Conjugated Linoleic Acids Alter Bone Fatty Acid Composition and Reduce *ex vivo* Prostaglandin E_2 Biosynthesis in Rats Fed n-6 or n-3 Fatty Acids

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ABSTRACT: This study evaluated the effects of conjugated linoleic acids (CLA) on tissue fatty acid composition and *ex vivo* prostaglandin E_2 (PGE_2) production in rats given diets varying in n-6 and n-3 fatty acids. Four groups of rats were given a basal semipurified diet (AIN-93G) containing 70 g/kg of added fat for 42 d. The fat treatments were formulated to contain CLA (0 vs. 10 g/kg of diet) and n-6 (soybean oil having an n-6/n-3 ratio of 7.3) and n-3 fatty acids (menhaden oil + safflower oil having an n-6/n-3 ratio of 1.8) in different ratios in a 2×2 factorial design. Fatty acids in liver, serum, muscle, heart, brain, spleen, and bone (cortical, marrow, and periosteum) were analyzed by capillary gas–liquid chromatography. The various dietary lipid treatments did not affect growth; however, CLA improved feed efficiency. The CLA isomers were found in all rat tissues analyzed although their concentrations varied. Dietary CLA decreased the concentrations of 16:1n-7, 18:1, total monounsaturates and n-6 fatty acids, but increased the concentrations of n-3 fatty acids (22:5n-3 and 22:6n-3), and saturates in the tissues analyzed. *Ex vivo* PGE_2 production in bone organ culture was decreased by n-3 fatty acids and CLA. We speculate that CLA reduced the concentration of 18:1 fatty acids by inhibiting liver Δ9-desaturase activity. The fact that CLA lowered *ex vivo* PGE_2 production in bone organ culture suggests that these conjugated fatty acids have the potential to influence bone formation and resorption.

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Conjugated linoleic acids (CLA) are positional and geometric isomers of linoleic acid that occur naturally in several foods but are highest in dairy products and beef (1). The CLA isomers have been suggested to possess potent beneficial health and biological effects (2–6). CLA isolated from extracts of grilled ground beef were found to exhibit anticarcinogenic activity against chemically induced skin cancer in mice (7). Recent experiments on prostate cancer cell lines demonstrated that CLA isomers are incorporated into cell lipids and that CLA compared to linoleic acid decreased cell proliferation (8). Further, CLA were reported to reduce prostaglandin E_2 (PGE_2) concentration in rat serum and spleen (9).

CLA isomers become enriched in tissue and cell lipids when administered in a diet or supplemented in a cell culture medium (8–10). As CLA incorporates into membrane phospholipids, it may compete in elongation and desaturation steps with other unsaturated fatty acids that are precursors of arachidonic acid (the precursor of PGE_2), and such competition in arachidonate synthesis may alter eicosanoid biosynthesis (11,12). The *cis*-9, *trans*-11 isomer of CLA was preferentially incorporated into the membrane phospholipids of mice (13) and rats (14), suggesting that *cis*-9, *trans*-11 is the biologically active isomer. Therefore, CLA may exert physiological effects by altering the formation of phospholipid-derived hormones, e.g., eicosanoids, or by modifying the signal transduction system (15).

This laboratory recently reported that milk fat stimulated bone formation rate in growing chicks possibly by modulating *ex vivo* PGE_2 production in bone (16). Having a concentration up to 30 mg CLA/g fat (17), milk fat is a primary dietary source of CLA. With regard to bone metabolism, PGE_2 is recognized as a mediator of bone formation and bone resorption *in vivo* (18). Although CLA recently has been shown to affect PGE_2 production (9) and to modulate cytokine production (19), the purpose of the present investigation was twofold: first, to quantify the effects of CLA on the fatty acid composition of tissue lipids in the presence of relatively high dietary n-6 or n-3 fatty acids; second, to document that CLA could alter PGE_2 production in mammalian bone in the presence of dietary lipids varying in an n-6/n-3 fatty acid ratio.

MATERIALS AND METHODS

Animals and diets. Forty male weanling rats (21-d-old, mean body weight 46 ± 2.0 g; Harlan Sprague-Dawley, Indianapolis, IN) were housed in individual cages under a 12-h light-dark cycle. Animal care was in compliance with applicable guidelines from the Purdue University (West Lafayette, IN) policy on animal care and use. The rats were randomly assigned to four groups and fed *ad libitum* the basal diet (AIN-93G without fat; Dyets, Inc., Bethlehem, PA) with one of the following fat treatments at 70 g/kg (Table 1): soybean oil (SBO, diet rich in n-6 fatty acids); soybean oil + CLA

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Abbreviations: BF_3, boron trifluoride; CLA, conjugated linoleic acids; FAME, fatty acid methyl esters; MSO, menhaden oil + safflower oil diet; MSOC, menhaden oil and safflower oil + CLA diet; PGE_2, prostaglandin E_2; PUFA, polyunsaturated fatty acid; SBO, soybean oil diet; SBOC, soybean oil + CLA diet.

TABLE 1
Fatty Acid (wt%) and Ingredient Composition of the Diets Given to Rats[a,b,c]

	Dietary lipid treatments[d]			
Fatty acid	SBO	SBOC	MSO	MSOC
12:0 (lauric)	Trace	Trace	0.08	0.03
14:0 (myristic)	0.20	0.19	3.64	3.07
15:0 (pentadecylic)	n.d.[e]	n.d.	0.34	0.29
16:0 (palmitic)	10.32	8.80	12.19	10.23
16:1n-7 (palmitoleic)	0.09	Trace	4.83	4.05
17:0 (margaric)	0.07	Trace	0.41	0.35
18:0 (stearic)	4.63	3.96	3.11	2.60
18:1 (octadecenoic)	23.18	19.75	12.25	10.47
18:2n-6 (linoleic)	52.84	45.33	36.67	32.38
18:3n-3 (α-linolenic)	7.26	6.24	1.03	0.85
18:4n-3 (stearidonic)	n.d.	n.d.	1.89	1.64
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	n.d.	6.52	n.d.	7.11
18:2(*t*-10,*c*-12)	n.d.	6.84	n.d.	7.34
18:2(*c*-9,*c*-11/*c*-10,*c*-12)	n.d.	0.55	n.d.	Trace
18:2(*t*-9,*t*-11/*t*-10,*t*-12)	n.d.	0.42	n.d.	0.43
20:0 (arachidic)	0.35	0.34	0.27	Trace
20:1n-9 (gondoic)	0.16	n.d.	0.73	0.65
20:4n-6 (arachidonic)	n.d.	n.d.	0.42	0.35
20:5n-3 (eicosapentaenoic)	n.d.	n.d.	8.02	6.72
22:0 (behenic)	0.47	0.42	Trace	Trace
22:5n-3 (docosapentaenoic)	n.d.	n.d.	1.51	1.27
22:6n-3 (docosahexaenoic)	n.d.	n.d.	8.57	7.20
SAT[f]	16.04	13.71	20.04	16.57
MONO[g]	23.44	19.75	17.81	15.17
PUFA[h]	60.09	51.57	58.10	50.41
n-6 PUFA	52.84	45.33	37.09	32.73
n-3 PUFA	7.26	6.24	21.01	17.67
n-6/n-3	7.28	7.27	1.77	1.85

[a]The semipurified basal diet contained the following (g/kg): casein, 200; corn starch, 397.486; DYETROSE, 132; sucrose, 100; cellulose, 50; L-lysine, 3; choline bitartrate, 2.5; salt mix, 35; vitamin mix, 10.
[b]Salt mix provided (mg/kg diet): $CaCO_3$, 12495; K_2HPO_4, 6860; $C_6H_5O_7K_3 \cdot H_2O$, 2477; NaCl, 2590; K_2SO_4, 1631; MgO, 840; $C_6H_5O_7Fe$, U.S.P., 212.1; $ZnCO_3$, 57.75; $MnCO_3$, 22.05; $CuCO_3$, 10.5; KIO_3, 0.35; Na_2SeO_4, 0.359; $(NH_4)_2MoO_4 \cdot H_2O$, 0.278; $Na_2O_3Si \cdot 9H_2O$, 50.75; $CrK(SO_4)_2 \cdot 12H_2O$, 9.625; LiCl, 0.609; H_3BO_3, 2.853; NaF, 2.223; $NiCO_3$, 1.113; NH_4VO_3, 0.231.
[c]Vitamin mix provided (mg/kg diet): thiamine HCl, 6; riboflavin, 6; pyridoxine HCl, 7; niacin, 30; calcium pantothenate, 16; folic acid, 2; biotin, 0.2; cyanocobalamin (B-12) (0.1%), 25; vitamin A palmitate (500,000 IU/g), 4000; vitamin E acetate (500 IU/g), 75; vitamin D_3, 1000; vitamin K_1, 0.75.
[d]Dietary treatments included soybean oil (SBO), or menhaden oil + safflower oil (MSO) and with (+) or without (–) conjugated linoleic acids [CLA; 41.9% 18:2(*c*-9,*t*-11/*t*-9,*c*-11), 44.3% 18:2(*t*-10,*c*-12), 13.6% 18:2(*t*-9,*t*-11/*t*-10,*t*-12), and <1% 18:2(*c*-9,*c*-11/*c*-10,*c*-12)]. Total fat content in each diet was 70 g/kg of diet and CLA was added (10 g/kg of diet) at the expense of SBO or MSO.
[e]n.d., not detected (peak detection at 10 ng).
[f]SAT, total saturated fatty acids.
[g]MONO, total monounsaturated fatty acids.

(SBOC); menhaden oil + safflower oil mixture (MSO, menhaden/safflower = 56:44 weight basis, diet rich in n-3 fatty acids); MSO + CLA (MSOC). The CLA was added at 10 g/kg at the expense of the SBO or MSO. All dietary lipids were stored at –80°C before use. Four 49-d-old rats were used in a preliminary study to evaluate the acute enrichment of tissues with CLA. Two rats were fed SBO and the other two SBOC, and the rats on the SBOC diet also were administered CLA intragastrically twice (0.5 g CLA mixed with 0.5 g SBO, at day six and eight) before being killed after 9 d of feeding. Fresh diets were mixed every 14 d, and all the diets were kept at –20°C until fed. Food cups were refilled three times weekly and feed consumption measured at the same time. Body weights were recorded weekly and feed efficiency (total gram weight gain/total gram feed consumed) calculated for each treatment group.

Sample collections. After 42 d of feeding (9 d of feeding for the four rats in the preliminary study), the rats were killed and tissue samples (liver, heart, thigh muscle, spleen, femur, blood, and brain) collected for lipid analysis. Blood was collected by bleeding from axillary vessels. All samples were kept on ice at the time of collection and then frozen at –20°C. Serum was separated by centrifuging the clotted (room temperature) blood at $1200 \times g$ for 20 min (Beckman GS-6; Palo Alto, CA) and stored at –20°C until analyzed.

Analysis of fatty acids. Lipids in the diet and tissue samples were extracted with chloroform/methanol (2:1, vol/vol). Fatty acid methyl esters (FAME) were prepared from dietary lipids by transesterification using boron trifluoride (BF_3) in methanol (14%, w/w; Supelco Inc., Bellefonte, PA). Cortical bone, freed of periosteum and marrow, was cooled with liquid nitrogen and pulverized to a fine powder with a mortar and pestle, placed in 7 mL methanol, and sonicated for 10 min to extract lipids (16). Lipids extracted from rat tissue samples were methylated (sodium methoxide) as follows: sample was dissolved in dry toluene (1 mL) in a test tube with a Teflon-lined screw cap, 0.5 M sodium methoxide in anhydrous methanol (2 mL) added, the solution maintained at 50°C for 10 min, and glacial acetic acid (0.1 mL) added followed by deionized water (5 mL). The FAME were extracted into hexane (2×3 mL), dried over anhydrous sodium sulfate, and filtered. For comparison, lipids from several replicate samples were methylated using BF_3-methanol. All FAME were analyzed using a gas chromatograph (HP 5890 series II, autosampler 7673, HP 3365 ChemStation; Hewlett-Packard Co., Avondale, PA) equipped with a DB 225 or DB 23 column (30 m, 0.53 mm i.d., 0.5 μm film thickness; J&W Scientific Co., Folsom, CA) and operated at 140°C for 2 min, temperature programmed 1.5°C/min to 198°C and held for 7 min. The injector and flame-ionization detector temperatures were 225 and 250°C, respectively. FAME were identified by comparison of their retention times with authentic standards [GLC-422, CLA (UC-59-A and UC-59-M); Nu-Chek-Prep, Elysian, MN; CLA (Cat# 1245, *c*-9,*t*-11 and Cat# 1181, *t*-9,*t*-11); Matreya Inc., Pleasant Gap, PA] and FAME prepared from menhaden oil (Matreya Inc.).

Ex vivo *PGE_2 production.* PGE_2 production by bone organ cultures was performed as previously described (16,20). Shafts from the right tibia and femur bones were removed and carefully flushed with 0.9% NaCl to remove marrow cells. A section of bone shaft was immersed in 20 mL of Hank's balanced salt solution (Sigma Chemical Co., St. Louis, MO) and incubated with shaking for 2 h at 37°C. After incubation, the bone culture medium was collected and stored at –80°C until analyzed for PGE_2 by radioimmunoassay. Values for PGE_2 were expressed per unit of bone wet or dry weight.

Statistical analysis. Data were statistically analyzed by a one-way or two-way analysis of variance, and where significant differences were found, a Tukey's range test was performed at a probability of $P < 0.05$ (SAS software package for UNIX; SAS Institute Inc., Cary, NC). Variation between treatment groups was expressed as the pooled standard error of the mean (SEM).

RESULTS

Body weights between the four treatment groups were not significantly different throughout the feeding period. Final mean body weights at 42 d ranged from 305–313 g (average weight gain in grams: 260 ± 8 SBO, 264 ± 4 SBOC, 267 ± 9 MSO, 263 ± 5 MSOC). Feed efficiency (total grams weight gain/total grams feed consumed), however, was significantly improved ($P < 0.01$) in rats fed CLA compared with those not given CLA (0.42 vs. 0.39). Dietary levels of n-6 and n-3 fatty acids did not influence mean body weights nor feed efficiencies.

Upon analysis of FAME by gas chromatography, the sodium methoxide method gave superior results for CLA isomer distribution compared with those obtained by the BF_3 method. The latter tended to overestimate the concentrations of 18:2(*c,c*) and 18:2(*t,t*) but underestimate those for 18:2(*c*-9,*t*-11/*t*-9,*c*-11) and 18:2(*t*-10,*c*-12). Therefore, all rat tissue fatty acid values reported herein were obtained by the sodium methoxide method.

The short-term feeding and gavage of rats with CLA enriched cortical bone (femur and tibia) with 18:2(*c*-9,*t*-11/*t*-9,*c*-11) and 18:2(*t*-10,*c*-12). The CLA content reached 2.57% of the total fatty acids in 9 d of treatment (data not shown). The enrichment of CLA in bone was accompanied by an increase in n-3 fatty acids, primarily 18:3n-3, and a decrease in 16:0.

After 42 d of feeding, CLA isomers were detected in femur (cortical bone, marrow, and periosteum), brain, heart, liver, muscle, serum, and spleen in rats given CLA (Table 2). The concentration of CLA isomers varied across the rat tissues analyzed with bone containing the greatest amounts. For bone, CLA were highest in bone periosteum (5.46% of total fatty acids), while only trace amounts were detected in brain. The 18:2(*c*-9,*t*-11/*t*-9,*c*-11) and 18:2(*t*-10,*c*-12) isomers were incorporated into all tissues of rats given CLA independent of the n-6 and n-3 fatty acid content of the diet. In contrast, the 18:2(*t*-9,*t*-11) isomer was only observed in bone marrow and periosteum. Liver, serum, bone, marrow, and periosteum had more 18:2(*c*-9,*t*-11/*t*-9,*c*-11) than 18:2(*t*-10,*c*-12), while spleen, muscle, and heart had more 18:2(*t*-10,*c*-12).

Significant differences in tissue fatty acid composition were observed between the dietary treatment groups. For example, rats given CLA had lower concentrations of 16:1n-7, 18:1, 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, and total monounsaturates in liver regardless of the level of n-6 and n-3 fatty acids in the diet (Table 3). Total liver n-6 fatty acid concentration was lower in the MSOC group compared to that in the MSO group, but it was not different between the SBO and SBOC groups. The concentrations of 22:5n-3, 22:6n-3, and total saturates were increased by CLA in liver of rats independent of the n-6 and n-3 fatty acid treatments. However, CLA increased the concentration of 18:0 in liver of rats given the n-6 but not n-3 fatty acid diet. CLA had a similar effect on serum and spleen fatty acid composition (data not shown) compared with liver except that the concentrations of 18:2n-6, 18:3n-6, 20:2n-6, and 18:0 were not affected.

When given CLA, the fatty acid composition of rat skeletal muscle (Table 4) and heart (data not shown) showed similar changes, that is, decreased 16:1n-7, 18:1, and total monounsaturates. Both tissues had increased 22:5n-3, but heart contained reduced concentrations of 18:0 and 22:6n-3. The biceps femoris of rats given diets rich in n-3 fatty acids (MSO and MSOC) had increased 20:5n-3 and 22:6n-3, but lower 20:4n-6 and total n-6 fatty acid concentrations.

Rat brain fatty acid composition was least affected by 42 d of CLA feeding. CLA decreased 16:1n-7 and 20:2n-6, but increased 22:5n-3, 22:5n-6 in rats given the n-6 fatty acid treatment (data not shown). The total n-3 fatty acid concentration was greatest in brain of rats on the MSO and MSOC treatments. Total monounsaturates, saturates, and polyunsaturated fatty acids (PUFA) were not affected by either CLA or n-6

TABLE 2
Tissue Level of CLA Isomers in Rats[a]

	n-6 PUFA + CLA			n-3 PUFA + CLA		
Tissue	CLA (*c*-9,*t*-11/*t*-9,*c*-11)	CLA (*t*-10,*c*-12)	CLA (*t*-9,*t*-11/*t*-10,*t*-12)	CLA (*c*-9,*t*-11/*t*-9,*c*-11)	CLA (*t*-10,*c*-12)	CLA (*t*-9,*t*-11/*t*-10,*t*-12)
Femur cortical bone	1.03 ± 0.20	0.81 ± 0.13	n.d.[b]	0.95 ± 0.13	0.86 ± 0.12	n.d.
Femur marrow	2.02 ± 0.03	1.34 ± 0.05	0.16 ± 0.01	2.13 ± 0.20	1.46 ± 0.20	0.19 ± 0.01
Femur periosteum	2.31 ± 0.06	1.98 ± 0.08	0.12 ± 0.04	2.86 ± 0.13	2.42 ± 0.13	0.18 ± 0.04
Brain	Trace[c]	Trace	n.d.	Trace	Trace	n.d.
Heart	0.31 ± 0.02	1.42 ± 0.05	n.d.	0.32 ± 0.02	1.34 ± 0.03	n.d.
Liver	0.95 ± 0.05	0.69 ± 0.03	n.d.	1.45 ± 0.15	0.70 ± 0.09	n.d.
Muscle	0.68 ± 0.15	1.32 ± 0.12	n.d.	0.56 ± 0.10	1.19 ± 0.06	n.d.
Serum	1.06 ± 0.13	0.78 ± 0.09	n.d.	1.08 ± 0.10	0.53 ± 0.10	n.d.
Spleen	0.67 ± 0.04	1.30 ± 0.03	n.d.	0.76 ± 0.07	1.24 ± 0.04	n.d.

[a]The enrichment of CLA in tissues is expressed as wt% ± SEM.
[b]n.d., not detected.
[c]Trace, less than 0.01% of total fatty acids. See Table 1 for abbreviations.

TABLE 3
Fatty Acid Composition (wt%) of Liver from Rats Fed Different Dietary Lipid Treatments[a]

	Dietary group[b]							
	SBO		MSO		Pooled	ANOVA *P* value		
Fatty acid	CLA +	CLA –	CLA +	CLA –	SEM	CLA	PUFA	CLA × PUFA
14:0	0.45^{n}	0.49^{n}	0.72^{m}	0.56^{m}	0.06	NS[c]	0.007	NS
16:0	21.37^{n}	21.45^{n}	23.77^{m}	22.41^{m}	0.34	NS	0.0001	NS
16:1n-7	$1.43^{n,y}$	$2.65^{n,x}$	$2.51^{m,y}$	$2.95^{m,x}$	0.27	0.007	0.02	NS
17:0	0.30^{n}	0.26^{n}	0.38^{m}	0.39^{m}	0.03	NS	0.002	NS
18:0	14.42^{A}	11.72^{B}	11.63^{B}	11.77^{B}	0.53	0.03	0.02	0.01
18:1	$10.81^{m,y}$	$14.69^{m,x}$	$9.27^{n,y}$	$10.93^{n,x}$	0.65	0.0004	0.0006	NS
18:2n-6	18.14^{y}	19.78^{x}	17.24^{y}	18.69^{x}	0.61	0.02	NS	NS
18:3n-6	$0.24^{m,y}$	$0.28^{m,x}$	$0.17^{n,y}$	$0.21^{n,x}$	0.01	0.001	0.0001	NS
18:3n-3	0.66^{B}	0.89^{A}	0.22^{C}	0.22^{C}	0.05	0.04	0.0001	0.046
20:2n-6	$0.48^{m,y}$	$0.54^{m,x}$	$0.23^{n,y}$	$0.30^{n,x}$	0.03	0.03	0.0001	NS
20:3n-6	$0.88^{n,y}$	$0.93^{n,x}$	$1.08^{m,y}$	$1.21^{m,x}$	0.04	0.04	0.0001	NS
20:4n-6	19.21^{A}	18.30^{A}	6.74^{B}	8.45^{B}	0.59	NS	0.0001	0.04
20:5n-3	0.37^{n}	0.41^{n}	5.68^{m}	5.70^{m}	0.10	NS	0.0001	NS
22:4n-6	0.61^{m}	0.52^{m}	$n.d.^{n}$	$n.d.^{n}$	0.03	NS	0.0001	NS
22:5n-6	0.13^{m}	0.04^{m}	$n.d.^{n}$	$n.d.^{n}$	0.04	NS	0.03	NS
22:5n-3	$2.29^{n,x}$	$1.60^{n,y}$	$5.06^{m,x}$	$4.01^{m,y}$	0.11	0.0001	0.0001	NS
22:6n-3	$5.94^{n,x}$	$4.77^{n,y}$	$11.92^{m,x}$	$10.86^{m,y}$	0.47	0.03	0.0001	NS
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	0.95^{B}	$n.d.^{C}$	1.45^{A}	$n.d.^{C}$	0.08	0.0001	0.006	0.006
18:2(*t*-10,*c*-12)	0.69^{x}	$n.d.^{y}$	0.70^{x}	$n.d.^{y}$	0.04	0.0001	NS	NS
SAT	36.53^{x}	33.91^{y}	36.50^{x}	35.14^{y}	0.55	0.002	NS	NS
MONO	$12.24^{m,y}$	$17.34^{m,x}$	$11.78^{n,y}$	$13.88^{n,x}$	0.89	0.0006	0.04	NS
PUFA	48.95	48.06	48.33	49.64	0.74	NS	NS	NS
n-6 PUFA	39.70^{A}	40.39^{A}	25.45^{C}	28.85^{B}	0.57	0.002	0.0001	0.02
n-3 PUFA	$9.26^{m,x}$	$7.67^{m,y}$	$22.88^{n,x}$	$20.80^{n,y}$	0.51	0.002	0.0001	NS
n-6/n-3	4.31^{A}	5.31^{A}	1.12^{C}	1.39^{B}	0.12	0.0001	0.0001	0.01

[a]Mean values for liver fatty acid composition ($n = 6$) within a row having different superscripts (m,n for PUFA effect; x, y for CLA effect; A, B, C for interaction effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 12$ in the statistical analysis of main effects.
[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of SBO or MSO.
[c]NS, not significant. See Table 1 for other abbreviations.

and n-3 fatty acids. Generally, rats fed high n-3 fatty acids (MSO and MSOC) had more 16:1n-7, 20:3n-6, 20:5n-3, 22:5n-3, and 22:6n-3. In contrast, rats fed high n-6 fatty acids (SBO and SBOC) had higher 20:2n-6, 20:4n-6, and 22:4n-6. The 18:2n-6 concentration in brain was not affected by the fatty acid treatments.

Consumption of CLA lowered the concentrations of 20:3n-6, 22:6n-3, and total n-6 in cortical bone (Table 5). The rats given n-3 fatty acids (MSO and MSOC) had higher concentrations of 16:0, 20:5n-3, 22:5n-3, 22:6n-3, total n-3, and saturates but lower 18:1, 18:3n-3, 22:4n-6, total n-6, and monounsaturates compared to those given n-6 fatty acids (SBO and SBOC). In bone marrow of rats supplemented with CLA, the concentrations of 12:0, 14:0, 16:0, and total saturates were higher while those for 16:1n-7, 18:1, 18:2n-6, 20:2n-6, 20:3n-6, 22:5n-6, total monounsaturates, PUFA, and n-6, were lower (Table 6). The n-3 fatty acid diets (MSO and MSOC) resulted in higher 12:0, 14:0, 15:0, 16:0, 16:1n-7, 17:0, 20:3n-6, 20:5n-3, 22:5n-3, 22:6n-3, total saturates, and n-3, but lower 18:1, 18:2n-6, 20:2n-6, 20:4n-6, 22:4n-6, total monounsaturates, PUFA, and n-6 concentrations in marrow. A CLA–PUFA interaction influenced the 18:3n-6, 18:3n-3, 20:1n-9, and 22:6n-3 concentrations in bone marrow. The periosteum of rats given CLA had higher 12:0, 14:0, 16:0, and total saturates but lower 18:1, 18:2n-6, total monounsaturates, PUFA, and n-6 (Table 7). Rats given n-3 fatty acids compared with those on the n-6 fatty acid diets (SBO and SBOC) had higher 14:0, 15:0, 16:0, 16:1n-7, 20:1n-9, 20:3n-6, 20:5n-3, 22:5n-3, 22:6n-3, total saturates, and n-3 although lower 12:0, 18:1, 18:2n-6, 18:3n-3, 20:2n-6, 20:4n-6, 22:4n-6, 22:5n-6, total monounsaturates, PUFA, and n-6 concentrations in periosteum.

In rats, CLA and n-3 fatty acids both reduced *ex vivo* PGE_2 production (Table 8). The *ex vivo* PGE_2 production by bone organ cultures from rats given n-3 fatty acids (MSO and MSOC) was lower than the values from those given n-6 fatty acids (SBO and SBOC). Interestingly, CLA consumption by rats further reduced *ex vivo* PGE_2 production in bone (femur and tibia) independent of n-6 and n-3 fatty acid intake.

DISCUSSION

The results from the feeding experiment showed that neither CLA nor n-6 and n-3 fatty acids (SBO high in n-6 and MSO high in n-3 fatty acids) influenced final body weight or weight gain of the rats. However, feed efficiency was significantly

TABLE 4
Fatty Acid Composition (wt%) of Muscle (biceps femoris) from Rats Fed Different Dietary Lipid Treatments[a]

	Dietary group[b]							
	SBO		MSO		Pooled	ANOVA *P* value		
Fatty acid	CLA +	CLA –	CLA +	CLA –	SEM	CLA	PUFA	CLA × PUFA
14:0	0.66	0.78	0.84	0.85	0.08	NS	NS	NS
16:0	21.96	21.62	23.36	22.41	0.30	NS	NS	NS
16:1n-7	1.17^{y}	2.74^{x}	1.71^{y}	2.28^{x}	0.20	0.02	NS	NS
17:0	0.13^{n}	0.15^{n}	0.28^{m}	0.31^{m}	0.01	NS	0.0001	NS
18:0	12.73	10.38	11.47	11.56	0.32	NS	NS	NS
18:1	9.91^{B}	16.63^{A}	7.44^{B}	9.76^{B}	0.52	0.0003	0.0002	0.046
18:2n-6	25.30^{m}	25.03^{m}	19.67^{n}	19.86^{n}	0.23	NS	0.0001	NS
18:3n-3	0.68^{B}	1.31^{A}	n.d.[C]	0.06^{C}	0.05	0.004	0.0001	0.02
20:3n-6	0.37	0.41	0.44	0.46	0.02	NS	NS	NS
20:4n-6	12.50^{A}	10.67^{A}	4.41^{B}	5.09^{B}	0.27	NS	0.0001	0.03
20:5n-3	n.d.[n]	n.d.[n]	1.74^{m}	1.80^{m}	0.03	NS	0.0001	NS
22:4n-6	0.83^{m}	0.07^{m}	0.10^{n}	0.05^{n}	0.03	NS	0.0001	NS
22:5n-6	0.46^{m}	0.43^{m}	n.d.[n]	n.d.[n]	0.02	NS	0.0001	NS
22:5n-3	3.11^{x}	2.32^{y}	2.90^{x}	2.51^{y}	0.09	0.004	NS	NS
22:6n-3	8.07^{n}	6.32^{n}	23.66^{m}	22.24^{m}	0.63	NS	0.0001	NS
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	0.68^{x}	n.d.[y]	0.56^{x}	n.d.[y]	0.05	0.0001	NS	NS
18:2(*t*-10,*c*-12)	1.32^{x}	n.d.[y]	1.19^{x}	n.d.[y]	0.03	0.0001	NS	NS
SAT	$35.48^{n,x}$	$32.94^{n,y}$	$35.96^{m,x}$	$35.14^{m,y}$	0.21	0.001	0.005	NS
MONO	$11.08^{m,y}$	$19.37^{m,x}$	$9.15^{n,y}$	$12.04^{n,x}$	0.70	0.0007	0.003	NS
PUFA	51.33	47.34	52.92	52.06	0.80	NS	NS	NS
n-6 PUFA	39.46^{A}	37.39^{B}	24.62^{C}	25.45^{C}	0.25	NS	0.0001	0.009
n-3 PUFA	11.86^{n}	9.95^{n}	28.30^{m}	26.61^{m}	0.68	NS	0.0001	NS
n-6/n-3	3.37^{m}	3.83^{m}	0.87^{n}	1.02^{n}	0.16	NS	0.0001	NS

[a]Mean values for muscle fatty acid composition ($n = 6$) within a row having different superscripts (m,n for PUFA effect; x, y for CLA effect; A, B, C for interaction effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 12$ in the statistical analysis of main effects.
[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of SBO or MSO. See Tables 1 and 3 for other abbreviations.

improved for rats given CLA independent of the dietary n-6 and n-3 fatty acid treatments. Our results corroborate those of Chin *et al.* (21) who observed that CLA given to rats improved feed efficiency. Sugano *et al.* (9) also found that CLA feeding to rats did not influence growth, but an experiment in mice revealed that CLA reduced body weights without influencing feed disappearance (22). Unfortunately, the authors of study in mice did not measure feed wastage (22) which could have explained the discrepancy in food disappearance and the 50% depression in growth.

CLA was reported to reduce the catabolic response from immune stimulation in animals without adversely affecting immune function (23,24). Cytokines and PGE_2 (21) are believed to mediate this catabolic response, and recently, CLA was found to lower *in vivo* and *ex vivo* production of cytokines in rats (9,19). The present investigation with rats is consistent with other findings that dietary CLA did not affect body weight but decreased feed consumption to improve feed efficiency. Further research is needed to characterize the biochemical and physiological actions of CLA on energy metabolism.

The pattern of CLA enrichment in rats showed certain tissue selectivity for the various isomers. For example, liver, serum, bone, marrow, and periosteum contained more 18:2(*c*-9, *t*-11/*t*-9,*c*-11) than 18:2(*t*-10,*c*-12), the two predominant isomers. Spleen, muscle, and heart, however, contained more 18:2(*t*-10,*c*-12) than the 9,11 isomer. Furthermore, only trace amounts of 18:2(*c*-9,*t*-11/*t*-9,*c*-11) and 18:2(*t*-10,*c*-12) were detected in brain. To our knowledge this is the first report demonstrating that CLA are found in bone tissue. Surprisingly, concentrations of CLA in bone tissues (cortical, marrow, and periosteum) were the highest of all tissues analyzed. Moreover, the various isomers of CLA incorporated into bone tissue lipids equally well for diets high in n-6 or n-3 fatty acids.

The fatty acid composition of rat tissues was significantly modified by the dietary lipids. The effects of dietary n-6 and n-3 enrichment (SBO and MSO, respectively) on fatty acid composition of bone in rats were similar to the results in chicks we have reported previously (20). Linoleate (18:2n-6) and its chain elongation and desaturation products (20:4n-6, 22:4n-6, and 22:5n-6) were higher in liver, spleen, serum, muscle, heart, bone (marrow and periosteum), and brain in rats given SBO. CLA consumption decreased the concentrations of 16:1n-7, 18:1, total monounsaturates, and n-6, but increased those for 22:5n-3, 22:6n-3, total n-3, and saturates in most tissues analyzed. CLA decreased the concentration of 18:2n-6 in bone marrow and periosteum, 18:3n-6, 20:2n-6, 20:3n-6, 22:5n-6 in marrow, and 20:3n-6 in cortical bone. Further, CLA decreased the ratio of n-6/n-3 in liver, brain,

TABLE 5
Fatty Acid Composition (wt%) of Femur Cortical Bone from Rats Fed Different Dietary Lipid Treatments[a]

	Dietary group[b]							
	SBO		MSO		Pooled	ANOVA *P* value		
Fatty acid	CLA +	CLA –	CLA +	CLA –	SEM	CLA	PUFA	CLA × PUFA
14:0	1.43	1.08	1.30	1.43	0.16	NS	NS	NS
15:0	0.27^{n}	0.31^{n}	0.41^{m}	0.51^{m}	0.04	NS	0.001	NS
16:0	27.78^{n}	26.06^{n}	28.66^{m}	30.18^{m}	1.12	NS	0.04	NS
16:1n-7	1.64	2.52	2.08	2.66	0.40	NS	NS	NS
17:0	0.30	0.14	0.13	0.20	0.12	NS	NS	NS
18:0	9.76	7.96	8.47	9.42	0.50	NS	NS	NS
18:1	18.65^{m}	16.09^{m}	11.69^{n}	13.83^{n}	1.23	NS	0.002	NS
18:2n-6	12.36	15.49	11.72	12.64	1.28	NS	NS	NS
18:3n-4	2.65	3.28	3.54	1.27	0.89	NS	NS	NS
18:3n-3	0.61^{m}	1.15^{m}	n.d.[n]	n.d.[n]	0.16	NS	0.0001	NS
20:2n-6	0.40	0.68	0.42	0.34	0.11	NS	NS	NS
20:3n-6	0.23^{y}	0.89^{x}	0.72^{y}	0.88^{x}	0.16	0.02	NS	NS
20:4n-6	7.91	9.28	6.87	8.59	0.80	NS	NS	NS
20:3n-3	0.83	0.90	1.31	0.44	0.36	NS	NS	NS
20:5n-3	0.75^{n}	0.54^{n}	2.76^{m}	2.52^{m}	0.16	NS	0.0001	NS
22:0	0.15	0.25	0.36	0.14	0.12	NS	NS	NS
22:1n-9	0.34	0.16	0.37	0.08	0.21	NS	NS	NS
22:4n-6	2.45^{m}	2.62^{m}	1.33^{n}	1.48^{n}	0.30	NS	0.002	NS
22:5n-3	0.58^{n}	0.80^{n}	3.16^{m}	3.10^{m}	0.15	NS	0.0001	NS
22:6n-3	$0.64^{n,y}$	$11.12^{n,x}$	$3.35^{m,y}$	$4.14^{m,x}$	0.20	0.007	0.0001	NS
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	1.03^{x}	n.d.[y]	0.95^{x}	n.d.[y]	0.13	0.0001	NS	NS
18:2(*t*-10,*c*-12)	0.81^{x}	n.d.[y]	0.86^{x}	n.d.[y]	0.09	0.0001	NS	NS
SAT	39.70^{n}	35.80^{n}	39.34^{m}	41.88^{m}	1.33	NS	0.046	NS
MONO	20.63^{m}	18.77^{m}	14.14^{n}	16.56^{n}	1.49	NS	0.01	NS
PUFA	29.42^{y}	36.73^{x}	35.18^{y}	35.40^{x}	1.77	0.049	NS	NS
n-6 PUFA	$23.36^{m,y}$	$28.95^{m,x}$	$21.06^{n,y}$	$23.93^{n,x}$	1.71	0.02	0.048	NS
n-3 PUFA	3.41^{n}	4.50^{n}	10.58^{m}	10.20^{m}	0.35	NS	0.0001	NS
n-6/n-3	7.33^{m}	6.74^{m}	1.99^{n}	2.35^{n}	0.85	NS	0.0001	NS

[a]Mean values for femur cortical bone fatty acid composition ($n = 5$) within a row having different superscripts (m, n for PUFA effect; x, y for CLA effect; A, B, C for interaction effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 10$ in the statistical analysis of main effects.
[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of SBO or MSO. See Table 1 for other abbreviations.

spleen, and serum of rats given MSO. The relative sparing of n-3 fatty acids in rats given CLA might be explained as either an increase in the utilization of n-6 or a conservation of n-3 fatty acids. The increase of total saturates in liver, marrow, periosteum, spleen, muscle, and serum of rats consuming CLA could indicate a depression of desaturase activity. Since liver is a major organ for fatty acid synthesis in mammals, and we observed a decrease of 18:1n-9 in rat tissues (liver, marrow, and periosteum), one would speculate that CLA affects the saturates/monounsaturates ratio by inhibiting liver Δ9-desaturase activity as proposed by Lee *et al.* (10).

The n-3 fatty acid treatment (MSO) significantly reduced *ex vivo* PGE_2 production in bone organ culture (tibia and femur) compared to the n-6 dietary treatment (SBO). In bone, dietary CLA lowered *ex vivo* PGE_2 production beyond that of n-3 fatty acid feeding. Sugano *et al.* (9) also reported that the concentration of PGE_2 in spleen tended to be reduced by CLA. Dietary supplementation with CLA may impact bone modeling in the young since PGE_2 mediates bone formation and bone resorption (18). Recent work from our laboratory demonstrated that modulation of *ex vivo* PGE_2 production in bone with dietary lipids was associated with changes in bone formation rate (16,20). PGE_2 is a potent agent regulating both bone modeling and remodeling, but its effect in bone may be concentration-dependent. For example, Raisz and Koolemans-Beynen (25) showed that PGE_2 inhibits matrix formation at high concentrations in bone organ culture. However, at lower doses, PGE_2 can stimulate bone formation *in vitro* and *in vivo* (26–29). Therefore, excessive production of PGE_2 may adversely affect bone modeling, and a lower level of PGE_2 is believed to stimulate bone formation in animals fed diets containing moderate levels of n-6 fatty acids. Chicks fed a diet containing a lower n-6/n-3 fatty acid ratio demonstrated reduced PGE_2 production in bone that was accompanied by an increased bone formation rate when compared with those given a higher n-6/n-3 ratio (20). Although different mechanisms may be operative for CLA and n-3 fatty acids to reduce *ex vivo* PGE_2 production, the fact that both fatty acids lowered PGE_2 by cultured bone may be significant in understanding their potential effects on bone health.

In summary, supplementing the diets of rats with CLA led to differences in CLA enrichment in various tissues, with

TABLE 6
Fatty Acid Composition (wt%) of Femur Bone Marrow from Rats Fed Different Dietary Lipid Treatments[a]

	Dietary group[b]							
	SBO		MSO		Pooled	ANOVA *P* value		
Fatty acid	CLA +	CLA –	CLA +	CLA –	SEM	CLA	PUFA	CLA × PUFA
12:0	$0.21^{n,x}$	$0.14^{n,y}$	$0.27^{m,x}$	$0.21^{m,y}$	0.01	0.0002	0.0002	NS
14:0	$2.37^{n,x}$	$1.76^{n,y}$	$3.24^{m,x}$	$2.90^{m,y}$	0.11	0.0004	0.0001	NS
15:0	0.25^{n}	0.23^{n}	0.35^{m}	0.37^{m}	0.01	NS	0.0001	NS
16:0	$29.15^{n,x}$	$25.17^{n,y}$	$31.48^{m,x}$	$28.32^{m,y}$	0.51	0.0001	0.0001	NS
16:1n-7	$4.12^{n,y}$	$4.94^{n,x}$	$5.02^{m,y}$	$6.10^{m,x}$	0.38	0.02	0.01	NS
17:0	0.21^{n}	0.19^{n}	0.30^{m}	0.32^{m}	0.01	NS	0.0001	NS
18:0	6.53	6.39	7.15	6.74	0.39	NS	NS	NS
18:1	$18.89^{m,y}$	$23.07^{m,x}$	$14.95^{n,y}$	$18.11^{n,x}$	0.42	0.0001	0.0001	NS
18:2n-6	$22.73^{m,y}$	$24.63^{m,x}$	$18.52^{n,y}$	$19.62^{n,x}$	0.48	0.006	0.0001	NS
18:3n-6	0.17^{C}	0.25^{A}	0.17^{C}	0.21^{B}	0.01	0.0001	0.003	0.003
18:3n-3	1.78^{A}	2.13^{A}	0.50^{B}	0.42^{B}	0.10	NS	0.0001	0.04
20:1n-9	0.19^{B}	0.25^{A}	0.20^{B}	0.24^{A}	0.01	0.0001	NS	0.007
20:2n-6	$0.62^{m,y}$	$0.82^{m,x}$	$0.54^{n,y}$	$0.60^{n,x}$	0.04	0.002	0.0004	NS
20:3n-6	$0.24^{n,y}$	$0.36^{n,x}$	$0.42^{m,y}$	$0.49^{m,x}$	0.03	0.01	0.0002	NS
20:4n-6	4.68^{m}	5.75^{m}	3.76^{n}	3.80^{n}	0.41	NS	0.002	NS
20:5n-3	0.13^{n}	0.06^{n}	1.44^{m}	1.82^{m}	0.13	NS	0.0001	NS
22:4n-6	0.77^{m}	0.96^{m}	0.27^{n}	0.26^{n}	0.06	NS	0.0001	NS
22:5n-6	0.07^{y}	0.14^{x}	0.07^{y}	0.13^{x}	0.02	0.01	NS	NS
22:5n-3	0.58^{n}	0.58^{n}	2.06^{m}	2.27^{m}	0.16	NS	0.0001	NS
22:6n-3	0.59^{C}	0.59^{C}	2.49^{B}	3.30^{A}	0.20	0.047	0.0001	0.048
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	2.02^{x}	n.d.^{y}	2.13^{x}	0.10^{y}	0.11	0.0001	NS	NS
18:2(*t*-10,*c*-12)	1.34^{x}	n.d.^{y}	1.46^{x}	0.08^{y}	0.11	0.0001	NS	NS
18:2(*t*-9,*t*-11/*t*-10,*t*-12)	0.16^{x}	n.d.^{y}	0.19^{x}	n.d.^{y}	0.01	0.0001	NS	NS
SAT	$38.73^{n,x}$	$33.87^{n,y}$	$42.80^{m,x}$	$38.86^{m,y}$	0.48	0.0001	0.0001	NS
MONO	$23.32^{m,y}$	$28.40^{m,x}$	$20.32^{n,y}$	$24.63^{n,x}$	0.70	0.0001	0.0001	NS
PUFA	$32.35^{m,y}$	$36.27^{m,x}$	$30.24^{n,y}$	$32.95^{n,x}$	0.93	0.002	0.008	NS
n-6 PUFA	$29.27^{m,y}$	$32.91^{m,x}$	$23.74^{n,y}$	$25.10^{n,x}$	0.77	0.004	0.0001	NS
n-3 PUFA	3.08^{n}	3.36^{n}	6.50^{m}	7.85^{m}	0.43	NS	0.0001	NS
n-6/n-3	9.87^{m}	9.83^{m}	3.84^{n}	3.26^{n}	0.49	NS	0.0001	0.01

[a]Mean values for femur bone marrow fatty acid composition ($n = 6$) within a row having different superscripts (m, n for PUFA effect; x, y for CLA effect; A, B, C for interaction effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 12$ in the statistical analysis of main effects.
[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of SBO or MSO. See Tables 1 and 3 for other abbreviations.

brain having the lowest concentrations of isomers but bone tissue containing the highest. Furthermore, CLA influenced the fatty acid composition of rat tissues leading to reduced 18:1 in liver, muscle, heart, and bone (marrow and periosteum). The data on tissue fatty acid composition presented here suggest that CLA may affect the formation of 18:1n-9 and metabolism of n-6 and n-3 fatty acids. Consistent with the hypothesis that CLA modifies arachidonate-derived eicosanoid production, we observed that CLA depressed *ex vivo* PGE_2 production in bone organ cultures. The reduction in PGE_2 by CLA might be explained as a competitive inhibition of n-6 fatty acid formation (30) that would lower substrate availability for cyclooxygenase. Since PGE_2 is a potent agent that stimulates both bone formation and resorption, research on CLA will be relevant to understanding the potential role of essential fatty acids in bone biology.

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TABLE 7
Fatty Acid Composition (wt%) of Femur Bone Periosteum from Rats Fed Different Dietary Lipid Treatments[a]

	Dietary group[b]							
	SBO		MSO		Pooled	ANOVA *P* value		
Fatty acid	CLA +	CLA –	CLA +	CLA –	SEM	CLA	PUFA	CLA × PUFA
12:0	$0.15^{m,x}$	$0.09^{m,y}$	$0.17^{n,x}$	$0.14^{n,y}$	0.02	0.01	0.03	NS
14:0	$1.65^{n,x}$	$1.12^{n,y}$	$2.57^{m,x}$	$2.33^{m,y}$	0.12	0.004	0.0001	NS
15:0	0.18^{n}	0.14^{n}	0.28^{m}	0.31^{m}	0.02	NS	0.0001	NS
16:0	$25.68^{n,x}$	$22.18^{n,y}$	$28.42^{m,x}$	$24.97^{m,y}$	0.54	0.0001	0.0001	NS
16:1n-7	2.80^{n}	2.77^{n}	3.94^{m}	4.57^{m}	0.30	NS	0.0001	NS
17:0	$0.21^{B,C}$	0.16^{C}	$0.31^{A,B}$	0.38^{A}	0.03	NS	0.0001	NS
18:0	8.38	8.92	7.86	8.29	0.47	NS	NS	NS
18:1	$17.14^{m,y}$	$20.70^{m,x}$	$13.46^{n,y}$	$17.10^{n,x}$	0.63	0.0001	0.0001	NS
18:2n-6	$25.38^{m,y}$	$28.16^{m,x}$	$20.85^{n,y}$	$22.54^{n,x}$	0.60	0.001	0.0001	NS
18:3n-3	1.69^{A}	2.07^{A}	0.50^{B}	0.42^{B}	0.10	NS	0.0001	0.04
20:1n-9	$n.d.^{n}$	$n.d.^{n}$	0.10^{m}	0.18^{m}	0.03	NS	0.0001	NS
20:2n-6	0.25^{m}	0.26^{m}	0.14^{n}	0.15^{n}	0.04	NS	0.01	NS
20:3n-6	0.27^{n}	0.30^{n}	0.34^{m}	0.42^{m}	0.04	NS	0.02	NS
20:4n-6	5.43^{m}	6.88^{m}	2.93^{n}	2.94^{n}	0.47	NS	0.0001	NS
20:5n-3	$n.d.^{C}$	$n.d.^{C}$	1.30^{B}	1.72^{A}	0.08	0.01	0.0001	0.01
22:4n-6	0.81^{m}	0.73^{m}	0.36^{n}	0.33^{n}	0.09	NS	0.0002	NS
22:5n-6	0.28^{m}	0.20^{m}	$n.d.^{n}$	0.10^{n}	0.06	NS	0.002	NS
22:5n-3	1.41^{n}	1.44^{n}	2.01^{m}	2.00^{m}	0.13	NS	0.0003	NS
22:6n-3	3.25^{n}	3.53^{n}	7.46^{m}	8.98^{m}	0.62	NS	0.0001	NS
18:2(*c*-9,*t*-11/*t*-9,*c*-11)	2.31^{x}	$n.d.^{y}$	2.86^{x}	$n.d.^{y}$	0.13	0.0001	NS	NS
18:2(*t*-10,*c*-12)	1.98^{x}	$n.d.^{y}$	2.42^{x}	$n.d.^{y}$	0.13	0.0001	NS	NS
18:2(*t*-9,*t*-11/*t*-10,*t*-12)	0.12^{x}	$n.d.^{y}$	0.18^{x}	$n.d.^{y}$	0.04	0.0003	NS	NS
SAT	$36.25^{n,x}$	$32.61^{n,y}$	$39.61^{m,x}$	$36.43^{m,y}$	0.52	0.0001	0.0001	NS
MONO	$19.94^{m,y}$	$23.47^{m,x}$	$17.50^{n,y}$	$21.85^{n,x}$	0.81	0.0001	0.02	NS
PUFA	$38.76^{m,y}$	$43.57^{m,x}$	$35.88^{n,y}$	$39.61^{n,x}$	1.17	0.002	0.008	NS
n-6 PUFA	$32.42^{m,y}$	$36.52^{m,x}$	$24.62^{n,y}$	$26.48^{n,x}$	0.86	0.003	0.0001	NS
n-3 PUFA	6.34^{n}	7.05^{n}	11.26^{m}	13.12^{m}	0.72	NS	0.0001	NS
n-6/n-3	5.29^{m}	5.28^{m}	2.24^{n}	2.06^{n}	0.28	NS	0.0001	NS

[a]Mean values for femur bone periosteum fatty acid composition ($n = 6$) within a row having different superscripts (m, n for PUFA effect; x, y for CLA effect; A, B, C for interaction effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 12$ in the statistical analysis of main effects.
[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of SBO or MSO. See Tables 1 and 3 for other abbreviations.

TABLE 8
Effect of Dietary CLA and PUFA Treatments on PGE_2 Production (mean ± SE) in Bone Organ Culture[a]

	Dietary groups[b]						
	SBO		MSO		ANOVA *P* value		
Tissue	CLA +	CLA –	CLA +	CLA –	CLA	PUFA	CLA × PUFA
Tibia, ng/g (dry weight)	$19.1 \pm 2.1^{m,y}$	$42.9 \pm 11.6^{m,x}$	$6.6 \pm 2.6^{n,y}$	$15.8 \pm 2.0^{n,x}$	0.02	0.007	0.25
Tibia, ng/g (wet weight)	$17.6 \pm 1.5^{m,y}$	$34.5 \pm 8.8^{m,x}$	$5.0 \pm 1.7^{n,y}$	$12.8 \pm 1.3^{n,x}$	0.02	0.003	0.35
Femur, ng/g (dry weight)	$21.2 \pm 3.6^{m,y}$	$30.8 \pm 4.7^{m,x}$	$6.6 \pm 0.8^{n,y}$	$16.0 \pm 4.3^{n,x}$	0.02	0.002	0.98
Femur, ng/g (wet weight)	19.8 ± 3.6^{m}	25.7 ± 4.2^{m}	5.6 ± 1.1^{n}	14.5 ± 4.3^{n}	0.06	0.004	0.69

[a]Mean values for *ex vivo* prostaglandin E_2 (PGE_2) production ($n = 6$) having different superscripts (m, n for PUFA effect; x, y for CLA effect) are significantly different by two-way ANOVA and Tukey's studentized range test ($P < 0.05$). The factorial design of dietary treatments allowed for an $n = 12$ in the statistical analysis of main effects.

[b]Dietary treatments included SBO, or MSO and with (+) or without (–) CLA. Total fat content in each diet was 70 g/kg of diet, and CLA was added (10 g/kg of diet) at the expense of kSBO or MSO. See Tables 1 and 3 for other abbreviations.

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