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Does maternal long chain polyunsaturated fatty acid status in pregnancy influence the bone health of children? The Southampton Women's Survey

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Abstract

Purpose—Maternal diet in pregnancy has been linked to childhood bone mass, but the mechanisms and nutrients involved are uncertain. Long-chain polyunsaturated fatty acids (LCPUFAs) have been shown to affect bone metabolism, but the relationship between maternal fatty acid status and bone mass in the offspring remains unknown.

Methods—We evaluated the association between maternal LCPUFA status in late pregnancy (34 weeks gestation) and bone density in their children at age four years, within 727 mother-child pairs taking part in the Southampton Women's Survey.

Results—Concentrations of the n-3 LCPUFA component of maternal plasma phosphatidylcholine were positively associated with a number of bone mineral measures at the age of 4 years; these associations persisted after adjustment for maternal body build, walking speed and infant feeding. Relationships were most evident for eicosapentaenoic acid (r=0.09, p=0.02 for whole body areal bone mineral density [aBMD] and r=0.1, p=0.008 for lumbar spine aBMD) and for docosapentaenoic acid (r=0.09, p=0.02 for whole body aBMD and r=0.12, p=0.002 for lumbar spine aBMD).

Conclusions—These findings suggest that variation in early exposure to n-3 and n-6 LCPUFA may have potential consequences for bone development and that the effects appear to persist into early childhood.

Keywords

Epidemiology; osteoporosis; development; nutrition; bone mass

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INTRODUCTION

The trajectory of postnatal childhood skeletal growth appears to be determined by the interaction of genetic and environmental factors in utero, with further modification by factors such as diet and physical activity during childhood [1-2]. Maternal diet in pregnancy has been linked to bone development in children [3-5], but the specific nutrients which modulate bone mineral accrual during intrauterine and postnatal life remain uncertain [4,5]. Data from animal models suggest a role for long chain polyunsaturated fatty acids (LCPUFAs) in the regulation of bone metabolism [6]. The metabolites of LCPUFAs have a wide range of metabolic functions, but in terms of bone health, the eicosanoids derived from the oxidation of the 20-carbon LCPUFAs may be of key importance. The major eicosanoid products of arachidonic acid (AA; 20:4n-6) are 2-series prostaglandins (PG) such as PGE_2 and 4-series leukotrienes, which have been shown to affect bone turnover in a dose-dependent manner: high levels stimulate bone resorption and low levels, bone formation [7]. Because there is competition between the n-3 and n-6 PUFA families for the enzymes involved in the conversion of the parent fatty acids to the bioactive LCPUFAs and their metabolites, increased availability of the n-3 LCPUFA eicosapentaenoic acid (EPA; 20:5 n-3) acts to reduce formation of eicosanoids from AA [8]. In keeping with this, n-3 LCPUFAs have been demonstrated to reduce bone loss and attenuate inflammation-induced bone resorption in ovariectomised rats [9]; in growing rats, the bone mineral content of the proximal femur has been shown to be positively associated with the total n-3 fatty acid concentration [10].

Little is known about effects of variation in maternal LCPUFA status in pregnancy on fetal bone mineral accrual. Experimental manipulation of the n-6:n-3 fatty acid ratio of the diet of rat dams in late gestation and lactation has been shown to alter bone growth of their offspring, with greater femur length, bone mineral content, cortical thickness and cortical cross-sectional area in offspring of mothers in the high n-6:n-3 compared with low n-6:n-3 ratio group. Importantly, these effects persisted into adult life suggesting a permanent influence of maternal diet in the perinatal period on the regulation of bone metabolism in the offspring [11]. In the only human study, variations in maternal and cord blood LCPUFAs were predictive of bone mass at birth [12], but the long-term consequences of variation in maternal LCPUFA status for bone mineral accrual in childhood are not known. Postnatally, an effect of variations in the balance of n-6 and n-3 LCPUFAs on bone mass has been described in studies of children, with a positive association between %AA and whole body aBMD, but an inverse relationship between % total n-6 fatty acids and lumbar spine aBMD in a cross-sectional study of children aged 8 years [13].

Given previous work suggesting that maternal diet may influence bone mineral accrual in the offspring, and that early growth may be an important determinant of later risk of osteoporosis, our objective was to evaluate the relationships between maternal LCPUFA status in this large cohort of women who were studied in late pregnancy, and bone size/density in their children at 4 years old. We hypothesised that total and %total maternal n-6 and n-3 fatty acid status in pregnancy would be positively related to offspring bone mass assessed by DXA in childhood.

METHODS

The Southampton Women's Survey

The Southampton Women's Survey (SWS) is a study of a population sample of non-pregnant women aged 20 to 34 years, resident in the city of Southampton, UK [14]. Its aim is to identify the maternal influences acting before and during pregnancy that determine fetal growth, and to characterize how maternal and intrauterine influences interact with the

offspring's genes and postnatal environment to determine subsequent growth, development and health. Assessments of lifestyle, diet and anthropometry were performed at study entry and, for women who became pregnant, again at 11 and 34 weeks gestation. The lifestyle information included details of smoking status and customary walking speed. In early and late pregnancy, measures of the woman's anthropometry, including skinfold thicknesses, were repeated and blood samples were taken.

The child's duration of breastfeeding was determined from milk feeding histories obtained when they were aged 6 and 12 months. At 3 years the children's diets were assessed using an administered food frequency questionnaire [15]. The key dietary pattern identified by principal components analysis at this age was a 'prudent' pattern, characterized by greater consumption of fruit, vegetables, water, wholemeal bread and fish, but by lower consumption of white bread, crisps, chips and processed meat. A prudent diet score was calculated for each child that indicated their compliance with the pattern, and therefore the quality of their diet. Thus, children with high prudent diet scores had high intakes of fruit, vegetables and fish whilst children with low scores ate less of these foods but had high intakes of white bread, chips and processed meat [15].

The SWS was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Southampton and South West Hampshire Research Ethics Committee approved all procedures. Written informed consent was obtained from all participants.

Fatty acid composition of maternal plasma phosphatidylcholine

Blood was taken into heparinised tubes at week 34 of pregnancy. Plasma was prepared and stored at −80°C until analysis. Dipentadecanoyl phosphatidylcholine was added to thawed plasma as internal standard prior to total lipid extraction with chloroform/methanol (2:1 vol/vol); butylated hydroxytoluene was added to the extraction as antioxidant. Phosphatidylcholine (PC), which contributes about 75% of plasma phospholipid, was isolated by solid phase extraction on aminopropylsilica cartridges using chloroform to elute triacylglycerol and cholesteryl ester fractions, which were discarded, and then chloroform/methanol (60:40 vol/vol) to elute the PC. Purified PC was dissolved in toluene and fatty acid methyl esters generated by reaction with methanol containing 2% (vol/vol) sulphuric acid at 50°C for 2 hours. After cooling and neutralisation, fatty acid methyl esters were extracted into hexane. Fatty acid methyl esters were separated by chromatography on a BPX-70 column (30 m × 220 μm; film thickness 0.25 μm) fitted to a Hewlett-Packard HP6890 gas chromatograph. Front inlet temperature was 300°C; initial column temperature was 115°C and was programmed to hold this temperature for 2 min, then to increase temperature at 10°C/minute to 200°C, to hold at 200°C for 10 min, to increase temperature at 10°C/min to 240°C, and then to hold this temperature for 2 min. Helium was used as the running gas and fatty acid methyl esters were detected by flame ionisation. Fatty acid methyl esters were identified by comparison with retention times of standards run previously and they were quantified using ChemStation software. Data were expressed as both absolute concentration (μg/ml plasma) and as percentage contribution to the total plasma PC fatty acid pool.

4-year follow-up

A subset of 900 participants was recruited sequentially from the SWS cohort. The mother (or father/ guardian) and child were invited to visit the Osteoporosis Centre at Southampton General Hospital for assessment. At this visit written informed consent for the DXA scan was obtained from the mother or father/ guardian. The child's height (using a Leicester height measurer [Seca Ltd, UK]) and weight (in underpants only, using calibrated digital scales [Seca Ltd, UK]) were measured. A whole body and lumbar spine DXA scan was obtained, using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA) in

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paediatric scan mode. To encourage compliance, a sheet with appropriate coloured cartoons was laid on the couch first; to help reduce movement artefact, the children were shown a suitable DVD cartoon. The total radiation dose for the scans was 4.7 microsieverts for whole body measurement (paediatric scan mode). The manufacturer's CV for the instrument was 0.75 % for whole body bone mineral density, and the experimental CV when a spine phantom was repeatedly scanned in the same position 16 times was 0.68%. The ability of DXA to measure bone mass in small subjects was demonstrated using miniature piglets, where correlation between DXA-derived BMC and ashed calcium content was 0.90 ($p<0.001$) [16].

Statistical analysis

All variables were checked for normality. T-tests and Mann-Whitney U-tests were used to test the difference in normally and non-normally distributed variables, respectively, by gender. Correlation and linear regression methods were used to explore the relationships between maternal LCPUFA status and childhood bone measurements at whole body minus head (WB) and lumbar spine sites using Stata V11.0 (Statacorp, Texas, USA) [17]. Bone outcomes at 4 years include bone area (BA), bone mineral content (BMC), which represent the absolute size and total mineral content of the bone respectively, areal bone mineral density (aBMD, a partly size-corrected measure) and estimated volumetric bone mineral density (vBMD: BMC adjusted for BA, height and weight to correct for body size as fully as possible [18]). In the models we took account of a number of maternal characteristics that have been shown to predict children's bone mass in previous work (maternal age, triceps skinfold thickness, walking speed [19]). To address the confounding effects of variations in the children's diets and postnatal exposure to LCPUFA, we also included the duration of breastfeeding and the child's prudent diet score in our models [15].

RESULTS

Characteristics of the mothers and their plasma PC concentrations of LCPUFA are presented in table 1. The children's characteristics and bone mineral measures (whole body and spine) at age 4 years are shown in table 2. The boys and girls were the same age at the time of the DXA scan, had comparable weights and heights, but differed in mean bone indices at the lumbar spine. Bone outcomes were therefore adjusted for the effect of the child's sex before analyses of the associations with maternal LCPUFA status.

Compared with mothers of children born to the SWS during the same time frame, but who did not have DXA scans at 4 years, the mothers of children who did have DXA assessments were, on average, slightly older at the birth of their child (mean age 31.2 years vs. 30.6 years respectively, $p=0.007$), better educated (24.8% with higher degree vs. 20.6% respectively, $p=0.002$) and smoked less (8.5% smoked before pregnancy vs. 17.4% respectively, $p<0.001$).

Maternal total LCPUFA status and childhood bone outcomes

We examined the associations between maternal LCPUFA status in late pregnancy and bone mineral measures in the children at age 4 years firstly after adjusting for the child's sex and age at DXA scan only, and secondly after additionally adjusting for a number of potential confounders [maternal age, triceps skinfold thickness, smoking status (yes/no), walking speed (6 groups) and duration of breastfeeding (5 groups)]. The pattern of associations was very similar before and after adjustment for the maternal factors; the adjusted correlations are presented in Table 3. Maternal plasma PC concentrations of the n-3 LCPUFAs were positively associated with a number of bone mineral measures at the age of 4 years. This was most evident for EPA which was positively related to aBMD and estimated vBMD at

whole body (aBMD: r=0.09, p=0.02; vBMD: r=0.08, p=0.048) and lumbar spine (aBMD: r=0.1, p=0.008; vBMD: r=0.08, p=0.047), and for docosapentaenoic acid (DPA; 22:5 n-3) which was associated with aBMD at both sites (whole body: r=0.09, p=0.02; lumbar spine: r=0.12, p=0.002). In comparison with EPA and DPA, there were fewer associations between total n-3 PUFA concentration and bone mineral measures. Total n-6 PUFA concentration was positively associated with spine aBMD (r=0.08, p=0.04), but unlike the associations with the individual n-3 LCPUFAs there were no associations between AA concentration and the measures of bone size or density. Total DHA and the ratio of n-6 to n-3 LCPUFAs were unrelated to the bone measures at 4 years (all p>0.05).

Maternal % LCPUFA status and childhood bone outcomes

To further explore the role of the balance of n-3 and n-6 fatty acids, we examined the associations between LCPUFA status assessed as a percentage of total fatty acids and bone measures at 4 years (Table 3). Compared to LCPUFA concentrations, LCPUFAs (% total fatty acids), showed fewer associations with the 4-year bone measures. However, the overall pattern of associations was comparable, and there were positive associations between individual n-3 LCPUFAs and whole body BMC and aBMD. In contrast with the findings for n-6 PUFA concentration, total n-6 PUFAs (as % total PUFAs) were not related to any bone measure at 4 years. The associations for AA also differed, as AA expressed as a % of total plasma PC fatty acids was inversely related to whole body BMC (r=−0.08, p=0.039) and aBMD (r=−0.08, p=0.037), and to spine BMC (r=−0.1, p=0.01). The relationships between maternal status and whole body bone measures are illustrated for the 20-carbon LCPUFAs, EPA and AA, in Figure 1. The importance of the balance of these individual LCPUFAs (EPA % and AA %) is shown clearly, with opposite patterns of association with whole body aBMD at 4 years.

Childhood dietary factors

In further analyses we considered the role of the child's diet in the relationship between maternal LCPUFA status and bone outcomes at 4 years. Inclusion of the children's prudent diet scores, as a measure of the quality of the childhood diet, resulted in few changes in the pattern of associations between maternal LCPUFA status and bone outcomes at the age of 4 years. After taking account of maternal characteristics, duration of breastfeeding and child's prudent diet score, the opposing associations between EPA (%) and AA (%) with whole body aBMD were still evident (both $p < 0.05$).

DISCUSSION

The purpose of this study was to determine how maternal LCPUFA status in late pregnancy related to measures of bone mineral in their children at age 4 years. A key finding is that maternal status of two LCPUFAs, EPA and DPA, were positively associated with a number of DXA-derived bone indices in the children. These associations were evident when EPA and DPA status were considered both as concentrations and as % total fatty acids, and were independent of potential confounding influences. A second finding was that although n-6 fatty LCPUFA status showed few associations with bone indices at 4 years, AA (% total fatty acids) was inversely related to whole body BMC and areal BMD, highlighting the importance of the balance of n-6 to n-3 fatty acids for bone mineral accrual. These relationships were largely unchanged when duration of breastfeeding and childhood diet were included in the models. Our findings therefore suggest that there are effects of maternal LCPUFA status on fetal bone metabolism that persist into postnatal life, and that these are independent of variations in childhood diet.

A strength of our study was that we obtained measures of fatty acid status and bone DXA measurements from a large number of mother-child pairs. Although the children who had DXA measurements were a subset of the SWS cohort, and tended to have mothers with higher levels of educational attainment [20], they represent a wide range of family backgrounds. Unless the associations between maternal LCPUFA status and bone mass in the children were different in the remainder of the cohort it is unlikely that selection bias could explain the relationships that we observed. Because our study was large we were able to take account of the effects of a number of confounding influences on bone mineral accrual, including variations in the child's pattern of diet in postnatal life. A limitation to our study was that the measurement of bone mineral in children is hampered by their tendency to move and also by their low absolute BMC. However, we used specific paediatric software, movement artefact was minimal and the small numbers of children with excess movement artefact were excluded. A further consideration is that the use of DXA does not allow measurement of true volumetric bone density, thus making it difficult to be certain about differential determinants of skeletal size and volumetric density. Within the limitations of DXA, we used a staged approach to size correction with bone area as a measure of skeletal size, bone mineral content as a measure of the total calcium mineral, areal bone mineral density as a partly size-corrected measure and estimated volumetric bone density to fully adjust for body size (bone mineral content adjusted for bone area, height and weight).

We observed positive associations between n-3 PUFA status and bone mineral measures in the children, which is consistent with other studies [6,10]. Högström and colleagues have recently reported a positive association between n-3 PUFA status and peak bone mineral density among healthy young men [21]. In this study, strong associations were observed between specific n-3 fatty acids at 16 years, particularly DHA, and bone density at age 22 years, as well as changes in spine BMD between the ages of 16 and 22 years. The authors postulated that n-3 LCPUFAs might increase calcium dependant ATPase activity and have a beneficial impact on calcium absorption through this mechanism. However the relationship between maternal n-3 PUFA status and fetal bone mineral accrual may be complex as in the only human study of maternal LCPUFA status in relation to bone mass in the offspring, there was an inverse association between maternal erythrocyte DHA concentration and bone mineral content of the infant femur and lumbar spine at birth [12]. Although the authors commented that the DHA status of the women studied was relatively low, it was very comparable to the status of the SWS women in our study, in which we observed no relationships between plasma DHA concentration and offspring whole body or lumbar spine bone measurements.

Some of the differences in findings may be explained by variations both in the amount and balance of n-3 and n-6 LCPUFAs in the habitual diets of the study participants. For example, in the mother-child pairs studied by Weiler and colleagues [12], cord blood erythrocyte AA was positively associated with whole body BMC, which is consistent with the findings of experimental animal studies [6,22]. In our study, we found the opposite association, and AA (% total) was inversely related both to whole body BMC and aBMD at 4 years. However, although the total n-3 PUFA and DHA status of the women in our study was comparable to the women studied by Weiler and colleagues [12], the AA status of the SWS women was considerably lower. It is therefore possible that variations in the balance of n-6 and n-3 LCPUFAs supplied to the fetus are key in determining effects on bone mineral accrual.

An effect of variations in the balance of n-6 and n-3 LCPUFAs on bone mass has been described in studies of children. For example, in a cross-sectional study of children aged 8 years, whilst AA (% total) was positively associated with whole body aBMD, there were

inverse associations between total n-6 fatty acids (% total) and lumbar spine aBMD, and between the ratio of n-6 to n-3 PUFAs and whole body aBMD [13]. These observations point to specific effects of individual LCPUFAs on bone mineral accrual. Our study also provided some evidence of this, as compared with total n-3 PUFA status, there were many more associations between bone mineral measures in the children and maternal status of EPA and DPA. Future studies will determine how varying amounts and balance of total and individual n-3 and n-6 LCPUFAs affect fetal bone metabolism and accrual. However, our observational data suggest that existing variations in maternal fatty acid status in UK women have implications for fetal bone development, which clearly merits further study.

The mechanisms that link maternal fatty acid status to bone health of the offspring are unknown. LCPUFAs are likely to have several effects on processes involved in bone mineral accrual and maintenance. These include their influence on intestinal calcium absorption; their inhibitory effects on osteoclastic bone resorption, and their potentiation of collagen synthesis by osteoblasts in animal studies [6-7, 9-11]. Animal studies have also confirmed that a high dietary intake of n-3 LCPUFAs is associated with both promotion of bone formation and inhibition of bone resorption [6]. Studies in rats and pigs have shown that maternal dietary supplementation with combinations of AA, EPA, and DHA has positive influences on offspring bone mass [10,11]. These and other studies have demonstrated that an appropriate fatty acid balance is important for skeletal health [6].

The observational data might suggest that supplementation with fatty acids could be a strategy to improve bone mineral accrual. We are not aware of any data relating supplementation of the pregnant mother to bone mass of the offspring, but in one study in which premature infants were allocated to formula either supplemented or unsupplemented with AA and DHA, bone size and density did not differ between groups [23]. Indeed, in another trial, adults administered a fatty acid supplement did not demonstrate any change in bone density over 1 year [24]. In 113 adults randomised to either n-3 PUFA supplementation or placebo there was no difference in bone resorption between the two groups [25]. In contrast, a small study of n-3 PUFA supplementation showed a reduction in bone resorption markers compared with standard diet in 23 adults in the US [26]; a similar effect was observed in studies in Spain [27] and Iran [28]. Thus the evidence from interventional studies is somewhat mixed, and the lack of any data relating to maternal supplementation in pregnancy prevents any definite inferences to be made regarding a possible benefit from this intervention to offspring bone mineral accrual in utero.

In conclusion, the present study demonstrates a positive association between blood levels of maternal n-3 fatty acids and bone mineral density of their children at the age of 4 years, but negative associations between aBMD and maternal % AA. These findings suggest a further possible pathway by which maternal nutrition might influence offspring skeletal development.

MINI ABSTRACT In this large, population-based, prospective, mother-offspring cohort study, maternal long chain polyunsaturated fatty acid (LCPUFA) status during pregnancy was found to be positively associated with bone mass in the offspring at age four years. The findings suggest that variation in intrauterine exposure to n-3 and n-6 LCPUFAs may have potential consequences for skeletal development.

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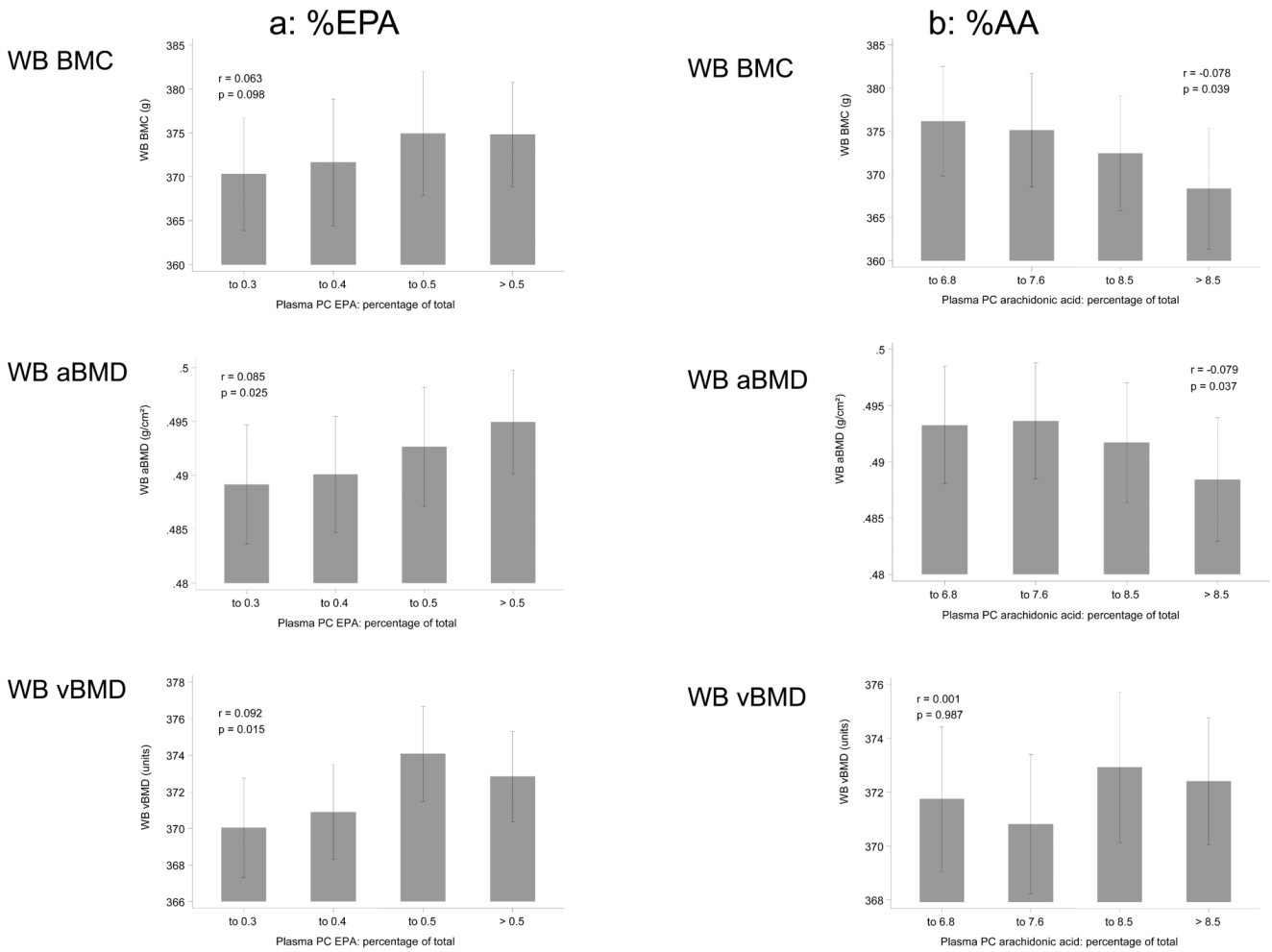

Figure 1.

Whole body BMC, aBMD and estimated vBMD at 4 years, and a) maternal %EPA status in late pregnancy; b) maternal %AA status in late pregnancy. [Data adjusted for child's age at DXA and sex, and maternal age, triceps skinfold thickness, walking speed and duration of breastfeeding; Pearson correlation coefficient (r) and statistical significance (p) are given for the continuous data)].

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Table 1

Characteristics of the 727 mothers studied

Maternal characteristics		
Age (years), mean (SD)	30.6	(3.7)
Height (cm), mean (SD) *	163.6	(6.6)
Triceps skinfold thickness in late pregnancy (mm), median (IQR) *	20.8	(16.9-25.8)
	%	**n**
Smoking during late pregnancy *	10.9	(79)
Duration of breastfeeding **		
Never tried	13.1	(95)
<1 month	20.0	(145)
1 to 3 months	18.4	(133)
4 to 6 months	20.4	(148)
7 to 11 months	15.7	(114)
12 or more months	12.3	(89)
Plasma PC fatty acid concentration (μg/ml), median (IQR)		
Total n-3 PUFAs	72.3	(52.6-97.3)
EPA	5.2	(3.5-7.9)
DPA	6.5	(4.5-8.7)
DHA	54.1	(39.7-72.5)
Total n-6 PUFAs	505.1	(387.4-622.9)
AA	106.7	(80.5-136.8)
Plasma PC n-6 to n-3 PUFA ratio	7.0	(5.8-8.4)
% of total plasma PC fatty acids, median (IQR)		
Total n-3 PUFAs	5.11	(4.37-5.95)
EPA	0.37	(0.27-0.51)
DPA	0.44	(0.37-0.55)
DHA	3.82	(3.25-4.51)
Total n-6 PUFAs	35.40	(2.20)
AA	7.67	(1.30)

* 5 missing values

** 3 missing values

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Table 2

Characteristics of the children studied at 4 years

	Boys (n = 385)		Girls (n = 342)		
	Mean/ median*	**sd/ IQR***	**Mean/ median***	**sd/ IQR***	**P**
Age (years)	4.1*	4.1-4.2*	4.1*	4.1-4.1*	0.16
Height (cm)	104.2	3.78	103.8	4.46	0.23
Weight (kg)	17.3*	16.1-18.7*	17.3*	15.9-18.9*	0.65
WB BA (cm^2)	747.6	43.5	766.5	50.7	<0.001
WB BMC (g)	369.8	41.8	376.3	48.0	0.059
WB aBMD (g/cm^2)	0.494	0.034	0.489	0.038	0.13
WB vBMD (units)	373.0	16.1	370.8	18.7	0.093
Lumbar spine BA (cm^2)	27.7	2.9	26.3	2.7	<0.001
Lumbar spine BMC (g)	13.2	2.1	13.0	2.1	0.21
Lumbar spine aBMD (g/cm^2)	0.476	0.049	0.492	0.054	<0.001
Lumbar spine vBMD (units)	13.0	1.1	13.3	1.3	<0.001

WB: Whole body minus head; BA: bone area; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: estimated volumetric bone mineral density.

Table 3

Adjusted correlations between long chain polyunsaturated fatty acid status in late pregnancy and bone mineral measures at 4 years in 727 mother-child pairs

	Whole body minus head				Lumbar spine			
	BA	**BMC**	**aBMD**	**vBMD**	**BA**	**BMC**	**aBMD**	**vBMD**
Maternal plasma PC concentration:								
Total n-3 PUFAs	0.030	0.055	0.068	0.046	0.021	0.071	**0.089** *	0.064
EPA	0.034	0.069	**0.086** *	**0.075** *	0.015	**0.075** *	**0.100** **	**0.075** *
DPA	**0.080** *	**0.092** *	**0.086** *	0.044	0.019	**0.085** *	**0.115** **	0.075
Total n-6 PUFAs	0.041	0.039	0.029	0.0	−0.012	0.045	**0.078** *	0.062
AA	0.004	0.002	−0.003	0.008	−0.055	0.004	0.058	0.056
Maternal percentage fatty acids:								
Total n-3 PUFAs	0.000	0.038	0.066	0.064	0.039	0.049	0.041	0.02
EPA	0.022	0.063	**0.085** *	**0.092** *	0.019	0.064	**0.081** *	0.06
DPA	0.071	**0.090** *	**0.091** *	0.061	0.034	0.066	0.074	0.033
Total n-6 PUFAs	0.005	−0.023	−0.047	−0.056	−0.049	−0.063	−0.053	−0.05
AA	**−0.078** *	**−0.078** *	**−0.079** *	0.001	**−0.111** **	**−0.098** *	−0.04	−0.01

BA: bone area; BMC: bone mineral content; aBMD: areal bone mineral density; vBMD: estimated volumetric bone mineral density. Bone outcome variables have been adjusted for child's age at DXA and sex, and maternal age, late pregnancy triceps, smoking, walking speed and duration of breastfeeding. Data shown are the adjusted

Pearson correlation coefficients;

* $p<0.05$

** $p<0.01$