Dietary Conjugated Linoleic Acid Does Not Adversely Affect Bone Mass in Obese *fa/fa* or Lean Zucker Rats

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Conjugated linoleic acid (CLA) elevates body ash in healthy animals. The objective of the present study was to determine if single or mixed CLA isomers improve bone mass in an obese and hyperinsulinemic state. Male ($n = 120$) lean and obese *fa/fa* Zucker rats (age, 6 weeks) were randomized to 8 weeks on a control diet or to 0.4% (w/w) *cis*-9, *trans*-11 CLA (Group 1); 0.4% (w/w) *trans*-10, *cis*-12 CLA (Group 2); 0.4% (w/w) *cis*-9, *trans*-11 CLA and 0.4% (w/w) *trans*-10, *cis*-12 CLA (Group 3); 0.4% (w/w) *cis*-9, *trans*-11 CLA, 0.4% (w/w) *trans*-10, *cis*-12 CLA, and traces of other CLA isomers (Group 4); and 0.4% (w/w) *cis*-9, *trans*-11 CLA, 0.4% (w/w) *trans*-10, *cis*-12 CLA, and 0.3% (w/w) other CLA isomers (Group 5). Bone area (BA), bone mineral content (BMC), and bone mineral density (BMD) of the whole body, spine, and femur were measured at baseline (6 weeks) and at 14 weeks of age. Effects of genotype, diet, and genotype × diet interactions were assessed using factorial analysis of variance. At 6 and 14 weeks, whole-body BA and BMC were lower in lean rats compared with *fa/fa* rats. Similarly, at 14 weeks, *fa/fa* rats had a higher spine and femur BMD despite a lower femur weight. The *fa/fa* rats in Groups 4 and 5 had higher adjusted whole-body BMC compared with Group 3, but not with Group 1, Group 2, or the control. In lean rats, Group 3 had a greater adjusted whole-body BMC than Groups 1 and 2, but not Group 4, Group 5, or the control. Thus, commercially available CLA mixtures and single CLA isomers do not affect bone mass in a hyperinsulinemic, obese state. Exp Biol Med 231:1602–1609, 2006

This research was supported by the Dairy Farmers of Canada and the Natural Sciences and Engineering Research Council of Canada. This paper was presented in part at the Canadian Federation of Biological Sciences Conference, June 2005, in Guelph, Ontario, Canada (Burr LL, Taylor CG, Weiler HA. Femur bone mass, but not whole body bone mass, is greater in fa/fa Zucker rats than in lean Zucker rats. CFBS Proc 48:83, 2005.

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Received February 17, 2006.
Accepted April 24, 2006.

1535-3702/06/23110-1602$15.00

Key words: conjugated linoleic acid; bone mass; obesity; hyperinsulinemia; Zucker rat

Introduction

Polyunsaturated fatty acids (PUFAs), including conjugated linoleic acid (CLA), are recognized as nutrients involved in bone mineral homeostasis (1). CLA is a group of positional and geometric isomers of linoleic acid (2), of which the *trans*-10, *cis*-12 (*t*10, *c*12) and the *cis*-9, *trans*-11 (*c*9, *t*11) CLA isomers are sold commercially and available in mixed or pure forms (3). Research has heightened interest in CLA as a positive modulator of several health outcomes associated with excess body weight. Because CLA may be recommended therapeutically to overweight individuals, including young adults and adolescents (4, 5), there is a need to clarify whether CLA improves or reduces bone mass during a period of bone mineralization and consolidation.

Animal studies have provided evidence that CLA may play a role in bone mineralization and turnover. In general, the effect of CLA on bone mass appears to be dependent on the CLA isomers included in the diet (6), the dietary total n-6 to n-3 PUFA ratio (7), and the period of life when CLA is fed (8). The effect of CLA on bone also may be dose dependent (9). A CLA mixture containing many isomers was observed to have no effect on bone mass (8), but supplementing the diet primarily with *t*10, *c*12 CLA isomers elevated body ash in mice (6). Studies that observed positive effects of CLA on bone mass used a high n-6 to n-3 PUFA ratio for the dietary lipid composition (8–11), whereas a lower n-6 to n-3 PUFA ratio frequently was used during studies in which CLA had no effect (7, 12, 13). Studies conducted during periods of rapid growth have found a response in bone to CLA treatment. A positive effect of CLA on bone mass has been observed in male chicks fed butter fat from birth (10) and in growing male and female mice (6, 8, 11). Research conducted during later stages of growth in swine, however, suggests that CLA has no effect on bone mass (14, 15). A recent epidemiologic study

conducted with postmenopausal women found that dietary CLA intake was positively associated with forearm bone mineral density (BMD; Ref. 16), but other studies in adults (17–19) have found no effect of CLA on biochemical markers of bone resorption and formation or osteoporotic risk factors. Based on these studies, CLA might best be used to enhance bone mass during childhood or adolescence, a time of continued bone growth and mineralization.

Osteoporosis may be a health risk (20) for the growing number of children and young adults in North America who are overweight or obese (21–23). In adults, BMD is positively associated with body weight (24, 25), but the protective effect of excess body fat on bone mass appears to diminish as body weight increases (26). In children, excess body weight (or, more specifically, fat mass) reduces bone mineral content (BMC; Refs. 27, 28) and increases the risk of bone fracture (29, 30). Because CLA has been reported in some studies to reduce body weight (31), dietary CLA may be useful in achieving a body weight that is supportive of optimal bone mineralization. Juvenile obesity also is associated with the development of hyperinsulinemia and type 2 diabetes mellitus in childhood (32, 33). Elevated bone mass is associated with hyperinsulinemia in adults (34, 35), but the effects on bone in the young are unknown.

Because it is premature to test for benefits of CLA on bone mass in children, the primary objective of the current study was to determine the effect of feeding *c*9, *t*11 or *t*10, *c*12 CLA isomers individually (0.4% w/w, where w/w is g/kg diet × 100), in combination (0.8% w/w CLA isomers), or in a mixture with other CLA isomers (0.8%–1.1% w/w CLA isomers) to young, male Zucker rats. The *fa/fa* Zucker genotype is an obese, leptin receptor–deficient model that exhibits hyperinsulinemia without hyperglycemia (36). This model also has been confirmed to be representative of juvenile obesity and bone metabolism (37). The *fa/fa* rat has reduced long bone longitudinal growth and whole-body bone mass, with lower bone formation and higher resorption, than lean Zucker rats by 12–24 weeks of age (37, 38). The *fa/fa* genotype, however, is responsive in bone to intervention, because previously, an exercise regime elevated bone mass between 3–6 months of age (39). Because of the potential of CLA to be a nutraceutical in the management of obesity and type 2 diabetes (40, 41), determining the effect of CLA isomers on bone mass in an obese, hyperinsulinemic model is a necessary investigation to further ascertain the benefits and side effects of CLA treatment.

Materials and Methods

Animals and Diet. All animal care procedures were based on guidelines from the Canadian Council on Animal Care (42) and approved by the University of Manitoba Fort Garry Campus Protocol Management and Review Committee.

Male lean and *fa/fa* Zucker rats were purchased from Harlan (Indianapolis, IN) at 5 weeks of age. Because of the potential confounding variables associated with female sex hormones and differences in pubertal onset between males and females, only male rats were used. Rats were housed individually in stainless steel, wire-bottomed cages in a controlled environment of 55% humidity and 21°–23°C with a 14:10-hr light:dark cycle. Rats followed longitudinally were acclimatized on a control diet for 5–9 days, and test diets were randomly assigned ($n = 10$ rats/diet group) and fed *ad libitum* for 8 weeks. Feed intake (corrected for spillage) and rat weight was measured weekly. To provide for baseline values, four lean and five *fa/fa* rats were terminated at 6 weeks of age (before receiving the test diet). These rats underwent the same analysis as those in the treatment groups (description follows).

All diets were based on the AIN-93G formulation and were nutritionally adequate for normal rat growth and development (43). Each diet contained 8.5% (w/w) lipid (Table 1). The control diet contained soybean oil exclusively. Test diets were designed to provide 0.4% (w/w) *c*9, *t*11 and/or *t*10, *c*12. Groups 1 and 2 received 0.4% (w/w) of pure *c*9, *t*11 and *t*10, *c*12, respectively, whereas Group 3 received both CLA isomers (0.4% w/w *c*9, *t*11 and 0.4% w/w *t*10, *c*12) or 0.8% (w/w) CLA isomers. Groups 4 and 5 also received both CLA isomers (0.4% w/w *c*9, *t*11 and 0.4% w/w *t*10, *c*12), but because of the presence of other CLA isomers in the oils used for these diets, those groups received 0.8% and 1.1% (w/w) CLA isomers, respectively. The two primary CLA isomers in the diets were chosen based on evidence that both are more effective at altering body composition than other CLA isomers and that both are present in commercially available CLA supplements. The amount of CLA added to each diet was determined based on a pilot study that indicated 0.4% (w/w) *c*9, *t*11 and *t*10, *c*12 improved lipid metabolism (44) and on previous research that found a positive effect of CLA on calcium metabolism using 0.45% (w/w) *c*9, *t*11 and 0.47% (w/w) *t*10, *c*12 (13).

At the end of the 8-week study duration, rats were euthanized by CO_2 asphyxiation and cervical dislocation for trunk blood collection.

Dual-Energy X-Ray Absorptiometry and Femur Measurements. Analysis of bone mass was performed using dual-energy x-ray absorptiometry (DXA; small animal software; 4500A; Hologic, Inc., Bedford, MA), because DXA has been confirmed to be an appropriate method to evaluate body composition in the adult rat (45). DXA also was used with a high degree of accuracy and precision in mice weighing less than 100 g (46), and the small animal software was shown to be valid for use with young rodents (47), such as the baseline rats weighing 130–170 g. Scans were performed on frozen carcasses to measure whole-body bone area (BA), BMC, and BMD. Whole-body BMC was adjusted for the weight of the rat (g/kg) because of the large difference in body mass between the two genotypes. Regional scans were performed using high-resolution software to determine BA, BMC, and BMD for vertebrae

Table 1. Lipid Composition of Diets (g/kg diet)

Diet[a]	Soybean oil	*c*9, *t*11 isomer	*t*10, *c*12 isomer	Other CLA isomers	Total CLA
Group 1					
*c*9, *t*11	80.85	4.00	0.03	0.12	4.15
Group 2					
*t*10, *c*12	80.71	0.19	4.00	0.10	4.29
Group 3					
*c*9, *t*11 and *t*10, *c*12	76.56	4.19	4.03	0.22	8.44
Group 4					
*c*9, *t*11 and *t*10, *c*12	76.23	3.94	3.99	0.84	8.77
Group 5					
*c*9, *t*11 and *t*10, *c*12	74.00	3.91	4.28	2.81	11.00
Control	85.00	0	0	0	0

[a] All ingredients were supplied by Harlan Teklad (Madison, Wisconsin) unless otherwise indicated. The diet composition for all treatment groups and control groups included: cornstarch (363 g/kg), maltodextrin (132 g/kg), sucrose (100 g/kg), egg white (212.5 g/kg), cellulose (50 g/kg), AIN-93G mineral mix (35 g/kg), AIN-93G vitamin mix (10 g/kg), choline (2.5 g/kg), biotin (10 g/kg of a 200 mg biotin/kg cornstarch mix), *tert*-butylhydroquinone (0.014 g/kg; Aldrich, Milwaukee, Wisconsin). The CLA isomer composition of all oils was confirmed by gas chromatography. The CLA isomers in the diets of Groups 1–3 were supplied by Natural ASA (Hovdebygda, Norway). CLA isomers in the 2-isomer mix diet (Group 4) supplied by Bioriginal Food and Science Corp. (Saskatoon, Saskatchewan); CLA isomers in the 4-isomer mix diet (Group 5) supplied by Nu-Chek Prep (Eysian, Minnesota). All were free fatty acid formulations.

1–4 of the lumbar spine and the right femur *in situ* (48). Following DXA scanning, the right femur was removed, cleaned of soft tissue, weighed, and measured in triplicate to the nearest 0.01 mm for femur length, neck, proximal femur epiphysis width, diaphysis width, and knee width (49, 50). DXA also was used to measure the BA, BMC, and BMD of excised femurs by placing the bone in a water bath aligned in an anteroposterior position with 3 cm of water covering the femur (51).

Bone Mineral and Biochemistry. Femur calcium and phosphorus concentrations were measured using inductively coupled plasma–optical-emission spectroscopy (Varian Liberty 200; Varian, Mississauga, ON, Canada) after digestion in nitric acid (52). Femurs were dried at 85°C for 72 hrs in glass test tubes. Concentrated, trace metal–grade nitric acid (1 ml) was added, and after complete digestion of the bone (72 hrs), deionized water was added to each tube to achieve a final volume of 20 ml (final concentration of 5% [v/v] nitric acid). No difference was observed for bone mass among the diet groups, thus serum osteocalcin was only measured in the control diet groups for each genotype using an ELISA (Rat-Mid Osteocalcin; Osteometer BioTech A/S, Herlev, Denmark).

Statistics. A factorial analysis of variance was used to determine differences among groups (genotype × diet; $P < 0.05$). Body weight and food intake were tested for covariance with other variables but were ruled out after no further information was gained from this more complex model. When appropriate, least squares means testing was used to determine differences ($P < 0.05$) among diet groups. All results are expressed as the mean ± SEM.

Results

Baseline Measurements. Six-week-old *fa/fa* rats had a higher body weight (169 ± 4 vs. 131 ± 3 g; $P < 0.0001$) and a shorter tail length (12.7 ± 0.2 vs. 14.0 ± 0.1 cm; $P < 0.0001$) compared with lean rats, but no differences in body length were found between genotypes (16.5 ± 0.2 vs. 16.9 ± 0.2 cm; $P = 0.09$). The *fa/fa* rats had a greater whole-body BA and BMC than lean rats, but BMD and regional scans of the lumbar spine (Table 2) did not differ significantly. Excised (Table 2) and *in situ* measurements of femur BA (0.701 ± 0.035 vs. 0.721 ± 0.037 cm^2), BMC (0.117 ± 0.009 vs. 0.129 ± 0.008 g), and BMD (0.166 ± 0.011 vs. 0.180 ± 0.010 g/cm^2) were not statistically different ($P > 0.05$) between *fa/fa* and lean rats. Femur dry weight did not differ between genotypes. Femur length, neck width, proximal femur epiphysis width, and knee width were lower in *fa/fa* rats than in lean rats, but diaphysis width as well as calcium and phosphorus concentrations were greater in the *fa/fa* rats.

Body Size, Tail Length, and Feed Intake. Fourteen-week-old *fa/fa* rats had a higher body weight than lean rats (547 ± 5 vs. 327 ± 2 g; $P < 0.0001$). Although it failed to reach statistical significance, a 4% difference in body weight was found among lean diet groups (328 ± 5 for control vs. 317 ± 6 to 336 ± 7 g for CLA diet groups). For *fa/fa* diet groups, a 2%–5% difference was found in body weight (560 ± 13 for control vs. 533 ± 14 to 567 ± 18 g for CLA diet groups). The *fa/fa* rats had shorter tail lengths (16.6 ± 0.1 vs. 18.4 ± 0.1 cm; $P < 0.0001$) than lean rats. No differences were found in tail length among diet groups or in body length between genotypes (21.9 ± 0.1 vs. 21.8 ± 0.1 cm; $P = 0.2$). The *fa/fa* rats had a greater feed intake over the 8-week study period than lean rats (1.52 ± 0.01 vs. 0.98 ± 0.01 kg; $P < 0.0001$). The *fa/fa* rats on the control diet and in Groups 1, 3, and 4 ate significantly more than those in Group 2 (Fig. 1). Within the lean treatment groups, Group 5 ate less than other diet groups.

Bone Mass. Whole-body BA and BMC were greater

Table 2. Whole Body, Femur, and Spine Lumbar Vertebrae Measures for Lean and *fa/fa* Zucker Rats at 6 or 14 Weeks of Age[a]

Measurement	6 weeks (baseline)			14 weeks		
	Lean rats	*fa/fa* rats	*P*	Lean rats	*fa/fa* rats	*P*
Whole body						
BA (cm^2)	36.9 ± 0.5	42.3 ± 0.7	<0.0001	58.6 ± 0.4	79.5 ± 0.7	<0.0001
BMC (g)	3.82 ± 0.10	4.42 ± 0.06	<0.0001	9.02 ± 0.07	11.9 ± 0.1	<0.0001
BMD (g/cm^2)	0.103 ± 0.001	0.105 ± 0.001	NS	0.153 ± 0.001	0.150 ± 0.001	NS
Spine						
In situ lumbar spine BA (cm^2)	1.02 ± 0.02	0.960 ± 0.022	NS	1.79 ± 0.04	1.78 ± 0.02	NS
In situ lumbar spine BMC (g)	0.091 ± 0.007	0.090 ± 0.006	NS	0.430 ± 0.010	0.450 ± 0.010	NS
In situ lumbar spine BMD (g/cm^2)	0.090 ± 0.007	0.094 ± 0.006	NS	0.244 ± 0.003	0.253 ± 0.001	0.04
Femur						
Excised femur BA (cm^2)	0.604 ±.024	0.548 ± .027	NS	1.67 ± 0.84	1.56 ± 0.79	<0.0001
Excised femur BMC (g)	0.067 ± 0.002	0.062 ± 0.004	NS	0.339 ± 0.007	0.338 ± 0.009	NS
Excised femur BMD (g/cm^2)	0.110 ± 0.001	0.113 ± 0.002	NS	0.202 ± 0.004	0.217 ± 0.006	<0.0001
Dry weight (g)	61.1 ± 0.1	62.3 ± 0.1	NS	0.486 ± 0.006	0.472 ± 0.007	0.03
Length (mm)	20.8 ± 0.3	28.2 ± 0.2	0.0008	35.8 ± 0.1	33.1 ± 0.1	<0.0001
Neck width (mm)	1.57 ± 0.02	1.75 ± 0.05	0.02	1.84 ± 0.03	1.81 ± 0.02	0.04
Proximal femur epiphysis (mm)	2.31 ± 0.05	2.64 ± 0.04	0.003	3.51 ± 0.01	3.38 ± 0.01	<0.0001
Diaphysis width (mm)	2.79 ± 0.04	2.29 ± 0.03	0.05	2.28 ± 0.02	3.00 ± 0.02	0.009
Knee width (mm)	4.45 ± 0.09	4.90 ± 0.03	0.008	6.47 ± 0.02	6.36 ± 0.02	0.0002
Calcium (mmol/g dry wt)	1.18 ± 0.01	1.30 ± 0.01	<0.0001	6.21 ± 0.01	6.36 ± 0.01	NS
Phosphorus (mmol/g dry wt)	0.760 ± 0.001	0.791 ± 0.001	<0.0001	3.75 ± 0.02	3.88 ± 0.02	0.0005

[a] Values are presented as the mean ± SEM (at 6 weeks: lean, $n = 4$; *fa/fa*, $n = 5$; at 14 weeks: $n = 60$ per genotype). Differences between groups are identified using factorial analysis of variance. At 14 weeks, no significant main effects for diet or diet × genotype interaction were observed, thus, only genotype effects are reported. BA, bone area; BMC, bone mineral content; BMD, bone mineral density; NS, not significant.

in the *fa/fa* rats than in lean rats (Table 2). After correcting for body weight (adjusted BMC), the lean rats displayed greater whole-body BMC (Fig. 2). Despite no significant difference in body weight between diet groups, lean rats from Group 3 had a greater adjusted BMC than those in Groups 1 and 2 but did not significantly differ from those in Group 4, Group 5, and the control. The *fa/fa* rats in Groups 4 and 5 had a higher adjusted BMC than *fa/fa* rats in Group 3 but did not significantly differ from those in Group 1, Group 2, and the control. BMD did not differ between genotypes or diet groups, but a trend toward higher whole-body BMD was observed in the lean rats ($P = 0.06$; Table 2).

No effects of diet were observed for DXA measures of the lumbar spine or femur; thus, only genotype differences are reported. Regional scans of the lumbar spine indicated greater BMD in the *fa/fa* rats than in the lean rats, but no differences were observed between *fa/fa* and lean rats for *in*

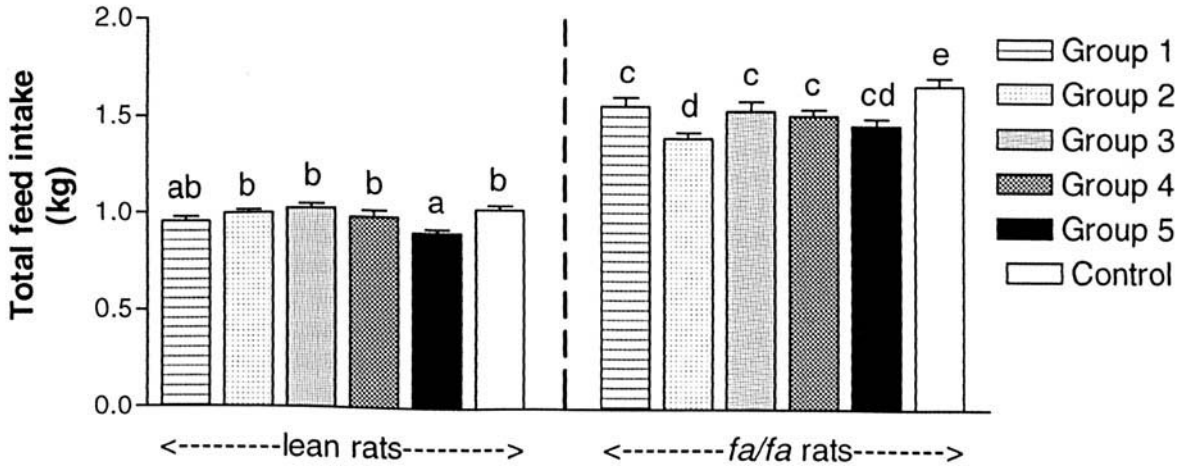

Figure 1. Total feed intake (kg) over the 8-week study duration. Values are presented as the mean ± SEM ($n = 10$ rats/group). Differences among dietary groups were identified using least squares means testing. Bars with a different letter indicates a significant difference between groups ($P < 0.05$). Diet groups are as follows: Group 1, 0.4% (w/w) *c*9, *t*11 CLA; Group 2, 0.4% (w/w) *t*10, *c*12 CLA; Group 3, 0.4% (w/w) *c*9, *t*11 CLA and 0.4% (w/w) *t*10, *c*12 CLA; Group 4, 0.4% (w/w) *c*9, *t*11 CLA, 0.4% (w/w) *t*10, *c*12 CLA, and traces of other isomers; and Group 5, 0.4% (w/w) *c*9, *t*11 CLA, 0.4% (w/w) *t*10, *c*12 CLA, and 0.3% (w/w) other CLA isomers.

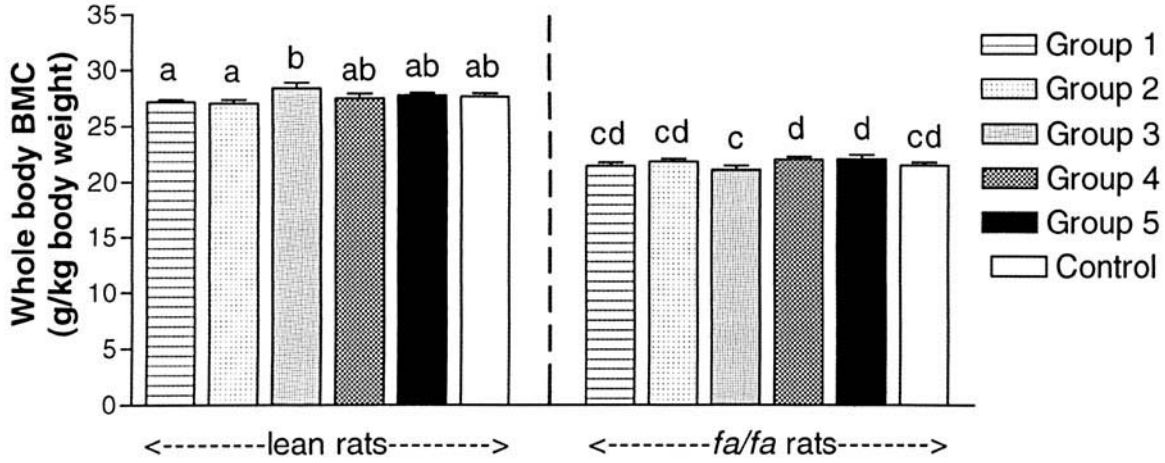

Figure 2. Adjusted whole-body BMC after 8 weeks of dietary treatment with *c*9, *t*11 or *t*10, *c*12 CLA isomers. Values are presented as the mean ± SEM ($n = 10$ rats/group). Differences among dietary groups were identified using least squares means testing. Bars with a different letter indicates a significant difference between groups ($P < 0.05$). Diet groups are as follows: Group 1, 0.4% (w/w) *c*9, *t*11 CLA; Group 2, 0.4% (w/w) *t*10, *c*12 CLA; Group 3, 0.4% (w/w) *c*9, *t*11 CLA and 0.4% (w/w) *t*10, *c*12 CLA; Group 4, 0.4% (w/w) *c*9, *t*11 CLA, 0.4% (w/w) *t*10, *c*12 CLA, and traces of other isomers; Group 5, 0.4% (w/w) *c*9, *t*11 CLA, 0.4% (w/w) *t*10, *c*12 CLA, and 0.3% (w/w) other CLA isomers and control diet.

situ DXA scans of the right femur (BA: 1.12 ± 0.05 vs. 1.00 ± 0.04 cm^2; BMC: 0.378 ± 0.180 vs. 0.391 ± 0.150 g; BMD: 0.378 ± 0.010 vs. 0.350 ± 0.010 g/cm^2) and lumbar spine BA and BMC (Table 2). Excised femurs from *fa/fa* rats exhibited a lower BA and greater BMD, but not BMC, than lean rats (Table 2).

Morphometric Measurements, Mineral Analysis, and Biochemistry. No significant effects of diet were observed for morphometric measurements, mineral analysis, or biochemistry; thus, only genotype effects are reported. The *fa/fa* rat femurs had a lower dry weight than those from lean rats and shorter femurs with smaller measurements for the femoral neck, proximal femur epiphysis, and knee width (Table 2). Diaphysis width was greater in the *fa/fa* rats than in the lean rats.

No differences were observed between genotypes in femoral calcium concentration. Femoral phosphorus concentration was greater in *fa/fa* rats than in lean rats (Table 2). Serum osteocalcin concentration did not differ significantly between genotypes (66.7 ± 8.3 vs. 60.4 ± 6.8 n*M*; $P = 0.50$), indicating no detectable difference in osteoblast activity or bone modeling.

Discussion

The rising rate of obesity and associated metabolic abnormalities among the adolescent population has severe negative health consequences. CLA is suggested to reduce fat mass and to improve insulin sensitivity in overweight, young individuals (4), whereas the effects of CLA on bone mass in young humans is presently unknown. Childhood, adolescence, and young adulthood, however, are periods of life in which optimal bone mineralization is critical for lifelong bone health (53). Therefore, an investigation was warranted to determine the effect of CLA on bone health in a young, obese state. The present study demonstrates that CLA has no positive or adverse effects on bone mass in a growing model of obesity and insulin resistance. This finding is an important contribution to CLA research, because it indicates that therapeutic CLA treatment does not adversely affect bone mineral accretion during an adolescent stage of growth in a male rodent model.

At 14 weeks of age, the *fa/fa* rats in the present study displayed genotypic manifestations, including obesity and hyperinsulinemia, without hyperglycemia (data not shown). Despite greater body weight, the *fa/fa* rats demonstrated lower adjusted whole-body BMC and reduced femoral bone growth (indicated by morphometry) in comparison to lean rats, a finding that is in agreement with previous reports of reduced long bone growth and bone mass in *fa/fa* Zucker rats (37, 39, 50). Similar to leptin-resistant *fa/fa* rats with elevated fat mass and reduced whole-body BMC, however, fat mass is negatively associated with whole-body bone mass in children (28) and adolescent females (54). In addition, bones of obese or overweight children have been reported to fracture more often than those of children of normal weight, which suggests disproportionate bone mineralization, similar to the observations in the *fa/fa* rat (29, 30).

At 6 weeks of age, whole-body BA, BMC, and femur size were greater in the *fa/fa* genotype, which also had a 29% higher body weight. By 14 weeks of age, the difference in body weight between genotypes had increased to 67%. This was accompanied a 7% and 4% higher BMD in the femur and lumbar spine, respectively, in the *fa/fa* rats. This suggests that gains in fat mass may be positively associated with BMD in the femur and spine, which has been reported in overweight children (55). This is a novel finding, because femoral BMD in *fa/fa* rats has been reported previously to be lower or the same as that of lean rats (39, 50, 56, 57). The differences among studies may be explained by the older age (39, 56, 57) and female sex (39, 56) of rats. The study by Mollard *et al.* (50) had less statistical power and used a

diet with 10% lipid, explaining in part why the conclusions differ from those of the present study despite a similar trend in femoral BMD.

Bone size was reduced and regional bone mass elevated in the *fa/fa* rats in comparison to lean rats, but serum osteocalcin did not differ between genotypes, indicating that the *fa/fa* and lean rats had similar rates of bone formation and modeling. Therefore, the reduced femur size but elevated bone mass may result from differences in the bone structure and formation of cortical and trabecular bone. In addition, the higher femoral BMD was accompanied by a higher concentration of phosphorus, not calcium, in the femur, suggesting differences in the bone matrix structure between genotypes. Areas that are richer in trabecular bone in the femur were reduced in size in the *fa/fa* rats (proximal epiphysis and femur neck), which has been observed previously (50). Lower femoral trabecular bone thickness and greater cortical bone width in *fa/fa* rats also has been reported (37).

It is not surprising that CLA did not modify bone mass in the *fa/fa* or lean rats. In young and ovariectomized rats, 0.25%–1.0% (w/w) CLA isomers did not increase femur or humerus length, BMC, and BMD (7, 58, 59). The present study provides further evidence that bone mass may not be altered by CLA treatment. Bone modeling, however, which was not assessed in the diet groups of the present study, may be improved with intakes of CLA. Reduced bone resorption and prostaglandin E_2 synthesis by bone, increased intestinal calcium absorption, and greater expression of insulin-like growth factor–binding proteins in bone were observed in healthy and ovariectomized rodents fed 1% (w/w) CLA isomers (7, 13, 58). Over time, all three mechanisms may serve to increase bone mass, but to our knowledge, this has not been investigated in a long-term animal study. In humans, a 2-year CLA supplementation trial conducted in males reported a positive change in bone mineral mass between 1 and 2 years of supplementation, but after 2 years, bone mass did not differ significantly from baseline measurements (19). Thus, it seems that sustained CLA supplementation has no negative effect on bone when fed in adulthood, but any long-term changes in bone mass when CLA is fed during early life are presently unknown. From the present study as well as others, however, it appears that during bone mineralization and consolidation, no negative effects are observed in bone, but this may be dependent on the amount of CLA that is fed and on the n-6 to n-3 PUFA ratio in the diet.

The effects of CLA on bone mass have been shown to be dependent on the amount of CLA provided in the diet. Feeding less than 0.5% (w/w) CLA has been shown to have no effect, whereas feeding 0.5%–1.0% (w/w) had a positive effect on bone in mice, rats, and pigs (6, 9, 58). Providing 0.4%–1.1% (w/w) CLA isomers resulted in no differences in bone mass in the present study, suggesting that the effects of CLA on bone are not dependent on the amount of CLA consumed by the male Zucker rat.

In the present study, soybean oil was used as the base lipid for all diets. Soybean oil has a n-6 to n-3 PUFA ratio that is moderately low (7:1) in comparison to those of other oils, such as corn (57:1), and to the ratio generally consumed in a Western diet, which is approximately 20:1 to 30:1 (60). Previous studies using a low ratio of n-6 to n-3 PUFA (~10:1 or less) have found minimal effects of CLA on bone mass (7, 12, 13). Therefore, the primary fat source in the present study may have limited the response in bone to CLA treatment. Also, the control diet group may have received a diet that marginally stimulated osteogenesis, because a low n-6 to n-3 PUFA ratio has been shown previously to be beneficial to bone mass (1). Thus, statistical differences between control and CLA diet groups may have been more difficult to elucidate.

In conclusion, at 6 and 14 weeks of age, *fa/fa* rats have reduced femoral growth, most notably in areas of trabecular bone. If reduced growth is accompanied by lower mineralization in these areas, fracture risk in the hip joint may be increased with age in this animal model. The *fa/fa* rat also appears to have disproportionate bone growth for body size, but greater body weight elevates BMD in the femur and spine. Supplementation with CLA did not benefit bone growth or whole-body mineralization in the *fa/fa* rats in comparison to lean rats. Further investigation is required to clarify the dietary conditions in which CLA may benefit bone mass.

We thank Danielle Defries, Natasha Ryz, Dielle Herchak, Rob Diakiw, Jennifer Zahradka, and the staff at the University of Manitoba Fort Garry Campus Animal Care Facility for assisting with animal feeding and care. We also thank the Manitoba Institute of Child Health for use of the DXA equipment and Shirley Fitzpatrick-Wong for assistance with the femur analysis. The kind gift of the CLA supplement from Bioriginal Food and Science Corporation (Saskatoon, Saskatchewan) is acknowledged.

1. Watkins BA, Li Y, Allen KG, Hoffmann WE, Seifert MF. Dietary ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats. J Nutr 130:2274–2284, 2000.
2. Yamasaki M, Ikeda A, Oji M, Tanaka Y, Hirao A, Kasai M, Iwata T, Tachibana H, Yamada K. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. Nutrition 19:30–35, 2003.
3. Baumgard LH, Corl BA, Dwyer DA, Saebo A, Bauman DE. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am J Physiol Regul Integr Comp Physiol 278:R179–R184, 2000.
4. Eyjolfson V, Spriet LL, Dyck DJ. Conjugated linoleic acid improves insulin sensitivity in young, sedentary humans. Med Sci Sports Exerc 36:814–820, 2004.
5. Song HJ, Grant I, Rotondo D, Mohede I, Sattar N, Heys SD, Wahle KW. Effect of CLA supplementation on immune function in young healthy volunteers. Eur J Clin Nutr 59:508–517, 2005.
6. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 34:235–241, 1999.
7. Li Y, Seifert MF, Ney DM, Grahn M, Grant AL, Allen KG, Watkins BA. Dietary conjugated linoleic acids alter serum IGF-I and IGF-

binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. J Bone Miner Res 14:1153–1162, 1999.
8. Park Y, Albright KJ, Storkson JM, Liu W, Cook ME, Pariza MW. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. Lipids 34:243–248, 1999.
9. Thiel-Cooper RL, Parrish FC Jr, Sparks JC, Wiegand BR, Ewan RC. Conjugated linoleic acid changes swine performance and carcass composition. J Anim Sci 79:1821–1828, 2001.
10. Watkins BA, Shen CL, McMurtry JP, Xu H, Bain SD, Allen KG, Seifert MF. Dietary lipids modulate bone prostaglandin E_2 production, insulin-like growth factor-I concentration and formation rate in chicks. J Nutr 127:1084–1091,1997.
11. Terpestra AH, Everts H, Kocsis S, Katan MB, Zock PL. The decrease in body fat in mice fed conjugated linoleic acid is due to the increases in energy expenditure and energy loss in the excreta. J Nutr 132:940–945, 2002.
12. Demaree SR, Gilbert CD, Mersmann HJ, Smith SB. Conjugated linoleic acid differentially modifies fatty acid composition in subcellular fractions of muscle and adipose tissue but not adiposity of post-weaning pigs. J Nutr 132:3272–3279, 2002.
13. Li Y, Watkins BA. 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. Lipids 33:417–425, 1998.
14. Swan JE, Parrish FC Jr, Wiegand BR, Larsen ST, Baas TJ, Berg EP. Total body electrical conductivity (TOBEC) measurement of compositional differences in hams, loins, and bellies from conjugated linoleic acid (CLA)-fed stress-genotype pigs. J Anim Sci 79:1475–1482, 2001.
15. Ostrowska E, Suster D, Muralitharan M, Cross RF, Leury BJ, Bauman DE, Dunshea FR. Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-energy x-ray absorptiometry. Br J Nutr 89: 219–229, 2003.
16. Brownbill RA, Petrosian M, Ilich JZ. Association between dietary conjugated linoleic acid and bone mineral density in postmenopausal women. J Am Coll Nutr 24:177–181, 2005.
17. Kreider RB, Ferreira MP, Greenwood M, Wilson M, Almada AL. Effects of conjugated linoleic acid supplementation during resistance training on body composition, bone density, strength, and selected hematological markers. J Strength Cond Res 16:325–334, 2002.
18. Doyle L, Jewell C, Mullen A, Nugent AP, Roche HM, Cashman KD. Effect of dietary supplementation with conjugated linoleic acid on markers of calcium and bone metabolism in healthy adult men. Eur J Clin Nutr 59:432–440, 2005.
19. Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H, Gudmundsen O. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. J Nutr 135:778–784, 2005.
20. Barker DJ. In utero programming of chronic disease. Clin Sci (Lond) 95:115–128, 1998.
21. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999–2000. JAMA 288:1728–1732, 2002.
22. Jolliffe D. Extent of overweight among US children and adolescents from 1971 to 2000. Int J Obes Relat Metab Disord 28:4–9, 2004.
23. Willms JD, Tremblay MS, Katzmarzyk PT. Geographic and demographic variation in the prevalence of overweight Canadian children. Obes Res 11:668–673, 2003.
24. Mazess RB, Barden HS. Bone density in premenopausal women: effects of age, dietary intake, physical activity, smoking, and birth-control pills. Am J Clin Nutr 53:132–142, 1991.
25. Nguyen TV, Center JR, Eisman JA. Osteoporosis in elderly men and women: effects of dietary calcium, physical activity, and body mass index. J Bone Miner Res 15:322–331, 2000.
26. De Laet C, Kanis JA, Oden A, Johanson H, Johnell O, Delmas P, Eisman JA, Kroger H, Fujiwara S, Garnero P, McCloskey E, Mellstrom D, Melton LJ, Meunier PJ, Pols HAP, Reeve J, Silman A, Tenenhouse A. Body mass index as a predictor of fracture risk: a meta-analysis. Osteoporos Int 16:1330–1338, 2005.
27. Goulding A, Taylor RW, Jones IE, McAuley KA, Manning PJ, Williams SM. Overweight and obese children have low bone mass and area for their weight. Int J Obes Relat Metab Disord 24:627–632, 2000.
28. Specker BL, Johannsen N, Binkley T, Finn K. Total body bone mineral content and tibial cortical bone measures in preschool children. J Bone Miner Res 16:2298–2305, 2001.
29. Goulding A, Jones IE, Taylor RW, Williams SM, Manning PJ. Bone mineral density and body composition in boys with distal forearm fractures: a dual-energy x-ray absorptiometry study. J Pediatr 139:509–515, 2001.
30. Skaggs DL, Loro ML, Pitukcheewanont P, Tolo V, Gilsanz V. Increased body weight and decreased radial cross-sectional dimensions in girls with forearm fractures. J Bone Miner Res 16:1337–1342, 2001.
31. Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. Am J Clin Nutr 79:1164S–1168S, 2004.
32. Ball GML. Childhood obesity in Canada: a review of prevalence estimates and risk factors for cardiovascular disease and type 2 diabetes. Can J Appl Physiol 28:117–140, 2003.
33. Molnar D. The prevalence of the metabolic syndrome and type 2 diabetes mellitus in children and adolescents. Int J Obes Relat Metab Disord 28(Suppl 3):S70–S74, 2004.
34. Barrett-Connor E, Holbrook TL. Sex differences in osteoporosis in older adults with non–insulin-dependent diabetes mellitus. JAMA 268: 3333–3337, 1992.
35. Abrahamsen B, Rohold A, Henriksen JE, Beck-Nielsen H. Correlations between insulin sensitivity and bone mineral density in non-diabetic men. Diabet Med 17:124–129, 2000.
36. Zucker LM, Antoniades HN. Insulin and obesity in the Zucker genetically obese rat "fatty." Endocrinology 90:1320–1330, 1972.
37. Foldes J, Shih MS, Levy J. Bone structure and calcium metabolism in obese Zucker rats. Int J Obes Relat Metab Disord 16:95–102, 1992.
38. Cifuentes M, Morano AB, Chowdhury HA, Shapses SA. Energy restriction reduces fractional calcium absorption in mature obese and lean rats. J Nutr 132:2660–2666, 2002.
39. Mathey J, Horcajada-Molteni MN, Chanteranne B, Picherit C, Puel C, Lebecque P, Cubizoles C, Davicco MJ, Coxam V, Barlet JP. Bone mass in obese diabetic Zucker rats: influence of treadmill running. Calcif Tissue Int 70:305–311, 2002.
40. Wahle KW, Heys SD, Rotondo D. Conjugated linoleic acids: are they beneficial or detrimental to health? Prog Lipid Res 43:553–587, 2004.
41. Tricon S, Burdge GC, Williams CM, Calder PC, Yaqoob P. The effects of conjugated linoleic acid on human health-related outcomes. Proc Nutr Soc 64:171–182, 2005.
42. Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals (2nd ed.). Ottawa: Bradda Printing Services, 1993.
43. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127:838S–841S, 1997.
44. Noto A, Zahradka P, Yurkova N, Xie X, Nitschmann E, Ogborn M, Taylor C. Conjugated linoleic acid (CLA) reduces hepatic steatosis, improves liver function, and favorably modifies lipid metabolism in obese insulin resistant rats. Lipids 41(in press), 2006.
45. Bertin E, Ruiz JC, Mourot J, Peiniau P, Portha B. Evaluation of dual-energy x-ray absorptiometry for body-composition assessment in rats. J Nutr 128:1550–1554, 1998.
46. Nagy TR, Clair AL. Precision and accuracy of dual-energy x-ray absorptiometry for determining in vivo body composition of mice. Obes Res 8:392–398, 2000.
47. Engelbregt MJT, Tromp AM, van Lingen A, Lips P, Popp-Snijders C, Delemarre-van de Waal HA. Validation of whole-body DXA in young and adult rats. Horm Res 51:248, 1999.

48. Grier SJ, Turner AS, Alvis MR. The use of dual-energy x-ray absorptiometry in animals. Invest Radiol 31:50–62, 1996.
49. Reichling TD, German RZ. Bones, muscles, and visceral organs of protein-malnourished rats (*Rattus norvegicus*) grow more slowly but for longer durations to reach normal final size. J Nutr 130:2326–2332, 2000.
50. Mollard RC, Gillam ME, Wood TM, Taylor CG, Weiler HA. (n-3) fatty acids reduce the release of prostaglandin E_2 from bone but do not affect bone mass in obese (*fa/fa*) and lean Zucker rats. J Nutr 135:499–504, 2005.
51. Kastl S, Sommer T, Klein P, Hohenberger W, Engelke K. Accuracy and precision of bone mineral density and bone mineral content in excised rat humeri using fan beam dual-energy x-ray absorptiometry. Bone 30:243–246, 2002.
52. Clegg M KC, Lonnerdal B, Hurley L. Influence of ashing techniques on the analysis of trace elements in animal tissues. I. Wet ashing. Biol Trace Elem Res 3:107–115, 1981.
53. Brown JP, Josse RG. 2002 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada. CMAJ 167:S1–S34, 2002.
54. Weiler HA, Janzen L, Green K, Grabowski J, Seshia MM, Yuen KC. Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. Bone 27:203–207, 2000.
55. Leonard MB, Shults J, Wilson BA, Tershakovec AM, Zemel BS. Obesity during childhood and adolescence augments bone mass and bone dimensions. Am J Clin Nutr 80:514–523, 2004.
56. Tamasi JA, Arey BJ, Bertolini DR, Feyen JH. Characterization of bone structure in leptin receptor–deficient Zucker (fa/fa) rats. J Bone Miner Res 18:1605–1611, 2003.
57. Picherit C, Horcajada MN, Mathey J, Chanteranne B, Puel C, Lebecque P, Davicco MJ, Coxam V, Barlet JP. Isoflavone consumption does not increase the bone mass in osteopenic obese female Zucker rats. Ann Nutr Metab 47:70–77, 2003.
58. Kelly O, Cusack S, Jewell C, Cashman KD. The effect of polyunsaturated fatty acids, including conjugated linoleic acid, on calcium absorption and bone metabolism and composition in young growing rats. Br J Nutr 90:743–750, 2003.
59. Kelly O, Cashman KD. The effect of conjugated linoleic acid on calcium absorption and bone metabolism and composition in adult ovariectomized rats. Prostaglandins Leukot Essent Fatty Acids 71:295–301, 2004.
60. Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 70:560S–569S, 1999.