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Dietary supplementation with long chain polyunsaturated fatty acids in pregnant guinea pigs has sex-dependent effects on growth and bone outcomes in offspring ☆

Z. Yin, S. Agellon, P. Lavery, H. Weiler *

School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, Ste. Anne-de-Bellevue, Québec H9 X3V9, Canada

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ABSTRACT

Long chain PUFA enhance bone mass in non-pregnant mammals. We examined the effects of arachidonic (AA; 20:4n-6) and docosahexaenoic (DHA; 22:6n-3) acid on bone mass of mothers and neonates. Guinea pig sows ($n=15$) were fed control, DHA or AA+DHA diets from mating to weaning. Measurements included: osteocalcin (OC), deoxypyridinoline (DPD), areal bone mineral density (aBMD) in sows and neonates; and volumetric density (vBMD) in neonates. Only vertebral aBMD and OC:DPD ratio declined during reproduction and only DHA reduced OC:DPD. Male pup weight was reduced by DHA and female weight elevated by AA+DHA. Whole body and femur aBMD were reduced by DHA and AA+DHA; whereas tibia vBMD was reduced by DHA in males. Female whole body, tibia and vertebrae aBMD plus tibia vBMD were elevated by AA+DHA; and DHA elevated whole body, tibia and vertebrae aBMD. Dietary AA+DHA and DHA elicit sex-dependent effects on neonatal bone, with minimal impact on mothers.

1. Introduction

Dietary recommendations for pregnant and lactating women in the USA and Canada stipulate that the acceptable macronutrient distribution range (AMDR) is 20 to 35% of energy for total fat and that an Adequate Intake (AI) of linoleic acid (18:2n-6) is 13 g/d, whereas AI of α-linolenic acid (18:3n-3) was set at 1.4 g/d in pregnancy and 1.3 g/d in lactation [1]. There is no specific recommendation for arachidonic acid (AA; 20:4n-6), or DHA (22:6n-3). However, 0.6 to 1.2% of energy is recommended for n-3 PUFA of which up to 10% of this can be consumed as EPA or DHA. This would correspond with 130 to 270 mg of EPA and/or DHA per day. Although it is still argued that current data are insufficient to make specific recommendations for long chain PUFA (LCPUFA) intakes during this period, one expert panel proclaims that women during pregnancy and lactation must ensure a DHA intake of 300 mg/d [2]. Similarly, a European consensus statement concludes that pregnant and lactating women should achieve a dietary DHA intake of at least 200 mg/d, but there were no specific recommendations for AA [3].

Dietary DHA can be obtained through consumption of fatty fish and seafood, while the major sources of AA are seed oils, eggs or meat [4]. Supplementation of single-cell DHA (260 mg/d) and AA (570 mg/d) to the maternal diet during pregnancy has been proven to be safe in humans and effectively enhances DHA levels in both plasma and erythrocyte phospholipids [5]. DHA is deposited in considerable amounts (30–45 mg/d in the third trimester) in the fetus, and adequate maternal intakes of the preformed LCPUFA are important for maintaining optimal tissue function [6]. Increased DHA concentrations in breast milk from maternal LCPUFA supplementation during pregnancy and lactation result in increased DHA concentration in blood lipids of infants [7,8]. The importance of LCPUFA on pregnancy outcomes, child growth, visual acuity and neurodevelopment is well established [9]. However, the role of dietary LCPUFA during pregnancy and lactation on bone physiology is a relatively new subject of study.

Li et al. [10] used an artificial rearing method to generate n-3 fatty acid-deficient rats and concluded that DHA is required for normal growth and mineralization of long bone in neonatal rat pups. High levels of dietary intake of n-3 LCPUFA (10% fish oil supplementation) reduced bone growth and tibia structural properties in growing rabbits [11]. One large cohort study confirmed that a higher dietary ratio of n-6 to n-3 PUFA was associated with lower bone mineral density (BMD) in the hips of men and women from 45 to 90 years of age [12]. These studies suggest that the dietary balance of n-6 to n-3 LCPUFA also has a profound influence on bone biology. Maternal supplementation of high n-3 LCPUFA has the potential of imposing imbalances in maternal-fetal transfer of n-6 LCPUFA in the fetus [13]. In humans, maternal blood DHA

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* Corresponding author. Tel.: +1 514 398 7905; fax: +1 514 398 7739.
E-mail address: hope.weiler@mcgill.ca (H. Weiler).

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negatively correlates with newborn femur and lumbar spine bone mineral content (BMC), while cord blood AA is positively correlated with newborn whole body BMC [14]. In both rats and guinea pigs, when maternal diets during lactation were supplemented with 0.5% of the fat as AA and 0.2% DHA, increased BMC of the lumbar spine and tibia were observed in the pups. In addition, BMC of the rat dams was protected by supplementation during lactation [15]. Whether benefits are observed throughout pregnancy and lactation has not been studied. The majority of intervention studies in pregnancy and lactation rely solely on DHA supplementation [16] or high-dosage DHA and EPA [17]. The effects of high amounts of DHA and sex effects of these fatty acids on bone require further investigation.

For a variety of ethical and methodological reasons, studying prenatal and postnatal bone growth in humans and dietary requirements therein is difficult [18]. The guinea pig is a small animal species with a gestation length of 63–69 days and litter size of 3 to 4 [19]. Throughout the second half of gestation, the fetal guinea pigs have physical and chemical characteristics similar to those of the human fetus, including relative rates of weight gain, as well as changes in energy, fat, protein, water, and mineral (sodium, calcium, and phosphorus) composition [19]. The guinea pig has been proposed and validated as a suitable model for studying fetal and postnatal bone development [20–22]. Therefore, the objectives of this study were to examine if maternal supplementation with DHA alone or a combination of DHA and AA during pregnancy and lactation has an effect on bone mass and metabolism in both mother and offspring using the guinea pig model for human nutrition.

2. Materials and methods

2.1. Ethics

Ethical approval was obtained from Macdonald Campus Facility Animal Care Committee of McGill University. All animal care procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care [23].

2.2. Experimental design

Pigmented guinea pigs sows ($n=21$) from our laboratory's breeding colony were mated at 8 months of age. All were proven breeders and had previously reared one litter. Successful breeding was noted upon presence of a vaginal plug. All sows were housed in a controlled environment (14 h light, and 10 h dark cycle) and provided with deionized water ad libitum. Sows were weighed weekly throughout the study.

Prior to mating, guinea pig sows were fed with a regular chow designed to support all life stages (Diet 2041, Harlan Teklad Diets, Madison, WI) containing 4.4% fat by weight as soybean oil. Sows were randomised to receive either control diet (C), DHA enriched diet (DHA), or a diet enriched with both AA and DHA (AA+DHA) during pregnancy and lactation. Upon successful mating, the study diet was provided until weaning, after which sows returned to the regular chow diet for 21 days of recovery from reproduction. The control and experimental diets were purchased from Purina Testdiet (Richmond, IN, USA). With the exception of amounts of AA and DHA, all diets were balanced for macro and micronutrient content and contained 6% of the diet by weight as soybean oil to meet essential fatty acid requirements (Table 1). The AA and DHA sources were ARASCO® and DHASCO® provided in kind (Martek Biosciences, Columbia, MD, USA). The AA+DHA diet contained 0.6% of the fat as AA and 0.2% as DHA whereas the DHA diet contained 0.8% of the fat as DHA alone. The dietary amounts were set to mimic 10% of the human AMDR as EPA and/or DHA; a 2000 kcal diet would yield 0.2 to 0.4% of the fat as DHA by weight. The value of 0.2% was used based on studies during lactation in the rat and guinea pig [15], whereas the higher value (0.4%) was doubled to achieve a higher DHA group and to balance the total LCPUFA in the two test diets. The percent of fat as AA was selected since 0.6% has been successfully used in studies of bone in piglets [24] and 0.5% in lactating rats and guinea pigs [15]. All diets were apple flavored to prevent the sows from perceiving changes in diet, which is known to cause stress in this species [25]. All test diets were kept frozen, prior to feeding, to preserve LCPUFA. Diet was provided fresh daily and food disappearance per cage was measured. Investigators were blinded to the identity of diet groups through use of letter coding.

Table 1
Composition of diets fed to guinea pig sows.

Ingredient[a]	Control	DHA	AA+DHA
Soy protein	161.0	161.0	161.0
Casein	53.0	53.0	53.0
L-Cystine	0.5	0.5	0.5
L-Methionine	3.0	3.0	3.0
Corn starch	217.9	217.9	217.9
Cellulose	140.0	140.0	140.0
Sucrose	290.0	290.0	290.0
Soybean oil	60.0	60.0	60.0
ARASCO® oil (38–44% AA)	None	None	0.8205
DHASCO® oil (40–45% DHA)	None	1.3128	0.3283
Ascorbic acid, coated (97.5%)	2.3	2.3	2.3
Vitamin mix	10.0	10.0	10.0
Potassium acetate	20	20	20
Calcium phosphate	14.8	14.8	14.8
Calcium carbonate	12.1	12.1	12.1
Potassium citrate	7.0	7.0	7.0
Magnesium oxide	5.0	5.0	5.0
Sodium chloride	3.0	3.0	3.0
Ferric citrate	0.6	0.6	0.6
Manganese sulfate	0.3	0.3	0.3
Zinc carbonate	0.11	0.11	0.11
Cupric sulfate	0.03	0.03	0.03
Chromium potassium sulfate	0.01	0.01	0.01
Potassium iodate	0.01	0.01	0.01
Sodium selenite	0.0004	0.0004	0.0004
tert-butylhydroquinone	0.012	0.012	0.012

[a] Ingredients g/kg of diet as prepared by Purina Testdiet (Richmond, IN, USA).

All sows had blood (heparinised) sampled from the saphenous vein for fatty acids and biomarker measurements, followed by dual-energy X-ray absorptiometry (DXA) scans at baseline (prior to mating), d3 and d21 postpartum, and then d42 postpartum in the anesthetized state. Sows' milk samples were manually expressed into capillary tubes on d7 post-partum under isoflurane anesthetic 20 min after an IP injection of 1–4 IU/kg oxytocin. Milk was flushed with nitrogen and stored at −80 °C for fatty acid analysis. Following d42 procedures, sows were transferred to another study; thus no other tissue samples were collected.

Offspring were considered to be in 1 of 3 study groups based on the maternal diet. Offspring were reared by their natural mothers, and were sexed and weighed on d3. For litters with more than 2 pups, litter size was reduced by randomizing pups to endpoints at d3 or d21 to ensure each sow nursed a maximum of 2 pups. Only litters with 2 or more live-born pups were studied so that the demands of lactation could be standardized to 2 per litter. At d3 and d21, all pups underwent blood collection (heparinised) from the saphenous vein, and then DXA scans for measurement of BMC and BMD in the anaesthetized state. Pups removed at d3 and d21 and were anaesthetized with isoflurane (Aerrane, Baxter, Deerfield, IL, USA) prior to exsanguinations by cardiac puncture. For all time-points, blood was separated into heparin plasma and red