

## Dietary *n*-6:*n*-3 fatty acid ratio in the perinatal period affects bone parameters in adult female rats

M. Korotkova<sup>1</sup>\*, C. Ohlsson<sup>2</sup>, L. Å. Hanson<sup>3</sup> and B. Strandvik<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Göteborg University, SE 41685 Göteborg, Sweden

<sup>2</sup>Research Centre for Endocrinology and Metabolism, Department of Internal Medicine, Göteborg University, Göteborg, Sweden

<sup>3</sup>Department of Clinical Immunology, Göteborg University, Göteborg, Sweden

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PUFA and their metabolites are important regulators of bone formation and resorption. The effect of PUFA on bone growth may be especially striking during the perinatal period. The aim of the present study was to investigate the effect of diets with different *n*-6:*n*-3 fatty acid (FA) ratios during the perinatal period on bone parameters in the adult offspring. During late gestation and throughout lactation, rat dams were fed an isoenergetic diet containing 70 g linseed oil (*n*-3 diet), soyabean oil (*n*-6+*n*-3 diet) or sunflower-seed oil (*n*-6 diet) per kg with *n*-6:*n*-3 FA ratios of 0.4, 9 and 216, respectively. The offspring were weaned onto an ordinary chow and followed until 30 weeks of age. Bone parameters were analysed using peripheral quantitative computerised tomography and dual-energy X-ray absorptiometry. Femur length and cortical cross-sectional bone area and bone mineral content were significantly higher in the *n*-6+*n*-3 group than in the other groups. Cortical bone thickness in the *n*-6+*n*-3 group was increased compared with the *n*-3 group, but most cortical bone parameters did not differ between the *n*-3 and *n*-6 groups. The results suggest that regulatory mechanisms were influenced by the *n*-6:*n*-3 FA ratio early in life and not compensated for by the introduction of an ordinary diet after weaning.

### Diet: Bone mineral density: Fatty acids: Insulin-like growth factor-1

Nutrient supply during critical stages of life such as the fetal and postnatal periods has important effects on the offspring, determining their health status in adulthood (Lucas, 1991, 1998). The mechanisms underlying these effects are supposed to be the programming of a range of metabolic and endocrine parameters via nutritional stimuli during early development. Recently, associations were reported between a number of nutrients in the maternal diet during pregnancy and later bone mass in the children (Jones *et al.* 2000). Maternal protein restriction in rat dams leads to decreased bone area and mineral content and widened epiphyseal growth plates in the offspring in late adulthood (Mehta *et al.* 2002). This implies that the maternal diet might be one factor modifying bone growth and mineralisation in the adult offspring.

The type of dietary lipids plays an important role in bone metabolism and diseases (Watkins *et al.* 2001). The *n*-6:*n*-3 fatty acid (FA) ratio in the diet has a profound influence on bone FA composition and biosynthesis of prostaglandins, which regulate bone formation and resorption (Kokkinos *et al.* 1993; Li *et al.* 1999). Dietary intake of long-chain *n*-3 PUFA in the form of fish oil has been shown to modulate

the *n*-6:*n*-3 FA ratio in tissues, decrease bone prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production and enhance bone formation in chicks and rats (Watkins *et al.* 1996, 1997, 2000) presumably by reducing osteoclastic activity and bone resorption (Iwami-Morimoto *et al.* 1999). Further increase of the dietary intake of (*n*-3) PUFA (10 g fish oil/100 g diet) resulted in reduced bone growth and bone structural properties in growing rabbits (Judex *et al.* 2000), but the dietary *n*-6:*n*-3 FA ratio required for optimising bone growth and bone parameters remains unknown.

The *n*-6:*n*-3 FA ratio in human milk mainly reflects the ratio in the maternal diet and varies considerably in different populations from 3:1 in an Inuit population (Innis & Kuhnlein, 1988) up to 53:1 in some African countries (Rocquelin *et al.* 2001). Whether the *n*-6:*n*-3 FA ratio in the maternal diet causes changes in neonatal bone metabolism, which persist into adulthood and influence bone health later in life, has not been reported.

The aim of the present study was to investigate the long-term effects of diets with different *n*-6:*n*-3 FA ratios during the perinatal period on bone parameters in the adult offspring.

**Abbreviations:** BMC, bone mineral content; BMD, bone mineral density; FA, fatty acid; IGF, insulin-like growth factor; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PL, phospholipid.

\* **Corresponding author:** Dr Marina Korotkova, fax +46 31 21 70 23, email Marina.Korotkova@cmm.ki.se

## Materials and methods

### Animals and diets

Pregnant Sprague–Dawley rats (BK Universal, Stockholm, Sweden) were received on day 7 of gestation and kept in our animal facility under constant conditions of humidity (70–80%), temperature (22–25°C), and light (12 h light–dark cycle). The rats were housed individually in plastic cages with food and water *ad libitum*. At 10 d before delivery the rats were randomly divided into three groups (nine or ten per group) and fed one of three experimental pellet diets (Morinaga Milk Industry Co. Ltd, Tokyo, Japan) during late gestation and throughout lactation. The diets differed only by lipid composition; linseed oil (*n*-3 diet), soyabean oil (*n*-6+*n*-3 diet) or sunflower-seed oil (*n*-6 diet) (each 70 g/kg) with the *n*-6:*n*-3 PUFA ratios of 0.4, 9 and 216, respectively. The composition of the three diets is given in Table 1. To be able to obtain a substantial difference in the *n*-6:*n*-3 FA ratios of the tissue phospholipids (PL) within the short neonatal period in rats, dietary intervention with extreme *n*-6:*n*-3 ratios in the diets was used. However, at 3 weeks of age the *n*-6:*n*-3 FA ratios in the serum PL of the offspring were within the levels found in human diets. The ordinary chow (rat and mouse standard diet; B&K Universal Ltd, Grimston, Aldbrough, Hull, UK) contained (g/kg): protein, 190; fat, 50; crude fibre, 40; ash, 55. The total metabolisable energy of the diets was 14.0 MJ/kg.

Litter size was adjusted to ten pups per litter. Some pups from each litter were studied for the perinatal effects of dietary FA (Korotkova *et al.* 2002). The rest of the pups were studied for possible long-term effects of the perinatal dietary FA. At 3 weeks of age, the serum FA composition

of the offspring was analysed and reflected the dietary intervention for the pups. In the present study, 3 week-old female pups (ten per group), separately randomised from each litter, were weaned onto ordinary chow and followed until 30 weeks of age. Blood samples for FA and insulin-like growth factor (IGF)-1 analysis were collected at 3 and 30 weeks of age. Sera were kept at –20°C. The study was approved by the animal ethics committee of Göteborg University.

### Fatty acid analysis

Total lipids from the serum of the rats were extracted according to Folch *et al.* (1957). Total serum lipids were fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Milford, MA, USA) according to the method described previously (Korotkova *et al.* 2001). The fractions of PL were transmethylated in 3 M-methanolic-HCl at 90°C over 4 h. The FA methyl esters were separated by capillary GLC in a Hewlett-Packard 6890 gas chromatograph equipped with a 30 m × 0.25 mm SP-2380 column; film thickness 20 µm. The carrier gas used was He at 2.0 ml/min. The injector and detector temperatures were 300°C and 250°C, respectively. The column oven temperature was programmed from 50°C to 230°C at a heating rate of 20°C/min up to 180°C, and thereafter 2°C/min. The separation was recorded with HP GC Chem Station software (HP GC, Wilmington, DE, USA). The FA 21:1 was used as the internal standard and the FA methyl esters identified by comparison with retention times of pure reference substances (Sigma Aldrich Sweden AB, Stockholm, Sweden).

### Analysis of insulin-like growth factor-1

Serum IGF-1 levels were measured by double antibody IGF-binding protein-blocked RIA using a commercial kit (Mediagnost, Tubingen, Germany).

### Peripheral quantitative computerised tomography

Computerised tomography was performed with the Stratec pQCT XCT Research M (v5.4B; Norland-Stratec, Fort Atkinson, WI, USA) operating at a resolution of 70 µm as previously described (Windahl *et al.* 1999). Trabecular volumetric bone mineral density (BMD) was determined with a metaphyseal peripheral quantitative computerised tomography scan of the distal femur and defined as the inner 45% of the total area. Cortical volumetric BMD, the cortical cross-sectional area, the periosteal circumference, endosteal circumference, cortical thickness, cross-sectional moment of inertia and cross-sectional moment of resistance were determined with a mid-diaphyseal peripheral quantitative computerised tomography scan of the femur. If the quality of the bone is unchanged then the bone strength as measured by three-point bending is directly proportional to the cross-sectional moment of inertia. Thus, the cross-sectional moment of inertia indicates the bending strength and is dependent on the periosteal and endosteal diameters of bone cross section. Cortical moment of resistance is an indicator of the resistance to

**Table 1.** Composition of the diets (g/kg)

Diet...	<i>n</i> -3 Diet	<i>n</i> -6 + <i>n</i> -3 Diet	<i>n</i> -6 Diet
Casein	200	200	200
Potato starch	540	540	540
Glucose	100	100	100
Cellulosal flour	40	40	40
Mineral mix*	40	40	40
Vitamin mix†	10	10	10
Linseed oil	70	–	–
Soyabean oil	–	70	–
Sunflower-seed oil	–	–	70
Fatty acids (mol %)			
16:0	14	12	8
16:1	0.3	0.1	0.1
18:0	6.1	3.9	4.2
18:1	32	21	22
18:2 <i>n</i> -6	14	56	65
20:0	0.3	0.4	0.3
18:3 <i>n</i> -3	33	6.2	0.3
<i>n</i> -6: <i>n</i> -3	0.4	9	216

\* Salt mixture containing (per 100 g): KH<sub>2</sub>PO<sub>4</sub>, 34.1; CaCO<sub>3</sub>, 35.9; KCl, 2.5; NaCl, 18; MgSO<sub>4</sub>·H<sub>2</sub>O, 5.1; FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·5H<sub>2</sub>O, 3.3; MnO, 0.27; Cu<sub>2</sub>C<sub>6</sub>H<sub>4</sub>O<sub>7</sub>·2.5H<sub>2</sub>O, 0.06; ZnCO<sub>3</sub>, 0.04; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.002; KAl(SO<sub>4</sub>)<sub>2</sub>·2H<sub>2</sub>O, 0.008; NaF, 0.025; KIO<sub>3</sub>, 0.009; Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, 0.002; Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O, 0.001; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.001.

† Vitamin mixture containing (per g): vitamin A, 3.57 µg; vitamin D<sub>3</sub>, 0.0375 µg; vitamin B<sub>1</sub>, 4 µg; vitamin B<sub>2</sub>, 12 µg; vitamin B<sub>6</sub>, 5 µg; calcium pantothenate 45%, 11 µg; niacin, 40 µg; vitamin B<sub>12</sub>, 0.02 µg; vitamin K<sub>3</sub>, 7.75 µg; biotin 2%, 3 µg; vitamin C, 500 µg; inositol, 30 µg; vitamin E, 42 µg; choline chloride 50%, 1 mg; folic acid, 0.5 µg.

torsion as calculated from the outer dimensions of the bone cross section.

#### Dual-energy X-ray absorptiometry

Bone mineral content (BMC), area and areal BMD (BMC per cm<sup>2</sup>) were measured with the pDEXA Sabre and Sabre Research software (both from Norland Medical Systems Inc., Fort Atkinson, WI, USA). *Ex vivo* measurements of femurs were performed on excised bones placed on a 10 mm-thick Plexiglas table (Vidal *et al.* 2000).

#### Statistical analysis

Results are presented as mean values with their standard errors. The data were analysed by one-way ANOVA followed by Fisher's *post hoc* protected least significant difference test. A *P* value of <0.05 was considered to be statistically significant.

## Results

#### Fatty acid composition of serum phospholipids

There were significant changes in the FA composition of serum PL in the offspring at 3 weeks of age receiving the different diets reflecting the dietary FA composition. The *n*-6:*n*-3 FA ratio in serum PL reflected the mothers' diet (Table 2). After feeding ordinary chow from weaning to 30 weeks of age there was no difference in the FA composition of the serum PL in the rats from the different dietary groups (data not shown). The *n*-6:*n*-3 FA ratio in

the serum PL of adult rats was 5.2 (SE 0.1), 5.6 (SE 0.2) and 5.6 (SE 0.3) in the *n*-3, the *n*-6+*n*-3 and the *n*-6 groups, respectively.

#### Body weight

At 3 weeks of age, the mean body weight of the offspring of the dams fed the *n*-6+*n*-3 diet was significantly higher (*P*<0.05) than that of the offspring of the dams fed the *n*-3, or the *n*-6 diets; the offspring of the dams fed the *n*-6 diet also had a significantly higher mean body weight than those of the dams fed the *n*-3 diet (53.7 (SE 1.3), 41.1 (SE 0.9) and 46.5 (SE 0.9) g for the *n*-6+*n*-3, *n*-3 and the *n*-6 diets, respectively). Still, at 30 weeks of age the mean body weight of the offspring of the dams fed the *n*-6+*n*-3 diet was significantly higher (*P*<0.05) than that of both the offspring of the dams fed the *n*-3, or the *n*-6 diets (379.2 (SE 4.9) v. 347.5 (SE 6.5) and 323.6 (SE 7.3) g, respectively). In contrast to 3 weeks of age, the offspring of the dams fed the *n*-6 diet had the lowest mean body weight at 30 weeks of age, lower than the mean body weights of both the offspring of the dams fed the *n*-6+*n*-3 and those fed the *n*-3 diet.

#### Analysis of insulin-like growth factor-1

The serum IGF-1 levels were significantly reduced in the 3-week-old offspring of the dams fed the *n*-3 diet compared with those fed the *n*-6+*n*-3 and the *n*-6 diets being 99 (SE 9), 156 (SE 12) and 137 (SE 16) ng/ml, respectively (*P*<0.05). The IGF-1 levels in the *n*-3+*n*-6 group were not

**Table 2.** Fatty acid composition of serum phospholipids of the rat pups at 3 weeks of age from dams fed diets with different *n*-6:*n*-3 fatty acid ratios\*

(Mean values with their standard errors)

Diet group ... Fatty acids (mol %)	<i>n</i> -3 ( <i>n</i> 10)		<i>n</i> -6 + <i>n</i> -3 ( <i>n</i> 10)		<i>n</i> -6 ( <i>n</i> 10)	
	Mean	SE	Mean	SE	Mean	SE
12:0	1.7	0.2	1.3	0.1	1.3	0.2
14:0	2.9 <sup>a</sup>	0.2	2.2 <sup>b</sup>	0.1	2.1 <sup>b</sup>	0.2
16:0	28.2 <sup>a</sup>	1.0	24.3 <sup>b</sup>	0.4	24.8 <sup>b</sup>	0.4
16:1 <i>n</i> -7	0.25 <sup>a</sup>	0.02	0.17 <sup>b</sup>	0.01	0.14 <sup>b</sup>	0.01
18:0	20.0	0.4	19.4	0.3	20.0	0.3
18:1 <i>n</i> -9	5.1 <sup>a</sup>	0.2	3.8 <sup>b</sup>	0.1	3.8 <sup>b</sup>	0.2
18:2 <i>n</i> -6	22.6 <sup>a</sup>	1.8	28.1 <sup>b</sup>	0.5	28.5 <sup>b</sup>	0.8
18:3 <i>n</i> -6	0.08 <sup>a</sup>	0.02	0.08 <sup>a</sup>	0.01	0.13 <sup>b</sup>	0.01
20:0	0.15 <sup>a</sup>	0.01	0.19 <sup>b</sup>	0.01	0.17 <sup>c</sup>	0.01
18:3 <i>n</i> -3	3.3 <sup>a</sup>	0.4	0.44 <sup>b</sup>	0.03	0.05 <sup>c</sup>	0.01
20:2 <i>n</i> -6	0.22 <sup>a</sup>	0.01	0.47 <sup>b</sup>	0.01	0.41 <sup>c</sup>	0.01
22:0	0.27 <sup>a</sup>	0.03	0.33 <sup>b</sup>	0.01	0.35 <sup>b</sup>	0.02
20:4 <i>n</i> -6	7.7 <sup>a</sup>	0.7	13.4 <sup>b</sup>	0.3	14.4 <sup>b</sup>	0.5
24:1 <i>n</i> -9	0.79 <sup>a</sup>	0.2	0.45 <sup>b</sup>	0.02	0.40 <sup>b</sup>	0.02
22:6 <i>n</i> -3	5.8 <sup>a</sup>	0.7	4.6 <sup>b</sup>	0.1	2.6 <sup>c</sup>	0.2
<i>n</i> -6: <i>n</i> -3	3.5 <sup>a</sup>	0.8	8.4 <sup>b</sup>	0.7	17.3 <sup>c</sup>	4.4
20:4 <i>n</i> -6:22:6 <i>n</i> -3	1.4 <sup>a</sup>	0.1	2.9 <sup>b</sup>	0.1	5.8 <sup>c</sup>	0.5
SFA	54.1 <sup>a</sup>	0.6	48.5 <sup>b</sup>	0.3	49.6 <sup>b</sup>	0.4
MUFA	6.1 <sup>a</sup>	0.3	4.4 <sup>b</sup>	0.1	4.3 <sup>b</sup>	0.2
PUFA	39.8 <sup>a</sup>	0.9	47.1 <sup>b</sup>	0.2	46.1 <sup>b</sup>	0.4
USI	2.4 <sup>a</sup>	0.03	3.0 <sup>b</sup>	0.03	2.7 <sup>c</sup>	0.02

SFA, saturated fatty acids; USI, unsaturation index (ratio  $\Sigma$ (mol % each unsaturated fatty acid  $\times$  number of double bonds of the same fatty acid)/SFA).

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different (ANOVA) (*P*<0.05).

\* For details of diets and procedures, see Table 1 and p. 644.

significantly different from those in the *n*-6 group. In the adult rats the serum IGF-1 levels were not significantly different between the groups, being 597 (SE 22), 638 (SE 22) and 590 (SE 25) ng/ml in the *n*-3, the *n*-6+*n*-3 and in the *n*-6 groups, respectively.

#### Bone size and mineral status

At 30 weeks of age the femur length was significantly increased ( $P < 0.05$ ) in adult rats from the *n*-6+*n*-3 group compared with those from the other dietary groups (Table 3). Peripheral quantitative computerised tomography analysis showed that the trabecular volumetric BMD in the metaphysis of the femur was not significantly different in the adult rats from the different diet groups. Analysis of the cortical bone parameters of the femur showed that the mineral content and area but not mineral density were significantly increased ( $P < 0.05$ ) in the *n*-6+*n*-3 group compared with the other diet groups. The increased cortical cross-sectional area in the *n*-6+*n*-3 group compared with the *n*-3 group was associated with an increased cortical thickness. In the *n*-6 group the cortical periosteal circumference, as well as the cortical endosteal circumference, was significantly reduced compared with the *n*-6+*n*-3 group ( $P < 0.05$ ), resulting in no significant changes in cortical thickness between these two groups. The changes in the cortical content and the cortical cross-sectional bone area resulted in a decrease of the cortical cross-sectional moment of inertia and the cortical cross-sectional moment of resistance, suggesting lower strength of the cortical bone in the *n*-6 than in the *n*-6+*n*-3 group. In addition, dual-energy X-ray absorptiometry analyses of femur similarly showed that BMC was significantly increased ( $P < 0.05$ ) in the *n*-6+*n*-3 group compared with that in the *n*-3 and *n*-6 groups (0.515 (SE 0.010) v. 0.475 (SE 0.01) and 0.448 (SE 0.010) g, respectively). This increase was associated with an enhanced bone area in the *n*-6+*n*-3 group (2.646 (SE 0.038) v. 2.411 (SE 0.038) and 2.33 (SE 0.029) cm<sup>2</sup> in the *n*-3 and *n*-6 group, respectively), while BMD was not different between the groups (data not shown).

#### Discussion

The dietary *n*-6:*n*-3 FA ratio during the perinatal period had significant long-term effects on cortical bone parameters in the adult rats. The normalisation of serum PL FA composition in the adult rats after the introduction of an ordinary diet suggested that permanent changes in bone status were initiated early in life and persisted into adulthood.

It has been shown that the dietary balance of *n*-6 and *n*-3 FA plays an important role in bone growth and metabolism due to a modulation of eicosanoid and growth factors production (Kokkinos *et al.* 1993; Watkins *et al.* 2001). Prostaglandins and leucotrienes participate in the local control of bone metabolism (Raisz & Fall, 1990; Takiguchi *et al.* 1999). PGE<sub>2</sub>, an important product of arachidonic (20:4*n*-6) acid, is the major prostaglandin affecting bone metabolism. Both bone formation and bone resorption are influenced by PGE<sub>2</sub> and its effect on bone is dose dependent. At low levels, PGE<sub>2</sub> enhances bone formation by osteoblasts, while at higher levels PGE<sub>2</sub> suppresses osteoblast differentiation (Takiguchi *et al.* 1999) and promotes bone resorption by osteoclasts (Okada *et al.* 2000). Eicosanoids derived from *n*-3 PUFA have much less biological potency than those derived from arachidonic acid. In addition, *n*-3 PUFA are potent inhibitors of cyclo-oxygenase (Knapp, 1990). Thus, the *n*-6:*n*-3 ratio defines the net balance of eicosanoids derived from *n*-6 and *n*-3 PUFA and the biological response elicited after eicosanoid release. Dietary reduction of (*n*-6) PUFA has been associated with lower PGE<sub>2</sub> synthesis and increased bone formation in growing rats (Watkins *et al.* 2000), decreased loss of bone weight and strength in ovariectomised adult rats (Sakaguchi *et al.* 1994) and reduced osteoclastic activity (Iwami-Morimoto *et al.* 1999). However, it has not previously been shown whether the balance between *n*-6 and *n*-3 FA in the perinatal period might influence bone growth and mineralisation in adult rats.

In the present study the femoral length and cortical bone content were increased in the adult offspring of the dams fed the *n*-6+*n*-3 diet compared with the *n*-6 group. Increased cortical bone content was accompanied by an

**Table 3.** Trabecular volumetric bone mineral density (BMD) and cortical bone parameters of the femur of the female adult rats fed diets with different *n*-6:*n*-3 fatty acid ratios during the perinatal period\* (Mean values with their standard errors)

Diet group...	<i>n</i> -3		<i>n</i> -6 + <i>n</i> -3		<i>n</i> -6	
	Mean	SE	Mean	SE	Mean	SE
Trabecular BMD (mg/mm <sup>3</sup> )	0.51	0.04	0.47	0.02	0.48	0.01
Femur length (mm)	38.1 <sup>a</sup>	0.3	39.3 <sup>b</sup>	0.2	37.6 <sup>a</sup>	0.1
Cortical BMC (mg/mm)	10.2 <sup>a</sup>	0.3	10.9 <sup>b</sup>	0.2	10.0 <sup>a</sup>	0.2
Cortical BMD (mg/mm <sup>3</sup> )	1.47 <sup>a</sup>	0.00	1.47 <sup>ab</sup>	0.00	1.48 <sup>b</sup>	0.00
Cortical bone area (mm <sup>2</sup> )	6.92 <sup>a</sup>	0.2	7.39 <sup>b</sup>	0.1	6.74 <sup>a</sup>	0.1
Cortical bone thickness (mm)	0.74 <sup>a</sup>	0.01	0.77 <sup>b</sup>	0.01	0.75 <sup>ab</sup>	0.01
Cortical periosteal circumference (mm)	11.6 <sup>ab</sup>	0.2	12.0 <sup>b</sup>	0.1	11.3 <sup>a</sup>	0.1
Cortical endosteal circumference (mm)	6.9 <sup>b</sup>	0.1	7.2 <sup>b</sup>	0.1	6.6 <sup>a</sup>	0.1
Cortical cross-sectional moment of inertia (mm <sup>4</sup> )	19.3 <sup>ab</sup>	1.2	21.9 <sup>b</sup>	0.8	17.4 <sup>a</sup>	0.7
Cortical moment of resistance (mm <sup>3</sup> )	8.6 <sup>ab</sup>	0.5	9.4 <sup>b</sup>	0.3	8.0 <sup>a</sup>	0.3

BMC, bone mineral content.

<sup>a,b</sup> Mean values within a row with unlike superscript letters were significantly different (ANOVA) ( $P < 0.05$ ).

\* For details of diets and procedures, see Table 1 and p. 644.

enhanced cortical cross-sectional bone area. Increased cortical bone radial growth, as well as indicators of increased bone strength, was observed in the *n-6+n-3* group. The serum FA compositions in the *n-6* and the *n-6+n-3* groups were very similar, except for the levels of *n-6* and *n-3* FA, whereas the bone parameters were significantly different. The *n-6+n-3* diet contained similar amounts of *n-6* FA but more *n-3* FA compared with the *n-6* diet. The more balanced dietary intake of *n-6* FA in the perinatal period might have reduced the production of PGE<sub>2</sub> and leucotriene B<sub>4</sub> in bone tissue and increased the cortical bone formation, which persisted into adulthood. The mechanism, however, could not be suggested from the present study.

It is less probable that the lower total amount of PUFA in the diet of the *n-3* group was responsible for the lower bone parameters compared with the *n-6+n-3* group. Similar bone parameters were seen in the *n-6* group, which did not differ from the *n-6+n-3* group on the composition of saturated FA and MUFA in the diets. Further increase in the level of *n-3* FA in the perinatal diet resulted in decreased femoral length and cortical cross-sectional bone content, area and thickness in rats compared with those in the *n-6+n-3* group. The inhibitory effect of a higher level of fish oil supplementation on cortical bone morphology and biomechanics has been observed in growing rabbits, though the FA composition of the diets was not analysed in that study (Judex *et al.* 2000). The effect of a high intake of *n-3* FA on cortical bone mass may include direct effects on the bone tissue via eicosanoids or indirect effects such as a regulation of systemic hormones. It has been shown that PGE<sub>2</sub> induces IGF-1 synthesis by rat osteoblasts *in vitro* (McCarthy *et al.* 1991). Indeed, the levels of arachidonic acid in serum PL, the substrate for eicosanoid production, and serum IGF-1 levels were significantly lower in 3-week-old offspring of the dams fed the *n-3* diet, compared with those in the other dietary groups. IGF-1 plays important roles in longitudinal bone growth and the maintenance of bone mass (Ohlsson *et al.* 1998). Reduction in serum IGF-1 levels has been associated with decreased cortical bone growth and decreased BMD in human subjects and animals (Moreira-Andres *et al.* 1995; Chlebna-Sokol & Rusinska, 2001; Sjogren *et al.* 2002). Additionally, we have recently shown serum leptin levels to be reduced in the offspring of dams fed the *n-3* diet compared with those in the offspring of dams fed the *n-6+n-3* diet (Korotkova *et al.* 2002). Leptin, an adipose-derived hormone, directly affects bone growth inducing osteoblastic cell proliferation, *de novo* collagen synthesis, and mineralisation (Gordeladze *et al.* 2002) and leptin deficiency in *ob/ob* mice is associated with decreased bone growth (Steppan *et al.* 2000). These data suggest that lower levels of IGF-1 and leptin in early life might have resulted in decreased bone growth in the *n-3* group, which persisted into adulthood.

At 30 weeks of age, body weight was higher in the rats from the *n-6+n-3* group than in any of the other dietary groups. It is possible that the different body weight of the offspring caused the differences in bone parameters. Therefore, the *n-6:n-3* PUFA ratio in the maternal diet

might have had an impact on bone parameters indirectly by influencing body weight.

In conclusion, variations in *n-6:n-3* FA ratio in the maternal diet during the perinatal period caused changes in bone growth in adult rats, indicating that regulatory mechanisms have been programmed early in life and were not compensated for by the introduction of a standard diet after weaning. The precise mechanisms by which the *n-6:n-3* FA ratio in the perinatal diet has long-term effects on skeletal growth remain to be investigated.

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