Long-Term Supplementation of Various Dietary Lipids Alters Bone Mineral Content, Mechanical Properties and Histological Characteristics of Japanese Quail

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ABSTRACT The purpose of this study was to investigate the effects of long-term supplementation of fat in the diets on the fatty acid composition, chemical, mechanical, and histological properties of tibial bone. Month-old male Japanese quail were fed a basal diet containing either soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), or menhaden fish oil (FO) at 50 g/kg of the diet and maintained on these diets for 7 mo. Lipid treatments did not affect body weight, food intake, tibial length, or diameter. The FO diet group had the highest percentage of tibial ash, and both the FO and HSBO significantly increased tibial mineral content compared to those given SBO or CF. The type and amount of fatty acids in the diets had a profound influence on fatty acid composition of lipids in tibial cortical bones. Quail fed FO had the highest concentration of (n-3) fatty acids, and those fed SBO were highest in (n-6) fatty acids. The HSBO diet, containing high level of trans-fatty acids, led to the accumulation of these fatty acids in bone. In quail, long-term supplementation of FO or HSBO increased tibial shear force and shear stress and improved histological cortical thickness and density when compared to those given SBO or CF. These results suggest that long-term exposure to a FO or HSBO diet have a significant beneficial effect on bone metabolism.

(*Key words*: bone, histology, lipid, mechanical property, quail)

2003 Poultry Science 82:831–839

INTRODUCTION

Osteoporosis is a major health care problem that is quickly becoming more severe in economically developed countries. Usually afflicting the aging population, it is estimated to cost $10 billion a year in the United States to treat such patients (Seifert and Watkins, 1997). It is reported that there are 300,000 new cases of osteoporotic hip fractures annually in the United States alone, and 1.5 million people around the world suffer from osteoporosis (Das, 1994).

Many nutrients influence the growth, development, modeling, and remodeling of bones. Among them, the effects of calcium, phosphorous, and 1,25-dihydroxyvitamin D_3 on bone growth and health are well studied. Dietary supplementation of calcium and vitamin D_3 are traditionally believed to be effective in prevention of bone loss. Therefore, an increase in dietary calcium intake is often recommended for reducing postmenopausal bone loss (Heaney, 1992; Cumming and Nevit, 1997). However, some evidence indicates that increased calcium intake above normal does not prevent bone loss in post-menopausal women (Riis et al., 1987; Dawson et al., 1990; Hosking et al., 1998).

The relationships between dietary fat, calcium metabolism, and bone development have only recently been studied. An early study indicated that the degree of mineralization in hypertrophic and calcified cartilage was closely related to the amounts of acidic phospholipids present in the tissue (Wuthier, 1968, 1975). A diet high in saturated fat adversely affected bone mineralization and consequently compromised structural and mechanical properties of bones in growing rats (Hou et al., 1990; Li et al., 1990; Salem et al., 1992; Zernicke et al., 1995) and chicks (Atteh et al., 1983; Wohl et al., 1998). In other studies, results indicated that dietary lipids, depending upon the type and amount ingested, enhanced or impaired bone growth and development and also modulated bone mineral content. Models that have been employed include rats (Alam et al., 1990; Claassen et al., 1995) and chicks (Xu et al., 1994; Watkins et al., 1996a,b; 1997a). Lipid sources that are most often used include

Received for publication November 11, 2002.
Accepted for publication February 6, 2003.
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Abbreviation Key: CF = chicken fat; CLA = total conjugated linoleic acids; FAME = fatty acid methyl esters; FO = menhaden fish oil; HSBO = hydrogenated soybean oil; HZ = hypertrophic zones; PGE_2 = prostaglandin E_2; PUFA = polyunsaturated fatty acids; SBO = soybean oil.

hydrogenated soybean oil (HSBO), soybean oil (SBO), menhaden fish oil (FO), and saturated fat (SF), which are enriched with *trans* fatty acids, n-6 fatty acids, n-3 fatty acids, and saturated fatty acids, respectively. Briefly, the results of these studies indicated that dietary lipids modify the fatty acid composition of cartilage and bone, which reflects the dietary lipid profile. Supplementation of the diet with FO elevates 20:5(n-3) and 20:6(n-3) fatty acids in phospholipids of bone, while decreasing the concentration of 20:4(n-6) fatty acids compared to supplementation with corn oil or SBO. Consequently, growing animals fed either (n-3) fatty acids or saturated fat enriched diets showed significantly greater bone formation rate compared to those given soybean oil (Watkins et al., 1996a, 1997b).

High-fat diets are pervasive in western countries, and dietary fat constitutes 30 to 40% of the food calories (Watkins et al., 1997a). The type of fat consumed may have a significant impact on the bone metabolism and growth in the young. There are no studies on how different dietary lipids affect adult bone following long-term supplementation. Therefore, the purpose of this study was to investigate the long-term effects of different dietary lipids, varying in the amounts of (n-3) and (n-6) fatty acids, saturated, and trans-fatty acids on the fatty acid composition of older bone tissue in Japanese quail. Bone biomechanical properties and histological measurements were performed to determine the effects of lipids on these bone parameters.

MATERIALS AND METHODS

Animals and Diets

Day-old Japanese quail from the university flock were placed in temperature-controlled battery brooders on continuous light and raised on identical dietary and managerial conditions until 4 wk old. Birds were then identified by sex, and the males were wing-banded, weighed, and randomly divided into four groups of 30 birds each. The quail were fed a basal diet containing either 50 g/kg diet soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), or menhaden fish oil (FO). The basal diet (Table 1) was formulated to meet or exceed the nutrient requirements of breeding quail (NRC, 1994). At 6 wk of age, quail were placed in individual cages where they remained throughout the rest of the experiment. Diets were mixed twice each month and stored in cool conditions to minimize oxidation. The quail were given free access to feed and water throughout the 7-mo experimental period. Body weight and feed consumption were recorded monthly.

Sample Collections

Twenty quail per dietary treatment were used for bone histological, mechanical, and mineral measurements. At 8 mo of age, quail were killed by cervical dislocation. Both tibial bones from each bird were excised and cleaned of surrounding soft tissue. The length of the right tibia of each bird was measured, and the diameter at the center of the diaphysis was determined (in mm) by measuring with calipers the widest and narrowest sites. The right tibiae were then sealed in plastic bags and frozen (–20°C) until the day of mechanical testing. Bone wall thickness was derived following the mechanical test. After recording the weight, 10 left tibiae from each treatment were fixed in a 10% phosphate-buffered formalin solution for 2 d and then decalcified with formic acid solution for histological analysis. The rest of the left tibiae were collected and frozen at –20°C for ash and mineral measurements. The remaining eight birds per treatment were sacrificed at the same age, and the tibiae were collected and kept at –80°C for fatty acid analysis.

Bone Mechanical Testing

On the day of testing, tibial bones were thawed at room temperature. It has previously been shown that freezing and thawing does not affect bone-breaking strength of chicken bones (Wilson and Mason, 1992). All mechanical tests were performed on a Universal Testing Machine[2]. A shear test was used to determine the mechanical properties of the tibial bone with a double shear block apparatus (Wilson and Ruszler, 1998). Tests were conducted at room temperature and specimens were kept moist during testing. Loaded at the midpoint of the shaft, the tibia was subjected to shear test to failure at a constant loading rate of 2 mm/min. The shear force, shear fracture energy, and maximal deflection before fracture were read directly or calculated from the computer recordings. The shear force is the force it takes to break the bone, whereas the shear fracture energy is the area under the load-deflection curve and represents the amount of energy absorbed by the bone before it fractures. A lower shear fracture indicates that the bone is more brittle. Because strength values are influenced by bone size, the ultimate stress was calculated using the following formula which normalizes values for bone size and is based on the fact that the bones are hollow: Stress = Force/2π(D*BW-BW^2), where D is the diameter of tibia, and BW is the thickness of bone wall at the failure (ASAE, 1999).

Histological Measurements

Histological measurements were made as described previously (Liu et al., 2003). Decalcified bone samples were split longitudinally in half and then cut into two parts at the center of the diaphysis. Proximal and distal parts of tibiae were embedded in paraffin and cut into 5-μm sections. Randomly selected sections were stained with hematoxylin and eosin, microscopically evaluated, and photographed without knowledge of treatment group. Using a stage micrometer and erythrocyte sizing for a secondary reference, dimensions of the cortical bone and widths of the proliferative, hypertrophic, and miner-

[2]MTS Systems Co., Cary, NC.

TABLE 1. Composition of the experimental diets fed to quail[1]

Ingredients	Dietary lipid treatment[2] (g/100 g)			
	SBO	HSBO	CF	FO
Ground yellow corn	53.55	53.55	53.55	53.55
Dehulled soybean meal	32.00	32.00	32.00	32.00
Soybean oil	5.00	—	—	—
Hydrogenated soybean oil	—	5.00	—	—
Chicken fat	—	—	5.00	—
Menhaden oil	—	—	—	5.00
Limestone	6.50	6.50	6.50	6.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Vitamin premix[3]	1.00	1.00	1.00	1.00
Trace mineral premix[4]	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.35	0.35

[1]The diet was formulated to contain 21.0 g/100 g crude protein, 12.43 MJ/kg metabolizable energy, 7.36 g/100 g crude fat, 2.43 g/100 g crude fiber, 2.81 g/100 g calcium, and 0.67 g/100 g phosphorous.

[2]Dietary lipid sources were soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), or menhaden fish oil (FO).

[3]Provided per kilogram of diet: retinol, 1,100 IU; cholecalciferol, 330 IU; α-tocopherol, 33 IU; menadione sodium bisulfite, 3.52 mg; thiamine HCl, 1.1 mg; riboflavin, 6.6 mg; calcium D-pantothenate, 16.5 mg; niacin, 44 mg; choline chloride, 374 mg; vitamin B_{12}, 0.0165 mg; folic acid, 1.1 mg; pyridoxine HCl, 1.1 mg; biotin, 0.055 mg; bacitracin, 22.0 mg; selenium, 0.20 mg; ethoxyquin, 0.124 mg.

[4]Provided per kilogram of diet: manganese, 44 mg; zinc, 47.5 mg; iron, 50.0 mg; copper, 6.25 mg; iodine, 2.0 mg; and selenium, 0.3 mg.

alized zones of the physis were measured. The cortical bone was measured from the periosteum to the maximum width of the internal (endosteal) side, at approximately 25 μ inside the physis towards the diaphysis. Cortical and trabecular density was an estimation of the variability of open spacing within the cortical bone dimensional limits and was given a score of 1 to 4 from low to high density, respectively.

Lipid Analysis

Lipids from diet samples were extracted using a Folch wash of chloroform/methanol (2:1, v/v). Methyl esters of fatty acids (FAME) in lipid extracts were prepared by transesterification using 0.5N NaOH in methanol and 14% boron triflouride (BF_3) in methanol (Watkins et al., 1997b). The FAME were separated using a Chrompack 100 m × 0.25 mm i.d. CP-Sil 88 column,[3] and analyzed using a gas-liquid chromatograph[4] with a flame ionization detector and ultra pure hydrogen as the carrier gas. Conditions for analysis of dietary lipid extracts were: split injection (80:1), 0.5 μL injection volume, initial oven temperature 150°C (held for 2 min), increased to 175°C at a rate of 10°C/min and held for 41 min, increased to 210°C at a rate of 20°C/min and held for 11 min, and a final increase to 225°C at a rate of 10°C/min and held for 11 min. Total run time was 70.75 min. Injector and detector temperatures were held at 250°C and 255°C, respectively.

The shaft portion of tibia was used for fatty acid analysis. After removing the periosteum and marrow, the cortical bone was frozen in liquid nitrogen, pulverized to powder with a mortar and pestle, weighed, and placed in 7 ml methanol and 14 ml chloroform for 24 h to extract the lipids. Chromatographic conditions for preparation and separation of FAME from bone lipid extracts were similar to those for the diets except as follows: splitless injection, 1.5 min purge valve closure, initial oven temperature 40°C and held for 1.5 min, increased to 100°C at a rate of 40°C/min and held for 10 min, increased to 175°C at a rate of 25°C/min and held for 70 min, and a final increase to 220°C at a rate of 10°C/min and held for 20 min. Total run time was 110.5 min. Injector and detector temperatures were held at 150 and 255°C, respectively. As an internal standard, 10-Undecenoate[5] was added to all samples. The amounts of total saturated fatty acids (SAF), monounsaturated fatty acids, polyunsaturated fatty acids (PUFA), total (n-3) and (n-6) PUFA, as well as the (n-6)/(n-3) ratios, were calculated from the gas-liquid chromatographic analysis. The amount of conjugated linoleic acid represented all detected isomers.

Ash, Calcium, and Phosphorous Analyses

Tibia samples were oven-dried at 105°C for 48 h, weighed, and ashed in a muffle furnace at 600°C for 14 h in porcelain crucibles. Tibial ash was expressed as a percentage of dry weight, and mineral content was represented by ash weight (mg) per unit of tibial length. Afterwards, the ash was digested with nitric acid:perchloric acid mixture (5:3 vol/vol), and the Ca contents of tibial ash were determined with an atomic absorption spectrophotometer,[6] and expressed as a percentage of dry weight of the tibiae. The P content was measured colorimetrically

[3]Varian, Inc., Walnut Creek, CA.
[4]HP 5890GC, autosampler, Sunnyvale, CA.
[5]Nu Check Prep, Elysian, MN.
[6]Perkin Elmer, 5100 PC, Norwalk, CT.

TABLE 2. Fatty acid composition (μg/100 μg) of the quail experimental diets

	Dietary lipid treatment[1]			
Fatty acids	SBO	HSBO	CF	FO
14:0	0.06	0.19	0.57	5.29
15:0	0.02	0.04	0.09	0.42
16:0	9.95	11.26	15.01	15.45
16:1(n-7)	0.08	0.15	2.30	6.95
18:0	3.54	11.11	6.53	3.26
t18:1	0.05	25.49	2.57	1.45
18:1	25.82	30.76	39.50	15.07
18:2(n-6)	54.79	18.11	30.00	19.16
18:3(n-3)	4.33	0.98	1.54	2.32
20:0	0.30	0.34	0.23	0.20
20:3(n-3)	ND[2]	ND	0.05	0.11
20:4(n-6)	0.01	0.01	0.16	0.51
20:5(n-3)	0.01	ND	0.02	18.49
22:1	ND	ND	0.03	0.24
22:4(n-6)	0.01	0.01	0.03	0.09
22:5(n-3)	0.02	0.03	0.02	1.47
22:6(n-3)	ND	ND	0.01	7.47
SAT[3]	13.87	22.94	22.42	24.62
MUFA[4]	25.95	56.42	44.43	23.91
PUFA[5]	59.18	19.15	31.82	49.61
(n-6) PUFA	54.81	18.13	30.19	19.75
(n-3)PUFA	4.37	1.02	1.63	29.86
(n-6)/(n-3)	12.55	17.85	18.47	0.66

[1]Dietary treatments include soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), and menhaden fish oil (FO) at 50 g/kg of the diet.

[2]ND = not detected.

[3]SAT = total saturated fatty acids.

[4]MUFA = total monounsaturated fatty acids.

[5]PUFA = total polyunsaturated fatty acids.

(AOAC, 1990) with the computer program "Microkinetics"[7] and a vertical photometer.[8]

Statistic Analysis

Data were analyzed by a one-way ANOVA of SAS software (SAS, 1998). Significant differences of means between treatments were tested using studentized Tukey test at the 5% probability level. Variation within treatment was expressed as the standard error of the treatment mean (SEM).

RESULTS

Fatty Acid Composition of the Diets and Bone Lipids

The fatty acid composition of the four diets (Table 2) demonstrated that the SBO diet provided the highest amount of 18:2(n-6) and 18:3(n-3), whereas the FO diet had the greatest levels of 14:0, 16:1(n-7), 20:5(n-3), 22:5(n-3), and 22:6(n-3). The HSBO diet contained the largest amount of 18:0 and trans-18:1, and the CF diet was the highest in 18:1. Consequently, the SBO diet had the highest levels of total polyunsaturates and (n-6) fatty acids, but the FO diet provided the largest amount of (n-3) fatty acids, total saturates, and the lowest ratio of (n-6)/(n-3). The HSBO diet contained the greatest level of total monounsaturates. The CF diet, however, also had nearly double the amount of monounsaturates compared to the FO diet and much higher levels of total (n-6) fatty acids compared to both the HSBO and FO diets. All diets contained 1.3 to 4.0% of 18:2(n-6), which was well above the recommended requirement (1.0%) for laying quail (NRC, 1994).

Significant differences in bone fatty acid composition were observed between the dietary treatment groups. Quail given SBO had the highest concentrations of 18:2(n-6), 18:3(n-3), 20:4(n-6), total polyunsaturates, and total n-6 PUFA in the tibiae, whereas those consuming FO were the highest in 14:0, 16:1(n-7), 20:5(n-3), 22:6(n-3), and total saturates but were lowest in the level of 20:4(n-6) and the ratio of (n-6):(n-3) (Table 3). Likewise, the tibiae from birds given the HSBO had the highest trans-18:1, 18:0, CLA, and total monounsaturates. The CF group was significantly higher in 20:4(n-6) and total (n-6) fatty acids than those in the FO and HSBO groups and also had higher levels of total monounsaturates, t18:1, CLA, and (n-6)/(n-3) than those in the SBO and FO groups.

Body Weight, Feed Intakes, and Bone Measurements

No significant differences among the lipid treatment groups were observed for BW and feed intake of quail

[7]Catalog no. 78-588-00, Flow Laboratories, Inc., McLean, VA.

[8]Titerek Multiskan MCC/340 serial no. 1EEE-448, Titertek, Huntsville, AL.

TABLE 3. Fatty acid composition (μg/100 μg) of tibial cortical bone from mature male quail fed different lipids[1]

Fatty acids	Dietary lipid treatment[2]				Pooled SEM	*P*-values
	SBO	HSBO	CF	FO		
14:0	0.43^{c}	0.57^{b}	0.69^{b}	4.43^{a}	0.06	0.0001
15:0	0.08^{b}	0.08^{b}	0.11^{b}	0.38^{a}	0.01	0.0001
16:0	15.74^{b}	18.25^{ab}	19.19^{a}	19.65^{a}	0.72	0.0155
16:1(n-7)	3.09^{c}	4.78^{b}	4.61^{b}	8.79^{a}	0.33	0.0001
18:0	5.49^{b}	6.92^{a}	5.61^{b}	5.58^{b}	0.36	0.0135
t18:1	0.32^{c}	7.05^{a}	1.47^{b}	0.41^{c}	0.19	0.0001
18:1	28.14^{b}	41.26^{a}	41.40^{a}	27.91^{b}	0.76	0.0001
18:2(n-6)	29.95^{a}	14.21^{b}	15.61^{b}	14.38^{b}	1.05	0.0001
CLA[3]	0.02^{c}	0.56^{a}	0.14^{b}	ND[4]	0.02	0.0001
18:3(n-3)	2.22^{a}	0.54^{c}	0.70^{c}	1.19^{b}	0.08	0.0001
20:0	0.11	0.12	0.08	0.12	0.02	0.6643
20:1(n-9)	0.20^{c}	0.24^{c}	0.30^{b}	0.71^{a}	0.01	0.0001
20:3(n-3)	0.11	0.40	0.09	0.14	0.02	0.4236
20:4(n-6)	12.10^{a}	4.10^{c}	8.75^{b}	3.71^{c}	0.48	0.0001
20:5(n-3)	0.10^{b}	0.08^{b}	0.06^{b}	6.14^{a}	0.27	0.0001
22:4(n-6)	0.11	0.14	0.11	0.07	0.03	0.6432
22:5(n-3)	0.07^{b}	0.05^{b}	0.04^{b}	1.14^{a}	0.07	0.0001
22:6(n-3)	0.16^{b}	0.12^{b}	0.14^{b}	3.83^{a}	0.15	0.0001
SAT[5]	22.26^{c}	26.42^{b}	26.23^{b}	30.93^{a}	0.92	0.0006
MUFA[6]	31.82^{d}	53.46^{a}	47.87^{b}	37.94^{c}	0.72	0.0001
PUFA[7]	45.75^{a}	19.89^{d}	25.64^{c}	30.58^{b}	1.25	0.0001
(n-6)PUFA	43.10^{a}	19.11^{c}	24.61^{b}	18.16^{c}	1.25	0.0001
(n-3)PUFA	2.65^{b}	0.88^{b}	1.03^{b}	12.43^{a}	0.52	0.0001
(n-6)/(n-3)	16.29^{b}	22.22^{a}	23.98^{a}	1.49^{c}	0.88	0.0001

[1]Mean values for bone fatty acid composition (n = 6) within a row having different superscripts are significantly different by Duncan multiple comparison test ($P < 0.05$).

[2]Dietary lipids include soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), and menhaden fish oil (FO) at 50 g/kg of the diet.

[3]CLA = total conjugated linoleic acids.

[4]ND = not detected.

[5]SAT = total saturated fatty acids.

[6]MUFA = total monounsaturated fatty acids.

[7]PUFA = total polyunsaturated fatty acids.

across the dietary treatments during the whole experimental period (Table 4). Accordingly, long-term dietary lipid supplementation with various fatty acid compositions had no pronounced effect on tibial bone length, diameter, or weight. However, the percentage of ash in the tibiae was significantly higher from quail fed FO than from the other treatment groups. Mineral content was significantly higher both in the FO and HSBO groups than in the SBO or CF group. In addition, birds from the FO treatment group also showed the highest Ca and P contents in the tibiae. There were no significant differences between the other groups.

Biomechanical Properties

There were significant differences in the biomechanical properties of tibial bones between dietary lipid treatment groups. Birds fed SBO had significantly lower values in shear force and stress than those fed FO or HSBO (Table 5). Similarly, the CF group was also significantly lower in these parameters except for shear force, which was still lower, but not statistically significant, compared to the FO or HSBO groups. There were no differences in these parameters between the SBO and CF groups or between the FO and HSBO groups. The shear fracture energy was significantly higher for birds fed SBO than those given HSBO but not different compared to those consuming the FO or CF diet (Table 5).

Anatomic and Histologic Parameters

There were no differences in the diameter and length of the tibiae subjected to histological analysis between dietary lipid treatments (Table 6). Cortical bone thickness in the proximal end of the tibia was not different between treatments, but birds fed either FO or HSBO showed a significantly higher cortical density compared to those given a CF or SBO diet. In the diaphysis, both cortical thickness and density in birds fed FO, and cortical density in birds fed HSBO were significantly higher compared to those consuming the SBO or CF diet. In the distal end of the tibiae, significantly higher cortical thickness was found in quail provided the FO or HSBO diet than those fed SBO, but there was no difference in cortical density in this area between treatments.

DISCUSSION

The long-term effect of different dietary lipid supplementation on fatty acid composition, mechanical, and histological properties in aged tibial bone, using the quail as an animal model, was investigated. These results dem-

TABLE 4. Body weight, tibial bone measurements, and mineral content of mature male quail fed different lipids[1]

Measurements	Dietary lipid treatment[2]				*P*-values
	SBO	HSBO	CF	FO	
Body weight (g)	114.5 ± 2.3	116.0 ± 2.1	115.6 ± 2.3	114.2 ± 1.9	0.9244
Feed intakes (g/d)	11.9 ± 0.2	12.1 ± 0.3	12.0 ± 0.3	11.9 ± 0.3	0.9211
Tibia length (mm)	46.8 ± 0.4	46.6 ± 0.4	46.4 ± 0.4	46.3 ± 0.3	0.7062
Tibia diameter (mm)	2.25 ± 0.02	2.22 ± 0.03	2.22 ± 0.04	2.22 ± 0.03	0.8420
Tibia weight (g)	0.34 ± 0.01	0.37 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.5102
Ash (% dry weight)	39.0 ± 1.0^{b}	39.0 ± 0.6^{b}	39.7 ± 0.6^{b}	43.7 ± 1.2^{a}	0.0023
Mineral content (mg/mm)	2.89 ± 0.06^{b}	3.10 ± 0.05^{a}	2.88 ± 0.09^{b}	3.27 ± 0.09^{a}	0.0011
Calcium (% dry weight)	14.0 ± 0.3^{b}	13.9 ± 0.3^{b}	14.1 ± 0.3^{b}	16.2 ± 0.5^{a}	0.0004
Phosphorus (% dry weight)	6.5 ± 0.2^{b}	6.6 ± 0.1^{b}	6.8 ± 0.1^{b}	7.5 ± 0.3^{a}	0.0059

[1]Mean values (means ± SEM) within rows having different superscripts are significantly different ($P < 0.05$). n = 20 for BW and bone measurements, n = 10 for ash and mineral measurements.

[2]Dietary lipids treatments included soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), and menhaden oil (FO) provided at 50 g/kg of the diet. All d-old quail were fed standard diets for 4 wk and then randomly divided into four experimental groups and fed the experimental diets for 7 mo before being killed.

onstrated that BW, tibial length, weight, and diameter were similar in all experimental groups, suggesting that dietary lipids had no significant effect on these parameters. However, the percentages of tibial ash, Ca, and P were significantly higher in quail fed FO, compared to those of the other groups. Mineral content was significantly higher in both FO and HSBO treatment groups. Although Watkins et al. (1996a; 1997b) reported that an (n-6)-enriched diet such as SBO impaired bone formation rate as measured by histomorphometric measurements in growing chicks, there was no difference in percent bone ash and mineral content between chicks fed dietary SBO and those given the other dietary lipids either at 21 or 42 d of age. The current results suggest that the effect of lipids on bone mineralization and metabolism is a cumulative process, and long-term dietary treatment amplifies the effects of lipids on bone turnover. Thus, dietary SBO and CF diets may eventually lead to negative bone turnover and mineral loss in aged quail compared to those fed FO diet. This hypothesis, however, needs to be confirmed by a more detailed study.

The fatty acid composition of lipids in bone was significantly altered by dietary lipids. Specifically, the concentrations of 18:2(n-6) and 20:4(n-6) were higher in tibial cortical bone of quail fed SBO, whereas dietary enrichment with (n-3) fatty acids greatly increased the levels of 20:5(n-3) and 22:6(n-3) and depressed the concentration of 20:4(n-6), the precursor of prostaglandin E_2 (PGE_2), compared with other groups. Alam et al. (1990) and Kokkinos et al. (1993) reported that feeding ethyl esters of (n-3) polyunsaturates for over 5 wk elevated the concentrations of 20:5(n-3) and 22:6(n-3) but lowered the level of 20:4(n-6) in alveolar bone of rats. Watkins et al. (1996a; 1997b) demonstrated that dietary SBO increased the concentrations of 20:4(n-6), whereas menhaden fish oil decreased the level of this fatty acid but increased the concentration of (n-3) fatty acids in the tibiae of growing chicks. In addition, *trans*-fatty acids (mainly t18:1) were incorporated into bone lipids in chicks fed HSBO (Watkins et al., 1991). The results of the present study are in agreement with these findings.

The study of mechanical properties revealed that in quail fed FO or HSBO compared to CF and SBO, bone shear force was increased by 16.6 to 29.3%. Furthermore, shear stress, which is a measure of the internal resistance of the molecular structure of a material to deformity (Wilson and Ruszler, 1998; Rath et al., 1999), was increased by 26.2 to 37.2%. Fracture energy, representing brittleness, was comparable between treatments, except that the SBO group had higher fracture energy than quail fed HSBO. Overall, these measurements showed that bones from quail fed FO or HSBO were stronger than those given SBO or CF.

TABLE 5. Mechanical properties of tibial bones in mature male quail fed different dietary lipids[1]

Measurements	Dietary lipid treatment[2]				*P*-values
	SBO	HSBO	CF	FO	
Shear force (N)[3]	52.6 ± 3.1^{b}	68.0 ± 3.2^{a}	57.8 ± 3.8^{ab}	67.4 ± 3.6^{a}	0.0098
Shear fracture energy (N-mm)	17.2 ± 4.1	15.1 ± 2.6	15.7 ± 4.6	14.6 ± 4.4	0.0819
Shear stress (N/mm^2)	19.6 ± 1.2^{b}	26.9 ± 1.4^{a}	21.0 ± 1.1^{b}	26.5 ± 1.6^{a}	0.0003

[1]Mean values (means ± SEM) within rows having different superscripts are significantly different ($P < 0.05$). n = 20 for mechanical measurements.

[2]Dietary lipid treatments included soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), and menhaden oil (FO) at 50 g/kg of the diet.

[3]N = Newtons.

TABLE 6. Histological measurements of tibial bones from mature male quail fed different dietary lipids[1]

Measurements	Dietary lipid treatment[2]				*P*-values
	SBO	HSBO	CF	FO	
Diameter (mm)	2.18 ± 0.03	2.20 ± 0.04	2.25 ± 0.04	2.24 ± 0.04	0.9230
Length (mm)	46.2 ± 0.5	46.3 ± 0.4	46.5 ± 0.5	46.2 ± 0.5	0.7662
	Proximal end of tibia				
Cortical thickness (mm × 10)	1.3 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.9 ± 0.2	0.3721
Cortical density[3]	1.2 ± 0.2^{b}	2.7 ± 0.3^{a}	1.7 ± 0.3^{b}	3.0 ± 0.3^{a}	0.0026
	Diaphysis of tibia				
Cortical thickness (mm × 10)	1.7 ± 0.2^{b}	2.3 ± 0.2^{ab}	1.6 ± 0.3^{b}	2.7 ± 0.2^{a}	0.0125
Cortical density[3]	3.0 ± 0.4^{b}	4.1 ± 0.2^{a}	3.3 ± 0.2^{b}	4.4 ± 0.2^{a}	0.0078
	Distal end of tibia				
Cortical thickness (mm × 10)	0.5 ± 0.02^{b}	0.8 ± 0.09^{a}	0.6 ± 0.07^{ab}	0.9 ± 0.08^{a}	0.0163
Cortical density[3]	3.1 ± 0.2	4.1 ± 0.3	4.1 ± 0.4	4.2 ± 0.4	0.2050

[1]Mean values (means ± SEM) within rows having different superscripts are significantly different ($P < 0.05$). n = 8 for histological measurements.

[2]Dietary lipid treatments included soybean oil (SBO), hydrogenated soybean oil (HSBO), chicken fat (CF), and menhaden oil (FO) at 50 g/kg of the diet. All 1-d-old quail were fed standard diets for 4 wk and then randomly divided into four experimental groups and fed above experimental diets for 7 mo before killed.

[3]An estimation of the variability of open spacing within the cortical bone dimensional limits, was scored in a range of 1 to 4 from low to high density.

The biomechanics of bone has been extensively investigated in both animals and humans (Aerssens et al., 1993; Barengolts et al., 1993; Grynpas et al., 1992; Yoshitake et al., 1993; Zernicke et al., 1995; Wohl et al., 1998; Wilson and Ruszler, 1998; Rath et al., 1999; Bailey et al., 1999). Bone strength is related to its structural (e.g., shear force, energy) and material properties (e.g., stress, matrix chemistry). The structural properties of bones depend in part on bone geometry (Zernicke et al., 1995). Our current study indicates that birds in all treatments had similar gross geometry and BW, suggesting that the deleterious effects of the SBO and CF diets were mainly related to changes in material properties of the cortical bones. Further, histological observations from this study demonstrate that both cortical bone density and thickness at 8 mo of age are markedly improved in quail fed FO or HSBO compared to those given SBO or CF. These data and the mineral measurements indicate that the SBO and CF diet-related changes in mechanical properties of cortical bones resulted from both reduced cortical bone quality and quantity.

Several past histomorphometric studies on rapidly growing animals have indicated that short-term supplementation of a high level of SBO in the diet decreased the rate of bone formation rate compared to FO (Watkins, 1996a; 1997b). These studies, however, did not assess the mechanical properties of the resulting bone. In the current study, mature quail were used as animal model, and the results, therefore, largely reflect the influence of lipids on bone remodeling (formation and resorption), which is the primary mechanism of mature bone turnover (Wohl et al., 1998). Thus, it can be assumed that long-term supplementation of SBO or CF in the diets caused a negative bone balance by inducing bone resorption, which resulted in decreased bone mass, mineral density, and impaired mechanical properties.

Mature bone remodeling is regulated partially by locally produced factors within the skeleton (Baylink et al., 1993; Marks and Miller, 1993; Mundy, 1993; Raisz, 1993). Prostaglandins have been extensively investigated and have been found to play an important role in bone metabolism. Certain fatty acids are precursors of prostaglandins, and, therefore, one mechanism by which dietary lipid influences bone formation and resorption is by altering prostaglandin production. PGE_2, which is produced from the 20-carbon polyunsaturated essential fatty acid (arachidonic acid) in osteogenic cells (Sardesai, 1992; Kikkinos et al., 1993; Watkins, 1997a), has powerful effects on bone metabolism. In vitro, although PGE_2 can stimulate bone formation at a low concentration, its main function over the long term is to stimulate bone resorption (Marks and Miller, 1993; Raisz et al., 1993; Fall et al., 1994). Recent studies indicate that diets enriched with n-6 PUFA increased the concentration of arachidonic acid [20:4(n-6)] in bone and elevated bone PGE_2 production in chicks (Watkins 1996a; 1997b) and rats (Kokkinos et al., 1993) but depressed bone formation rate. In this study, quail fed SBO or CF not only had a significantly higher level of arachidonic acid but also maintained a higher n-6/n-3 ratio in the tibiae than those consuming FO or HSBO. While PGE_2 was not measured in the present study, we have previously shown that dietary FO and HSBO can decrease tibial PGE_2 compared to quail fed SBO or CF (Liu and Denbow, 2001).

In summary, our current study demonstrated that in mature quail, long-term supplementation of lipids in the diets altered fatty acid composition of bone lipids, which reflected the fatty acid profile of the diet. Specifically, quail fed SBO or CF had elevated bone arachidonic acid concentrations, a precursor of PGE_2. In a previous study using quail, it was shown that there is a direct relationship between 20:4(n-6) concentration and PGE_2 production

(Liu and Denbow, 2001). The beneficial effects of FO and HSBO on bone mineral content, mechanical and histological properties in this study might be, at least in part, attributable to their decreased PGE_2 production-induced bone resorption. Further study on the effects of dietary lipids on bone should include biochemical assays and histomorphometrical measurements to determine the effects of lipids on bone chemistry.

ACKNOWLEDGMENTS

The authors thank B. Self for laboratory and editorial assistance. Special thanks are given to Omega Protein, Inc., Reedville, Virginia 22539 for donation of the menhaden oil and to the John Lee Pratt Animal Nutrition Program for financial support.

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