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Bone Mass in Indian Children—Relationships to Maternal Nutritional Status and Diet during Pregnancy: the Pune Maternal Nutrition Study

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Context/Objective: Bone mass is influenced by genetic and environmental factors. Recent studies have highlighted associations between maternal nutritional status during pregnancy and bone mass in the offspring. We hypothesized that maternal calcium intakes and circulating micronutrients during pregnancy are related to bone mass in Indian children.

Design/Setting/Participants/Main Outcome Measures: Nutritional status was measured at 18 and 28 wk gestation in 797 pregnant rural Indian women. Measurements included anthropometry, dietary intakes (24-h recall and food frequency questionnaire), physical workload (questionnaire), and circulating micronutrients (red cell folate and plasma ferritin, vitamin B12, and vitamin C). Six years postnatally, total body and total spine bone mineral content and bone mineral density (BMD) were measured using dual-energy x-ray absorptiometry (DXA) in the children (n = 698 of 762 live births) and both parents.

Results: Both parents' DXA measurements were positively correlated with the equivalent measurements in the children ($P < 0.001$ for all). The strength of these correlations was similar for fathers and mothers. Children of mothers who had a higher frequency of intake of calcium-rich foods during pregnancy (milk, milk products, pulses, nonvegetarian foods, green leafy vegetables, fruit) had higher total and spine bone mineral content and BMD, and children of mothers with higher folate status at 28 wk gestation had higher total and spine BMD, independent of parental size and DXA measurements.

Conclusions: Modifiable maternal nutritional factors may influence bone health in the offspring. Fathers play a role in determining their child's bone mass, possibly through genetic mechanisms or through shared environment. **(*J Clin Endocrinol Metab* 91: 2994–3001, 2006)**

OSTEOPOROSIS IS AN increasing public health problem worldwide through its association with age-related fractures (1). Fracture risk depends on the mechanical strength of bone and the forces applied to it (2). Bone mass (a composite measure of bone size and mineral density) is a determinant of bone strength, and adult bone mass depends on the mass acquired during skeletal growth (3). Although heritability estimates for bone mass range up to 80%, currently identified genetic markers explain only a small proportion of the variation in individual bone mass and fracture risk (4). Environmental factors during childhood and puberty influence bone mineral accrual (5). The rapid rate of mineral gain during intrauterine life, coupled with the plasticity of skeletal development *in utero*, offers the possibility of profound effects of the environment at this early stage of development (6).

Epidemiological evidence that the risk of osteoporosis might be modified by the intrauterine environment has emerged from several sources. Retrospective cohort studies have demonstrated associations between birth weight and adult bone mass assessed by dual-energy x-ray absorptiometry (DXA) (7, 8). Mother-offspring studies in Western populations have shown associations of maternal body build, diet, nutritional status, smoking, and physical activity with bone mass in the newborns and children (9–14). Mothers with suboptimal vitamin D status have offspring with reduced intrauterine and postnatal skeletal development (13, 14). Supplementation of pregnant mothers with vitamin D (15), calcium (16, 17), and other micronutrients (18) is associated with increased skeletal growth and/or bone mass/density in the offspring. The extent to which these factors operate in different populations and the contributions of paternal and maternal bone mass are unknown. We have addressed some of these issues in a community-based observational study of parents and children taking place in India (the Pune Maternal Nutrition Study). Maternal anthropometry, diet, and circulating micronutrients were measured during pregnancy, and bone mass was measured in the children and both parents 6 yr later. We examined the associations of maternal nutritional and lifestyle factors during pregnancy and maternal and paternal bone mass to the child's bone mass. We specifically hypothesized that higher maternal intakes of calcium-rich foods would be associated with higher bone mass in the children.

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Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; GLV, green leafy vegetable; MMA, methylmalonic acid; SGA, smallness for gestational age; tHcy, homocysteine.

Subjects and Methods

The Pune Maternal Nutrition Study methodology has been described (19). The study was established in six rural villages (total population 35,000) near the city of Pune. All married women aged 15–40 yr were identified, and of 2675 women eligible for the study (excluding sterilized couples), 2466 (92%) agreed to participate. Last menstrual period dates were recorded every month, and women who missed two successive periods were examined by ultrasound at 15–18 wk to confirm pregnancy and record sonographic gestational age. Of 2466 women, 1102 became pregnant. After excluding those with abortions, major fetal anomalies detected on ultrasound, multiple pregnancies, terminations of pregnancy, and pregnancies detected later than 21 wk, 797 pregnant women were enrolled between June 1994 and April 1996. According to national policy, women were given 100 tablets of iron (60 mg) and folic acid (500 μg) at 18 wk gestation.

Nutritional assessment

Anthropometry (prepregnancy). Nonpregnant women were measured every 3 months, and the last set of measurements before pregnancy was used to define prepregnant anthropometry. Weight was measured to the nearest 0.5 kg (SECA scales; CMS Instruments, London, UK) and height to the nearest 0.1 cm (Harpenden portable stadiometer, CMS Instruments). Biceps, triceps, and subscapular and suprailiac skinfold thicknesses were measured to the nearest 0.1 mm using Harpenden skinfold calipers (CMS Instruments). Fat mass was calculated from the four skinfolds (20, 21). For women who became pregnant, the height of the husband was measured.

Dietary intakes and physical workload (18 and 28 wk gestation). Dietary assessment methods were developed specifically for this community (19). A modified semiweighed 24-h recall method was used to estimate energy, protein, carbohydrate, and fat intakes in a single 24-h period. A food frequency questionnaire was administered to obtain the frequency of consumption of 111 foods in 17 food categories [beverages, chapatis/rotis (unleavened bread), rice, pulses (lentils), legumes, green leafy vegetables (GLVs), other vegetables, chutneys, fasting foods, fruits, meat/fish, milk products, bakery items, spicy snacks, sweet snacks, festival foods, and special foods] over the preceding 3 months on an 8-point scale ranging from never to more than once daily. Fasting foods are foods rich in simple carbohydrates, eaten by many women for religious reasons once or twice per week rather than normal meals. Special foods are sweet and/or oily foods consumed on religious festivals once or twice per year.

For this analysis we created a variable for calcium-rich foods by summing the frequencies of intake of milk, milk products (milk in tea and other beverages, yogurt, buttermilk, ice cream, and other milk-based preparations), pulses (lentils) and pulse-containing foods, legumes, nonvegetarian foods (fish, meat, and eggs), GLVs, fruit, sesame seeds, and coconut. We estimated the total intake of calcium, phosphorus, and magnesium using average portion sizes (from the 24-h recall), frequency of intake (food frequency questionnaire), and nutrient content tables for raw Indian foods (22). Estimations were impossible for rarely eaten foods whose portion size was not available. For mixed and/or cooked foods, we made assumptions based on the predominant ingredients and allowed for changes in moisture content during cooking. The women's physical activity was recorded using a questionnaire from which a daily score was derived; higher scores indicate more activity (23). Women were asked whether they chewed or smoked tobacco.

Biochemical measures of maternal nutritional status (18 and 28 wk gestation). Maternal erythrocyte folate, plasma ferritin, vitamin B12, vitamin C, methylmalonic acid (MMA), and homocysteine (tHcy) concentrations were measured in fasting blood samples, as previously described (19, 24), taking all necessary precautions for the transportation and processing of samples.

Measurement of the newborn babies

Of 797 pregnant women enrolled, 12 had spontaneous late abortions, 14 had late terminations, and one died. There were 770 deliveries, including eight stillbirths. The babies were measured by one of five trained fieldworkers. Birth weight and placental weight were measured to the nearest 25 g using a Salter spring balance. Birth weight was used only if measured within 72 h of birth. Crown-heel length was measured to the nearest 0.1 cm using a portable Pedobaby Babymeter (ETS J.M.B., Brussels, Belgium) up to 1 wk after birth. Birth weight was available for 702 and birth length for 727 of the 762 live births, which formed the sample for this study. Eighty-four babies (11%) were preterm (<37 wk gestation).

Investigations in the children

Data on infant feeding (duration of exclusive breast-feeding) were recorded at 1-yr follow-up. Thirty-nine children died between birth and 6 yr. Of 723 available for follow-up at 6 yr, 698 (97%) and their parents (682 mothers and 643 fathers) attended for investigations between December 2000 and January 2003. Both parents' bone outcomes were available for 631 children; mother-only data for 51 children (some fathers were unwilling to forgo work commitments to attend); father-only data for 12 children (the mother was either sick or no longer alive); and no parental data for four children. Paternity was assumed. Socioeconomic status was assessed using a questionnaire that derives a composite score based on occupation and education of the head of the household; caste; type of housing; and ownership of animals, land, and material possessions. Higher scores indicate better socioeconomic status (25). Anthropometry was carried out by one of four observers using standardized protocols. Weight was measured using electronic weighing scales with a least count of 10 g (ATCO Healthcare Ltd., Mumbai, India) and height to the nearest 0.1 cm using a wall-mounted Microtoise (CMS Instruments). A pediatrician examined the children for clinical evidence for rickets.

DXA scans

Total body DXA scans were carried out on the parents (adult mode) and children (pediatric mode) using a DPX-IQ 240 pencil beam machine (Lunar Corp., Madison, WI), adhering to the manufacturer's guidelines for data acquisition and analysis (software version 4.7) (26). Scans were analyzed by positioning the cuts to measure total bone mineral and fat and lean mass for anatomical regions (total body = head + arms + legs + trunk including ribs, spine, and pelvis) (26). Bone outcomes were bone mineral content (BMC) and bone mineral density (BMD, defined as BMC divided by bone area) for total body, and total spine (T1 to L5). Quality assurance tests were conducted daily using the phantom and standard protocol provided by the manufacturer (26). Interobserver variation studies were conducted to maintain quality; coefficients of variation for all outcomes were less than 0.6% for adults and less than 0.5% for children.

Ethical permission was granted by the KEM Hospital Ethical Committee and local village leaders. Informed written consent was obtained from the parents.

Statistical methods

The following continuous variables were transformed to normality using standard transformations: maternal prepregnant fat mass, frequency of consumption of pulses, GLVs, fruit, milk/milk products and calcium-rich foods, intakes of energy, protein, fat and calcium, concentrations of folate, MMA, tHcy and vitamin B12, and the children's age at DXA measurements. Maternal intakes of nonvegetarian foods (fish, meat, and eggs), which are expensive and therefore rarely consumed, were converted to binary variables describing whether they were ever consumed and combined into a single group. Pearson correlation coefficients were used to assess associations between potential determinants and DXA measurements. Smallness for gestational age (SGA) was defined as a birth weight below the within-cohort gestation and sex-specific 10th percentile. Multiple linear and logistic regression and ANOVA models were used to assess whether associations were independent of potential confounding factors. Analyses were performed using Stata (version 8.2; Stata Corp., College Station, TX).

Results

The children were studied at a median age of 6.2 yr; 53% were boys (Table 1). Boys were heavier and taller than girls and had higher BMC and BMD. Compared with National

TABLE 1. Body size and bone mass measurements for the children and their parents

Children	Boys			Girls			*t* test
	n	Median	IQR	n	Median	IQR	
Birth							
Weight (g)	349	2700	2500, 2950	308	2550	2300, 2800	<0.001
Length (cm)	363	48.0	46.5, 49.4	323	47.2	46.0, 48.3	<0.001
Placental weight (g)	323	355	315, 410	283	350	295, 400	0.06
Gestation (wk)	370	39.1	38.1, 40.3	328	39.1	38.3, 40.1	0.5
6 yr							
Age (yr)	369	6.2	6.1, 6.3	326	6.2	6.1, 6.3	0.4
Socioeconomic score	360	28	21, 33	308	27	22, 33	0.7
Weight (kg)	370	16.4	15.2, 17.6	328	15.7	14.6, 16.9	<0.001
Height (cm)	370	109.9	107.1, 113.2	328	109.4	106.5, 112.5	0.01
6-yr DXA measurements							
Total BMC (g)	369	655.0	597.6, 724.1	326	615.3	560.6, 690.6	<0.001
Total BMD (g/cm^2)	369	0.79	0.76, 0.82	326	0.77	0.75, 0.80	<0.001
Spine BMC (g)	369	50.8	44.7, 57.7	326	49.3	42.9, 56.8	0.002
Spine BMD (g/cm^2)	369	0.67	0.63, 0.71	326	0.67	0.63, 0.70	0.5
Lean mass (kg)	369	13.0	12.3, 14.2	326	12.1	11.3, 13.0	<0.001
Fat mass (kg)	369	2.9	2.3, 3.5	326	3.3	2.6, 4.0	<0.001
Parents	Fathers			Mothers			
Age (yr)	639	33.9	31.5, 37.0	681	27.5	25.6, 29.6	<0.001
Height (cm)	643	164.6	160.6, 168.6	682	153.0	148.5, 155.4	<0.001
DXA measurements							
Total BMC (g)	641	2413	2210, 2626	682	1883	1738, 2042	<0.001
Total BMD (g/cm^2)	641	1.15	1.10, 1.20	682	1.11	1.06, 1.15	<0.001
Spine BMC (g)	641	192.4	172.5, 214.3	682	157.8	142.0, 175.2	<0.001
Spine BMD (g/cm^2)	641	1.06	0.99, 1.15	682	1.04	0.97, 1.13	0.004
Lean mass (kg)	641	43.7	40.6, 46.9	682	30.4	28.4, 32.2	<0.001
Fat mass (kg)	641	8.9	5.2, 15.6	682	10.2	7.7, 14.7	<0.001

All children's 6-yr values were adjusted to an exact age of 6 yr. *P* values for differences between the sexes were derived using two-sided unpaired *t* tests. IQR, Interquartile range.

Centre for Health Statistics references (http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/datafiles.htm.), the children were light, short, and thin. Mean weight, height, and body mass index SD scores were −2.16, −1.24, and −2.06 for boys and −2.32, −1.37, and −2.12 for girls, respectively.

The children's age, socioeconomic status, weight, and height were positively related to all bone outcomes ($P < 0.01$ for all). The parents' heights and DXA measurements were positively correlated with bone outcomes in the children (Table 2 and Fig. 1). The strength of these correlations was similar for fathers and mothers and greater for size than for density measurements. Maternal height and bone measurements were positively correlated with paternal height and equivalent bone measurements (height: r = 0.20 $P < 0.001$; total body BMC: r = 0.20 $P < 0.001$; total body BMD: r = 0.09 $P = 0.003$). Pregnancy is associated with reduced maternal bone mass. A total of 419 mothers had at least one child since the birth of the study child; all maternal bone outcomes were positively related to the interval, and inversely related to the number of children born, since the birth of the last child ($P < 0.01$ for both). Even after adjusting for these factors, mother-child and father-child correlations remained statistically significant ($P < 0.05$ for all) and similar to each other.

The children's birth weight, birth length, and placental weight were positively associated with all bone outcomes ($P < 0.001$ for all). Fifty-nine (9%) children were SGA; total body BMC was lower in the SGA children than the other children (614 *vs.* 651 g; $P = 0.01$, not statistically significant after adjusting for the child's 6-yr weight and height). There were no differences between children born preterm and full term.

TABLE 2. Correlations between the parents' and children's DXA measurements

	Child's bone outcomes at 6 yr			
	Total BMC	Total BMD	Spine BMC	Spine BMD
Mother's DXA data (n = 681)				
Total BMC (g)	0.36	0.31	0.32	0.29
Total BMD (g/cm^2)	0.28	0.39	0.23	0.28
Spine BMC (g)	0.33	0.26	0.36	0.29
Spine BMD (g/cm^2)	0.23	0.27	0.22	0.28
Father's DXA data (n = 640)				
Total BMC (g)	0.38	0.32	0.32	0.30
Total BMD (g/cm^2)	0.26	0.33	0.20	0.23
Spine BMC (g)	0.30	0.25	0.30	0.25
Spine BMD (g/cm^2)	0.18	0.24	0.14	0.22

All correlations shown were statistically significant at the $P < 0.001$ level.

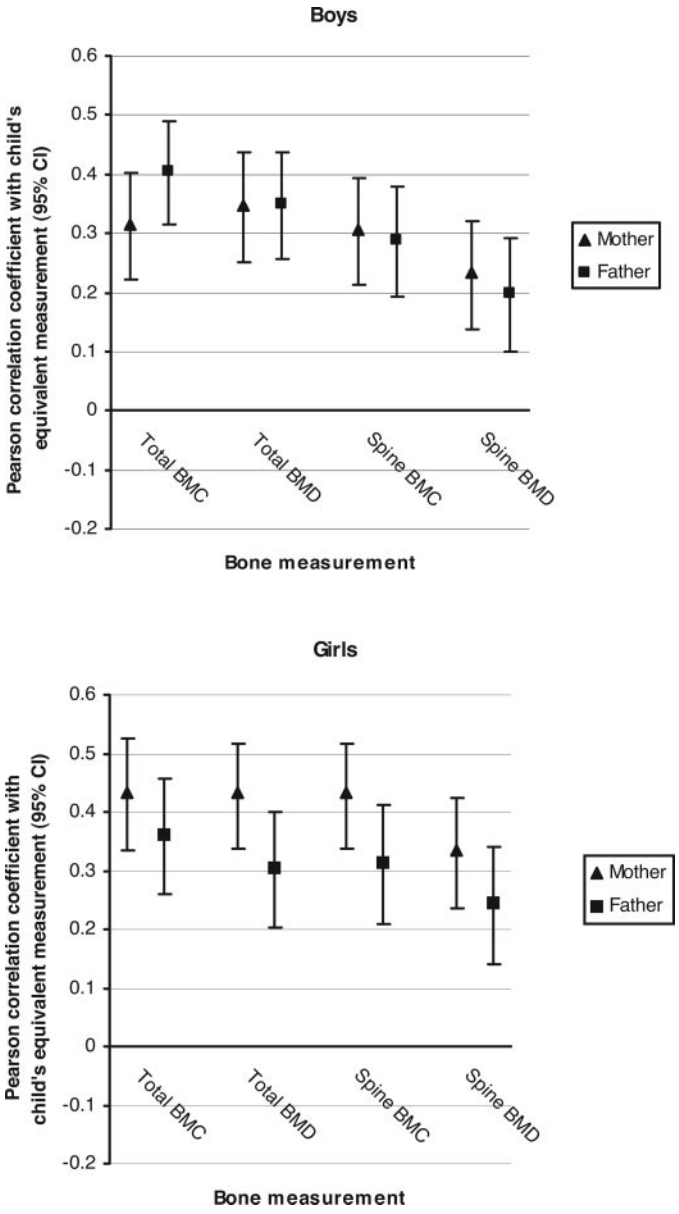

FIG. 1. Plot of Pearson correlation coefficients [95% confidence interval (CI)] between equivalent bone measurements in child and both parents separately for boys and girls. Six hundred thirty-one of the fathers (98%) were a subset of the partners of the 682 mothers; in 12 cases (in which the mother was sick or no longer alive), the father alone was studied, and in 51 cases (in which the father did not participate in the study), the mother alone was studied. For four children, no parental DXA data were available.

Maternal characteristics and nutritional status during pregnancy

Approximately one third of the women were primiparous. The women's energy, protein, and calcium intakes were low, compared with Indian Council of Medical Research recommended daily allowances for pregnant women (22) (median intakes in Pune: 1726 kcal, 45 g, and 274 mg daily at 18 wk; 1637 kcal, 42 g and 268 mg at 28 wk). The main protein source was pulses, which were consumed almost twice a day on average (Table 3). GLVs were consumed on average twice a week and fruit every day. Whole milk was consumed less than twice a week, whereas milk products (mainly milk in tea) were consumed twice a day. A third of women never ate meat, fish, or eggs, and a further quarter consumed them less than once a week. Higher socioeconomic status was associated with higher intakes of milk and calcium ($P < 0.001$ for both). More than 60% of women had low vitamin B12 concentrations (<150 pmol/liter), approximately half had low ferritin status (<12 ng/ml), and two thirds had low vitamin C concentrations (<23 μmol/liter). Only one had low folate status (<283 nmol/liter). Twenty-eight percent of mothers chewed tobacco (none smoked).

In univariate analyses, the following maternal nutritional variables were positively related to bone outcomes in the children (Table 3): prepregnant fat mass; frequency of intake of milk, milk products, pulses, fruit, all calcium-rich foods at 18 and 28 wk gestation and GLVs at 28 wk (Fig. 2); total calcium intake at 18 and 28 wk; and folate concentrations at 28 wk. Maternal parity, tobacco use, and physical workload score at 28 wk were negatively related to the children's bone outcomes. Maternal energy; protein, fat, and carbohydrate intakes; and plasma ferritin, vitamin C, vitamin B12, MMA, and tHcy concentrations were unrelated to bone outcomes in the children. Children who were exclusively breast-fed for a longer duration had lower spine BMD.

In multiple regression analyses (Table 4), larger parental BMC (both parents), lower maternal parity, higher maternal intakes of milk and milk products at 28 wk gestation, and longer birth length independently predicted higher total body and/or spine BMC in the child. Both parents' BMD, maternal milk and milk product intakes at 28 wk, and maternal folate status at 28 wk were independent predictors of the child's total and/or spine BMD. Socioeconomic status, paternal height, maternal prepregnant fat mass, physical workload, tobacco use during pregnancy, and duration of exclusive breast-feeding were no longer significant predictors of bone outcomes. Maternal height and the child's gestational age at delivery were negatively related to total BMC. The addition of placental weight to the models did not alter the above associations, although placental weight was a significant independent predictor of all bone outcomes. Maternal consumption of milk and milk products and folate concentration at 28 wk each accounted for around 1% of the variance in total BMD, compared with 5% for paternal and 11% for maternal BMD. The full regression model accounted for 31% of the variance (Table 4). When estimated calcium intakes at 28 wk were substituted for milk and milk products in the regression models, calcium intake was positively associated with total body and spine BMC ($P = 0.056$ and $P = 0.064$); the other associations, and the variance explained, were little changed.

Discussion

We report a large community-based study exploring determinants of bone mass among rural Indian children. This is the first such study, to our knowledge, from a developing country. BMC and BMD were higher among heavier, taller children and those whose parents had higher BMC and BMD.

TABLE 3. Correlations between maternal characteristics and infant feeding and bone mass outcomes in the children

	n	Median	IQR	Total BMC	Total BMD	Spine BMC	Spine BMD
Prepregnant fat mass (kg)	698	8.5	6.9, 10.2	0.21[a]	0.15[a]	0.20[a]	0.15[a]
Parity (0, 1, >1)	698			−0.7	−0.08[b]	−0.11[c]	−0.12[c]
Frequency of intake (per month)							
Milk, 18 wk	690	6	0, 16	0.06	0.09[b]	0.02	0.02
Milk, 28 wk	667	6	0, 16	0.07	0.13[c]	0.04	0.05
Milk products, 18 wk	690	61	36, 66	0.10[b]	0.14[a]	0.08[b]	0.09[b]
Milk products, 28 wk	667	61	34, 66	0.06	0.09[b]	0.09[b]	0.04
Pulses, 18 wk	661	52	35, 73	0.09[b]	0.07	0.08[b]	0.06
Pulses, 28 wk	667	47	31, 69	0.09[b]	0.05	0.08[b]	0.09[b]
Fish, meat, or eggs, 18 wk	690	2	0, 8	0.04	0.05	0.03	0.04
Fish, meat, or eggs, 28 wk	667	1	0, 8	0.05	0.06	0.05	0.05
GLVs, 18 wk	690	10	5, 21	0.05	0.04	0.05	0.03
GLVs, 28 wk	667	6	2, 14	0.11[b]	0.07	0.10[b]	0.06
Fruit, 18 wk	690	28	14, 47	0.07	0.14[a]	0.07	0.10[b]
Fruit, 28 wk	667	20	10, 37	0.08[b]	0.07	0.10[b]	0.07
All calcium-rich foods, 18 wk	690	145	109, 182	0.13[a]	0.13[a]	0.11[c]	0.07
All calcium-rich foods, 28 wk	667	134	103, 169	0.13[a]	0.12[c]	0.13[a]	0.10[c]
Calcium intake, 18 wk (mg/d)	690	274	223, 354	0.13[a]	0.15[a]	0.09[b]	0.06
Calcium intake, 28 wk (mg/d)	667	268	208, 332	0.11[c]	0.11[c]	0.10[c]	0.07
Magnesium intake, 18 wk (mg/d)	690	477	416, 548	0.02	0.01	0.01	−0.02
Magnesium intake, 28 wk (mg/d)	667	477	416, 545	0.07	0.04	0.08[b]	0.06
Phosphorus intake, 18 wk (mg/d)	690	1033	911, 1120	0.06	0.05	0.04	0.01
Phosphorus intake, 28 wk (mg/d)	667	1016	890, 1174	0.07	0.05	0.07	0.05
Red cell folate, 18 wk (nmol/liter)	615	879	693, 1097	0.06	0.08	0.05	0.06
Red cell folate, 28 wk (nmol/liter)	554	960	739, 1258	0.12[c]	0.13[c]	0.13[c]	0.17[a]
Maternal workload score, 18 wk	690	79	50, 92	−0.08	−0.07	−0.06	−0.03
Maternal workload score, 28 wk	668	63	43, 86	−0.14[a]	−0.11[c]	−0.13[a]	−0.11[c]
Tobacco chewing [n (%)]	698	196 (28.1%)		−0.07	−0.07	−0.07	−0.08[b]
Exclusive breast-feeding (months)	697	6	4, 7	−0.03	−0.04	−0.06	−0.08[b]

IQR, Interquartile range.
[a] $P < 0.001$.
[b] $P < 0.05$.
[c] $P < 0.01$.

Maternal and paternal measurements showed positive associations, of equal strength, with offspring measurements. The mothers had low calcium intakes, consistent with other low-income groups in India (27). Greater maternal consumption of calcium and calcium-rich foods, especially milk and milk products, in mid- to late pregnancy was associated with higher total body and spine BMC and/or BMD in the children. Higher maternal folate status was associated with higher total body and spine BMD. The associations of maternal calcium and milk intakes and folate concentrations with bone outcomes in the children were statistically significant after adjustment for newborn size (19). They remained significant after adjustment for age, sex, socioeconomic status, current size, parental size and bone measurements, infant feeding, and maternal energy and protein intake, tobacco use, and parity.

These data indicate the importance of both genetic and environmental factors in determining bone mineral accrual during childhood. The association between paternal and offspring bone density, even after adjusting for maternal effects, could reflect genetic influences, epigenetic effects, or the influence of shared family environment and lifestyle. Potential candidate genes that might mediate genetic effects include those influencing placental calcium transport as well as vitamin D receptor, PTH, PTHrP, and PTH receptor genes.

Experimentalists have demonstrated that altering the diet of pregnant animals can produce lasting changes in the size and metabolism of the offspring (28). Cohort studies in Western populations have shown that birth weight and weight in infancy predict adult bone mass, independently of known environmental risk factors for osteoporosis during adulthood (smoking, alcohol consumption, exercise, calcium nutrition, and reproductive variables in women) (5, 8, 11, 29). Physiological studies suggest a potential role of the PTH-vitamin D axis as a mediator of these epidemiological associations (30). Furthermore, recent evidence suggests interactions between birth weight and genetic markers of the PTH/vitamin D axis with adult bone mass (31).

Although a family history of osteoporosis is a risk factor for the disorder, most studies have focused on maternal, rather than paternal, history. A study from California of osteoporotic parents and their sons showed correlations between the BMD of offspring and both parents (32). Our study suggests associations of approximately equal magnitude of paternal and maternal bone outcomes with those of the offspring, the strongest being with bone size and the most attenuated for bone density. This suggests that bone size may be more heritable than BMD.

Several other studies have examined associations between maternal nutrition and bone outcomes in the children. Spine BMC and BMD were higher in U.K. neonates born to mothers with higher calcium intakes in pregnancy (11). Studies in children have shown associations of bone density with maternal vitamin D status (14) and intakes of magnesium (10, 12), potassium (10, 12), phosphorus (10), folate (12), protein (10), and milk (10) during pregnancy. As in Pune, the proportion of variance explained by maternal dietary variables was small. Two randomized, controlled trials of calcium

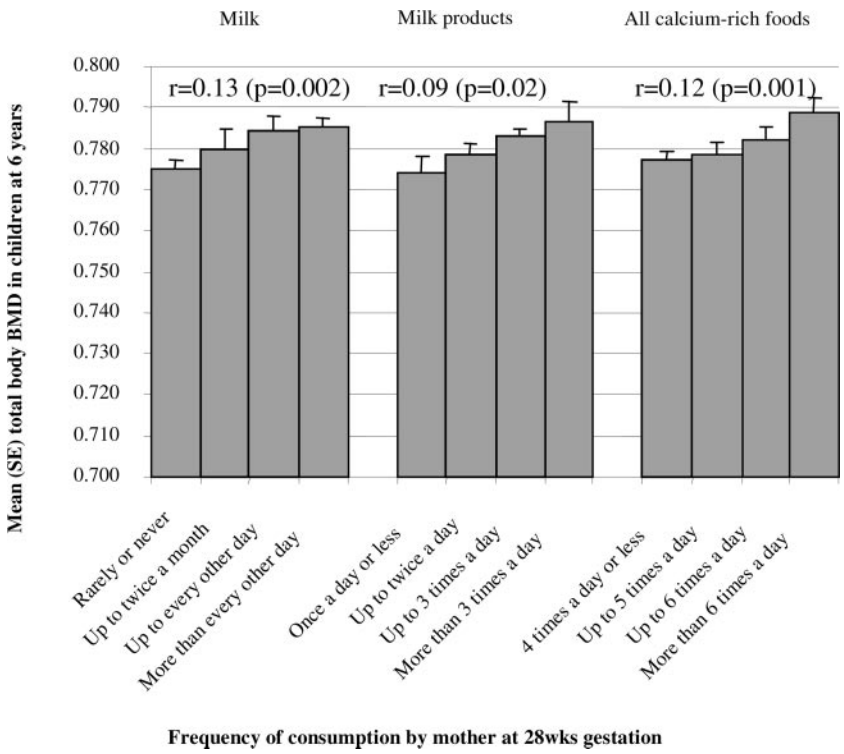

FIG. 2. Total body BMD (means and SE values) in the children according to the mother's frequency of intake of milk, milk products, and calcium-rich foods at 28 wk gestation (r = Pearson correlation coefficient and P value for continuous predictor and outcome variables).

supplementation in pregnancy have demonstrated increased BMD in the children (16, 17). Animal studies have shown permanently impaired bone mineralization in offspring of mothers on low calcium diets (33). Our findings are therefore in accord with other evidence that maternal calcium status influences bone outcomes in the children and with one study linking maternal folate status to bone density in the children (12).

Other maternal factors that have been related to bone outcomes in the children (both associated with lower bone mass outcomes) include smoking (9, 11, 14) and vigorous activity (11). In Pune, there were negative associations for maternal workload score and tobacco chewing with bone outcomes, although these associations were not statistically significant after adjusting for other variables. There have been few studies of bone measurements in children in relation to maternal parity. In our study, higher maternal parity was associated with lower total BMC and spine BMD. A study of premature newborns in Egypt showed lower cord blood collagen propeptides, markers of bone formation, in babies born to mothers of higher parity (34).

There are caveats to our findings. We did not have measurements of the diet and activity of the children at 6 yr, and because family diets tend to remain similar over time, the child's current diet, rather than the mother's nutrition during pregnancy, may explain our findings. Our study relied on DXA for measurement of bone mass. Although validated in adults, its use in children raises some technical problems. The smaller amounts of bone mineral, and variability between the proportions of intraosseous marrow fat and that present in lean tissue, lead to greater precision errors than in adults (35, 36). Our spine measurements were derived from total body scans, and although these are strongly correlated with values derived from specific spine scans (37), the latter are considered superior (38). Finally, there is debate about the use of pencil-beam (used in this study) as opposed to fan-beam scanners for bone measurements (39). However, it is difficult to see how these inaccuracies would lead to spurious relationships between maternal nutrition and bone outcomes.

We cannot infer that maternal calcium intakes and folate status caused our observed associations. Associations of bone outcomes in the children with estimated maternal calcium intakes were weak, compared with those with milk and milk product intakes. Calcium intakes were, however, approximate, based on average portion sizes and assumptions about raw weights. Some calcium-rich foods were also high in protein. Although the associations with calcium-rich foods were independent of protein intakes, protein quality may be an important factor. Any causal associations between calcium-rich foods and bone mass could reflect postnatal rather than intrauterine effects, related to breast milk quality or postnatal diet. Indian studies (including studies of pregnant women) have shown a very high prevalence (66–84%) of vitamin D insufficiency or deficiency in rural and other low-income populations (40, 41). We did not have measurements of calcitrophic hormone status in either the mothers or children. The assessment of maternal serum 25-hydroxyvitamin D and umbilical venous calcium concentrations would be helpful in evaluating intrapregnancy mechanisms for the association between maternal milk intakes and childhood bone mass.

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TABLE 4. Multiple regression analysis of predictors of the children's BMC and BMD (as continuous variables)

	Total BMC				Total BMD				Spine BMC				Spine BMD			
	b	β	P	Var, %	b	β	P	Var, %	b	β	P	Var, %	b	β	P	Var, %
Child's age (yr)	48.2	0.36	<0.001	12.1	0.009	0.20	<0.001	3.5	3.7	0.297	<0.001	8.11	0.018	0.241	<0.001	5.33
Child's sex (boys 0, girls 1)	−28.5	−0.14	<0.001	1.7	−0.009	−0.12	0.006	1.3	−0.82	−0.042	0.2	0.16	0.004	0.031	0.5	0.09
Child's current height (cm)	11.7	0.51	<0.001	16.8	0.001	0.15	0.005	1.4	1.19	0.563	<0.001	20.36	0.005	0.383	<0.001	9.45
Child's birth length (cm)	4.3	0.10	0.019	0.5	0.0007	0.05	0.4	0.1	0.28	0.068	0.1	0.26	0.0009	0.038	0.5	0.08
Mother's height (cm)	−2.3	−0.11	0.006	0.7	−0.0002	−0.03	0.6	0.1	−0.15	−0.077	0.06	0.40	−0.0004	−0.035	0.5	0.09
Mother's parity (0, 1, > 1)	−8.5	−0.07	0.048	0.4	−0.003	−0.06	0.2	0.3	−0.66	−0.055	0.1	0.26	−0.006	−0.084	0.058	0.61
Mother's intake of milk and milk products (frequency per month), 28 wk	4.3	0.08	0.017	0.5	0.002	0.11	0.014	1.1	0.34	0.066	0.064	0.38	0.0009	0.030	0.5	0.08
Mother's equivalent bone outcome	0.058	0.18	<0.001	2.5	0.12	0.23	<0.001	11.4	0.035	0.112	0.005	3.69	0.096	0.180	<0.001	3.66
Father's equivalent bone outcome	0.10	0.22	<0.001	2.0	0.21	0.37	<0.001	4.8	0.089	0.235	<0.001	0.90	0.11	0.209	<0.001	2.96
Gestation at birth (wk)	−4.8	−0.07	0.049	0.4	0.0002	0.01	0.9	0.0	−0.23	−0.038	0.3	0.10	0.00003	0.001	0.99	0.00
Folate at 28 wk gestation (nmol/liter)	3.4	0.01	0.7	0.0	0.009	0.10	0.036	0.8	0.92	0.037	0.3	0.12	0.015	0.098	0.024	0.87
Constant term	−484.4		0.001	1.0	0.249		0.001	1.7	−75.8		<0.001	2.96	−0.10		0.3	0.19
n = 394	Adj R^2 = 0.63				Adj R^2 = 0.31				Adj R^2 = 0.56				Adj R^2 = 0.33			

Standardized regression coefficients are those obtained when all variables are standardized to have a mean of 0 and a SD of 1. They can be interpreted as the number of SD values by which the dependent variable changes when the independent variable increases by 1 SD. They give an indication of the relative sizes of effects within a regression model because all variables now have the same scale. The following variables were included in all models but showed no statistically significant associations with bone outcomes: socioeconomic status, paternal height, maternal energy and protein intakes at 28 wk gestation, physical workload score, tobacco use, prepregnant fat mass, and duration of exclusive breast-feeding in months. b, Regression coefficient; β, standardized regression coefficient; Var, %, percentage of variance explained.

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