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Dietary conjugated linoleic acid in the *cis*-9, *trans*-11 isoform reduces parathyroid hormone in male, but not female, rats

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Abstract

Previously, a mixture of conjugated linoleic acid (CLA) isoforms reduced parathyroid hormone (PTH) in male rats over 8 weeks. The objective herein was to determine which isoform caused the reduction in PTH; whether the effect was sex specific; and whether CLA-induced reductions in PTH were sustained. Male and female weanling rats (n=48) were randomized to a control diet or one made with 0.5% of the diet as *cis*-9, *trans*-11 (c9,t11) CLA, 0.5% of the diet as *trans*-10, *cis*-12 (t10,c12) CLA or these CLA in a mixture. Measurements made after 4, 8 and 16 weeks were body weight, bioactive PTH, ionized Ca, whole-body and regional bone mineral density (BMD) using dual-energy X-ray absorptiometry. With the use of a factorial design, a sex×c9,t11 CLA interaction was observed that reduced PTH (139.5±63.9 vs. 95.8±42.4 pg/ml, P=.02) in male rats only. No other effects of c9,t11 CLA were observed. Regarding t10,c12 CLA, no interaction effects were observed, but a main effect was observed to reduce lumbar spine BMD (0.265±0.044 vs. 0.255±0.044 g/cm^2, P<.01) along with reduced retention of Ca and P at Week 4. No other dietary effects were observed. In summary, the c9,t11 CLA isoform is responsible for reduced PTH and this effect is sex specific; this was true whether fed as a pure isomer or mixed with an equal amount of t10, c12 CLA. Whether such reductions in PTH might be observed in females lacking sex hormones such as ovariectomized rats and also in humans is required to expand health implications of dietary CLA.

Keywords: Conjugated linoleic acid; Parathyroid hormone; Rat; Bone mineral density

1. Introduction

To date a main focus of research related to dietary conjugated linoleic acid (CLA) has been on body composition, particularly lean and fat mass [1], and less so regarding bone mass and metabolism as the primary outcomes. There are a number of mechanisms by which CLA might affect bone. Dietary supplementation with CLA is reflected in tissue fatty acid composition and displaces linoleic acid and arachidonic acid [2]. One result is reduced synthesis of prostaglandin E_2 (PGE_2) that depends on release of arachidonic acid from the plasma membrane phospholipids [2]. Prostaglandins play major roles in cell signaling in organs such as bone [2] and the parathyroid gland [3]. The parathyroid gland has a major impact on the endocrine control of bone metabolism and mineralization. Recently, we conducted a study whereby feeding a CLA mixture (1% of the diet by weight) to male rats for 8 weeks did not alter bone formation and resorption, femur bone mineral density (BMD) or release of PGE_2 from femur, but suppressed parathyroid hormone (PTH) to 60% of control values in both normal and hyperparathyroid states [4]. The mixture of CLA used in the study was 19% *cis*-9, *trans*-11 (c9,t11) and 28% *trans*-10, *cis*-12 (t10,c12) CLA. Thus the results may have been due to combined effects of the two CLA isomers or specific to only one isomer. Additionally, the reduced PTH may or may not have been sustained and to date whether females experience a similar reduction in PTH following exposure to CLA has not been tested.

Other studies of dietary CLA have not yet included measurement of PTH and thus whether PTH is reduced in association with bone or other health outcomes is not clear.

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Nonetheless, it has long been accepted that continuously elevated PTH leads to bone loss whereas intermittent elevations lead to increases in bone mass [5]. PTH follows a circadian rhythm reaching the lowest nadir between 0930 and 1100 hours followed by a small peak in the afternoon, another smaller nadir at 2100 and the primary peak at 0314 hours [6,7]. These diurnal patterns do not seem to change with age in premenopausal women and men [8]. Reductions in PTH in the order of 30% to 40% in the rodent CLA study were measured at the morning nadir [4]. The reduced PTH might be related to the effects of CLA on calcium absorption since another study in rats reported higher net and fractional calcium absorption with CLA supplementation [9]. In women fed a high calcium diet (2414 vs. 815 mg/day) PTH was 40% lower [10]. In another study, 150 mg of calcium supplementation with each meal plus 450 mg prior to bed in healthy men also results in reduced PTH in the morning compared to placebo [11]. Enhanced calcium absorption plus lower PTH would support higher bone mass.

Higher dietary CLA is associated with higher BMD of the forearm in postmenopausal women [4]. In mice, feeding mixed CLA increases bone mass in young males [12] and reduces age-associated bone loss in females [13]. In osteoblast-like cells, the c9,t11 CLA isomer increases the number and size of mineralized bone nodules while the t10, c12 CLA isomer did not [14]. Based on these studies it appears that the dietary form of CLA, c9,t11, might be responsible for reduced PTH and enhanced bone mass.

The objectives of the proposed research in male and female rats fed CLA (c9,t11 and/or t10,c12 CLA) from 4 to 20 weeks of life were to (1) determine which isoform caused the reduction in PTH; (2) whether the effect was sex specific; and (3) whether CLA-induced reductions in PTH were sustained over longer periods of time. A secondary objective was to determine the physiological response to CLA by measuring other variables including bone mass, PGE_2, ionized serum Ca and Ca/P retention to help explain any reductions in PTH or alterations in bone.

2. Materials and methods

2.1. Study protocol and diets

This study was approved by the University of Manitoba Committee on Animal Care and conformed to Canadian Council on Animal Care guidelines [15]. Sprague-Dawley rats (24 male and 24 female) were randomized at 3 weeks of age to receive one of four diets between 4 and 20 weeks of age. Between Weeks 3 and 4 of life, animals were acclimatized to the housing conditions and fed the control diet. Animals were housed in same-sex pairs and food disappearance monitored three times weekly over the 16-week period. The diets all contained 84 g total fat/kg diet to ensure that essential fatty acids were not compromised at the expense of adding CLA. The diets were as follows: (1) control AIN-93 diets [16] made with soybean oil (n-6/n-3 ratio ×7:1); (2) control diet combined with 0.5% c9,t11 CLA; (3) control diet combined with 0.5% t10,c12 CLA and (4) control diet combined with 0.5% c9,t11 CLA+0.5% t10,c12 CLA in fatty acid form. These CLA mixtures were adjusted so that a single isomer represented 0.5% of the diet by weight. The CLA isomers were all in free fatty acid form and provided in kind from Lipid Nutrition, a division of Loders Croklaan (Channahon, IL, USA). The t10,c12 CLA product was 69.8% t10,c12 with total CLA at 83.1%; the c9,t11 product was 61.7% c9,t11 and total CLA at 73.2%; and the pre-mixed CLA was 74.5% c9,t11 and t10,c12 CLA with total CLA at 80.6% (Loders Croklaan Inc., certificate of analyses). Total Ca/kg diet was 5.1 g/kg and total P/kg diet was 46.08 g/kg based on mineral content of casein and mineral mix (Harland Teklad, certificate of analyses).

2.2. Study measurements: growth and bone

Weight was measured weekly for assessment of growth. At 4, 8 and 16 weeks after consuming the diets, rats were then anaesthetized using isofluorane gas for measurement of bone mass including whole body, lumbar spine, femur and tibia using a small animal program and dual-energy X-ray absorptiometry (DXA; 4500A Elite Series, Hologic, Bedford, MA, USA). Whole-body length from tip of nose to base of tail was also measured in the anesthetized state. The DXA measurements have been validated using identical hardware and software for rats 130 g in weight and higher for whole-body assessment [17] and in rodents as small as mice for high-resolution regional scans [18]. After each blood sampling and DXA, the rats continued on the feeding trial until 20 weeks of age.

2.3. Biomarkers of bone metabolism

At each of the three time points, a blood sample of no more than 10% blood volume was taken from the saphenous vein, between 0800 and 1000 hours to control for diurnal variations, and separated to obtain serum for determination of serum PTH, osteocalcin and C-telopeptide of Type 1 collagen (CTx). Both bioactive and intact PTH were measured using an ELISA (Alpco Diagnostics, Windham, NH, USA), osteocalcin using an ELISA (Osteometer, Nordic Bioscience, Herlev, Denmark), in addition to urinary CTx using an ELISA (Ratlaps, Osteometer, Nordic Bioscience). All of these protein assays are specific to rodents. Regarding serum ionized Ca, samples were measured within 4 h of collection using a Nova analyzer (Model 11, Nova Biomedical) and a CV <1.6 over the study period. In the last 5 days of each study phase, rats were housed in metabolic cages and mass balance studies conducted by measuring disappearance of food and excretion of nutrients over the last 3 days; the first 2 days are adaptation to the new housing. Minerals (Ca, P) were measured in the 72-h pooled samples of urine and feces following digestion in nitric acid and using inductively coupled plasma optical emission spectroscopy (Varian Liberty 200, Varian Canada).

2.4. Ex vivo release of prostaglandin E_2

During the study, ex vivo release from bone was not performed since this requires termination of the animals. At the end of 16 weeks, cortical bones from tibia and femur (×0.5 g) were freed of periosteum, marrow and soft tissue, and incubated at 37°C for 2 h in Hank's solution (Sigma Diagnostics) [19]. Liver was also assessed for PGE_2 biosynthesis using the same technique with exception of shorter incubation times (1 h). Prostaglandin E_2 was determined using ELISA (R&D Scientific) and data expressed to weight of the tissue segment studied.

2.5. Statistics and sample size estimate

Since the estimate of the sample size is intended to estimate the minimum sample size to detect differences among groups, it was reasonable to use values of PTH from the preliminary study [4]. Based on a difference in PTH values of 12.6 pmol/L (×3 S.D.) between the CLA and control diet groups with a standard deviation of 4.0, an alpha of 0.05 and power of 0.80, the estimated sample size was four per group. However, six males plus six females per dietary group were used to yield a balanced design and were large enough (n=12 rats per diet) to detect smaller reductions in PTH than previously observed.

Main and interaction effects were identified using factorial (end-point) and mixed (repeated measurements) model ANOVAs; all pair-wise differences in diet means were tested using Bonferroni correction and a P value of .5 or less was accepted as significant. Data are presented as groups by diet, sex and time. When main or interaction effects were observed due to a dietary effect, data are presented to show the differences. However, differences due to sex or time alone are not shown. Data are presented as mean±S.D. unless otherwise stated for n=12 per dietary group with a balanced number of males and females.

3. Results

3.1. Growth and feed intakes

There were no main effects of feeding either t10,c12 or c9,t11 CLA on weight. Main effects of sex and time were observed for weight as anticipated with male rats being heavier than female rats and growth resulting in higher weights by Weeks 8 and 16 of study (Table 2). No interaction effects were observed for weight. For body length, a main effect of t10,c12 resulted in 0.3 cm shorter rats in the CLA group (22.3±2.6 vs. 22.0±2.6 cm, P=.03). In addition, main effects of sex and time were observed with male rats being longer than female rats and length at 4 weeks less than at 8 and 16 weeks of study (Table 1). No interaction effects were observed for length.

Main effects of t10,c12, c9,t12, sex and time were observed for feed intake (Table 1). A t10,c12×c9,t12 interaction (P<.001) resulted in the lowest intake observed in the combined CLA group compared to all other groups (control, 165.4±32.3; t10,c12, 162.6±36.2; c9,t11, 163.6±30.3; and mixed CLA, 137.1±30.3 g/week, P<.001). As well, a t10,c11×sex interaction indicated that intakes were similar between male and female rats when fed control diet, but not when fed t10,c11, and that intakes were reduced by feeding t10,c12 CLA (control male, 167.7±36.5; control female, 161.3±25.7; t10,c12 male, 158.4±38.3; and t10,c12 female, 141.4±30.8 g/week, P<.005). No other interaction effects were observed.

Table 1
Growth and dietary characteristics of male and female rats fed a control diet or a diet made with CLA isomers at 4, 8 and 16 weeks of study

Measurement	Gender	Week of study	Control	t10,c12	c9,t11	Mixed CLA	Main effect			
							t10,c12	c9,t11	Sex	Time
Weight (g)	Male	4	318.9±22.1	311.5±19.0	304.3±31.56	293.9±24.5	0.21	0.18	<0.01	<0.01
		8	473.0±39.9	467.1±31.8	462.2±35.0	447.4±28.8				
		16	622.1±56.7	633.2±55.4	611.9±61.6	585.2±40.9				
	Female	4	209.3±15.0	202.5±18.2	218.4±17.3	201.6±19.6				
		8	265.8±22.6	268.7±20.1	277.0±18.8	263.5±37.4				
		16	317.3±31.6	323.3±27.3	329.3±28.9	325.7±53.1				
Body length (g)	Male	4	21.1±1.8	21.0±0.6	21.2±1.0	21.1±0.6	0.03	0.82	<0.01	<0.01
		8	24.1±1.2	23.7±0.8	24.1±1.2	24.1±1.3				
		16	27.0±0.7	26.4±0.7	26.6±0.8	26.0±1.0				
	Female	4	19.0±1.0	18.9±1.5	19.4±0.8	18.6±0.9				
		8	21.4±0.6	20.8±1.5	21.3±0.9	20.8±1.4				
		16	21.6±0.5	21.6±0.3	21.5±0.5	21.3±0.8				
Food intake (average g/week)	Male	4	122.5±5.5	118.7±7.1	120.0±4.1	103.2±3.7	<0.01	<0.01	<0.01	<0.01
		8	179.3±10.8	176.2±5.6	174.9±8.8	164.8±7.4				
		16	212.7±4.1	214.9±4.4	199.6±11.9	172.6±6.3				
		4	134.6±13.0	123.6±14.0	135.1±8.4	104.9±7.1				
		8	154.4±10.3	155.0±13.1	160.7±1.2	131.4±27.4				
	Female	16	191.7±1.1	187.3±16.7	191.3±19.1	145.8±18.3				

Data are mean±S.D. Main and interaction effects determined using mixed model ANOVA with Bonferroni post hoc comparisons. Only main effects presented herein with any interaction effects described in the text of the results and figures.

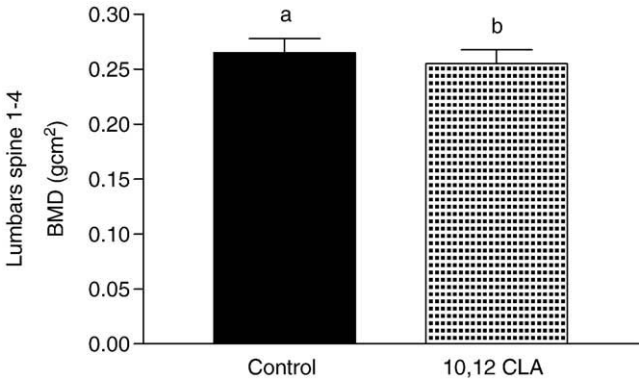

Fig. 1. Main effect of t10,c12 CLA for Lumbar spine vertebrae 1 to 4 BMD in male and female rats fed diets either without or containing t10,c12 CLA for 16 weeks. Differences detected using mixed model ANOVA with post hoc testing using Bonferroni correction. Different letters indicate significant differences ($P<.001$).

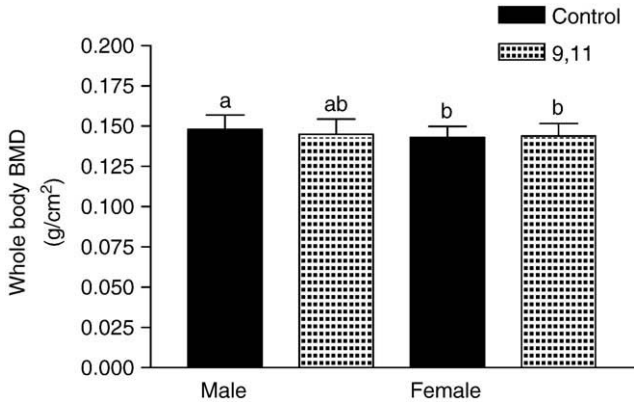

Fig. 2. Interaction effects between c9,t11 CLA and sex for whole-body BMD in male and female rats fed diets either without or containing 9,11 CLA for 16 weeks. Differences detected using mixed model ANOVA with post hoc testing using Bonferroni correction. Different letters indicate significant differences ($P<.01$).

3.2. Bone mass

Main effects of sex and time were observed for all BMD measurements (Table 2) except for tibia BMD where the effect of sex was not significant ($P=.06$). Regarding t10,c12, main effects were observed for Lumbar spine 1–4 BMD (Fig. 1), but not for whole body, femur or tibia (Table 2). No main effects of c9,t11 were observed for any measurement of BMD (Table 2). However, an interaction between c9,t11 and sex ($P=.02$) suggested that in males whole body BMD was lower in the c9,t11 group, but this was not significant upon post hoc testing (Fig. 2).

3.3. Biochemistry

For bioactive PTH, main effects of time were observed (Table 3), but not for t10,c12, c9,t11 or sex. However, a c9, t11×sex interaction revealed that bioactive PTH was reduced in male rats fed c9,t11 by 31.3%, but not female rats and that

Table 2
Bone mineral density of male and female rats fed a control diet or a diet made with CLA isomers at 4, 8 and 16 weeks of study

BMD (g/cm2)	Gender	Week of study	Control	t10,c12	c9,t11	Mixed CLA	Main effect			
							t10,c12	c9,t11	Sex	Time
Whole body	Male	4	0.121±0.003	0.121±0.003	0.119±0.007	0.117±0.002	0.10	0.16	<0.01	<0.01
		8	0.151±0.006	0.148±0.003	0.148±0.005	0.145±0.005				
		16	0.176±0.008	0.172±0.004	0.173±0.003	0.171±0.006				
	Female	4	0.124±0.001	0.121±0.003	0.122±0.004	0.121±0.005				
		8	0.146±0.004	0.147±0.003	0.145±0.007	0.146±0.005				
		16	0.161±0.004	0.161±0.004	0.164±0.006	41.0±5.5				
Lumbar vertebra 1–4	Male	4	0.199±0.013	0.195±0.014	0.200±0.011	0.185±0.011	<0.01	0.19	<0.01	<0.01
		8	0.260±0.020	0.247±0.009	0.250±0.014	0.239±0.018				
		16	0.319±0.020	0.304±0.018	0.308±0.018	0.292±0.014				
	Female	4	0.239±0.013	0.222±0.016	0.225±0.014	0.223±0.017				
		8	0.282±0.012	0.262±0.017	0.279±0.031	0.277±0.019				
		16	0.309±0.018	0.301±0.011	0.303±0.025	0.314±0.013				
Femur	Male	4	0.297±0.026	0.274±0.028	0.266±0.035	0.287±0.020	0.65	0.58	<0.01	<0.01
		8	0.389±0.021	0.406±0.019	0.394±0.027	0.391±0.008				
		16	0.492±0.044	0.491±0.038	0.483±0.036	0.464±0.051				
	Female	4	0.263±0.028	0.264±0.030	0.278±0.034	0.278±0.031				
		8	0.354±0.018	0.372±0.017	0.375±0.018	0.374±0.026				
		16	0.383±0.035	0.392±0.026	0.375±0.041	0.382±0.042				
Tibia	Male	4	0.170±0.022	0.148±0.026	0.146±0.010	0.159±0.015	0.33	0.73	0.06	<0.01
		8	0.201±0.038	0.227±0.019	0.211±0.034	0.216±0.021				
		16	0.253±0.027	0.244±0.017	0.243±0.030	0.238±0.038				
	Female	4	0.151±0.014	0.152±0.025	0.162±0.026	0.170±0.022				
		8	0.195±0.021	0.210±0.010	0.216±0.015	0.216±0.017				
		16	0.218±0.022	0.230±0.026	0.215±0.032	0.233±0.037				

Data are mean±S.D. Main and interaction effects determined using mixed model ANOVA with Bonferroni post hoc comparisons. Only main effects presented herein with any interaction effects described in the text of the results and figures.

Table 3
Biomarkers of calcium metabolism in male and female rats fed a control diet or a diet made with CLA isomers at 4, 8 and 16 weeks of study

Measurement	Gender	Week of study	Control	t10,c12	c9,t11	Mixed CLA	Main effect			
							t10,c12	c9,t11	Sex	Time
Bioactive PTH (pmol/L)	Male	4	15.8±6.0	14.4±4.3	9.8±5.4	9.5±3.3	0.72	0.31	0.09	<0.01
		8	15.7±8.5	17.3±7.4	12.5±6.0	12.9±3.5				
		16	13.5±6.5	11.2±8.7	8.4±3.7	7.5±3.0				
	Female	4	10.7±4.9	9.5±6.5	13.0±9.6	18.4±11.3				
		8	11.4±4.5	8.7±5.1	10.5±6.7	12.1±4.8				
		16	7.6±1.6	8.3±4.7	6.7±3.6	10.2±4.6				
Total intact PTH (pmol/L)	Male	4	24.5±7.9	24.5±4.1	19.3±7.4	18.9±5.1	0.91	0.85	<0.01	0.29
		8	25.4±8.8	27.9±7.6	25.2±8.4	24.0±3.5				
		16	27.3±6.6	24.3±10.3	21.5±7.5	18.1±3.8				
	Female	4	18.5±4.1	16.7±4.6	22.5±11.5	26.3±8.6				
		8	19.3±7.4	16.9±3.5	19.9±7.8	23.0±5.2				
		16	16.8±5.6	18.6±9.4	18.3±7.0	22.5±8.7				
Ionized Ca (mmol/L)	Male	4	1.38±0.10	1.37±0.09	1.37±0.12	1.38±0.10	0.89	0.42	0.15	<0.01
		8	1.41±0.06	1.40±0.03	1.40±0.04	1.44±0.02				
		16	1.45±0.03	1.46±0.04	1.46±0.04	1.45±0.03				
	Female	4	1.45±0.04	1.42±0.04	1.44±0.05	1.41±0.06				
		8	1.42±0.04	1.42±0.02	1.40±0.03	1.40±0.03				
		16	1.46±0.05	1.46±0.03	1.42±0.04	1.44±0.03				
Serum osteocalcin (nmol/L)	Male	4	92.8±13.6	95.9±14.1	108.3±26.3	84.6±16.4	0.20	0.11	<0.01	<0.01
		8	54.1±6.6	56.9±12.4	62.4±17.8	57.8±9.9				
		16	28.4±11.7	25.6±7.9	31.1±8.2	29.9±4.7				
	Female	4	72.0±25.7	67.6±32.0	81.2±10.4	82.1±10.9				
		8	42.7±15.3	39.9±3.2	42.1±5.8	39.8±19.1				
		16	19.7±4.3	18.1±4.8	21.2±3.6	20.2±5.0				
Urinary ratlaps (nmol/day) *	Male	4	4.74±2.34^{a}	5.93±2.17^{a}	10.59±5.91^{d}	5.76±2.16^{a}	0.31	0.052	<0.01	<0.01
		8	1.94±1.19^{a}	2.90±1.89^{a}	2.44±0.92^{a}	2.04±0.88^{a}				
		16	0.58±0.26^{b}	0.81±0.45^{b}	1.21±0.84^{b}	0.73±0.19^{b}				
	Female	4	2.32±0.97^{a}	2.11±0.60^{a}	2.27±0.76^{a}	2.42±0.63^{a}				
		8	0.76±0.34^{b}	0.62±0.14^{b}	0.62±0.22^{b}	0.85±0.38^{b}				
		16	0.13±0.04^{c}	0.12±0.05^{c}	0.12±0.03^{c}	0.22±0.12^{c}				

Data are mean±S.D. Main and interaction effects determined using mixed model ANOVA with Bonferroni post hoc comparisons. Only main effects presented herein with any third-order interaction effects described in the text of the results and figures.

* All pair-wise interaction detected (P=.004); differences between means indicated by superscripts (P<.01).

male rats had higher values than female rats (Fig. 3). The same pattern was observed for total PTH, but differences did not reach significance with post hoc Bonferroni correction (P=.06).

For ionized serum Ca, the only effect was that of time whereby values increased over the study (Table 3). Regarding osteocalcin, no effects of either dietary CLA were observed while main effects of both sex (males had higher values) and time (decreased with time) were observed (Table 3). For urinary ratlaps expressed per day, the same pattern was observed as for osteocalcin except that an all pair-wise interaction was observed; in the male rats fed c9, t11, values were highest at Week 4 only (Table 3).

No effects of either CLA isoform or due to sex or time were observed for release of PGE_2 from liver (274.9±215 ng/g, all rats) or tibia (27.1±15.4 ng/g, all rats). For femur PGE_2, the only effect was that of sex (16 vs. 20 ng/g, P=.004).

3.4. Mass balance

For this study, mass balance was conducted at Weeks 4, 8 and 16 of study. However, since many male rats were too large for the metabolic cages at week 16, the dataset was not complete and thus not analyzed for Week 16. Results are therefore reflective of only Weeks 4 and 8 of study.

In the mass balance, main effects of sex were observed for higher food intake as a total gram over the 3-day collection period, and higher Ca and P retention in male rats over female rats (P<.0001, data not shown). There was no effect of week of study on food intake (P=.49), but retention of Ca and P was lower at Week 8 compared to Week 4 (P<.0001, data not shown).

Regarding main or interaction effects of CLA isomers, a main effect of t10,c12 was observed to reduce food intake (P=.0004) along with a week×t10,c12 interaction (P=.0095) resulting in lower intakes at Week 4 due to t10,c12 CLA (54 vs. 44 g, P<.0001) but not at Week 8 (52 vs. 49 g, P=.46). Additionally, a c9,t11×t10,c12 interaction (P=.0258) such that compared to control, the mixed CLA group consumed less (P=.0057). Within the CLA containing groups, all intakes were significant with P<.05 (control=52, c9,t11=55, t10,c12=49, mixed CLA=45 g). A t10,c12×week of study interaction was observed for both Ca (P=.0038) and P (P=.0253) retention. Post hoc comparisons demonstrated that retention was reduced by t10,c12 at Week 4, but not

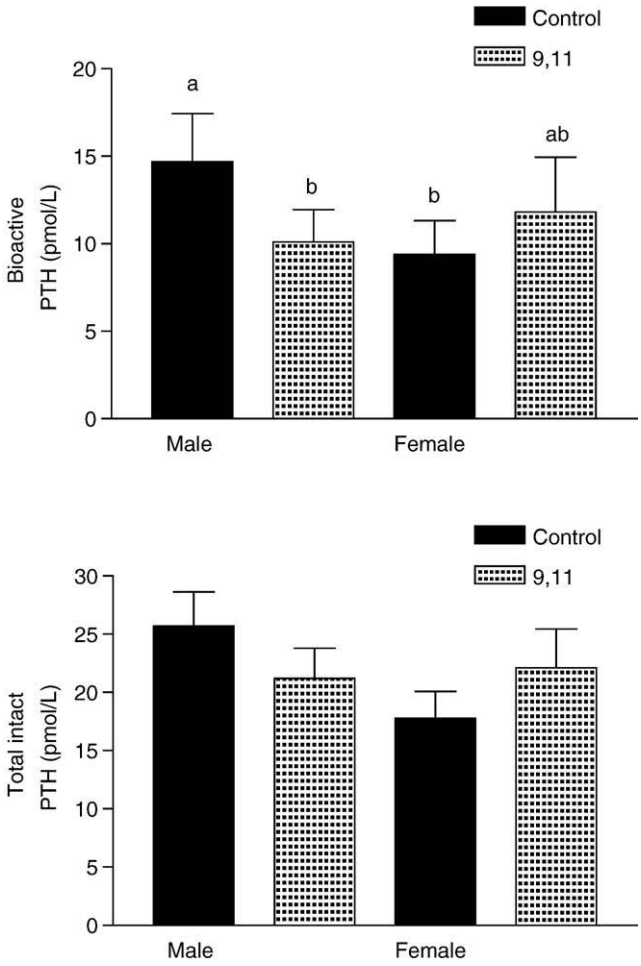

Fig. 3. Interaction effects between c9,t11 CLA and sex for bioactive (top panel) or total (bottom panel) PTH in male and female rats fed diets either without or containing 9,11 CLA for 16 weeks. Differences detected using mixed model ANOVA with post hoc testing using Bonferroni correction. Different letters indicate significant differences ($P<.01$).

Week 8. Values for Ca retention at Week 4 were 222±56 vs. 173±33 mg/balance and at Week 8 were 110±33 vs. 111±52 mg/balance. Values for P retention at Week 4 were 100±28 vs. 79±34 mg/balance and at Week 8 were 47±23 vs. 52±17 mg/balance.

4. Discussion

This study confirms that dietary CLA reduced PTH in male rats and that the effect is due to c9,t11 CLA. The magnitude of the reduction was 31% overall in line with previously observed reductions using mixed CLA whereby a 40% reduction was observed over 8 weeks [4]. While interactions with time were not observed, the reductions in PTH by c9,t11 at 4 weeks were 36.1%; at 8 weeks, 23%; and by 16 weeks, 35.6%, suggesting PTH is reduced as early as 4 weeks with overall sustained lower values throughout the study. It is interesting that c9,t11 did not have the same effect in female rats, although PTH was notably lower in females compared to male rats and thus further reductions were not likely possible given the nature of the dietary intervention (i.e., small amounts of CLA). To compare with previous work whereby intact PTH assays were used that might also reflect PTH fragments, intact total PTH was also measured. The magnitude of differences followed the same pattern but differences among males fed c9,t11 did not reach significance. The use of the Bonferroni correction in post hoc testing with our relatively small sample size, however, might have resulted in Type II errors. Dietary CLA did not alter ionized Ca or any biomarker of bone metabolism by the end of study, including PGE_2 release from tibia and femur as previously observed [4], and thus the mechanism for reduced bioactive PTH remains elusive.

While bioactive PTH was reduced in male rats, c9,t11 CLA did not affect BMD. Very few studies have directly compared the major CLA isomers with respect to BMD. In both lean and fa/fa Zucker rats, 0.4% of either c9,t11 or t10,c12 CLA compared to control did not affect whole body, femur or spine bone mass [20]. In mice fed these isomers, only the t10,c12 CLA enhanced whole body ash weight while reducing body fat [21]. Thus it is possible that the dietary amount of CLA needed to suppress PTH is different than the amount needed to alter BMD or other dietary qualities confound the relationship.

Seven of 12 controlled studies reviewed reported that CLA has effects on bone mass or metabolism. In male chicks fed butterfat (1.5% CLA of which 1.2% was *cis*-9, *trans*-11 CLA) from roughly birth to 6 weeks, bone formation rate was elevated [22]. Similarly, in young growing male and female mice whole body ash was elevated with CLA treatment over 28–49 days [21,23,24]. Both young growing male Balb/C mice [12] and aging female C57BL/5 mice [13] fed CLA have elevations in bone mass. Lastly, loin bone weight was higher after feeding CLA to male barrows (pigs, 26–116 kg) [25]. Other studies where no effect of CLA was observed were likely too short in duration given the stage of growth or maturation (pigs 40–57 kg at inception [26,27]; adult humans [28]) or did not study bones in a way that a response would be evident such as tail length or ribs alone [29,30]. One other study reported that humerus ash weight was unaltered by dietary CLA despite many alterations in biochemistry [2,31].

While the species used in these studies were different, each studied CLA supplementation up to 1% of the diet by weight. One difference among these studies was the base diet having either moderate [2,31] or high [24,25] n-6/n-3 fatty acid ratios. Of the studies with positive observations in bone, the sources of CLA were different (butterfat vs. chemical courses), but each diet would have had a high n-6/n-3 fatty acid ratio (×21–58) [21–25]. The studies where CLA did not demonstrate an effect on bone ash or weight used lower n-6/n-3 ratios (<10) [2,12,13,29,31]. Overall, the current study adds that c9,t11 CLA at least in low amounts and with a total

dietary n-6/n-3 fatty acid ratio of 7:1 does not affect BMD, but reduces PTH in males.

It is important to consider that certain CLA isoforms might lead to an adverse outcome in bone. In fact, a main effect of feeding t10,c12 was observed to reduce lumbar spine BMD by 3.8% regardless of sex or time. Reduced bone mass due to t10,c12 CLA might be due to impaired mineral retention since a 20% reduction in retention of both Ca and P was observed at week 4, but not week 8. This, however, was not reflected in biomarkers of bone formation or resorption. In a similar study, bone formation rate was reduced in tibia after 6 weeks of feeding CLA to weanling rats, but ash weight of the humerus was not altered [2,31]. This suggests that the response of reduced bone formation might be bone specific (i.e., tibia).

To date, the effect of c9,t11 on PTH in humans has not been reported. Most studies have examined bone mass and metabolism in humans fed mixed sources of CLA or examined the relationship between dietary CLA with bone metabolism in descriptive studies. For example, Doyle et al. [32] fed mixed CLA at 3 g/day for 8 weeks resulting in no changes to bone formation or resorption, but did not report on PTH. In a longer study using 2 g CLA/day over 2 years in overweight humans, no change to bone mass was observed and PTH was not measured [33]. However, in a descriptive study of postmenopausal women, dietary CLA (×63 mg/day) was positively predictive of BMD in the hip after correction for other dietary and lifestyle factors [34]. Serum PTH in menopause and aging is often elevated [35–38]. Some investigators postulate that menopausal women have a change in the PTH set-point and that their bones are more sensitive to the resorptive effects of PTH [39]. In rodent [13] and cell culture studies [40], CLA is associated with reduced receptor activator of NF-kB ligand (RANKL). RANKL is intimately linked with osteoclastogenesis, and lower levels would be consistent with lower capacity for bone resorption [41]. Conversely, c9,t11 CLA enhances alkaline phosphatase activity suggestive of early osteoblast differentiation and increases bone nodule formation [14]. Thus dietary c9,t11 CLA may have direct effects on osteoclastogenesis and osteoblast differentiation plus indirect effects through reduced PTH with potential application to suppression of bone loss during menopause. Studies in menopausal women randomized to c9,t11 containing CLA should be designed to measure PTH at various intervals over at least 2 years in combination with bone mass of lumbar spine and hip.

In summary, this study confirmed that dietary CLA reduced PTH in growing male rats and clarifies that such reduction is due to the c9,t11 CLA isomer. No effects on PTH were observed in growing female rats. Based on the results herein, a dose-effect study using c9,t11 and the effects on PTH along with bone metabolism and mass is well warranted. Likewise, studies in adult rodents and adult humans should be conducted to establish whether reduced PTH is only possible when CLA is fed during rapid growth vs. adulthood.

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