Dietary Lipids Modulate Bone Prostaglandin E_2 Production, Insulin-Like Growth Factor-I Concentration and Formation Rate in Chicks[1,2]

Bruce A. Watkins,[3] Chwan-L. Shen, John P. McMurtry,* Hui Xu, Steven D. Bain,† Kenneth G. D. Allen** and Mark F. Seifert‡

*Department of Food Science, Lipid Chemistry and Metabolism Laboratory, Purdue University, West Lafayette, IN 47907-1160; *USDA, Growth Biology Laboratory, Livestock and Poultry Institute, Beltsville, MD 20705; †ZymoGenetics, Inc., Seattle, WA 98105; **Department of Food Science and Human Nutrition, Colorado State University, Ft. Collins, CO 80523; and ‡Department of Anatomy, Indiana University Medical Center, Indianapolis, IN 46202*

ABSTRACT This study examined the effects of dietary fat on the fatty acid composition of liver and bone, and on the concentration of insulin-like growth factor-I (IGF-I) in liver and bone, as well as the relationship of these factors to bone metabolism. Day-old male broiler chicks were given a semipurified diet containing one of four lipid sources: soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), or menhaden oil + corn oil (MEC) at 70 g/kg of the diet. At 21 and 42 d of age, chicks fed MEC had the highest concentration of (n-3) fatty acids [20:5(n-3), 22:5(n-3) and 22:6(n-3)] in polar and neutral lipids of cortical bone but the lowest amount of 20:4(n-6) in polar lipids. Diets containing *t*-18:1 fatty acids (MAC and BC) resulted in *t*18:1 accumulation in bone and liver. Bone IGF-I concentration increased from 21 to 42 d in chicks given the SBO and BC diets. Tibial periosteal bone formation rate (BFR) was higher in chicks given BC compared with those consuming SBO and MEC at 21 d. The higher BFR and concentrations of hexosamine in serum and IGF-I in cartilage, but lower 20:4(n-6) content in bone polar lipids in chicks given BC compared with those given SBO suggest that BC optimized bone formation by altering the production of bone growth factors. A second study confirmed that dietary butter fat lowered ex vivo prostaglandin E_2 production and increased trabecular BFR in chick tibia. These studies showed that dietary fat altered BFR perhaps by controlling the production of local regulatory factors in bone. J. Nutr. 127: 1084–1091, 1997

KEY WORDS: • *lipids* • *bone* • *prostaglandin E_2* • *insulin-like growth factor-I* • *chicks*

The importance of prostaglandin E_2 (PGE_2)[4] in bone biology was realized with the discovery that this derivative of (n-6) polyunsaturated fatty acids (PUFA) caused resorption of bone mineral and the release of calcium into bone organ culture (Klein and Raisz 1970). Since then, considerable clinical and experimental evidence has revealed that PG are potent stimulators of bone formation (Marks and Miller 1993, Norrdin et al. 1990, Raisz 1993). The PG are believed to mediate part of the anabolic effects of biomechanical forces (Chow and Chambers 1994), parathyroid hormone (Yang et al. 1987), cytokines (Raisz 1993), and insulin-like growth factors (IGF) (Baylink et al. 1993, McCarthy and Centrella 1993) in bone. Even though PGE_2 was reported to increase metaphyseal and cortical bone mass of growing animals (Marks and Miller 1993), the response may be concentration dependent (Raisz and Fall 1990). On the other hand, excess inflammatory production of PGE might contribute to bone pathology, as in osteomyelitis and avian osteopetrosis (Norrdin et al. 1990).

Bone tissue and cells produce IGF (Isgaard 1992, McCarthy and Centrella 1993), and appreciable amounts of IGF-I and IGF-II are stored in skeletal tissue of vertebrates, including chickens and humans (Bautista et al. 1990). Although the concentration of IGF-I in bone is lower than that of IGF-II, IGF-I appears to be under greater regulatory control (Canalis et al. 1991, McCarthy et al. 1991).

Prostaglandin E_2 was reported to increase IGF-I transcript and polypeptide levels in rat calvaria cells (McCarthy et al. 1991, Schmid et al. 1992) and stimulate the expression of mRNA for IGF binding protein-3 (BP-3) to enhance the IGFBP-3 binding affinity to rat calvaria (Schmid et al. 1992). Thus, some of the effects exerted by PGE_2 on bone formation/resorption may be mediated locally by inducing the biosynthesis of IGF-I. Recent studies on bone modeling in chicks demonstrated that diets enriched with saturated fat or vitamin E

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[3] To whom correspondence should be addressed.

[4] Abbreviations used: ABO, anhydrous butter oil; BC, butter + corn oil; BFR, bone formation rate; BP, binding protein; FAME, fatty acid methyl esters; GLC, gas-liquid chromatography; IGF-I, insulin-like growth factor-I; IGFBP-3, insulin-like growth factor binding protein-3; LTB, leukotriene B; MAC, margarine + corn oil; MAR, mineral apposition rate; MEC, menhaden oil + corn oil; MONO, monounsaturated fatty acids; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; PUFA, polyunsaturated fatty acids; SAT, saturated fatty acids; SBO, soybean oil; STD, standardized differences.

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stimulated bone formation (Xu et al. 1995). In addition, diets enriched with (n-6) PUFA elevated ex vivo bone PGE_2 production and lowered the rate of trabecular bone formation (Watkins et al. 1996).

At this time it is unclear how dietary PUFA can influence endogenous PGE_2 production and bone formation. Therefore, the present study was designed to evaluate the effects of dietary lipids, varying in amounts of (n-3) and (n-6) PUFA, saturated and trans-18:1 fatty acids, on the fatty acid composition of chick bone tissue and to ascertain if the concentration of 20:4(n-6), the precursor of PGE_2, is modulated in bone by dietary lipids. Histomorphometry was performed to quantify static and dynamic events of bone modeling, and IGF-I was measured to determine the effects of the treatments on the concentration of this growth factor produced in bone.

MATERIALS AND METHODS

Animals and diets. Hubbard cockerel broiler chicks (160 d-old, average body weight, 41 g) were purchased from a local hatchery (Fairview Farm, Remington, IN). Animal care was in compliance with applicable guidelines for the policy on animal care and use at Purdue University. The chicks were individually wing-banded, weighed and randomly placed in temperature-controlled battery brooders on continuous light. Each treatment group contained four replicate pens of 10 chicks. The chicks were fed a basal semipurified diet (**Table 1**) containing one of the following dietary lipid treatments (70 g/kg diet): soybean oil (SBO); butter (52 g) + corn oil (18 g) (BC); margarine (55 g) + corn oil (15 g) (MAC); or menhaden oil (40 g) + corn oil (30 g) (MEC). The dietary ingredients were purchased (Dyets, Bethlehem, PA) or donated (ME Zapata Haynie, Reedville, VA). The addition of corn oil was made to the butter, margarine and menhaden oil treatments to supplement the essential fatty acid [18:2(n-6)] content of those diets. The semi-purified diet (Table 1) was formulated to meet or exceed the nutrient requirements of the growing chick (NRC 1994). Chicks were given free access to food and water throughout the 56-d feeding period. The diet containing MEC was kept at 4°C until fed, and fresh diet was provided every 24 h. Body weights and feed consumptions were recorded weekly to determine weight gains and feed conversions (total g gain/total g feed consumed). In a second study, day-old Hubbard cockerel broiler chicks (16 per treatment group) were fed for 16 d the basal diet containing SBO or anhydrous butter oil (ABO, Level Valley Dairy, West Bend, WI) at 100 g/kg. The amounts of cornstarch and dextrose were reduced by 15 g/kg to accommodate the extra fat. The higher level of dietary fat was used to better represent the amount of fat energy contained in a human diet, and ABO was selected as the saturated fat source because it is the fat found in milk and, unlike butter, it is free of food additives.

Sample collections. Two chicks per pen (eight per treatment) were used for bone histomorphometric measurements and tissue analyses at 21 and 42 d of age. At 5 and 3 d before killing, chicks were injected intraperitoneally with calcein green (15 mg/kg body weight) to label mineralizing bone surfaces. On d 21 and 42, chicks were killed by exsanguination. Tibia bones were excised, freed of surrounding soft tissue and their lengths measured with a vernier caliper. A 3 to 5-cm thick section of cortical bone was cut from the mid-diaphysis of the right tibia with a low speed diamond wheel saw (Model 11-1180, Buehler, Evanston, IL), fixed in 70% ethyl alcohol, and processed undecalcified for bone histomorphometry. In the second study, tissue samples were collected from chicks at 16 d. Right tibia bones were processed undecalcified for histomorphometry (Watkins et al. 1996), and livers and left tibia were collected and samples either kept on ice and frozen at −20°C for lipid and fatty acid analyses or immersed in liquid nitrogen and stored at −80°C for IGF-I analysis.

Blood was collected by cardiac puncture at 14, 28, 42 and 56 d of age. Blood containing EDTA (2 g EDTA/L blood) was centrifuged at 1000 × *g* for 20 min to obtain plasma and stored at −80°C for later IGF-I analysis. Hematocrits were determined on blood collected from the wing vein of chicks.

Analytical procedures. Histomorphometric analyses were performed on cross sections of tibia cortical bone (Watkins et al. 1989) or on frontal sections of proximal tibia bone in the second study (Watkins et al. 1996). Lipids in liver and tibia cortical bone were extracted with chloroform/methanol (2:1, v/v). 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 prior to the extraction of lipids. Polar (phospholipids) and neutral (triacylglycerols) lipids in cortical bone and liver were isolated by solid-phase extraction (Hamilton and Comai 1988). Fatty acid methyl esters (FAME) from lipid fractions were prepared by transesterification using 14% BF_3 in methanol. The FAME were extracted in isooctane for gas-liquid chromatographic (GLC) analysis by an HP 5890A gas chromatograph equipped with a flame ionization detector, autosampler, and workstation (Hewlett-Packard, Avondale, PA). A DB 23 (50% cyanopropyl-50% methyl) fused silica capillary column (30 m × 0.25 mm i.d., J & W Scientific, Rancho Cordova, CA) was used with helium as the carrier gas. The initial oven temperature of 175°C was held for 10 min and increased at a rate of 1°C/min until the final temperature of 210°C was reached

TABLE 1

Fatty acid composition and ingredient composition of the chick basal diet[1,2,3]

	Dietary lipid treatment[4]			
Fatty acid	SBO	BC	MAC	MEC
	mol/100 mol			
14:0	0.08	7.29	0.09	3.74
16:0	9.72	24.50	9.78	13.20
16:1(n-7)	0.09	0.34	0.08	5.56
18:0	4.34	11.51	8.50	2.68
*t*18:1	ND[5]	2.19	14.85	ND
18:1	24.92	26.57	24.22	17.08
18:2(n-6)	50.00	18.38	34.17	26.66
18:3(n-3)	6.55	0.90	3.03	1.05
20:0	0.37	0.29	0.41	ND
20:1(n-9)	0.24	0.11	0.18	0.72
20:5(n-3)	ND	ND	ND	9.36
22:5(n-3)	ND	ND	ND	1.99
22:6(n-3)	ND	ND	ND	5.83
SAT[6]	15.06	45.03	19.27	21.06
MONO[7]	25.27	31.29	39.37	25.19
PUFA[8]	56.55	19.99	38.03	47.58
(n-6)PUFA	50.00	19.09	35.00	27.61
(n-3)PUFA	6.55	0.90	3.03	19.97
[n-3]:[n-6]	0.13	0.05	0.09	0.72

[1] The semipurified basal diet contained the following (g/kg): isolated soybean protein, 250; cornstarch, 283.75; dextrose, 283.75; lipid, 70; cellulose, 30; $CaHPO_4 \cdot 2H_2O$, 25; $CaCO_3$, 15; DL-methionine, 7.5; mineral premix, 30; vitamin premix, 5. The diet was formulated to contain 22.5% crude protein and 14.88 MJ/kg metabolizable energy. The analyzed crude protein was 22.5–23.8% and lipid content ranged from 6.5 to 7.8% for the four dietary treatments.

[2] Mineral premix provided (mg/kg diet): ZnO, 90; $MgSO_4 \cdot 7H_2O$, 6000; $MnSO_4 \cdot H_2O$, 300; $CuSO_4 \cdot 5H_2O$, 60; $FeSO_4 \cdot 7H_2O$, 500; KIO_3, 6; $Na_2MoO_4 \cdot 2H_2O$, 10; $CoCl_2 \cdot 6H_2O$, 5; Na_2O_3Se, 0.43; KCl, 1500; K_2HPO_4, 6000; NaCl, 6000.

[3] Vitamin premix provided per kg of diet: retinyl palmitate, 2.47 mg; cholecalciferol, 112 μg; menadione sodium bisulfite, 1.5 mg; DL-α-tocopheryl acetate, 50 mg; thiamine hydrochloride, 15 mg; riboflavin, 15 mg; sodium pantothenate, 50 mg; niacin, 50 mg; pyridoxine hydrochloride, 6 mg; folic acid, 6 mg; cyanocobalamin, 0.02 mg; D-biotin, 0.3 mg; choline chloride, 600 mg; BHT, 200 mg.

[4] Dietary lipid treatments included soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), and menhaden oil + corn oil (MEC) at 70 g/kg of the diet.

[5] ND, not detected.

[6] SAT, total saturated fatty acids.

[7] MONO, total monounsaturated fatty acids.

[8] PUFA, total polyunsaturated fatty acids.

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and held for 5 min. The total time of gas chromatographic analysis was 50 min. An external standard mixture prepared from known amounts of triacylglycerols and methylated fatty acids (Nu Check-Prep, Elysian, MN) was used to obtain retention times and to develop the calibration table. Retention times for 18:4(n-3), 20:5(n-3) and 22:5(n-3) were obtained from the analysis of menhaden oil.

Total *cis* and *trans* isomers of 18:1 were determined by combined GLC and argentation-TLC as previously described (Watkins et al. 1991). The isomers of 18:1 eluted from 8.3 to 8.9 min. The reported values for 18:1 fatty acids in the diets and tissues included all positional and geometric isomers.

Fatty acid composition of the diets and tissue samples was expressed as mol/100 mol of FAME in the lipids. The amounts of total saturated fatty acids (SAT), monounsaturated fatty acids (MONO), PUFA, total (n-6) and (n-3) PUFA, and the (n-3):(n-6) ratios were calculated from the GLC analyses.

Plasma IGF-I levels were determined as previously described (Ballard et al. 1990). ^{125}I-labeled-IGF-I tracer was obtained from Amersham (Arlington, IL), and the primary antibody (rabbit anti-bovine IGF-I) was kindly provided by G. Francis (CSIRO, Adelaide, Australia). Human sequence IGF-I (GROPEP, Adelaide, Australia) was employed as the standard. Tissue IGF-I concentrations were determined following the method of McMurtry et al. (1994).

Plasma, liver and muscle hemoglobin concentrations were measured (Morrison 1965), and hemoglobin values were used to correct for the analyzed concentrations of IGF-I in those tissues. Liver and muscle protein concentrations were determined with a bicinchoninic acid protein assay kit purchased from Pierce (Rockford, IL) using bovine serum albumin as the standard. The values for IGF-I were expressed as nanograms or micrograms of IGF-I per milligram protein, except for bone.

Other analytical procedures included the measurement of hexosamine concentrations in bone and serum (Boas 1953), employing glycocyamine hydrochloride as the standard. Plasma vitamin E was extracted with ethanol (20 mg BHT/L) as described by MacCrehan (1990) and tocopherols quantified (Pascoe et al. 1987) by HPLC using a reversed-phase C18 column and electrochemical detector. Separation was achieved by isocratically eluting with 96% methanol and 4% 50 mmol/L $NaClO_4$ at a flow rate of 1 mL/min and comparing peaks with known tocopherol standards (Sigma Chemical, St Louis, MO). The activity of phospholipase A_2 (PLA_2) in serum and liver cytosol preparations from chicks at 42 d was measured by RIA (Glaser and Jacobs 1986) using *dl*-α-phosphatidyl-cholinedipalmitoyl as a substrate in the presence of 5 mmol/L $CaCl_2$. For bone ash and calcium concentration, samples of tibia bone were dried, weighed, dry-ashed at 600°C for 48 h, and ash content calculated by weight loss on a dry basis (Watkins et al. 1996). The dried bone was digested with 15.9 mol/L HNO_3 and calcium concentration (mmol/g) measured by atomic absorption spectroscopy (Model 2380 Perkin-Elmer, Norwalk, CT). Ex vivo PGE_2 production was quantified in tibia bone organ culture from 16-d-old chicks fed SBO or ABO (Watkins et al. 1996).

Data analyses. Data were statistically analyzed by a one-way or two-way ANOVA, and significant differences between treatment means were tested using Student-Newman-Keuls test at the 5% probability level (Neter and Wasserman 1974). Variation within treatments was expressed as the SEM, pooled SD for unequal sample size or pooled SEM for equal sample size.

RESULTS

The fatty acid composition of the four diets presented in Table 1 indicated that the SBO diet provided the highest amounts of 18:2(n-6) and 18:3(n-3), and the MEC diet contained the greatest levels of 16:1(n-7), 20:5(n-3), 22:5(n-3) and 22:6(n-3). The BC diet was highest in 14:0, 16:0, and saturates, and the MAC diet contained the largest amount of *trans*-18:1. Total PUFA and (n-6) fatty acid levels were greatest in the SBO diet, but the total amount of (n-3) fatty acids and the ratio of (n-3):(n-6) PUFA were highest in the MEC diet. Based on the GLC analysis, the semipurified diets provided an adequate amount of 18:2(n-6) (12–35 g/kg of diet) for the growing chick, an amount well above the required 10 g/kg of diet (NRC 1994).

There was no significant difference ($P > 0.05$) in feed conversions for chicks at 42 d across the dietary treatments (mean values ranged from 0.69 to 0.72). The growth rates of chicks did not differ among diet groups and the mean body weights at 42 d (mean values ranged from 1280 to 1450 g) were not significantly different ($P > 0.05$).

The fatty acid composition of polar and neutral lipids in tibial cortical bone of chicks at 21 d presented in **Table 2** showed significant differences between dietary treatments. Chicks fed the SBO diet had higher concentrations of 18:2(n-6), 20:4(n-6), 22:4(n-6) and total (n-6) PUFA in bone polar lipids compared with those fed the MEC diet. Chicks fed the MEC diet had the lowest concentration of 20:4(n-6) in bone polar lipids but the highest concentrations of 20:5(n-3), 22:5(n-3), 22:6(n-3), total (n-3) PUFA and ratio of (n-3):(n-6) PUFA in bone polar and neutral lipids. The concentration of 20:4(n-6) in bone polar lipids was lower in the BC group compared with the SBO group. The concentration of total MONO was highest in bone polar lipids, and the concentrations of 18:1 and total MONO were greatest in neutral lipids of chicks fed the BC and MAC diets. The neutral lipids in bone of chicks fed the BC diet had the highest amounts of 14:0, 15:0, 16:0, and total SAT but the lowest amounts of 18:2(n-6) and total (n-6) PUFA.

The fatty acid composition of polar and neutral lipids in cortical bone of chicks at 42 d (data not shown) were consistent with the results from 21 d. Those given the MEC diet maintained the lowest concentration of 20:4(n-6) in polar lipids but the highest amounts of (n-3) PUFA [20:5(n-3), 22:5(n-3), and 22:6(n-3)] in bone polar and neutral lipids at 42 d. The concentrations of 20:(4n-6) and total (n-6) PUFA decreased in bone polar lipids for chicks given the SBO diet from 21 to 42 d. Consistent with the data from chicks at 21 d was the trend for a higher concentration of 18:1 and MONO in bone lipids of those given the BC and MAC diets. The amount of *trans*-18:1 in bone was higher in chicks given the MAC diet (6.7% in polar and 11.2% in neutral lipids) than in those given the BC diet (1.5% in polar and a trace amount in neutral lipids).

The proportion of SAT/MONO/PUFA in bone neutral lipids of chicks fed the BC treatment was 2.86:3.1:1.0 and 2.61:2.43:1.0 at 21 and 42 d, respectively. In contrast, these proportions were 1.2:1.3:1.0 at 21 d and 0.97:1.06:1.0 at 42 d in bone neutral lipids of chicks in the SBO group. The proportion of SAT/MONO/PUFA in the polar lipid fraction was 0.96:0.48:1.0 and 0.65:0.21:1.0 for the BC and SBO groups at 21 d, respectively. However, the ratios were similar for the two treatment groups at 42 d. These ratios reflect a greater proportion of (n-6) PUFA to SAT and MONO in bone lipids of chicks given SBO.

Consumption of the MEC diet also elevated the (n-3) PUFA in polar and neutral lipids in liver of chicks as illustrated in **Figure 1** at 21 and 42 d (data not shown). The values for fatty acids are presented as standardized differences (STD) based on statistical analysis (number of standard error of the mean units). The STD values for (n-6) and (n-3) PUFA of chicks given the MEC diet show that 20:4(n-6) and total (n-6) PUFA were decreased in polar lipids, but 20:5(n-3), 22:6(n-3) and total (n-3) PUFA were increased in neutral and polar lipids compared with chicks given the other treatments. The liver polar lipids of chicks given the BC diet had the highest amount of 18:1 at 21 d, but those fed BC and MEC had the lowest amount of 18:2(n-6) at 21 and 42 d. The amount of *trans*-18:1 was highest in liver of chicks fed the MAC diet

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TABLE 2

Fatty acid composition of polar lipids and neutral lipids isolated from proximal tibiotarsal cortical bone of 21-d-old chicks fed different lipids[1]

	Polar lipids					Neutral lipids				
	Dietary lipid treatment[2]				Pooled	Dietary lipid treatment				Pooled
Fatty acid	SBO	BC	MAC	MEC	SEM	SBO	BC	MAC	MEC	SEM
	mol/100 mol					*mol/100 mol*				
14:0	*0.23*^{b}	*0.42*^{ab}	*0.31*^{b}	*0.56*^{a}	*0.06*	*0.43*^{c}	*2.62*^{a}	*0.44*^{c}	*2.01*^{b}	*0.06*
15:0	*ND*[3]	*ND*	*0.10*	*ND*	*0.05*	*0.04*^{c}	*0.30*^{a}	*0.04*^{c}	*0.13*^{b}	*0.02*
16:0	*17.57*^{b}	*22.10*^{a}	*22.98*^{a}	*25.15*^{a}	*1.30*	*20.76*^{c}	*26.64*^{a}	*21.00*^{c}	*23.60*^{b}	*0.72*
*t*16:1	0.06^{b}	0.33^{a}	0.21^{ab}	0.35^{a}	0.05	0.41	0.57	0.37	0.48	0.05
16:1	0.55^{b}	0.56^{b}	0.64^{b}	1.03^{a}	0.11	2.60^{b}	4.55^{a}	3.20^{b}	5.26^{a}	0.23
17:0	ND	0.04	0.14	ND	0.07	0.20^{c}	0.33^{b}	0.15^{d}	0.56^{a}	0.02
18:0	14.82	14.89	14.83	12.98	0.68	9.24	10.28	9.11	8.58	0.51
18:1	11.98	13.02	13.86	12.01	0.69	32.56^{b}	38.18^{a}	39.59^{a}	26.72^{c}	0.54
18:2(n-6)	14.38^{a}	10.60^{b}	14.05^{a}	10.43^{b}	0.60	24.87^{a}	11.27^{d}	19.84^{b}	16.79^{c}	0.81
18:3(n-3)	0.09	ND	ND	ND	0.05	0.54^{ab}	0.47^{ab}	1.38^{a}	0.16^{b}	0.28
18:4	ND	ND	0.29	ND	0.04	0.12^{ab}	0.32^{a}	0.28^{a}	ND	0.07
20:0	ND	ND	0.11	ND	0.06	ND	0.05^{b}	0.03^{b}	0.71^{a}	0.04
20:1(n-9)	ND	ND	0.08	ND	0.04	0.22	0.34	0.30	0.23	0.08
20:2(n-6)	0.88	0.93	0.73	ND	0.20	0.42	0.29	0.21	ND	0.12
20:3(n-6)	1.44	1.35	1.68	1.21	0.24	0.30	0.36	0.36	0.20	0.08
20:4(n-6)	23.68^{a}	18.14^{b}	16.12^{b}	7.61^{c}	1.60	0.72	1.03	0.92	0.87	0.16
20:5(n-3)	0.75^{b}	0.33^{b}	0.41^{b}	10.44^{a}	0.25	0.02^{b}	ND	ND	4.23^{a}	0.21
22:1	0.81^{b}	4.80^{a}	4.49^{a}	ND	0.92	ND	ND	ND	ND	—
22:4(n-6)	4.91^{a}	4.58^{a}	4.02^{a}	1.42^{b}	1.20	0.22	0.33	0.31	ND	0.04
22:5(n-3)	2.31^{b}	1.33^{b}	1.50^{b}	5.08^{a}	0.31	0.10^{b}	ND	0.05^{b}	2.46^{a}	0.10
22:6(n-3)	2.48^{b}	2.11^{b}	1.97^{b}	8.66^{a}	0.27	0.09^{b}	ND	0.04^{b}	3.19^{a}	0.16
SAT[4]	32.62^{b}	37.45^{a}	38.47^{a}	38.69^{a}	1.30	30.67^{c}	40.22^{a}	30.77^{c}	35.59^{b}	1.18
MONO[5]	13.40^{b}	18.71^{a}	19.28^{a}	13.39^{b}	1.21	35.79^{b}	43.64^{a}	43.46^{a}	32.69^{c}	0.60
PUFA[6]	50.92^{a}	39.37^{b}	40.77^{b}	44.85^{b}	2.29	27.40^{a}	14.07^{b}	23.39^{a}	27.90^{a}	1.31
(n-6)PUFA	45.29^{a}	35.60^{a}	36.60^{a}	20.67^{b}	2.07	26.53^{a}	13.28^{d}	21.64^{b}	17.86^{c}	0.89
(n-3)PUFA	5.63^{b}	3.77^{b}	3.88^{b}	24.18^{a}	0.57	0.75^{b}	0.47^{b}	1.47^{b}	10.04^{a}	0.60
[n-3]:[n-6]	0.12^{b}	0.11^{b}	0.11^{b}	1.17^{a}	0.03	0.03^{b}	0.03^{b}	0.07^{b}	0.57^{a}	0.02

[1] Mean values ($n = 4$) within rows for each variable having different superscripts are significantly different ($P < 0.05$). The 18:1 values include all *cis* and *trans* isomers.

[2] Dietary lipid treatments included soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), and menhaden oil + corn oil (MEC) at 70 g/kg of the diet.

[3] ND, not detected.

[4] SAT, saturated fatty acids.

[5] MONO, monounsaturated fatty acids.

[6] PUFA, polyunsaturated fatty acids.

(5.8% in polar and neutral lipids), and a trace amount was present in liver of those consuming the BC diet (0.8% in polar and neutral lipids).

Histomorphometric data on cortical bone modeling in the tibia revealed that the BC diet increased the bone formation rate (BFR) in chicks at 21 d (**Table 3**). Higher values for periosteal BFR (mm^2/d), total new BFR (mm^2/d) and intracortical porosity (mm^2) were observed in chicks fed the BC diet compared with values in chicks given the SBO and MEC diets. The values for total bone area (mm^2) and cortical bone area (mm^2) were lowest in chicks fed the SBO diet. There were no differences in values for cortical bone width (mm), periosteal mineral apposition rate (MAR) (μm/d), percentage of cortical bone, and percentage of medullary cavity among the diet groups. Chick tibia bone length was not influenced ($P > 0.05$) by the dietary treatments at 21 d (Table 3) or 42 d (lengths ranged from 111.8 to 113.6 mm). The histomorphometric values were not significantly different in chicks at 42 d ($P > 0.05$), and the periosteal BFR and MAR in the tibia ranged from 0.029 to 0.054 mm^2/d and 0.031 to 0.039 μm/d, respectively.

The plasma levels of IGF-I (μg/L) in chicks given the dietary treatments increased from 14 to 56 d ($P < 0.05$) (**Fig. 2**). At 14 d of age, the plasma concentration of IGF-I was highest ($P < 0.05$) in chicks consuming the MEC diet (23.3 μg/L). Plasma IGF-I concentration in chicks given the MEC diet was 138% of that measured in chicks fed the SBO diet. The level of IGF-I in plasma significantly increased in chicks fed the SBO, BC and MAC diets from 14 to 28 d of age but not in those given the MEC diet. The concentration of IGF-I in plasma was greatest (30.1 μg/L) ($P < 0.05$) in 28-d-old chicks given the MAC diet. There were no significant treatment differences in plasma IGF-I concentration at 42 and 56 d.

The mean concentration for IGF-I in liver tended to increase in all chicks except in the MAC group from 21 to 42 d of age (**Table 4**). Chicks fed the MEC diet had the highest IGF-I concentrations at 21 d. A similar trend of higher IGF-I concentration for chicks fed the MEC diet was observed in cortical bone and epiphyseal cartilage at 21 d, but no differences were observed between the dietary treatments for IGF-I in bone at 42 d. Chicks fed the SBO and MAC diets had a higher IGF-I concentration in cartilage compared with those

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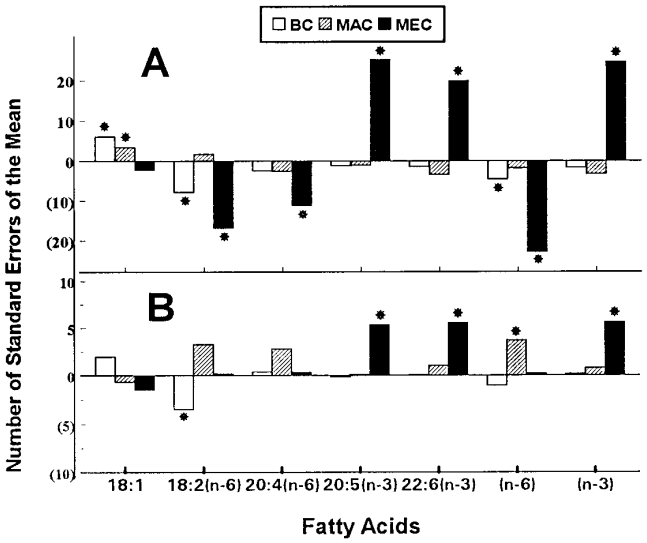

FIGURE 1 Liver fatty acid values presented as standardized differences (STD) for polar lipids (*panel A*) and neutral lipids (*panel B*) in 21-d-old chicks. The STD for fatty acid values were calculated as a difference in treatment mean [butter + corn oil (BC), margarine + corn oil (MAC), or menhaden oil + corn oil (MEC)] from the mean for those given soybean oil (SBO) divided by the pooled SEM ($n = 4$). Bars having a * are significantly different than the SBO treatment ($P < 0.05$).

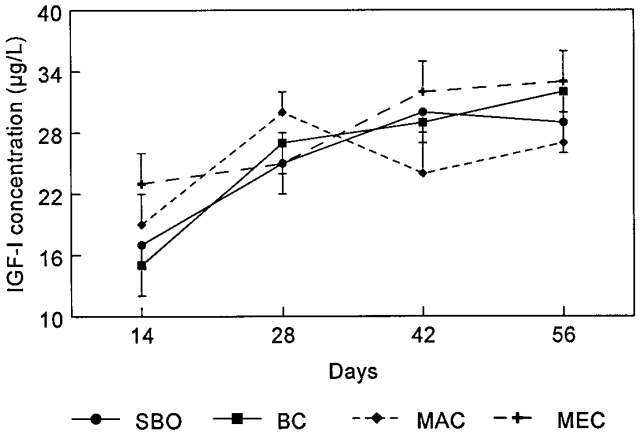

FIGURE 2 Plasma insulin-like growth factor (IGF)-I concentrations (mean ± SEM) in chicks ($n = 6–8$) fed soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), or menhaden oil + corn oil (MEC) at 70 g/kg of the diet at 0, 14, 28 and 42 d of age.

fed the BC and MEC diets at 42 d. It is interesting that IGF-I concentration in liver, bone and cartilage increased in chicks consuming SBO from 21 to 42 d. The same was true for chicks fed BC except that IGF-I decreased in cartilage.

The hexosamine concentration in chick cortical bone was not influenced by the dietary treatments at 21 or 42 d; however, serum levels of hexosamines were highest in chicks fed the BC diet at 21 d (**Table 5**). The PLA_2 activity in serum at 42 d was higher in chicks fed SBO and MEC [0.37 nmol/(min · mg protein)] compared with the activity in serum of those given BC and MAC (0.25). PLA_2 activity in liver of 42-d-old chicks was not influenced by the dietary treatments [mean values ranged from 5.6 to 7.2 pmol/(min · mg protein)]. Chicks given the BC diet had the highest level of plasma vitamin E at 21 d and maintained a higher level compared with those fed MAC and MEC at 42 d (Table 5). The ash and calcium concentrations in tibia were not affected by the dietary treatments.

In the second study, chicks given ABO had a significantly lower amount of 20:4(n-6) and reduced ex vivo PGE_2 production in tibia compared with chicks fed SBO (**Table 6**). The ABO treatment also resulted in a higher concentration of IGF-I in bone and greater trabecular BFR.

DISCUSSION

These studies demonstrated that dietary lipids modified the fatty acid composition of tibial cortical bone. Chicks given the BC diet maintained lower concentrations of 18:2(n-6) and 20:4(n-6) in bone polar lipids compared with the SBO group at 21 d. The BC diet, which contained moderate levels of PUFA but greater SAT, produced a higher ratio of SAT/PUFA in bone neutral lipids of chicks (2.86 at 21 d, and 2.61 at 42 d) compared with the ratio in those given SBO (1.2 at 21 d,

TABLE 3

Bone length and histomorphometric measurements in the tibia of 21-d-old chicks fed different lipids[1]

Measurement	Dietary lipid treatment[2]				Pooled SEM
	SBO	BC	MAC	MEC	
Tibia length, *mm*	76.7	78.4	78.0	77.4	1.0
Total bone area, mm^2	27.6[b]	31.3[a]	33.2[a]	33.3[a]	1.8
Medullary cavity area, mm^2	12.62[b]	13.52[ab]	15.42[a]	14.45[ab]	0.91
Cortical bone, area, mm^2	14.95[b]	17.73[a]	17.88[a]	18.80[a]	1.12
Cortical bone width, *mm*	0.93	1.01	0.96	1.02	0.04
Periosteal MAR,[3] $\mu m/d$	0.036	0.054	0.051	0.039	0.042
Periosteal BFR,[4] mm^2/d	0.049[b]	0.077[a]	0.067[ab]	0.046[b]	0.008
Total new BFR, mm^2/d	0.94[b]	1.58[a]	1.40[ab]	0.93[b]	0.17
Intracortical porosity, mm^2	0.027[b]	0.047[a]	0.043[a]	0.033[b]	0.005
% Cortical bone	54.4	56.6	53.5	56.5	1.5
% Medullary cavity	45.6	43.4	46.5	43.5	1.5

[1] Mean values ($n = 8$ for tibia bone length, all histomorphometric measurements $n = 6$) within rows having different superscripts are significantly different ($P < 0.05$).

[2] Dietary lipid treatments included soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), and menhaden oil + corn oil (MEC) at 70 g/kg of the diet.

[3] MAR, mineral apposition rate.

[4] BFR, bone formation rate.

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TABLE 4

Concentrations of insulin-like growth factor-I in chicks fed different lipids[1]

Tissue	d	Dietary lipid treatment[2] SBO	BC	MAC	MEC	Pooled SD
Liver, *pg/mg protein*	21	5.21[b]	5.52[b]	6.09[ab]	10.66[a]	4.24
	42	13.83	15.69	7.71	17.88	9.55
Cortical bone, *pg/μg dry weight*	21	0.37[b]	0.36[b]	0.49[a]	0.50[a]	0.10
	42	0.45	0.48	0.36	0.45	0.11
Cartilage, *pg/μg protein*	21	0.08[b]	0.15[a]	0.12[ab]	0.17[a]	0.04
	42	0.15[a]	0.08[b]	0.14[a]	0.07[b]	0.04

[1] Mean values within rows having different superscripts are significantly different ($P < 0.05$) (n = 6–8 for liver and cortical bone; n = 3 for cartilage).
[2] Dietary lipid treatments included soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), and menhaden oil + corn oil (MEC) at 70 g/kg of the diet.

and 1.0 at 42 d). In the second study, the concentrations of total (n-6) PUFA and 20:4(n-6) also were lower in cortical bone of chicks fed ABO compared with the values in the SBO group. The predominant effect produced by (n-3) PUFA in the MEC treatment group was a significant decrease in the concentration of 20:4(n-6), the precursor of PGE_2, and a concomitant increase in the amount of several (n-3) PUFA in bone polar and neutral lipids. In a study with rats, Alam et al. (1993) reported that feeding ethyl esters of (n-3) PUFA for 10 wk elevated the concentrations of 20:5(n-3) and 22:5(n-3) but lowered them for 20:4(n-6) in alveolar bone of the mandible.

Trans-fatty acids were incorporated into bone polar (1.5–6.7%) and neutral lipids (trace to 11.2%) in chicks fed the BC and MAC diets, and the uptake was dependent upon the level fed. In a previous study with chicks fed hydrogenated vegetable oil (44 wt% of *t*18:1), *trans*-18:1 fatty acids accumulated in liver and in epiphyseal cartilage of the tibia (Watkins et al. 1991).

Histomorphometric measurements made in cortical bone revealed that saturated fat significantly increased BFR in chicks at 21 d. Chicks given BC had higher values for periosteal BFR, total new BFR and intracortical porosity in cortical bone, and a higher level of circulating hexosamines compared with chicks fed the SBO and MEC diets. The higher BFR [158% (periosteal) and 168% (total new bone) of the SBO group] and level of serum hexosamines (component of bone matrix proteins) would reflect an increase in bone turnover (modeling and remodeling) in chicks fed the BC diet. These chicks also maintained the highest serum concentration of vitamin E, which was associated with greater trabecular BFR reported by our laboratory (Xu et al. 1995). The BC diet led to a higher SAT/PUFA ratio in bone and may spare serum vitamin E to enhance bone formation. A significant fat × vitamin E interaction was responsible for the higher trabecular BFR in chicks (Xu et al. 1995). A higher value for trabecular BFR was confirmed in chicks fed ABO in the second study. The fact that bone length, and bone ash and calcium contents were not different among the treatment groups suggests that the higher BFR in the BC group was perhaps accompanied by a corresponding increase in bone resorption that did not lead to a significant change in bone growth.

The plasma concentration of IGF-I increased from 14 to 56 d in all chicks, which corroborates earlier findings for growing

TABLE 5

Serum hexosamine and vitamin E levels and bone measurements in chicks fed different lipids[1]

Measurement	d	Dietary lipid treatment[2] SBO	BC	MAC	MEC	Pooled SD
Serum						
Hexosamine, *mmol/L*	21	3.78[c]	5.33[a]	3.87[c]	4.72[b]	0.11
	42	4.36	4.72	4.32	4.71	0.18
Vitamin E, *μmol/L*	21	45.8[b]	66.0[a]	33.8[c]	37.2[bc]	13.3
	42	45.8[ab]	50.9[a]	40.8[b]	31.2[c]	6.5
Bone						
Hexosamine, *μmol/g*	21	23.4	21.9	24.3	21.1	3.6
	42	18.7	21.9	21.9	23.4	4.5
Ash, *g/100 g*	21	52.8	54.2	55.6	55.9	1.53
	42	61.6	60.8	60.8	62.2	0.75
Calcium, *mmol/g*	21	5.89	5.61	6.04	5.96	0.11
	42	6.21	6.11	6.29	6.31	0.09

[1] Mean values within rows having different superscripts are significantly different ($P < 0.05$); n = 8 for all measurements except for vitamin E (n = 5–8) and hexosamine (n = 5–6).
[2] Dietary lipid treatments included soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), and menhaden oil + corn oil (MEC) at 70 g/kg of the diet.

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TABLE 6

Body weights and tibia bone measurements from 16-d-old chicks fed soybean oil (SBO) or anhydrous butter oil (ABO)[1]

Measurement	Dietary lipid[2]		Pooled SEM
	SBO	ABO	
Body weight, *g*	387	383	12
Bone length, *mm*	59.6	58.5	0.7
20:4(n-6), *mol/100 mol*	9.33[a]	4.51[b]	0.55
Total (n-6) PUFA, *mol/100 mol*	38.2[a]	18.0[b]	1.8
Ratio (n-6) PUFA/SAT, *mol/mol*	0.89[a]	0.38[b]	0.01
PGE_2,[3] *ng/g protein*	91.9[a]	67.0[b]	7.0
IGF-I,[4] *pg/mg protein*	290[b]	354[a]	25
BFR, *mm²/d*	1.09[b]	1.31[a]	0.07

[1] Mean values within rows having different superscripts are significantly different ($P < 0.05$); $n = 10$ for all measurements except bone formation rate (BFR), $n = 8$.

[2] Dietary lipid treatments included soybean oil (SBO) or anhydrous butter oil (ABO) at 100 g/kg of the diet.

[3] PGE_2, prostaglandin E_2.

[4] IGF-I, insulin-like growth factor-I.

chicks (Ballard et al. 1990). Chicks fed the MEC diet had the highest amount of IGF-I in plasma at 14 d, but at 28 d, the concentration in plasma of chicks fed the MAC diet was highest and then declined.

The IGF-I data for bone, cartilage and liver are more difficult to interpret because IGF-I was not consistently affected by diet; however, the IGF-I content of tissues tended to increase from 21 to 42 d in most groups. In contrast to bone, the higher concentration of IGF-I in cartilage of chicks fed BC compared with the value in those given SBO at 21 d might suggest a possible stimulatory effect of BC on early bone modeling; however, the IGF-I values in cartilage were reversed at 42 d for these two groups and yet no difference was observed in BFR. In the second study, the ABO diet resulted in a higher amount of IGF-I in tibial cortical bone, which coincided with the higher trabecular BFR. Even though these findings might suggest that dietary lipids influence the production of IGF-I, it is premature to explain the effects of dietary lipids on the action of IGF-I in skeletal tissues until measurements are performed for binding proteins and cell receptors that regulate its action. Furthermore, it is not known in what compartment of long bone (cortical and trabecular bone or epiphyseal cartilage) containing IGF-I that dominates bone formation. However, Pash and Canalis (1996) recently reported that PGE_2 induced IGFBP-5 synthesis by a transcriptional mechanism in osteoblast-enriched cells from fetal rat calvaria. Our data and those of Pash and Canalis (1996) suggest that dietary lipids alter PGE_2 production and may regulate the effects of IGF in bone by inducing IGFBP-5 synthesis. Future studies that examine dietary lipid effects on PGE_2 and mRNA synthesis for IGF and IGFBP in bone are needed.

Existing evidence indicates that eicosanoids (especially PGE_2) work in concert with growth factors and cytokines to regulate the local events of bone modeling (Norrdin et al. 1990, Raisz 1993). Further, the concentration of PGE_2 produced locally in bone is critical; at moderate levels, it is stimulatory for bone formation but inhibitory at a higher level (Raisz and Fall 1990), and excess production of PGE_2 is perhaps associated with bone pathology (Norrdin et al. 1990). In the present study, chicks given BC had a higher cortical BFR and lower concentration of 20:4(n-6) compared with chicks given SBO. A possible explanation for this response is that SBO led to an excess production of PGE_2 from the higher 20:4(n-6) content in bone polar lipids to depress bone formation. The second study confirmed in the tibia that feeding ABO to chicks reduced 20:4(n-6) concentration, increased trabecular BFR, decreased ex vivo PGE_2 production and elevated IGF-I concentration. **Figure 3** illustrates the relationship between dietary (n-6) PUFA intake and modulation of locally produced PGE_2 and IGF-I in bone, and the resulting effect on bone formation.

Relevant to the findings reported here is whether other eicosanoids besides PGE_2 are produced in bone to maintain normal bone resorption and formation activities. Because 20:5(n-3) is the substrate for the biosynthesis of leukotriene B_5 (LTB_5), the enrichment of 20:5(n-3) observed in bone lipids found in this study could presumably increase the formation of LTB_5 in bone. In mouse calvaria in vitro, LTB_4 was found to stimulate bone resorption in a biphasic manner and to a greater extent than for PGE_2 (Meghji et al. 1988). It is possible that LTB_5 exhibits effects similar to PGE_2 in bone, that is, stimulation of bone formation as well as bone resorption. The relationships between dietary PUFA and eicosanoid biosynthesis and level of mRNA for IGF-I in bone must be investigated.

In summary, the data demonstrated that dietary lipids altered the fatty acid composition of bone polar and neutral lipids. Notably, changes were observed in the concentration of PG precursors (20:4(n-6) and 20:5(n-3) and the ratio of SAT/PUFA. The increase in cortical bone formation in chicks fed butter suggests that the concentration of 20:4(n-6), PGE_2 precursor, is important for proper bone modeling in the young because a moderate level of PGE_2 stimulated bone formation, but a higher production of PGE_2 was associated with reduced BFR. Indeed, the second study showed that saturated fat resulted in lowered bone 20:4(n-6) but raised IGF-I content, moderated ex vivo PGE_2 production and increased trabecular BFR. In addition, a higher cortical BFR was accompanied by an increase in hexosamines in serum and IGF-I in epiphyseal cartilage of chicks fed butter. Although it is still unclear how

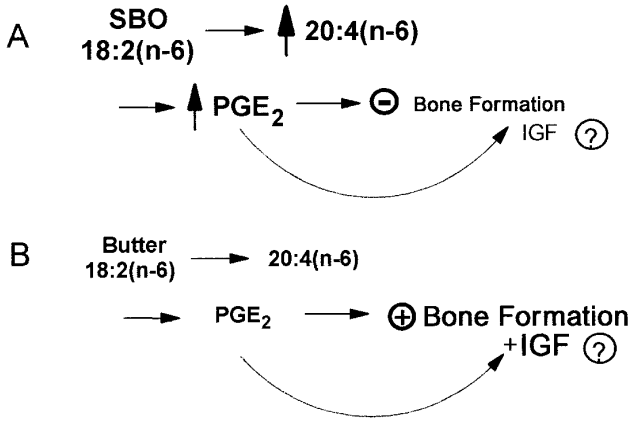

FIGURE 3 Effects of (n-6) polyunsaturated fatty acids and saturated fat on the concentration of 20:4(n-6) and ex vivo prostaglandin E_2 (PGE_2) production in tibia. *Panel A* illustrates how soybean oil elevates both 20:4(n-6) and PGE_2 in bone to reduce bone formation. *Panel B* shows how butter fat results in a moderate level of 20:4(n-6) and PGE_2 in bone to support higher bone formation. The observations in chicks suggest that moderating PGE_2 production in bone may increase the concentration or alter the action of insulin-like growth factor (IGF)-I in cartilage and bone to support greater bone formation.

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dietary PUFA can alter the action of IGF-I in bone, recent research indicated that IGFBP-5 synthesis was induced by PGE_2 in rat osteoblasts. Studies designed to characterize the effect of lipids on modulating PGE_2 production and its subsequent effect on IGF-I expression in bone will explain important relationships between dietary fat and bone modeling in the young.

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