormerveer, The Netherlands) and the extra virgin olive oil (Cibaria International Inc. CA, USA) is shown in Table 1. Both were encapsulated in amber opaque soft gel capsules identical in taste, smell, and appearance. Each participant took a total of 6 capsules per day (placebo and/or CLA capsules) with lunch (3) and dinner (3) meals to arrive at their respective dosage of CLA (0, 1.5, or 3 g/d). During the 8- and 16-week visits, all unused capsules were recovered for compliance assessment and a new supply was provided. Participants were defined as compliant if they took 80% or more of the supplements provided. Participants were asked to maintain their usual dietary and physical activity habits throughout the study.

2.3. Clinical assessments
Anthropometry data (height, weight, BMI, waist circumference) as well as systolic and diastolic blood pressure and heart rate (HR) were recorded at baseline and weeks 4, 8, 12, and 16. Body composition was assessed at baseline and at week 16. All 5 visits (baseline and 4, 8, 12, and 16 week) took place after a 12-hour fast at 7:00 to 10:00 AM during which a blood sample (15 mL) was collected in lithium heparin and plasma separated and stored at −80 °C. Erythrocytes were washed twice with saline then stored at −80 C in an equal weight of water/methanol/Butylated hydroxytoluene solution to prevent polyunsaturated fatty acid oxidation, as described by Magnusardottir and Skuladottir [19]. Plasma was used to analyze intact (LIAISON N-TACT PTH assay 310660), bioactive (LIAISON 1-84 PTH assay 310630) PTH, 25(OH)D (LIAISON 25 OH Vitamin D Total 310600), osteocalcin (OC; LIAISON Osteocalcin 310950), and bone-specific alkaline phosphatase (BAP; LIAISON BAP Ostease 310970) at McGill University using chemiluminescence assays (Liaison, DiaSorin, Stillwater, MN, USA). In addition, blood was analyzed for ionized calcium (iCa) (ABL800 FLEX analyzer; Radiometer, Copenhagen, Denmark), phospho-(PO4), and lipid profile including serum high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, TG, and total cholesterol (SYNCHRON LX20 PRO Clinical System; Beckman Coulter, Brea, CA, USA) at the McGill University Health Centre (Montreal General Hospital, QC, Canada). The coefficient of variation for all parameters (interassay and intra-assay) was less than 10%.

2.4. Diet and physical activity
Usual dietary intake was assessed during the screening visit by a nutritionist using an interviewer-administered modified semiquantitative food frequency questionnaire (FFQ) (Harvard/Willett [20]). The modification captured supplement use and frequency. Before use, the FFQ was translated to French and back-translated to ensure consistency of data collection among the English- and French-speaking participants. The FFQ was a 131-item semiquantitative FFQ designed to classify individuals according to their level of daily intake of multiple nutrients [20]. Questions that originally combined beef and lamb were separated to improve estimation of CLA. Furthermore, dietary assessment was performed using a 24-hour food recall at each visit plus every 2 weeks by telephone, for a total of 9 recalls for the duration of the study. Nutrient analysis of listed food items was determined using Nutritionist Pro version 2.2 (Nutritionist Pro; Axxya Systems, Stafford, TX, USA) and the 2010 Canadian Nutrient File. Estimates for CLA intake for both the FFQ and food recalls were calculated by multiplying the fat content of a food item by the concentration of CLA [21–23]. Physical activity was assessed during the
screening visit using the Paffenbarger Physical Activity Questionnaire that estimates physical activity during a 12-month period and is valid in several population groups including healthy men [24–30].

2.5. Anthropometry and body composition

Blood pressure was measured using a standard radial cuff and sphygmomanometer and HR ((beats/15 s) × 4) was assessed manually via palpation of the radial artery in a seated position after 5 minutes of rest. Height and weight were measured and standardized by having participants wear thin cotton pants and T-shirt without any metal. Weight was measured using a standard balance platform beam scale (Detecto, Webb City, MO, USA), height was measured using a stadiometer (Seca 213, Birmingham, UK), and BMI was calculated according the Health Canada guidelines [31]. Waist circumference was measured between the lower costal margin and the iliac crest according to Health Canada guidelines [31]. In addition, although the 16-week study would likely not enable sufficient time to detect minimal changes in BMC and BMD, we conducted the measures at both baseline and week 16 for the purpose of safety monitoring. Whole body composition, whole body lean mass (WBL), whole body fat (WBF), and measurement of bone area, BMC, and BMD of the whole body, spine vertebrae lumbar 1 to 4, and hip (total hip plus femoral neck) were assessed using dual-energy x-ray absorptiometry with Hologic Apex v3.2 analysis software (QDR 12.3:5, 4500A Discovery Series; Hologic Inc, Bedford, MA, USA). The coefficient of variation for BMD, BMC, and area (assessed using a spine phantom over the course of the study) was 0.325%, 0.498%, and 0.383%, respectively.

2.6. Fatty acid analyses of lipids in plasma and RBCs

Conjugated linoleic acid was measured from fasting samples using a modified method of Bondia-Pons et al [32] to determine short (plasma c9, t11 CLA) and long-term (RBC c9, t11 CLA) supplement compliance in all groups, as well as basal c9, t11 CLA status. Modifications included the use of 100 μL of plasma or 200 μL of RBC combined with 10 μL of heptadecanoic acid (internal standard) and the addition of 300 μL of hexane (600 μL for RBC) after methylation. Fatty acid methyl esters were separated using a 100-m CP-Sil-88 capillary column (Varian-Chrompack, CP7489), installed in a Varian CP-3800 Gas Chromatograph, (Varian, Inc, Walnut Creek, CA, USA) with a flame-ionization detector. For identification and quantification of the fatty acid methyl ester peaks, authentic standard 461 (catalogue no. GLC-461; Nu-Chek Prep, Inc, Elysian, MN, USA) was used. In addition, c9, t11 octadecadienoic acid and t10, c12 octadecadienoic acid (catalogue no. UC60M & UC61M; Nu-Chek Prep) and standards of known concentration were used to calculate recovery (>90%). The fatty acids were expressed as a percentage of the total fatty acids identified from docosahexaenoic acid (C12:0) to docosahexaenoic acid (C22:6).

2.7. Adverse effects and safety monitoring

At each visit, participants were asked using a questionnaire if they experienced any illness or been treated for acute illnesses. Frequency of headaches, dizziness, constipation, cramping, nausea, vomiting, diarrhea, or abdominal pain was documented. The study physician provided safety monitoring throughout this study including objective assessment of iCa, PO4, or PTH concentrations.

2.8. Statistical analyses

A sample size of 10 was calculated for an analysis of variance design with 3 groups based on an SD of 10.43 pg/mL [33] combined with an $\alpha$ value set at .05 and power at 0.80 and a detectable difference of 10.34 pg/mL from the means. Data were analyzed using SAS statistical package software version 9.2.0 (SAS Institute Inc, Cary, NC, USA) and was tested for normality and homogeneity of variances before using a mixed model with Tukey post hoc comparisons. A mixed-model repeated-measures design included time, CLA dosage, and plasma 25(OH)D concentration as a fixed effect and individual participants nested for treatment and block randomization as random effects. Pearson correlation coefficients ($r$) were calculated as preliminary evaluation to determine the degree of association between plasma and RBC CLA vs body composition and bone-related assessments for the cross-sectional analysis. Multiple regression models controlled for energy (in kilocalories) and dietary protein intake (in grams), WBL (in percent), BMI (in kilograms per meter squared), and plasma 25(OH)D concentration (in nanomoles per liter), all of which are known to affect bone. Data are presented as means ± SEMs.

3. Results

3.1. Cross-sectional analysis of the baseline assessment

3.1.1. Conjugated linoleic acid status, bone, and body composition

Anthropometric, body composition, and dietary measurements for all men were within healthy ranges (Table 2).
When participants were stratified on RBC CLA level above and below the median RBC CLA, whole body BMD (1.359 ± 0.024 g/cm² vs 1.287 ± 0.023 g/cm²; P = .04) and WBL (78.8% ± 0.9% vs 75.3% ± 1.0%; P = .01) were higher in the above median group, whereas BMI (24.8 ± 0.5 kg/m² vs 27.3 ± 0.9 kg/m²; P = .01) and WBF (17.3% ± 0.9% vs 21.3% ± 1.1%; P = .007) were lower. Waist circumference and specific regions examined for BMD such as hip and spine were not different above and below the median RBC CLA.

Body composition measurements that correlated with RBC CLA included BMI (r = −0.29, P = .04), whole body BMD (r = 0.30, P = .03), WBF (r = −0.34, P = .01), and WBL (r = 0.32, P = .02). Plasma CLA was not associated with body composition measures but was associated with dietary intake of CLA (r = 0.40, P = .05). In a regression model containing energy and dietary protein intakes, BMI, plasma 25(OH)D concentration, and RBC CLA status, the latter two were significant predictors of whole body BMD. The model accounted for 22.0% of the total variance in BMD where RBC CLA alone accounted for 10.0% of the total variance in whole body BMD based on the partial correlation coefficient (Table 3). The model did not

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<th>Table 3 – Multiple regression model for whole body BMD</th>
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Data are represented as means ± SEMs; total n = 31. Differences detected using mixed model with post hoc testing using Tukey honestly significant difference (HSD) test. Different letters indicate significant differences (P < .05). NA, not available.

1 Dietary intake assessed using FFQ.

2 Total vitamin D includes both dietary and supplemental vitamin D intake.
reach significance when age, waist circumference, and exercise were included \((P = .21)\).

3.2. Dose-response trial

3.2.1. Participants and lifestyle characteristics

Of the participants enrolled, 30 (96.8%) completed the study and 1 withdrew due to new employment. Compliance with the supplements was greater than 80% for all groups. There were no significant differences among groups at baseline (Table 4), except for exercise, which was higher in the 3.0CLA compared with the 1.5CLA and placebo groups \((P = .04)\). Throughout the study, 5 participants reported mild gastrointestinal discomfort as related to the supplement and placebo.

3.2.2. Conjugated linoleic acid levels in plasma and RBCs

There were no differences among the plasma and RBC CLA baseline values. In plasma, CLA proportion was significantly increased in both the 1.5CLA and the 3.0CLA groups compared with baseline for all time points, except week 16 in the 1.5CLA group \((P = .61)\). Furthermore, CLA proportion at week 12 in 3.0CLA was significantly greater than all other time points in the 1.5CLA and placebo groups (Fig. 2). For RBC CLA, the proportion was significantly increased from baseline at all time points in both 1.5CLA and 3.0CLA. In addition, CLA proportion in the 3.0CLA group was significantly greater at week 8 than all other time points of all groups, except for week 16 (Fig. 3). There were no time or treatment differences for other fatty acids including linoleic acid, \(\alpha\)-linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid.

3.2.3. Vital signs and blood lipid profile

There were no differences among groups for HR (44-82 beats/min), diastolic blood pressure (60-94 mm Hg), HDL (0.77-2.25 mmol/L), iCa (1.03-1.53 mmol/L), PO4 (0.62-1.56 mmol/L), TG (0.2-3.45 mmol/L), and total cholesterol (2.99-6.49 mmol/L) throughout the study. Across the study, higher values were observed in the 3.0 CLA vs placebo groups for systolic blood pressure (117.9 ± 1.2 mm Hg vs 112.5 ± 1.0 mm Hg, \(P < .0001\)) and LDL (2.96 ± 0.13 mmol/L vs 2.76 ± 0.10 mmol/L, \(P < .0001\)). Nevertheless, there was no significant change from baseline to week 16 in any of the parameters, and they were all within reference ranges throughout the study.

3.2.4. Anthropometry and body composition

Higher values were observed in the 3.0CLA group at all time points for weight \((P < .0001)\), waist circumference \((P < .0001)\), and BMI \((P = .0019)\) (Table 4). There were no group or time differences for WBF, WBL, whole body BMC, whole body BMD, hip BMC, and hip BMD. Spine BMD was significantly higher in the 3.0CLA group vs the 1.5CLA \((P = .05)\) group across the duration of the study, whereas placebo was not different from any group (Table 4). There was no significant change in any variable from baseline to week 16.

3.2.5. Calcitropic hormones and biomarkers of bone metabolism

Plasma intact PTH concentration was lower \((P = .006)\) in the 3.0CLA group vs 1.5CLA and placebo groups across the study and did not change significantly over time. At baseline and week 12, plasma bioactive PTH concentration was significantly \((P = .0002)\) lower in 3.0CLA vs 1.5CLA and placebo. In addition, there was no significant group or time differences in plasma 25(OH)D, bioactive PTH, intact PTH, OC, or BAP concentrations when expressed as percent change relative to baseline values (Fig. 4). Plasma BAP concentration was significantly higher in the 1.5CLA group compared with the 3.0CLA and placebo groups across the study but did not change significantly over time. There

![Fig. 2 – Plasma c9, t11 CLA content in a dose-response study. Differences detected using mixed model with post hoc testing using Tukey HSD test. Different letters indicate significant differences \((P < .05)\). Values are means ± SEMs; \(n = 10\) per diet.](image-url)

![Fig. 3 – Erythrocyte c9, t11 CLA content in a dose-response study. Differences detected using mixed model with post hoc testing using Tukey HSD test. Different letters indicate significant differences \((P < .05)\). Values are means ± SEMs; \(n = 10\) per diet.](image-url)
were no treatment or time differences for plasma 25(OH)D, iCa, OC, and PO₄ concentrations.

4. Discussion

This is the first study to demonstrate a relationship between long-term CLA status (based on diet) and body composition assessments (WBF, WBL, BMI) in adult men. Rosell et al. [34] also found an inverse association between BMI and dairy intake of CLA in 19,352 Swedish women aged 40 to 55 years studied for 9 years. The authors argued that dietary CLA could be partly responsible for this relationship; however, when adjusted for CLA intake, dairy intake still remained a significant covariate in the model. In the present cross-sectional analysis, RBC CLA was also positively associated

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Fig. 4 - Percent change in plasma 25(OH)D (A), bioactive PTH (B), intact PTH (C), OC (D), and BAP (E) concentrations relative to baseline. Change in 25(OH)D (in nanomoles per liter) (F), bioactive PTH (in nanograms per liter) (G), intact PTH (in nanograms per liter) (H), OC (in nanomoles per liter) (I), and BAP (in micrograms per liter) (J) concentrations over time. Placebo (−), 1.5CLA (−−), 3.0CLA (−−−). Differences detected using mixed model with post hoc testing using Tukey HSD test. Values are means ± SEMs; n = 10 per diet.
with whole body BMD. In a multiple regression model accounting for important confounders known to affect BMD including plasma 25(OH)D concentration, RBC CLA accounted for 10.0% of the total variance in whole body BMD. This is in agreement with a cross-sectional analysis in postmenopausal women (n = 136, 68.6 ± 7.1 years) by Brownbill et al [8], who documented an association between dietary CLA (63.1 ± 46.8 mg/d) and Ward triangle BMD (r = 0.04). Thus, we accept our first hypothesis where dietary CLA status appears to be associated with enhanced body composition and BMD.

This study is also the first to assess the effects of CLA on PTH in healthy men. c9, t11 CLA did not appear to significantly affect intact or bioactive PTH during a 16-week supplementation; therefore, we reject our second hypothesis. All men had low normal healthy concentrations of bioactive PTH ranging from 7.2 to 34.0 pg/mL throughout the study, which may have diminished our ability to document further reduction after CLA supplementation. These results do not agree with observations in rats where a 36.1% reduction in bioactive PTH was observed in males fed a diet of 0.5% c9, t11 CLA after 16 weeks of supplementation [12]. A study in rats with polycystic kidney disease (PKD) demonstrated that a 1% CLA supplementation resulted in a 60% attenuation in PTH in PKD and non-PKD animals [13]. Notably, in both these rat studies, CLA intake expressed as a percentage of total fat intake ranged from 6.0% and 14.3%, whereas in this study, CLA intake represented 0.17% of total fat intake. Moreover, bioactive PTH was greater (>100 pg/mL) than that in the healthy men. Hence, greater CLA intake and physiological values could allow for a greater decrease as seen with the CLA supplementation in the rats. Furthermore, chronic high PTH concentrations are known to promote bone loss, whereas intermittent or pulsatile PTH secretion or administration increases BMD [16]. Reductions in PTH have been observed in human studies where the participants were fed or supplemented with calcium, and it was concluded that greater calcium absorption combined with lower PTH could support higher BMD [14,17]. A randomized, double-blind, placebo-controlled trial that included 48 healthy primigravidas with a family history of preeclampsia and with diastolic notch were supplemented with daily oral doses of calcium carbonate (1484 mg) and 450 mg of CLA (50% c9t11, 50% t10c12) or a lactose-starch placebo from weeks 18 to 22 of gestation until delivery. Bioactive PTH (3.7-54.7 pg/mL) was decreased by 8.0% in the calcium-CLA group from baseline to postsupplementation, although these results did not reach significance [11]. Hence, CLA combined with calcium supplementation could potentially decrease PTH given elevated physiological concentrations.

This dose-response trial did not observe any differences in measurements of bone or biomarkers of bone metabolism. This is in agreement with a study by Gaullier et al [35], who supplemented obese (BMI, 32-35 kg/m²) men (n = 21) and women (n = 84) with 4.5 g CLA (3.4 g CLA; 37.5% c9, t11; 38% t10, c12) for 6 months and found no changes in BMC between or within groups. Also, in a study with overweight and obese (BMI percentile, 94.6 ± 3.2-96.1 ± 2.8) children (age, 8.1 ± 0.6-9.3 ± 0.8 years) supplemented with 3.0 g of CLA (50% c9, t11; 50% t10, c12) for 7 months resulted in no change in whole body BMC accretion in the CLA group [10]. Moreover, a double-blind, placebo-controlled trial by Doyle et al [36] in which 60 healthy adult men (age, 39-64 years) were randomly assigned to receive 3.0 g/d CLA (50% c9, t11; 50% t10, c12) for 8 weeks found no significant differences in OC, BAP, and several biomarkers of bone resorption or on serum or urinary calcium levels, which is consistent with our findings. This study assessed BMC and BMD in healthy men over a relatively short period (16 weeks); therefore, significant changes were not expected.

We also did not observe any significant differences in body composition over time and among groups. This is in agreement with Zambell et al [37], who supplemented 17 healthy women (BMI <25 kg/m²) with 3.0 g/d of CLA (22.6% t10, c12; 17.6% c9, t11 isomer) for 64 days and reported no change in weight and percent body fat, and Petridou et al [38], who gave 16 young healthy sedentary women 2.1 g/d of CLA (mixed isomers) for 45 days and found no differences in body fat (measured by skinfold thickness at 10 sites).

However, Gaullier et al showed that BW, WBF, and BMI were reduced in middle-aged men and women (BMI, 25-30 kg/m²) after 24 months of CLA supplementation. It is possible that the longer duration of 24 months, higher dose of CLA (4.5 g), and higher baseline BMI were needed to see changes in body composition. Two other studies using higher dosages (3.6-4.2 g/d) of CLA for shorter durations (6-12 weeks) in healthy men and women resulted in decreased WBF [39,40]. Thus, it is likely that a dosage of 3.0 g/d CLA was lower than required to observe changes in body composition within a 16-week timeframe.

The effect of CLA on lipid metabolism has been studied in many clinical trials, often as safety parameters, most of which do not report any significant differences in blood total, HDL and LDL cholesterol, and TG, which is congruent with the present results [38,39,41,42]. However, Smedman and Vessby [40] reported a significant increase in LDL cholesterol compared with baseline but not to placebo after 12 weeks of CLA supplementation (4.2 g/d) in 53 healthy subjects. High-density lipoprotein cholesterol decreased relative to baseline in some studies [43-45]; nevertheless, the changes in HDL cholesterol were all within the normal healthy population range and did not have any clinical significance. Triacylglycerol levels were increased in a study by Whigham et al [46]; however, this change was also considered within the normal healthy range.

The different outcomes for body composition, bone, and lipid profile among the CLA intake studies may be explained by differences in sex [47], study duration [48], isomeric specificity [49], and intake source (dietary vs supplemental) of CLA [41,49]. The study most similar to ours in terms of sex and CLA supplementation showed similar body composition and blood lipid outcomes [50]. Animal studies have demonstrated that CLA isomers incorporate in multiple tissues including the bone (cortical, marrow, and periosteum), brain, heart, liver, skeletal muscle, serum, and spleen, leading to their involvement in the metabolism of these tissues [51,52]. Despite no differences in the quantified bone biomarkers and an observed relationship between RBC CLA and BMD, this suggests that CLA affects other factors, most likely cell-derived mediators of inflammation such as eicosanoids and cytokines [53-55]. Limitations of our study include the small number of participants and issues with outcome variables (weight, BMI, intact and bioactive PTH, exercise) being significantly different among groups at baseline and across
the study. However, this study demonstrated an adequate method for estimating dietary CLA intake based on its positive association with plasma CLA content.

In summary, we demonstrated an association between RBC CLA and body composition and bone-related outcomes but did not show an effect of CLA supplementation on PTH. Based on other studies where PTH was reduced by CLA in humans (trend) [11] and rodents [12,13], future studies should consider a larger-scale study of the effects of CLA in older individuals with elevated PTH or in patients with secondary hyperparathyroidism or PKD.

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