Are early growth and nutrition related to bone health in adolescence? The Copenhagen Cohort Study of infant nutrition and growth\textsuperscript{1–4}

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ABSTRACT

Background: It is generally accepted that peak bone mass affects later fracture risk in the elderly. The extent to which early nutrition and growth can program later bone health has been examined in only a few studies. In the Copenhagen Cohort Study we showed that breastfed infants had significantly higher serum (s)-osteocalcin concentrations than did formula-fed infants.

Objective: We investigated whether early nutrition and early growth are associated with later bone mass in adolescence.

Design: Participants were examined at birth; at ages 2, 6, and 9 mo (n = 143); and at age 17 y (n = 109) with anthropometric and s-osteocalcin measures and whole-body dual-energy X-ray absorptiometry (DXA) scanning (age 17 y only). Total body (T) and lumbar spine (LS) DXA values were used.

Results: The duration of exclusive breastfeeding was positively correlated with the sex-adjusted LS bone mineral content (BMC), LS bone area (BA), and LS bone mineral density (BMD) (all \(P < 0.03\)) and with size-adjusted LS-BMC (\(P = 0.075\)) at 17 y of age. s-Osteocalcin at 6 mo was positively correlated with sex-adjusted LS-BMC and LS-BMD (both \(P < 0.04\)) and with size-adjusted LS-BMC (\(P = 0.047\)) at 17 y of age. Weight and length at 9 mo and increase in weight and length during the first 9 mo of life were positively correlated with sex-adjusted T-BMC and T-BA at age 17 y (all \(P < 0.04\)).

Conclusions: Early body size and growth in infancy are related to bone mass in late adolescence. Furthermore, the duration of exclusive breastfeeding and the markers of bone turnover at 6 mo seem to be positively related to LS bone mass at age 17 y. \textit{Am J Clin Nutr} 2011;94(suppl):1865S–9S.

INTRODUCTION

It is generally accepted that a higher peak bone mass obtained after cessation of growth soon after the age of \(\approx 20\) y is important for a later reduction in fracture risk in the elderly \((1)\), although some do not agree \((2)\). A higher bone mass in adulthood seems to be maintained later in life \((3)\). Knowledge about early predictors of peak bone mass may therefore be important to optimize later bone mass and thereby reduce fracture risk later in life.

It has been shown previously that body size at 1 y is related to bone mineral content (BMC) but not to bone mineral density (BMD) in adulthood \((4)\) and in the elderly \((5, 6)\). In Gambians aged 17–21 y, de Bono et al \((7)\) showed that birth weight (BW) predicted the attained cross-sectional area in cortical sites for males and in trabecular sites for females. However, others have shown that size-corrected BMC in prepubertal children was negatively associated with size and growth in infancy in both premature children \((8)\) and in those born at term \((9)\), indicating that a high growth rate in infancy may have a negative influence on bone mineralization before puberty. Concerning early nutrition and later bone mass, only few studies exist \((10–12)\). Jones et al \((10)\) showed that breastfeeding for \(>3\) mo was positively associated with bone mass in 8-y-old children born at term. In preterm-born adults, Fewtrell et al \((11)\) observed no effect of different mineral supplementations in infancy on bone mass at 20 y of age. The only significant relation was a positive association between whole-body bone mass and human milk intake. Similarly, Bäckström et al \((12)\) showed no effect of early mineral supplementation to prematurely born infants on bone mass at age 9–11 y. The mechanisms behind a possible association between early nutrition and later bone mass are not known. In the Copenhagen Cohort Study of Infant Nutrition and Growth (CCS), we showed that breastfed infants had significantly higher serum (s)-osteocalcin concentrations than did formula-fed infants \((13)\). Whether early bone turnover is important for later bone mass is not known. Furthermore, an inverse association between insulin-like growth factor I (IGF-I), which is associated with linear growth and thereby bone growth, in infancy and adolescence at 17 y has been described in the CCS \((14)\), suggesting a programming of the IGF-I axis. The aim of this study was to evaluate, based on the CCS cohort, whether there are relations between early nutrition, early growth, and later bone mass in Danish adolescents.

SUBJECTS AND METHODS

Subjects

A random sample of infants born between 1987 and 1988 at Hvidovre Hospital (Hvidovre, Denmark) was followed from birth

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\textsuperscript{2} Presented at the conference “The Power of Programming: Developmental Origins of Health and Disease,” held in Munich, Germany, 6–8 May 2010.

\textsuperscript{3} Supported by the Danish Heart Association, the Lundbeck Foundation, the Ville Heises Foundation, and the Strategic Research Foundation, Programme Commission on Food and Health (FØSU).

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First published online August 17, 2011; doi: 10.3945/ajcn.110.001214.

to 12 mo of age and examined again at 10 y and 17 y of age. Of 560 newborn infants, 251 fulfilled the inclusion criteria: healthy, parents of Danish origin, singleton birth, term (born between 37 and 42 wk), and BW for gestational age between the 10th and 90th percentiles. During the first year of life, 142 infants (63 were boys) completed the examinations. At 10 y of age, 136 were traced and invited, 106 accepted, and 104 completed the examinations (Figure 1). At the second follow-up, 140 subjects from the original study were invited, and 109 agreed to participate and completed the examinations. Only data from subjects who participated in the 17-y follow-up examination were used in the present analyses. The Ethics Committee of Copenhagen and Frederiksberg approved the protocol (KF01-226/97). For more details regarding the selection and characteristics of the study group, see previous publications (15–17).

Anthropometric measurements and dietary assessment

For this study, selected anthropometric data collected at birth and at 9 mo and 17 y of age were used. Birth data were obtained from birth records. At 9 mo, weight to the nearest gram and recumbent length to the nearest millimeter were measured. At 17 y of age, weight to the nearest 0.1 kg and height to the nearest millimeter were measured. Duration of exclusive breastfeeding and any breastfeeding up to 9 mo were registered. If the infants were breastfed at least once a day, they were classified as breastfed. For more detailed descriptions, see previously published data (15, 18).

Bone measurements

Bone mass was assessed by dual-energy X-ray absorptiometry (DXA) scanning at 17 y. Whole-body BMC (amount of hydroxyapatite in grams), bone area (BA, anterior-posterior projected area, cm²), and BMD (BMC/BA) were measured by a Hologic 1000/W DXA scanner (Hologic Inc, Waltham, MA). Hologic DXA software whole-body v5.73 was used. Subjects wore only T-shirts and underwear during the scan. For quality control, spine phantoms were scanned daily during the examination periods and CVs were <0.5%. The entrance radiation dose was 15 μSv with an effective dose ≤10 μSv per scan, equal to ≈1 d of background radiation in Denmark. Total body (T) and lumbar spine (LS) values were used in the analyses.

Biochemical analysis

Venous blood samples were obtained at 2, 6, and 9 mo, and serum was stored at −20°C until analysis. Serum bone γ-carboxyglutamate acid protein (s-BGP), also known as osteocalcin, was measured by radioimmunoassay (13). At the 17-y follow-up examination, fasting blood samples were drawn from vein puncture. Serum was stored at −80°C until analysis. s-Osteocalcin was analyzed by automated chemiluminescence immunoassay (IMMULITE 1000; DPC Biermann GmbH, Bad Nauheim, Germany) (16).

Statistical analyses

To estimate the relation between early nutrition, early growth, bone turnover, and later bone mass, sex-adjusted partial correlation analyses were used. Furthermore, the associations of early nutrition and bone turnover with size-adjusted bone mass were analyzed in multiple regressions in which BMC was controlled for BA, height, weight, and sex, as recommended by Prentice et al (19). BMC, BA, height, and weight were transformed to natural logarithms before analyses (19). SPSS (version 18.0; SPSS Inc, Chicago, IL) was used for the statistical analyses. P values <0.05 were considered significant.

RESULTS

Subject characteristics are shown in Table 1. The correlation between sex-adjusted T and LS BMC, BA, and BMD at 17 y and early growth variables, months of exclusive breastfeeding and any breastfeeding up to 9 mo, and s-BGP at 6 mo are shown in Table 2. Sex-adjusted T-BMC and T-BA were positively correlated with growth in weight and length in the first 9 mo (all P < 0.04) and weight and length at 9 mo (all P ≤ 0.004), but not with BW or length (BL). Sex-adjusted LS-BMC was correlated with BW (P < 0.05) and LS-BA to both BW and BL (both P ≤ 0.004). Sex-adjusted BMD was not related to weight or length in infancy (all P > 0.14). Sex-adjusted LS-BMC, LS-BA, and LS-BMD at 17 y were positively correlated with number of

FIGURE 1. Trial profile summarizing recruitment and participant flow.
months of exclusive breastfeeding (all \( P < 0.03 \)). Sex-adjusted LS-BMC, and LS-BMD at 17 y, were positively correlated with s-BGP at 6 mo of age (both \( P < 0.04 \), s-BGP at 2 and 9 mo were not correlated with bone mass at 17 y of age.

There was no association between s-osteocalcin in infancy and late adolescence (Table 3). However, there was a trend for a positive association between s-BGP at 9 mo and s-osteocalcin at 17 y (\( P = 0.06 \)).

Size-adjusted LS-BMC at 17 y was borderline associated with number of months of exclusive breastfeeding (\( P = 0.075 \)) but not with any breastfeeding (\( P = 0.38 \)). Furthermore, size-adjusted LS-BMC at 17 y was significantly associated with s-BGP at 6 mo (\( P = 0.047 \)) but not at 2 mo (\( P = 0.34 \)) or 9 mo (\( P = 0.79 \)) (data not shown).

**DISCUSSION**

We showed that T-BMC and T-BA at 17 y are positively related to body size and growth in infancy. Furthermore, breastfeeding in infancy seems to have a positive influence on later lumbar bone mass in adolescence. In this study early bone turnover as measured by s-BGP at 6 mo may also have a positive influence on later lumbar bone mass in adolescence.

<table>
<thead>
<tr>
<th>Variable</th>
<th>T-BMC</th>
<th>T-BA</th>
<th>T-BMD</th>
<th>LS-BMC</th>
<th>LS-BA</th>
<th>LS-MD</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
<td>( P )</td>
</tr>
<tr>
<td>Birth weight (( n = 102 ))</td>
<td>0.073</td>
<td>0.47</td>
<td>0.14</td>
<td>0.16</td>
<td>0.000</td>
<td>0.998</td>
</tr>
<tr>
<td>Weight at 9 mo (( n = 102 ))</td>
<td>0.28</td>
<td>0.004</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>0.13</td>
<td>0.2</td>
</tr>
<tr>
<td>( \Delta )Weight: 9 mo − birth (( n = 102 ))</td>
<td>0.26</td>
<td>0.008</td>
<td>0.33</td>
<td>&lt;0.001</td>
<td>0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>Birth length (( n = 102 ))</td>
<td>0.13</td>
<td>0.19</td>
<td>0.19</td>
<td>0.06</td>
<td>0.052</td>
<td>0.60</td>
</tr>
<tr>
<td>Length at 9 mo (( n = 102 ))</td>
<td>0.30</td>
<td>0.002</td>
<td>0.39</td>
<td>&lt;0.001</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>( \Delta )Length: 9 mo − birth (( n = 102 ))</td>
<td>0.21</td>
<td>0.035</td>
<td>0.26</td>
<td>0.010</td>
<td>0.11</td>
<td>0.27</td>
</tr>
<tr>
<td>Exclusive BF (( n = 104 ))</td>
<td>0.10</td>
<td>0.3</td>
<td>0.11</td>
<td>0.25</td>
<td>0.09</td>
<td>0.38</td>
</tr>
<tr>
<td>Any BF up to 9 mo (( n = 104 ))</td>
<td>0.001</td>
<td>0.99</td>
<td>0.034</td>
<td>0.73</td>
<td>0.004</td>
<td>0.97</td>
</tr>
<tr>
<td>s-BGP at 6 mo (( n = 46 ))</td>
<td>0.097</td>
<td>0.52</td>
<td>0.095</td>
<td>0.53</td>
<td>0.104</td>
<td>0.49</td>
</tr>
</tbody>
</table>

1. T-BMC, total bone mineral content; T-BA, total bone area; T-BMD, total bone mineral density; LS-BMC, lumbar spine bone mineral content; LS-BA, lumbar spine bone area; LS-BMD, lumbar spine bone mineral density.
2. For s-BGP at ages 2 and 9 mo, there was no significant association.
To our knowledge, the relation between intake of human milk and later bone mass has been reported in only 2 studies (10, 11). We believe our study is the first to have term infants followed to adolescence. Jones et al (10) examined a cohort of children from Tasmania at 8 y of age. They showed that children born at term had higher lumbar, hip, and whole-body BMD at age 8 y if breastfed >3 mo compared with children breastfed <3 mo. The difference was not shown in children born preterm. In a 20-y follow-up by Fewtrell et al (11) of infants born preterm and randomized to different early diet the first postnatal month, the effect on bone mass was examined. The infants were randomized to different combinations of human milk and formula, depending on whether the mother wanted to breastfeed or not. Despite very different nutrient compositions in the diets in the different groups, there were no differences in bone mass 20 y later. However, in an observational analysis of the data, whole-body bone mass was associated with the early intake of human milk. The authors speculated that the effect could be due to some nonnutrient factors in human milk, such as growth factors or hormones. These observations are in accordance with our finding of a positive correlation between duration of exclusive breastfeeding and LS bone mass. The mechanism is not known. However, in our study an association between early bone formation markers, in this case s-BGP/s-osteocalcin, and later bone mass was shown. Furthermore, it was shown earlier in the same cohort that s-BGP concentrations were higher in exclusively breastfed infants (13). Therefore, we speculate that human milk may influence early bone formation and thereby possibly have long-term effects. It is well known that the IGF-I concentration is lower in breastfed infants compared with formula-fed infants and that the stature later in life of earlier breastfed children is higher compared with earlier formula-fed children (20, 21). In the CCS we showed that the IGF-I concentration at 9 mo was negatively associated with the IGF-I concentration at 17 y (14). Whether this is one of the reasons that breastfeeding in infancy has a positive influence on bone mass later is not known.

Body size and growth in body size (weight and length) in infancy were associated with later whole-body BMC and BA but not BMD. This has also been shown by others (4–6), indicating that growth in infancy predicts later bone size more than bone density. It has also been shown by Oliver et al (6) that infant growth, apart from the relation to bone mass in the elderly, is also positively related to bone strength as measured by peripheral quantitative computed tomography. It seems, therefore, that growth early in life may be important for later fracture risk because of tracking in body size has influence on bone size. We showed previously in the CCS that both BMC and BA track from 10 y to 17 y of age (17). Furthermore, it was also shown that size-adjusted BMC tracks from 10 y to 17 y of age (17). In the CCS there were no significant associations between BW, BL, and whole-body bone mass; however, there were significant associations between BW and LS-BMC and LS-BA and BL and LS-BA, indicating that prenatal growth may also influence later lumbar bone mass. Recently it has been shown that fetal growth, expressed as growth in femur length from 19 to 34 wk, was related to bone size but not to bone volumetric density at 4 y (22). Another study showed that BW was an independent predictor of whole-body BMC and BMD in young Gambian girls (23). In young Gambian adults, BW predicted bone size in both men (cortical bone) and women (trabecular bone) (7). These studies, together with our CCS study, underline that both prenatal and postnatal growth in infancy may be important for bone health and fracture risk later in life.

The study has some obvious limitations. The major limitation is the relatively small sample size, especially at blood sampling at 2 and 6 mo of age. Different methods were used to analyze s-BGP/s-osteocalcin in infancy and at age 17 y. Furthermore, LS DXA values were from whole-body scans. The strengths are that we have detailed registrations during the first year of life and follow-up up to 17 y of life.

In conclusion, early body size and growth in infancy are related to later bone mass. Furthermore, duration of exclusive breastfeeding seems to be positively related to later lumbar bone mass.

The authors’ responsibilities were as follows—KFM: designed and completed the original infant study; KFM and AL: performed the statistical analyses and prepared the first draft. All authors contributed to the final manuscript. The sponsors were not influential in the study design, data collection, analysis, interpretation of results, or writing of the manuscript. None of the authors had any economic or personal conflicts of interest.

REFERENCES