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Breastfeeding as the sole source of milk for 6 months and adolescent bone mineral density

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Abstract

Summary—Little is known regarding the relationship between early life factors and bone mineral density (BMD). We found a positive association between breastfeeding for at least 6 months, without formula supplementation, and whole body adolescent BMD z-score.

Introduction—The aim of the study is to assess the role of breastfeeding BF on adolescent bone mineral density (BMD) in a cohort prospectively followed since infancy.

Methods—We studied 679 participants from an infancy iron deficiency anemia preventive trial in Santiago, Chile, followed to adolescence. Breast and bottle feeding were ascertained weekly from 4 to 12 months. At 16 years, whole body BMD was assessed by DEXA. Using linear regression, we evaluated associations between BF duration and BF as the sole source of milk and adolescent BMD z-score, adjusting for possible infancy, adolescent, and background confounders.

Results—Mean birth weight and length were 3.5 (0.3) kg and 50.7 (1.6) cm. For at least 6 months, BF was the sole source of milk for 26.3% and with supplementation for 36.7%. For 37%, BF was provided for less than 6 months. Mean 16-year BMD z-score was 0.25 (1.0). Covariates included male sex, birth length, and gestational age. BF as the sole source of milk ≥ 6 months, compared to BF < 6 months, was associated with higher adolescent BMD z-score adjusting for covariates ($\beta = 0.29$, $p < 0.05$). Mixed BF was not significantly related to adolescent BMD z-score ($\beta = 0.06$, $p = 0.47$). For every 30 days of BF as the sole source of milk, adolescent BMD z-score increased by 0.03 ($p = 0.01$).

Conclusion—BF without formula supplementation for at least 6 months was associated with higher adolescent BMD z-score and a suggestive trend in the same direction for BMD suggests that exclusivity and duration of BF may play a role in adolescent bone health.

Compliance with ethical standards The study was approved by the Institutional Review Boards at the University of California, San Diego, University of Michigan, Ann Arbor, and the Institute of Nutrition and Food Technology (INTA), University of Chile, the study site in Chile.

Conflicts of interest None.

Keywords

Bone health; Developmental origins of disease; Lactation; Osteoporosis

Introduction

Low bone mineral density (BMD) is responsible for considerable morbidity in older men and women. Prevention of adult osteoporosis requires optimal peak bone mass. As bone mass peaks in young adulthood, it is reasonable to examine early life factors that could relate to peak bone mass and BMD. Breastfeeding (BF) in infancy is one factor that may relate to higher BMD in childhood and adolescence [1–5]. However, some studies do not show this association [6–11]. A recent review concluded that there is evidence that BF in infancy is positively associated with bone mass in adolescence [12]. The authors also suggest that the influence of BF on bone mass appears to be more robust when measured in adolescence compared to earlier. As these conclusions are based on a small number of studies, more research is needed. Chile provides an ideal setting to address such questions. In Chile, BF is common. Nationally representative data indicates that 80% of Chilean children are exclusively breastfed for 1 month and 56% for 6 months [13]. Early supplementation with cow milk or infant formula, in conjunction with continued BF, is also common. One study showed that at 6 months, 27% of babies were breastfed and receiving formula, along with solid foods [14]. The WHO recommends exclusive BF for 6 months and continued BF with appropriate complementary foods for 2 years or longer [15]. However, for many women, 6 months of exclusive BF is difficult. Although many women in Chile, and other countries, have an extended lactation period, many also provide early formula supplementation. Recent reports indicate approximately 30% of babies in upper-middle income countries are exclusively breastfed between 0 and 5 months, yet >70% are receiving some breastmilk at 6 months [16]. It is still unclear if and how long-term effects of BF relate to early supplementation. The purpose of this study was to evaluate associations between BF and adolescent whole body BMD and BMD z-score in a sample of healthy Chilean youth followed since infancy.

Methods

We studied adolescents who were part of a longitudinal cohort followed since infancy as part of either an iron deficiency anemia (IDA) preventive trial or a neuromaturation study. Details of the original preventive trial have been previously described [17]. In brief, healthy infants from uncomplicated singleton vaginal births at term were recruited at 4–6 months of age from four low- to middle-socioeconomic neighborhoods in Santiago, Chile, between 1991 and 1996. Infants weighing ≥ 3 kg at birth without IDA at 6 months entered the preventive trial. Infants received either iron supplementation or usual nutrition between 6 and 12 months. Iron supplementation was provided via infant formula or liquid drops depending on breast/bottle feeding at the time of recruitment. Infants who, at study enrollment, were found to have IDA and the next nonanemic infant, were treated with medicinal iron and participated in a separate neuromaturation study [18]. Between 16 and 17 years, a subset of the original infancy cohort (preventive trial and neuromaturation participants) was invited to

participate in a study of cardiovascular risk ($n = 679$). The cardiovascular risk sample did not differ from the original infancy participants with respect to: birth weight and length and maternal and paternal total years of education. BF habits did differ. The date of the first bottle and date of last BF were later in the cardiovascular risk sample compared to the infancy cohort: 117 vs. 100 days and 244 vs. 227, respectively, both $p < 0.05$. For this secondary data analysis, we included all participants with complete information on the exposure (BF) and outcome (BMD and BMD z-score). Of the 679 total sample, 9 (1.3%) were excluded for not having information on date of first bottle or date of last BF and 8 (1.1%) were excluded for having no information from the DXA scan. Thus, the sample size for this analysis was 662. The study was approved by the Institutional Review Boards at the University of California, San Diego, University of Michigan, Ann Arbor, and the Institute of Nutrition and Food Technology (INTA), University of Chile, the study site in Chile.

Infancy study

Beginning at 6 months, infants were followed with monthly anthropometric measurements at INTA; previous anthropometric data were obtained from medical records. For those receiving formula/cow milk supplementation at 4–6 months, the date of the first bottle was recorded based on maternal recall. For all other infants, this information was asked prospectively, on a weekly basis, between 6 and 12 months of age. Mothers were also asked date of last BF. In Chile, at the time of this study, bottle feeding was equivalent to formula feeding, as feeding expressed breastmilk by bottle was exceedingly rare. Almost the entire sample was initially breastfed, with only five participants never being breastfed. Using date of first bottle of milk/formula and date of last BF, we computed three mutually exclusive BF groups: (1) BF as the sole source of milk 6 months, (2) mixed BF, and (3) <6 months BF. BF as the sole source of milk 6 months was defined as first bottle and last BF after 6 months or more. Mixed BF was defined as date of first bottle before 6 months of age and date of last BF after 6 months or more. Finally, <6 months BF was defined as first bottle and last BF before 6 months.

Adolescent evaluation

For the adolescent evaluation, participants were weighed and measured at INTA in minimal clothing according to standard methods by a research physician. Weight was measured to the closest 0.1 kg, using a Seca scale, and height to the closest 0.1 cm, using a Holtain stadiometer. Measurements were taken twice, with a third measurement if the difference between the first two exceeded 0.3 kg for weight and 0.5 cm for height. Body composition and bone measurements were evaluated using Lunar Prodigy dual energy X-ray absorptiometry (DEXA) scan using standard protocols on the same machine calibrated every other day. Whole body BMD z-scores were based on National Health and Nutrition Examination Survey III and adjusted for age and sex [19].

As physical activity—especially weight-bearing exercise— and diet have been associated with bone mineral density [20]; we controlled for these possible confounders in our model. Adolescents answered questions regarding physical activity habits using a questionnaire that was validated in a previous study using accelerometer-based activity monitors in children aged 8–13 [21]. Participants were asked about time spent in planned school-based physical

education, extracurricular sports and sedentary activities (e.g., class and computer time and TV watching), and walking for transportation. Responses were summed (maximum score of 10) and higher scores indicated greater physical activity. Participants answered questions on their dietary habits using a questionnaire previously used with school-aged Chileans. [22] The questionnaire evaluated the nutritional quality of meals and snacks, with higher scores (maximum score of 10) assigned for better nutritional quality (e.g., low/no-fat dairy consumption assigned a higher score than full-fat dairy consumption). Both questionnaires are available by request.

Statistical analysis

We evaluated differences in adolescent BMD and BMD z-score by BF group with an ANOVA test with Bonferroni post hoc tests for differences between groups. We then conducted multivariable linear regression to test the same relationship adjusting for relevant covariates. In initial models, we adjusted for sex, birth weight and length, gestational age, maternal education, age at adolescent visit, adolescent diet, and physical activity habits. In order to evaluate the effects of BF in a dose-response context, we also evaluated a continuous measure of BF (date of first bottle), in our multivariable models. Covariates that were significantly related to the outcome (BMD or BMD z-score) or that altered the association between BF and outcome were maintained in the final multivariate model for parsimony. A p value <0.05 denoted statistical significance.

We did not control for iron supplementation in infancy because it is confounded with breastfeeding, by design. Inclusion criteria changed midway through the original infancy study, such that infants with longer breastfeeding were more likely to belong to the usual nutrition group. Additional details of the changes in inclusion criteria have been published elsewhere [17]. A subset of mothers ($n = 359$) reported on smoking habits at the infancy wave. We provided information on maternal smoking in our sample for descriptive purposes but were unable to control for maternal smoking in multivariable models, as the reduced sample may introduce selection bias.

Results

At birth, participants had mean weight 3.5 (0.3) kg and length 50.7 (1.6) cm and, on average, received the first bottle at 118 (97) days. Over a quarter (26.2%) of the infants were BF as the sole source of milk ≤ 6 months, 36.7% mixed BF, and 37% BF < 6 months. Average adolescent BMD z-score was 0.25 (1.0). Table 1 provides additional descriptive information on the sample overall and by BF group.

Figure 1 shows the relationship between BF group and adolescent whole-body BMD z-score. There was an overall difference in adolescent BMD z-score by BF group (ANOVA, $p = 0.01$). Post hoc tests showed statistically significant differences between BF < 6 months versus BF sole source of milk ≤ 6 months. The difference in adolescent BMD z-score between BF ≤ 6 months and mixed BF showed a suggestive trend ($p = 0.09$). A statistically significant difference was not observed between mean BMD z-score of the mixed BF versus the BF < 6 months group. We found no evidence of a significant bivariate association between BMD and BF group (Fig. 2).

Using a multivariate linear regression model, we found that BF sole source of milk ≥ 6 months compared to BF < 6 months was independently associated with higher adolescent whole-body BMD z-score ($\beta = 0.29$ $p < 0.05$), adjusting for sex, birth length, and gestational age (Table 2, model 1). Mixed BF was not significantly related to adolescent BMD z-score, compared to BF < 6 months. Birth weight, maternal education, age at adolescent visit, adolescent dietary habits, and adolescent physical activity were not found to be significantly related to whole-body BMD z-score and were thus removed for parsimony. We observed a non-significant trend between BF sole source of milk ≥ 6 months, compared to BF < 6 months, and whole-body BMD, adjusting for the same covariates ($p = 0.09$, Table 2, model 2). As with the BMD z-score model, male sex, higher birth length, and gestational age were significantly related to higher adolescent BMD.

Table 3 shows the results of the multivariable linear regression models testing the association between date of first-bottle BMD z-score (model 1) and BMD (model 2). Date of first bottle was significantly related to BMD z-score ($p < 0.05$) adjusting for male sex, birth length, and gestational age. Adjusting for covariates, for every 30 days of BF as the sole source of milk, adolescent BMD z-score increased by 0.03. We observed no statistically significant effect of a continuous measure of BF on adolescent BMD (Table 3, model 2).

Discussion

In this sample of Chilean adolescents born at term, BF was related to adolescent bone health after adjusting for important confounders. We observed a positive association between BF as the sole source of milk ≥ 6 months, but not mixed BF (BF ≥ 6 months, with formula/milk introduction < 6 months), and adolescent standardized whole-body bone mineral density. We also found a trend in a similar direction for BMD. Our findings suggest that BF intensity may be more important than duration with respect to future bone health.

Some studies show a positive relationship between BF and bone health outcomes at different time points, but the association is complex given different definitions of BF, lengths of follow-up, and focus of reports [1–5]. Among Australian children, positive associations of BF for 1 month, but not longer (3 months), with total body and spine BMD and decreased risk of fracture in adolescence were observed [4]. Associations were not modified by length of gestation. Differences in findings may relate to the distribution of BF in the samples. Our cohort of healthy infants born at term was largely breastfed, with only five participants never being breastfed. Over a quarter were BF as the sole source of milk ≥ 6 months and age at first bottle was, on average, 4 months.

At least one study has reported the opposite effect. Van de Hooven and colleagues found that among breastfed infants, non-exclusive BF for 4 months or longer related to higher BMD and area-adjusted bone mineral content and lower bone area at 6 years of age compared to exclusive BF [23]. The same study showed that compared to children who were ever breastfed, those who never breastfed had lower BMD, bone mineral content and bone area at 6 years. Taken together, these results imply that overall BF has positive effects but seemingly better results for those children who had non-exclusive BF. Our study shows the opposite results, with protective effects of BF only for adolescents who received BF as the sole source

of milk for 6 months or longer, and not for those with mixed BF. These seemingly disparate results highlight the fact that the underlying mechanisms of breast milk and bone health are not well understood. Differing results may also relate to the different contexts of BF: average duration, exclusivity, and supplementation habits in the sample studied. Another explanation for the differences may relate to the outcome variable. We presented results for BMD and BMD z-score. We observed a trend between BF and adolescent BMD alone and found a statistically significant protective effect of BF for BMD z-score. BMD z-score allows for comparison between research studies and, importantly, adjusts for important confounders of bone size—age and sex.

There are plausible biological mechanisms for associations between BF and better bone health. Some researchers posit that the lower mineral content of breast milk, compared to formula, may program bone cells to be conservative with minerals in childhood resulting in higher bone mass later [24]. Others suggest that nutrients received from breast milk may be better absorbed by infants [3] or that growth factors and hormones present in breast milk might have mechanistic effects. A study that similarly had follow-up in adolescence and was also conducted with a healthy sample (mostly term infants) suggested that BF provides long-term protection by altering bone accrual trajectory early in life [2]. There also may be a role for insulin-like growth factor I (IGF I)—that is hypothesized to mediate the relationship between protein and growth. IGF-I has been shown to be inversely associated with linear growth [25] and breast milk contains lower levels of IGF-I.

Strengths of our study include a long follow-up (16.8 years) specifically at an age when peak bone mass is nearly obtained and BF information reported prospectively between 4 and 12 months of age. We also report results from a large cohort of term infants, recruited in infancy from healthy mothers with singleton, uncomplicated, vaginal births. These characteristics make the cohort an optimal one for studying long-term bone health effects of early nutrition in term infants. In this research area, term infants have received far less attention than preterm infants, as 80% of fetal bone mineral accumulation occurs during the last trimester and metabolic immaturity for preterm infants might limit nutrient intake. Consequently, findings from those studies are difficult to generalize to infants born at term. Additionally, our results come from a country experiencing rapid economic development. During the period of our study, Chile progressed from low to high income with a widening income disparity [26]. Our results strengthen the generalizability of the body of literature suggesting infant programming of bone health.

Limitations of our study include lack of detailed information on maternal health during pregnancy and maternal pre-and post-natal BMI. Recent research considers associations between maternal diet and bone outcomes of children using maternal-offspring cohorts. Maternal diet, physical activity, smoking history, and body composition may all influence bone mineralization of offspring [27]. Enrollment criteria for the original infancy trial included healthy pregnancy with no birth complications. However, we do not have information on specific lifestyle components (diet, physical activity, and smoking during pregnancy) or body composition of mothers. Another limitation is the lack of other bone parameters, such as measurements of lumbar spine and femoral neck. DEXA was assessed as only one component of a larger research study of cardiovascular risk and obesity and was

employed primarily to measure body composition. Additionally, data on introduction of complementary food were not collected in infancy (though pureed fruits were typically introduced after 4 months) [28] nor are there data on consumption of water or fruit juices. Thus, in our study, infants may have received water or juice and were likely to have started complementary foods after 4 months. Therefore, BF in our study is not equivalent to exclusive BF as defined by WHO [15]. Our study would have also benefitted from complete information on maternal smoking in infancy and comprehensive pubertal data. A final limitation is that due to the fact that BF was confounded with iron supplementation by study design, we could not adjust for iron supplementation in our models. We studied adolescents who were part of a longitudinal cohort followed since infancy as part of either an IDA preventive trial or a neuromaturation study. In the first two and a half years of the preventive trial, infant formula fortified with iron was the supplementation vehicle. Thus, infants who were exclusively breastfed at 6 months (trial entry) were excluded from participation. Mid-enrollment, the investigators changed the study eligibility criteria to allow exclusively breastfed infants to be randomized. At the same time, a no supplementation randomization group was added to the study. Due to these circumstances, those in the no supplementation group were more likely to be breastfed than infants randomized in earlier stages of the study, producing an artificial association between supplementation and breastfeeding. Although it is worth considering that our reported effects might be attributed to differences in infancy supplementation, there is no literature to support a link between lack of iron supplementation and improved bone density.

Conclusion

In our sample of low-middle income Chilean adolescents, BF as the sole source of milk for at least 6 months related to a higher BMD z-score 17 years later. Our findings add to the literature that suggest early metabolic programming via infant nutrition. Additional studies are needed to confirm our findings among populations with similar BF habits.

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Abbreviations

BMD	Bone mineral density
BF	Breastfeeding
DEXA	Dual energy X-ray absorptiometry
IGF I	Insulin-like growth factor I
NHANES	National Health and Nutrition Examination Survey

WHO World Health Organization

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Fig. 1.

Adolescent whole body BMD z-score by BF group ($n = 662$). Overall difference in adolescent whole body BMD z-score by BF group (ANOVA, $p = 0.01$). Bonferroni Post hoc tests: BF sole source of milk ≥ 6 months versus BF < 6 months, $p = 0.01$; BF sole source of milk ≥ 6 months versus mixed BF, $p = 0.09$; BF < 6 months versus mixed BF, $p = 1.00$. BF as the sole source of milk ≥ 6 months was defined as first bottle and last BF after 6 months or more. Mixed BF was defined as date of first bottle < 6 months and BF ≥ 6 months. BF < 6 months BF was defined as first bottle and last BF before 6 months. * $p < 0.10$, ** $p < 0.05$

Fig. 2.

Adolescent whole body BMD by BF group ($n = 662$). Overall difference in adolescent whole body BMD by BF group (ANOVA, $p = 0.37$). Bonferroni post hoc tests: BF sole source of milk ≥ 6 months versus BF < 6 months, $p = 0.48$; BF sole source of milk ≥ 6 months versus mixed BF, $p = 1.00$; BF < 6 months versus mixed BF, $p = 1.00$. BF as the sole source of milk ≥ 6 months was defined as first bottle and last BF after 6 months or more. Mixed BF was defined as date of first bottle < 6 months and BF ≥ 6 months. BF < 6 months BF was defined as first bottle and last BF before 6 months

Table 1

Descriptive information on sample of Chilean adolescents overall and by BF group

	Overall N = 662	BF sole source of milk N = 174	6 months Mixed BF N = 243	BF < 6 months N = 245
Infancy variables				
Male	351 (53%)	89 (51.1%)	127 (52.3%)	135 (55.1%)
Birth weight (kg)	3.5 (0.3)	3.5 (0.3)	3.5 (0.3)	3.5 (0.3)
Birth length (cm)	50.7 (1.6)	50.7 (1.5)	50.8 (1.7)	50.6 (1.7)
Gestational age (weeks)	39.4 (1.1)	39.4 (1.0)	39.5 (1.1)	39.3 (1.1)
Maternal education (y)	9.5 (2.5)	9.7 (2.6)	9.6 (2.4)	9.4 (2.6)
Maternal smoking ^a	82 (22.8%)	11 (18.6%)	31 (23.7%)	40 (23.7%)
Age at first bottle (days)	118 (97)	249 (62)	89 (57)	53 (44)
Age at last BF (days)	246 (139)	357 (64)	323 (83)	86 (49)
Adolescent variables				
Age at adolescent evaluation	16.8 (0.2)	16.7 (0.2)	16.8 (0.2)	16.8 (0.2)
Average weight (kg)	65.6 (14.0)	67.8 (15.0)	64.8 (13.7)	64.8 (13.4)
Average height (cm)	165.8 (8.4)	165.6 (8.5)	166.0 (8.6)	165.3 (8.3)
Physical activity score ^b	4.1 (1.6)	4.0 (1.6)	4.1 (1.7)	4.0 (1.5)
Nutrition score ^c	5.2 (1.2)	5.2 (1.1)	5.2 (1.2)	5.1 (1.2)
Bone mineral content (kg)	2.5 (0.4)	2.5 (0.5)	2.5 (0.4)	2.6 (0.4)
BMD	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)
BMD z-score	0.2 (1.0)	0.4 (1.0)	0.2 (1.0)	0.1 (1.0)

^aAmong 359 respondents^bScore ranges between 0 and 10, higher scores indicate higher physical activity^cScore ranges between 0 and 10, higher numbers represent higher nutritional quality

Table 2

Multivariable linear regression model testing associations between BF group, BMD z-score (model 1) and BMD (model 2)

	Model 1 ^a		Model 2 ^b	
	β	<i>p</i> value ^c	β	<i>p</i> value ^c
BF group (ref. BF <6 months)				
BF 6 months	0.291	<i>0.004</i>	0.016	0.092
Mixed BF	0.066	0.472	0.005	0.529
Male	0.389	<i><0.001</i>	0.080	<i><0.001</i>
Birth length	0.111	<i><0.001</i>	0.100	<i><0.001</i>
Gestational age	-0.091	<i>0.013</i>	-0.008	<i>0.019</i>

^a *R* [2] of BF only = 0.013, overall *R* [2] = 0.09

^b *R* [2] of BF only = 0.003 overall *R* [2] = 0.20

^c *p*-values <0.05 in italics

Table 3

Multivariable linear regression model testing associations between date of first bottle, BMD z-score (model 1) and BMD (model 2)

	Model 1 ^a		Model 2 ^b	
	β	<i>p</i> value ^c	β	<i>p</i> value ^c
Age (per 30 days) of first bottle	0.030	<i>0.018</i>	<0.001	0.22
Male	0.382	< <i>0.001</i>	0.080	< <i>0.001</i>
Birth length	0.111	< <i>0.001</i>	0.100	< <i>0.001</i>
Gestational age	-0.094	<i>0.010</i>	-0.008	<i>0.017</i>

^a *R* [2] of date of first bottle only = 0.009, overall *R* [2] = 0.089

^b *R* [2] of date of first bottle only = 0.002 overall *R* [2] = 0.20

^c *p*-values <0.05 in italics