Original Research

Maternal beef and postweaning herring diets increase bone mineral density and strength in mouse offspring

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Abstract

The maternal diet during gestation and lactation affects the long-term health of the offspring. We sought to determine whether maternal and postweaning crossover isocaloric diets based on fish or meat affect the geometry, mineral density, and biomechanical properties of bone in mouse offspring in adulthood. During gestation and lactation, C57BL/6 dams were fed a herring- or beef-based diet. After weaning, half of the pups in each group were fed the same diet as their dams, and half were fed the other diet. Areal bone mineral density (aBMD) and bone mineral content (BMC) of the whole body and lumbar spine were measured in the offspring by dual X-ray absorptiometry at 9 and 21 weeks of age. At 22–26 weeks, tibia bone geometry (length, cortical volumetric (v) BMD, BMC, area and thickness) was analyzed by peripheral quantitative computed tomography, and the biomechanical properties of the tibia were analyzed by the three-point bending test. Plasma insulin-like growth factor-1 was analyzed at 12 weeks. In comparison to the maternal herring diet, the maternal beef diet increased aBMD and BMC in the whole body and lumbar spine of adult offspring, as well as cortical vBMD, BMC, bone area, and thickness at the mid-diaphyseal region of the tibia and the biomechanical properties of tibia strength. In contrast, a postweaning beef diet decreased aBMD in the lumbar spine and BMC in the whole body and lumbar spine compared with a postweaning herring diet, which instead increased plasma insulin-like growth factor-1 levels. The change from a maternal beef diet before weaning to a herring diet after weaning decreased body weight and increased the cortical area, vBMD, BMC, thickness, and strength of the tibia. These significant crossover effects indicate that a preweaning maternal beef diet and a postweaning herring diet are optimal for increasing BMC and bone strength in offspring in adulthood.

Keywords: Maternal and postweaning diet, red meat, fatty fish, bone mineral density and content

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Introduction

Associations between birth weight and cardiovascular disease and diabetes later in life led to the developmental origins of health and disease hypothesis; that adverse environmental influences at a critical stage of early development lead to persisting changes in structure and function.[1] Increasing evidence that perinatal programming influences disease risk in humans has prompted interest into understanding how the *in utero* and postnatal environments, including the maternal diet, influence skeletal development and maintenance in adulthood. The rapid rate of bone mineral accrual and skeletal plasticity during intrauterine and early postnatal life make this period a potential window into the external exposures that have consequences in adult life.

Bone mineral growth is determined by environmental factors during childhood and puberty.[2] Calcium homeostasis and the vitamin D status of the mother are linked to childhood areal bone mineral density (aBMD) in the whole body and spine and to bone mineral content (BMC), independent of parental height.[3,4] Although the roles of vitamin D and calcium in bone development have been widely studied, the importance of dietary fatty acids (FAs) has only emerged as an interesting area of research in the last decade or so.[5]

The FA content of Western diets mainly consists of saturated and n-6 polyunsaturated fatty acids (PUFAs) with a low content of n-3 PUFAs. A high dietary n-6/n-3 ratio is associated with the loss of bone minerals in animals[6] and humans,[7] while a high fish intake, rich in n-3 PUFAs, inhibits bone mineral loss.[8]

Few studies have investigated how variations in PUFAs in the maternal diet during pregnancy affect bone mineralization during development and in adult offspring. In rodents, a maternal diet with a high n-6/n-3 ratio during late gestation and lactation increases femur length, cortical thickness, and cortical cross-sectional area in adult progeny.[9] In humans, PUFA levels in cord and maternal blood predict whole body and spine BMC at birth,[10] and elevations in the n-6 FA concentration and the n-6/n-3 ratio generally have a negative effect on bone mineralization in young children.[11] However, the effects of variation in maternal PUFA levels on bone mineral development in the perinatal period are not known. It seems likely that changes or imbalances in early exposure to n-6 and n-3 PUFAs influence bone development in early childhood and have persistent effects in later life.

In many studies, the maternal diet has been considered in terms of the intake of specific nutrients, but these are, of course, parts of broader dietary patterns. A recent study showed that women who consume a ''healthy'' diet in pregnancy, with a low intake of sugar and fat, have offspring with greater whole body and lumbar spine aBMD and larger bones than the offspring of women who consume more processed foods.[12] This finding was independent of socioeconomic factors, maternal height, smoking, and vitamin D status, as well as childhood weight, height, and exercise. Also, maternal dietary fat intake in late pregnancy is inversely related to aBMD of the lumbar spine and femoral neck in adolescent children.[13]

Thus, during pregnancy, exposure to high-fat diets with alterations in FAs seems to have both negative and positive effects on cortical and trabecular bone architecture. However, it is unclear how different diets affect intrauterine and postnatal skeletal development. Furthermore, other factors might influence the effects of perinatal diets on skeletal development, including the insulin-like growth factor (IGF-1)/growth hormone axis and leptin.[14,15]

In this study, we used a crossover design to investigate how the preweaning maternal diet and the postweaning diet of the offspring affect the development of cortical and trabecular bone geometry and the mineral density and biomechanical properties of bone in adult mice. Specifically, we studied the effects of a beef diet rich in n-6 PUFAs and a herring diet rich in n-3 PUFAs.

Materials and methods

Animals and study design

Fifty-six female C57 BL/6 mice (Charles River, Germany), aged eight weeks, were acclimatized for one week before the experiment. The mice were housed at 21°C with a 12-h light/12-h dark cycle. The experiment included a crossover diet intervention. On the second day of breeding, the dams were randomly separated into two diet groups; one was fed a beef-based diet and the other a herring-based diet (*Clupea harengus*). Within one week after delivery, pups were redistributed so that each experimental group was approximately equal in the gender and number of pups. After weaning at three weeks of age, half of the male offspring in each diet group were switched to the other diet leading to four postweaning diet groups: (1) herring-based maternal diet and herring-based postweaning diet (HH), (2) herring-based maternal diet and beef-based postweaning diet (HB), (3) beef-based maternal diet and beef-based postweaning diet (BB), and (4) beef-based maternal diet and herring-based postweaning diet (BH). The offspring were sacrificed at 22–26 weeks of age. The experiments were approved by the ethical committee for animal research at the University of Gothenburg.

Experimental diets

The diets consisted of 205 g of casein (catalogue number 160030, Harlan Laboratories, Madison, WI, USA), 115 g of wheat flour (Kungsörnen, Järna, Sweden), 90 g of butter (Arla, Stockholm, Sweden), 90 g of white sugar (Dansukker,

Table 1 Fatty acid composition of the diets analyzed by GC-MS

	% of total fatty acids (mg/g)		
Fatty acids	**Herring-based diet**	**Beef-based diet**	***P***
Saturated fatty acids			
12:0	3.0 ± 0.01	2.3 ± 0.1	<0.05
14:0	13.8 ± 0.2	9.5 ± 0.1	<0.05
15:0	nd	Nd	–
16:0	34.2 ± 1.0	33.7 ± 0.2	ns
17:0	nd	Nd	–
18:0	9.2 ± 0.2	17.8 ± 0.0	<0.001
20:0	nd	nd	–
Monounsaturated fatty acids			
14:1n-5	nd	nd	–
16:1n-7	2.7 ± 0.2	1.3 ± 0.3	<0.05
17:1n-7	nd	nd	–
18:1n-9	22.9 ± 0.4	32.3 ± 0.5	<0.001
18:1n-7	1.1 ± 0.4	nd	<0.05
20:1n-9	4.9 ± 0.2	nd	<0.05
Polyunsaturated fatty acids			
LA; 18:2n-6	2.7 ± 0.4	3.0 ± 0.2	ns
ALA; 18:3n-3	nd	nd	–
DGLA; 20:3n-6	nd	nd	–
ARA; 20:4n-6	nd	nd	–
AA; 22:4n-6	nd	nd	–
EPA; 20:5n-3	1.6 ± 0.4	0	<0.05
DPA; 22:5n-3	nd	d	–
DHA; 22:6n-3	3.9 ± 0.6	nd	<0.05
n-6/n-3 ratio	0.4 ± 0.03	12.9 ± 0.1	<0.001
Unsaturated/saturated ratio	0.3 ± 0.01	1.7 ± 0.04	<0.001

nd: not detectable; ns: not significant. Values are mean ± SEM. Three samples were analyzed for each diet. Differences were analyzed by Student's *t* test.

Malmö, Sweden), 50 g of mineral mix (catalogue number AIN-76, Harlan Laboratories, Madison, WI, USA), 50 g cellulose (Harlan Laboratories), and 20 g of vitamin mix (catalogue number 40060, Harlan Laboratories), which was formed into a dough, mixed with 500 g of minced herring or beef (from the local fish market or butcher [Gothenburg, Sweden], stored in 500 g portions at −80°C), shaped into regular pellets, baked for 3–6 min at 1000 W in a microwave oven, and frozen at −80°C. Every other day, a portion of food was defrosted and provided to the mice *ad libitum*. The test diets were isocaloric (~300 kcal/100 g) and had equal percentages of energy from protein, fat, and carbohydrates. The diet composition was a result of pilot studies that led to a healthy breeding process. The herring-diet had 10.0 g/100 g fat (30.3 E%), 26.8 g/100 g protein (36.0 E%), and 25.0 g/100 g carbohydrates (33.7 E%). The beef diet had 10.7 g/100 g fat (32.0 E%), 28.3 g/100 g protein (38 E%), and 22.4 g/100 g carbohydrates (30.0 E%). Food intake was measured in an additional study in which mice were fed either herring or beef throughout their life (during gestation and the postweaning period). These food intake measurements showed no difference in energy intake between the diet groups (results not shown).

The total FA contents in the two diets were analyzed by GC-MS, using a method slightly modified from that of Khoomrung et al.[16] The diet samples were mixed with 100 μg of an internal standard (nonadecanoic acid), 4 mL of hexane, and 2 mL of 14% boron trifluoride (in MeOH) in a tube and flushed with nitrogen for 30 sec. The tube was heated with a microwave digestion system (Milestone Start D, Sorisole Bergamo, Italy) equipped with rotor PRO-24. The temperature was increased from room temperature to 120°C within 6 min and maintained for 10 min. After the tube had cooled to room temperature, 2 mL of MilliQ water was added. The tube was shaken vigorously for 1 min and centrifuged at 1328 *g* for 5 min. The upper phase containing FA methyl esters (FAMEs) was analyzed by GC-MS. Unknown FAMEs from the samples were identified by comparing their mass spectrum profiles and retention times with commercial standards (Sigma-Aldrich, St. Louis, MO, USA). The data processing was performed with Xcalibur software (version 2.0, Thermo Fisher Scientific). Individual FAs were quantified as mg/g sample dry weight; FA composition was then calculated and plotted in a Weibull scale (Sigma Plot software) together with the n-6/n-3 ratio.

The compositions and FA profiles of the diets are shown in Table 1. The proportions of palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1n-9) were highest in the beef diet. The herring diet contained a higher proportion of n-3 PUFAs and a higher content of ALA (18:3n-3), EPA (20:5n-3), DPA (22:5n-3), and DHA (22:6n-3) than the beef diet. As expected, the herring diet contained a higher proportion of long-chain n-3 PUFAs than the beef diet. The n-6/n-3 ratio was 13 in the beef diet and 0.4 in the herring diet.

Body composition, bone geometry, and mineral density

At 9 and 21 weeks of age, aBMD and BMC of the headless whole body was measured by dual-energy X-ray absorptiometry (DEXA) (Lunar PIXImus2 GE Lunar; Madison, WI, USA) with PIXImus software version 2.0. The same software also measured aBMD in the lumbar spine (L2–L5). Before DEXA measurement, the mice were anesthetized with isoflurane (2% in 1:1 mixture of oxygen and air; Abbott Scandinavia, Solna, Sweden). Data on body composition (% of fat and lean body mass) was also collected from the DEXA measurements. After sacrifice, the left tibia was collected, dissected, and stored in ethanol (70%). The tibias were later scanned by peripheral quantitative computed tomography (pQCT; XCT Research M, version 4.5B, Norland, Fort Atkinson, WI, USA), at a resolution of 70 μm, to assess trabecular and cortical bone parameters ex vivo as previously described.[17,18] Trabecular volumetric BMD (vBMD) of the proximal tibia was determined by pQCT scan of the proximal tibia. The scan encompassed the metaphysis distal to the proximal growth plate corresponding to 3% of the total length of the tibia, and the trabecular bone region was defined as the inner 45% of the total cross-sectional area. The vBMD, BMC, area, and thickness of cortical bone were determined by pQCT scan of the mid-diaphyseal region of the tibia. Tibia length was measured to the closest 0.1 mm.

Bone biomechanical properties

The biomechanics of the tibia were tested with the universal mechanical testing machine (Instron 3366, Bluehill 2 software, version 2.6; Instron, Norwood, MA, USA). In the three-point bending test, the span length of the supporter was 5.5 mm and the bones were loaded at a rate of 0.155 mm/sec until failure. Biomechanical measurements were acquired from the load-deformation curves recorded by the software.

Plasma IGF-1 and leptin

For measurement of plasma IGF-1 and leptin, tail vein blood samples were drawn at 12 weeks of age into tubes containing EDTA, snap-frozen in liquid nitrogen, and stored at −80°C. Leptin was analyzed with the mouse Leptin Quantikine ELISA Kit, and IGF-1 was analyzed with the IGF-1 Quantikine ELISA Kit (both from R&D Systems, Minneapolis, MN, USA).

Statistical analyses

SPSS version 21 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The results are presented as means ± SEM. The FA compositions of the two diets were compared by Student's *t* test. The effects of the preweaning and postweaning diets and potential interactions between them were analyzed by two-way ANOVA. Differences were considered significant at $P < 0.05$.

Results

Body weight and body composition

The interaction between the maternal and postweaning offspring diets showed that the effect of the postweaning diet on body weight at 9 and 21 weeks of age was dependent of

maternal diet, i.e. body weight was higher in offspring fed the same diet as their dams (BB and HH) than in the crossover groups (BH and HB) (Table 2). Neither the maternal diet nor the postweaning diet affected body composition (fat percentage) at 21 weeks of age as measured by DEXA (data not shown).

Bone geometry and mineral density

At 9 and 21 weeks of age, aBMD and BMC in the whole body and lumbar spine were higher in the maternal beef diet groups (BB and BH) than in the maternal herring diet groups (HH and HB) (Table 2). However, at 21 weeks, offspring fed a postweaning beef diet (BB and HB) had a lower whole-body BMC and lower aBMD and BMC in the lumbar spine than offspring fed a postweaning herring diet (BH and HH).

Tibia length was similar in all groups. Compared to the maternal herring diet, the maternal beef diet induced higher cortical vBMD, BMC, thickness, and area, as well as polar moment of inertia and resistance in the tibia in adult offspring (22–26 weeks of age). Analysis of the interaction between the maternal and postweaning diets revealed that the postweaning diet had opposite effects in the maternal diet groups. Postweaning, herring diet induced higher cortical bone geometry and mineral density in the maternal beef diet group, but lower values in the maternal herring diet group.

Bone biomechanical properties

The maximal breaking load [F(max)] of the tibia was higher in the maternal beef diet groups (BB, BH) than in the maternal herring groups (HH, HB) (Table 2). Stiffness and F(max) of the tibia were higher in the crossover diet groups (HB and BH) than in groups remaining on their dams' diet.

IGF-1 and leptin

The postweaning herring diet induced higher levels of plasma IGF-1 at 12 weeks of age (HH: 405.8 ± 25.3 ng/mL, $n = 8$; BH: 410.5 ± 24.7 ng/mL, $n = 8$) than the postweaning beef diet (BB: 381.7 ± 13.8 ng/mL, $n = 8$; HB: 303.5 ± 17.7 ng/mL, $n = 10$) ($P < 0.01$). Plasma leptin at 12 weeks of age did not differ between the groups (results not shown).

Discussion

The main findings of this study show that, in comparison to a maternal herring diet, a maternal beef diet before weaning increases aBMD and BMC in the whole body and lumbar spine in the offspring, as well as cortical vBMD, BMC, bone area, and thickness of the proximal tibia and the biomechanical properties of tibia strength. In contrast, a postweaning beef diet decreased serum IGF-1, aBMD in the lumbar spine, and BMC in the whole body and lumbar spine compared with a postweaning herring diet. Mice exposed to a preweaning maternal beef diet and switched to a postweaning herring diet had higher tibia cortical vBMD, BMC, and thickness than mice exposed to a maternal herring diet.

The diets were isocaloric, had the same proportion of protein, carbohydrate and fat, but based on herring or beef, were Westernized with a high content of fat and sucrose, were given ad libitum, and provided complete nutrition. Therefore, the differences in bone properties between the two diets are thought to be mainly induced by the fish or meat in the preweaning diet, the postweaning diet, or both.

Effects of the maternal diets on offspring bone development

In comparison to the maternal herring diet, the maternal beef diet, rich in n-6 PUFAs, increased aBMD and BMC in the whole body and lumbar spine, cortical vBMD, BMC, bone area, thickness, and F(max) of the tibia in adult offspring. Previous studies of the effects of maternal diets on bone development in the offspring have mostly focused on general caloric restriction and macronutrient imbalances, demonstrating that perturbations of maternal intake during gestation have lasting effects on bone mass and bone geometry in the offspring.[19–21] In a recent study, artificial rat milk in which n-3 was replaced by n-6 PUFAs alpha-linoleic acid (ALA; C18:3n-3) and supplemented with docosahexaenoic acid (DHA, 22:6n-3) or docosapentaenoic acid (DPA, C22:5n-3)[22] was given to newborn pups. After weaning, the pups were fed a diet with a similar total dietary fat content (10%) and followed to 15 weeks of age. The findings showed that DPA n-6 could not replace DHA, which seems to be critical for the development of the periosteum and marrow of healthy bone.[22] Both total n-3 PUFAs and DHA strongly correlated with maximal BMC and BMD in the femur, indicating a very important role in bone health.

The study was technically impressive, but the results are difficult to compare with ours, as the rats were exposed prenatally to a maternal diet containing adequate concentrations of n-3 PUFAs, fed a low-fat diet, and were not followed to the time of peak bone growth. The same group previously showed that there seems to be a compensatory response with accelerated bone modeling and improved biomechanical properties of the long bones (femur and tibia) in rats made n-3 PUFA deficient during perinatal life and then given a diet with adequate n-3 levels from 7 to 12 weeks of age.[23] After 1 week of the n-3-adequate diet, the n-6/n-3 ratio decreased markedly in both tibia and femur cortical bone. These findings point to a surprisingly rapid bone metabolism of FAs but suggest that the body places a high priority on restoring deficient n-3 PUFAs to normal levels, indicating their importance for bone strength and functionality.

In our study, the mice had a relative deficiency of n-3 or n-6 PUFAs, provided in a diet comparable to a Westernized human diet based on fish or meat. It seems that the bone metabolism of FAs also can compensate for this imbalance by improving BMC and bone growth and strength when the mice are switched from one diet to another and probably restores the levels of FAs required for optimal bone development.

Table 2 Crossover effects of maternal preweaning and postweaning diets on bone minerals (whole body, lumbar spine, and tibia), bone geometry (tibia) and biomechanical properties (tibia) by DEXA, pQCT, and biomechanical testing

Maternal preweaning diet	Herring		Beef		Effects					
Offspring postweaning diet	Herring (n = 9–10)	Beef (n = 11)	Herring (n = 6–8)	Beef (n = 7–9)	Maternal diet Main effect Beef-herring	*P*	Postweaning diet Main effect beef-herring	*P*	Maternal x Postweaning diet	*P*
Bone minerals (DEXA, week 9)										
Whole body aBMD (g/cm^2)	0.047 ± 0.001	0.045 ± 0.001	0.049 ± 0.000	0.048 ± 0.004	0.003	<0.01	−0.001	ns	0.003	ns
Whole body BMC (g)	0.406 ± 0.016	0.368 ± 0.012	0.408 ± 0.012	0.423 ± 0.011	0.030	<0.05	−0.012	ns	0.054	ns
Lumbar spine aBMD (g/cm^2)	0.055 ± 0.002	0.047 ± 0.001	0.057 ± 0.002	0.0566 ± 0.001	0.003	<0.01	−0.001	ns	0.003	ns
Lumbar spine BMC (g)	0.031 ± 0.001	0.0248 ± 0.001	0.033 ± 0.003	0.031 ± 0.001	0.030	<0.05	−0.012	ns	0.054	ns
Body weight at 9 weeks (g)	26.8 ± 0.6	24.2 ± 0.6	24.8 ± 0.5	26.0 ± 0.5	−0.238	ns	−0.745	ns	3.557	<0.01
Bone mineral (DEXA, week 21)										
Whole body aBMD (g/cm^2)	0.052 ± 0.001	0.051 ± 0.001	0.0056 ± 0.001	0.054 ± 0.001	0.003	<0.01	0.002	ns	−0.001	ns
Whole body BMC (g)	0.460 ± 0.020	0.429 ± 0.013	0.515 ± 0.011	0.453 ± 0.011	0.040	<0.05	−0.047	<0.01	−0.032	ns
Lumbar spine aBMD (g/cm^2)	0.053 ± 0.002	0.048 ± 0.002	0.063 ± 0.003	0.054 ± 0.003	0.008	<0.01	−0.007	<0.05	−0.005	ns
Lumbar spine BMC (g)	0.029 ± 0.002	0.024 ± 0.002	0.039 ± 0.003	0.028 ± 0.002	0.007	<0.01	−0.008	<0.01	−0.007	ns
Body weight (g)	33.5 ± 1.1	30.1 ± 0.9	31.5 ± 1.1	32.7 ± 1.1	0.231	ns	−1.086	ns	4.558	<0.05
Bone minerals and geometry (pQCT, weeks 22–26)										
Cortical vBMD (mg/cm^3)	1122.6 ± 5.6	1130.1 ± 6.9	1160.6 ± 6.1	1136.0 ± 7.7	21.963	<0.01	−8.556	ns	−32.229	<0.05
Cortical BMC (mg)	0.97 ± 0.03	1.01 ± 0.02	1.14 ± 0.02	1.06 ± 0.03	0.109	<0.001	−0.017	ns	−0.118	<0.05
Cortical area (mm^2)	0.87 ± 0.02	0.90 ± 0.02	0.98 ± 0.01	0.93 ± 0.02	0.078	<0.001	−0.011	ns	−0.077	<0.05
Cortical thickness (mm)	0.20 ± 0.003	0.22 ± 0.004	0.24 ± 0.003	0.23 ± 0.003)	0.022	<0.001	0.001	ns	−0.030	<0.001
Trabecular vBMD 45% (mg/cm^3)	209.7 ± 19.2	238.7 ± 17.7	257.1 ± 28.3	266.7 ± 17.2	37.664	ns	19.325	ns	−19.422	ns
Polar moment of inertia (mm^4)	0.43 ± 0.022	0.42 ± 0.011	0.49 ± 0.014	0.46 ± 0.021	0.051	<0.05	−0.023	ns	−0.025	ns
Polar moment of resistance (mm^3)	0.31 ± 0.01	0.31 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.042	<0.01	−0.014	ns	−0.023	ns
Tibia length (mm)	18.6 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	−0.005	ns	−0.016	ns	0.134	ns
Biomechanical proterties (weeks 22–26)										
Tibia stiffness (N/mm)	63.7 ± 4.9	105.0 ± 8.7	117.9 ± 9.0	73.3 ± 6.7	11.253	ns	−1.655	ns	−85.934	<0.001
Tibia toughness (mJ)	6.95 ± 1.12	7.38 ± 0.82	7.53 ± 1.24	5.69 ± 1.14	−0.550	ns	−0.703	ns	−2.269	ns
F(max)	15.6 ± 0.70	17.5 ± 0.77	20.5 ± 0.79	16.7 ± 1.01	2.106	<0.05	−0.956	ns	−5.564	<0.01
E(max)	4.29 ± 0.52	3.08 ± 0.23	4.07 ± 0.44	3.75 ± 0.31	0.227	ns	−0.761	ns	0.895	ns

DEXA: dual X-ray absorptiometry; pQCT: peripheral quantitative computed tomography, aBMD: areal bone mineral density, BMC: bone mineral content, vBMD: volumetric bone mineral density; F(max): maximal load to break the bone; E(max): maximum energy absorption. Results from two-way ANOVA and interaction effects. Values for bone parameters are mean ± SEM Significance $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Effects of the postweaning diets on offspring bone development

Compared with the postweaning herring diet, the postweaning beef diet decreased BMC in the whole body and lumbar spine and aBMD in the lumbar spine but did not affect the geometry or mechanical properties of bone. Thus, the postweaning herring diet rich in n-3 PUFAs had a positive effect on bone mineral acquisition and bone strength. In several studies, n-3 PUFAs, especially EPA and DHA, had positive effects on bone formation in growing rats.[24] In adult mice, a high consumption of n-6 PUFAs, at the expense of n-3 PUFAs, increased endogenous production of prostaglandin E2, which had negative effects on bone metabolism by increasing the resorptive activity of osteoclasts.[25] In middle-aged and elderly women, intake of LA, total n-6 FAs, and total PUFAs were positively associated with BMD and total fracture risk, but no significant associations were found between total n-3 FAs and fracture risk.[26,27] Administration of n-3 PUFAs and fish oil in adults improves bone quality[28] and decreases osteoclastogenesis.[8] In epidemiological studies, a high ratio of n-6/n-3 PUFAs correlates negatively with BMD in the hip. These results suggest that it is important to maintain an appropriate balance between n-6 and n-3 PUFAs to preserve skeletal health and reduce the risk of osteoporosis in adulthood.[7] Studies in animals have shown positive effects of n-3 PUFAs, especially EPA and DHA on bone formation in growing rats.[24]

Circulating IGF-1 levels were higher in mice fed the postweaning herring diet. IGF-I is believed to be the most important systemic and local growth factor for bone tissue.[29] Osteoblasts can synthesize IGF-I, but the production and action of IGF-I can be altered by modulating prostaglandin E2 and can be reduced by conjugated LA in vitro.[30] In growing rats, ALA decreases circulating levels of IGF-1, leading to reduced mineral acquisition and a lower rate of bone formation in the tibia.[28] On the other hand, in adolescent boys given DHA-rich fish oil, no association with bone mass or markers of bone formation were found, but IGF-1 increased with increasing DHA-levels.[31]

Thus, FAs may affect local and circulating levels of IGF-1, depending on the ratio of n-6/n-3 PUFA in the diet, as well as bone metabolism through IGF mechanisms, at least in rodents.

Interaction effects of pre and postweaning diets on bone development

Significant interaction effects were found in mice exposed to a maternal beef diet before weaning and fed a herring diet afterward. This combination of diets gave the highest cortical vBMD, BMC, area, and thickness at the proximal tibia and was also favorable for the stiffness and F(max) of the tibia. The crossover groups weighed less than groups fed the same postweaning diet as their dams before weaning. We did not find any significant differences in trabecular bone between the different groups. In other studies, high-fat diets, rich in saturated FAs, were linked to inhibition of osteogenic differentiation of marrow stromal cells in C57BL/6 mice.[32–34] The unfavorable consequences are thought to depend on the unhealthy effects on the absorption of dietary calcium from a high-fat diet rich in saturated FAs. These results have only been found in trabecular bone and might be explained by the fact that calcium absorbed from the intestine has a faster rate of turnover in trabecular bone than in cortical bone.[33]

The effects of fish oil or omega-3 FAs have mostly been studied in adult rodents. To our knowledge, no study has yet assessed the effects of a fish diet on bone development from the gestational and early postnatal period to mature adult age. It is therefore interesting to notice that a diet can have different effects depending on time of exposure. These observations suggest that separate critical periods of bone development are susceptible to external influences. Therefore, what is regarded as a healthy diet might differ during different periods of perinatal life and bone development. Similarly, it might be possible to compensate for periods of deficiency in important nutrients or FAs by a change in nutrition that provides the missing parts of an optimal and healthy diet later in bone development. Whole foods are complex, and the effects of several nutrients may be additive, synergistic, or antagonistic.

Dietary lipids and potential mechanisms of bone programming

Different dietary lipids might directly influence the FA composition of cartilage and bone tissue during development and thereby influence bone health and growth in humans and experimental animals.[27,28] Increasing evidence suggests that certain families of PUFAs affect bone formation and restoration by modulating bone cell functions and altering the metabolic activities of osteoblasts and osteoclasts.[32,33] Endogenous tissue concentrations of n-3 or n-6 PUFAs can be modified by exogenous administration through the diet and change the FA composition of cell membrane phospholipids to influence the cellular biosynthesis of prostaglandins and in this way regulate IGF-1 levels to stimulate or suppress bone formation.[20,28,32] Thus, depending on the concentrations, prostaglandin E2 increases IGF-1 levels and thereby stimulates bone formation.[35] n-3 PUFAs also inhibit the production of pro-inflammatory cytokines that activate bone resorption and osteoclast differentiation.[36]

Epigenetic changes, such as modifications in the methylation of DNA promoter regions, are thought to occur during early development and might explain how perinatal influences cause lifelong effects in the phenotype of the offspring. The effects of the maternal diet that influence BMD and bone strength in adulthood might act through these mechanisms. In humans, perinatal exposure to famine alters methylation of the IGF-1 gene,[37] which could influence bone mass in adulthood. Diet also changes the postnatal transcriptional activity of multiple genes that might influence bone quality and strength after exposure to perinatal calorie restriction or to a high-fat diet.[38,39] It will be of great importance to determine whether epigenetic regulation directly affects bone development and whether

detrimental changes can be altered postnatally to reduce the risk of disease.

Limitations and future studies

A strength of our study is that the mice were followed to ~6 months of age, longer than in most other studies of bone development in mice. This is important because cortical bone thickness (cortical bone area) seems to increase until 6 months of age and then decreases slightly and slowly until 24 months of age.[40] These age-related changes in skeletal mass and structure in mice are considered to be very similar to those in humans, even though rodents have growth plates until adult age.[40] The timing of exposure to the diet also seems to be critical. Our study provides evidence that BMD and bone strength are affected by the maternal diet during lactation and gestation and by the postweaning diet of the offspring; however, it will be important to study these effects during each of these periods separately. In addition, some of the differences we found might be a consequence of the complexity of the whole foods in the diet rather than of single FAs.

Diet also influences other endocrine and metabolic pathways, and thus the phenotype of the bones in the offspring might result from perinatal programming of the endocrine axis rather than from direct effects of the diet on the skeleton. Other studies should therefore consider the effects of hormones important for skeletal acquisition and energy metabolism, such as glucocorticoids and osteocalcin. Also, since gender differences in adult bone characteristics and strength have been reported in programming studies,[41] it will also be important to look for sex differences. Finally, the effects of the levels and profile of FA intake during early life on the risk of osteoporosis at older age in humans should be thoroughly investigated.

Conclusion

In conclusion, the significant crossover effects in this study indicate that the combination of a maternal beef diet before weaning and a herring diet after weaning is optimal for increasing BMC and bone strength in adult mice. We can only speculate on the mechanisms of these effects, but variation of n-6/n-3 ratio in the diets may be important for bone development in the offspring. Additional studies are therefore required to elucidate the mechanisms, which are of great interest in preventive medicine and in recommending nutrition, where emphasis on whole foods and entire diets is important.

Author contributions: The authors' contributions were as follows – AyH, HO and AH designed research; AyH, IN, SK, AK, LA and HO conducted research; AyH, HO, IN, SK and SN analyzed data; AyH, HO, SN, IK, SK, LA, AK, JT, CO and AH did data interpretation; AyH, HO and AH wrote the paper; AyH and AH had primary responsibility for final content. All authors read and approved the final manuscript.

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