Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC)\textsuperscript{1–4}

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ABSTRACT

We examined associations between proxy measures of in utero nutrition and total body bone mineral content (BMC), bone area (BA), and bone mineral density (BMD) assessed at age 9.9 y in the Avon Longitudinal Study of Parents and Children (ALSPAC). There were positive relations between birth weight and BMC, BA, and BMD. These associations were explained by the co-association of birth weight with body size in later childhood. In height- and weight-adjusted analyses, an inverse association was observed between birth weight and BMD at age 9.9 y, which suggests that birth weight had a negative influence on bone mass after relations with bone and body size were taken into account. In analyses of associations between bone mass at age 9 y and background ultraviolet B exposure during the third trimester of pregnancy (a proxy measure for maternal vitamin D status), maternal ultraviolet B exposure was positively related to BMC, BA, and BMD. After adjustment for height, these associations were only partially attenuated, which suggests that maternal ultraviolet B exposure affected skeletal size and mass independently of longitudinal growth, possibly by the increase of periosteal expansion. There was a positive relation between maternal folate intake and BMD of the spine subregion independent of body size. Although a co-association with folate intake in childhood could explain this relation, the maternal methylenetetrahydrofolate reductase (MTHFR) genotype affected spine BMD independently of the child MTHFR genotype, which suggests that maternal folate status has an independent effect on bone development of offspring. Together, these results confirm that there is a relation between bone development in childhood and several proxy measures for nutritional status in utero. \textit{Am J Clin Nutr} doi: 10.3945/ajcn.110.001495.

INTRODUCTION

Nutritional deprivation during pregnancy has been suggested to increase risk of developing a range of chronic diseases in later life, including osteoporosis, as a result of programming (1). According to this hypothesis, adverse environmental conditions during pregnancy limit growth and weight gain during pregnancy and the trajectory of subsequent skeletal growth and development, which result in suboptimal bone structure and increased risk of osteoporotic fracture in later adult life. In support of this hypothesis, several measures related to nutritional status in utero appear to be associated with bone development as assessed in neonates and later childhood. For example, low maternal vitamin D status has been reported to be associated with splaying of the femoral metaphysis as assessed by ultrasound at 19 wk gestation (2) and with reduced total body and spinal bone mineral content (BMC) as assessed by dual-energy X-ray absorptiometry (DXA) at age 9 y (3), the velocity of fetal abdominal growth between 19–34 wk gestation was positively related to estimated volumetric bone mineral density (BMD) as measured by DXA at age 4 y (4), and dietary patterns in late pregnancy were associated with whole-body and lumbar bone mass as measured by DXA at age 9 y (5). In addition, there are several reports of associations between birth weight as a proxy for nutritional status and subsequent skeletal development, which may primarily reflect a relation between birth weight and the bone cross-sectional area as suggested by a recent peripheral quantitative computer tomography study in 120 17–21-y-olds from the Gambia (6).

Although these previous reports provided some evidence that early life exposures in utero influence subsequent bone development in childhood, to what extent these influences persist into later life and affect risk of osteoporotic fracture is currently unclear. Moreover, several of these studies were based on relatively small numbers and require replication in larger studies. Furthermore, the interpretation of several of these studies is complicated by methodologic limitations. For example, measures of nutritional status in utero and subsequent bone mass are both closely related to indexes of body size, such as height and weight, which are frequently adjusted for incompletely. Associations between measures of nutritional status and bone mass may also be confounded by other factors such as socioeconomic influences, which are known to have profound effects on bone development (7).

AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a unique birth cohort investigating factors that influence the health, growth, and development of children. All pregnant women who were resident within a defined part of the

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former county of Avon in southwest England with an expected date of delivery between April 1991 and December 1992 were eligible for recruitment of whom ∼14,000 women were enrolled (8) (http://www.alspac.bristol.ac.uk). Information was collected by using a combination of questionnaires administered during pregnancy and at regular intervals throughout subsequent childhood and repeated research clinic assessments to which all children were invited. As part of these research clinics, children underwent total body DXA scans (research clinics at ages 9, 11, 13, 15, and 17 y), hip DXA scans (research clinics at ages 13 and 17 y), and peripheral quantitative computer tomography scans of the mid-tibia (research clinics at ages 15 and 17 y). Information was also collected on a range of biomarkers. Ethical approval was obtained from the ALSPAC Law and Ethics committee and relevant local ethics committees, and written informed consent was provided by all parents.

Because of the detailed information available on early life factors and skeletal development in childhood, ALSPAC provides an ideal study population for extending our understanding of the role of programming by addressing outstanding research questions and replicating previous observations. ALSPAC has been used to study relations between skeletal development during childhood and different factors related to nutritional status in utero, namely birth weight, background ultraviolet B exposure in late pregnancy, and maternal diet.

**RELATIONS BETWEEN BIRTH WEIGHT AND BONE DEVELOPMENT**

Because birth weight partly reflects nutritional status during pregnancy, reports of an association between birth weight and subsequent skeletal development may provide evidence of an influence of programming on bone development. We initially explored these relations in ALSPAC on the basis of an analysis of relations between birth weight and total body DXA scan results at age 9 y. Total body DXA data in ALSPAC were provided as total body less head (TBLH) BMC, bone area (BA), and BMD. The area-adjusted bone mineral content (aBMC), in which BMC was adjusted for area by linear regression, was also calculated (unlike BMD, the aBMC was fully adjusted for bone size and provided a more accurate estimate of volumetric BMD).

As shown in Table 1 for the basic model (model A), strong positive associations were observed between birth weight and weight and height at age 9 y, which indicated that birth weight was closely related to subsequent body size. BMC was closely related to BA, which, in turn, was closely related to body size. Therefore, it was perhaps not surprising that birth weight shows a similar relation with TBLH BMC at age 9 y to that seen for height and weight as did related measures such as BA and BMD. In contrast, an inverse association was seen between birth weight and aBMC, which suggested that birth weight was negatively related to bone mass accrual after accounting for effects of body size.

It is likely that any relation between birth weight and size at age 9 y was related, at least in part, to shared genetic factors. In an attempt to adjust for these, we repeated our analyses adjusted for parental height and weight (Table 1, model B). As shown, this led to an attenuation between birth weight and outcomes at age 9 y by ∼50%, which implied that associations between birth weight and bone and body size at age 9 y were only partly explained by shared genetic factors. Subsequently, we explored the influence of body size on associations between birth weight and bone mass as measured at age 9 y by adjusting results for height and weight of the child as well as those of their parents (Table 1, model C). In these further analyses, there was no longer any evidence of a positive association between the birth weight and bone mass of the child. In contrast, the inverse association between birth weight and aBMC persisted, and there was also evidence for an equivalent inverse relation with BMD. Together, these findings suggested that the positive association in ALSPAC between birth weight and subsequent bone mass accrual of the child was explained by shared influences on body size; to the extent that birth weight was related to subsequent skeletal development independent of body size, this appeared to comprise a negative influence.

### TABLE 1

Association of birth weight with height, weight, and TBLH (total body less head) dual-energy X-ray absorptiometry at 9 y of age

<table>
<thead>
<tr>
<th></th>
<th>Model A (n = 6876)</th>
<th></th>
<th>Model B (n = 4507)</th>
<th></th>
<th>Model C (n = 4425)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>P</td>
<td>r</td>
<td>β (95% CI)</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Height</td>
<td>0.538 (0.489, 0.587)</td>
<td>&lt;0.001</td>
<td>0.249</td>
<td>0.293 (0.236, 0.349)</td>
<td>&lt;0.001</td>
<td>0.149</td>
</tr>
<tr>
<td>Weight</td>
<td>0.464 (0.413, 0.514)</td>
<td>&lt;0.001</td>
<td>0.212</td>
<td>0.257 (0.198, 0.316)</td>
<td>&lt;0.001</td>
<td>0.126</td>
</tr>
<tr>
<td>BMD</td>
<td>0.292 (0.241, 0.343)</td>
<td>&lt;0.001</td>
<td>0.134</td>
<td>0.137 (0.074, 0.200)</td>
<td>&lt;0.001</td>
<td>0.064</td>
</tr>
<tr>
<td>BMC</td>
<td>0.459 (0.409, 0.509)</td>
<td>&lt;0.001</td>
<td>0.213</td>
<td>0.242 (0.183, 0.300)</td>
<td>&lt;0.001</td>
<td>0.120</td>
</tr>
<tr>
<td>BA</td>
<td>0.518 (0.466, 0.567)</td>
<td>&lt;0.001</td>
<td>0.240</td>
<td>0.282 (0.225, 0.340)</td>
<td>&lt;0.001</td>
<td>0.142</td>
</tr>
<tr>
<td>aBMC</td>
<td>−0.216 (−0.268, −0.163)</td>
<td>&lt;0.001</td>
<td>0.097</td>
<td>−0.159 (−0.225, −0.092)</td>
<td>&lt;0.001</td>
<td>0.070</td>
</tr>
</tbody>
</table>

1. *P* partial correlation; BMD, bone mineral density; BMC, bone mineral content; BA, bone area; aBMC, area-adjusted BMC. Effect sizes (β) were numbers of SDs for each outcome per kilogram of birth weight. Model A was adjusted for sex, age at dual-energy X-ray absorptiometry scan, and gestation; model B included model A plus additional adjustment for parental height and weight; and model C included model B plus additional adjustment for height and weight at 9 y of age.
meteorologic office data and validated against third-trimester 25 (OH)D concentrations in the small subsample of mothers available (9). This variable was analyzed in relation to total body DXA variables at age 9 y. Ultraviolet B exposure was positively related to TBLH BMC, BA, and BMD, whereas no relation was seen with aBMC (10). Together, these findings suggested that maternal ultraviolet B exposure may affect the subsequent bone development of the child by influencing bone growth, whereas there is little evidence of a separate effect on volumetric density.

Overall, the relation between maternal ultraviolet B exposure and DXA variables that we observed in ALSPAC was broadly similar to that reported by Javaid et al (3) for the relation with maternal 25(OH)D concentrations, and together, these findings provide good evidence that maternal vitamin D status exerts persisting effects on the subsequent trajectory of bone mass accrual. Although the strength of the association was somewhat weaker in ALSPAC (eg, \( r = 0.04 \) for maternal ultraviolet B exposure compared with BA in ALSPAC compared with \( r = 0.17 \) for maternal ultraviolet B exposure compared with BA in the Southampton study), this may be explained by the fact that ultraviolet B exposure is only a proxy for maternal 25(OH)D concentrations.

We also observed a positive association in ALSPAC between background ultraviolet B amounts and height at age 9 y, in keeping with the known season of birth effects on adult height. Therefore, we subsequently examined what proportion of the association between background ultraviolet B amounts and bone size that we observed reflected an effect on longitudinal growth. A path analysis suggested that, whereas the majority of the association between background ultraviolet B exposure and bone size was explained by longitudinal growth, a substantial minority was independent of longitudinal growth, which presumably reflected an influence on periosteal relative to longitudinal growth (10). Because periosteal expansion may have a greater effect on biomechanical strength of the skeleton compared with the effect of longitudinal growth, these findings suggested that the maternal 25(OH)D status in pregnancy influences subsequent skeletal development in ways that, if they persist into later life, are likely to affect fracture risk.

**RELATIONS BETWEEN MATERNAL DIET AND BONE DEVELOPMENT**

We also used ALSPAC to examine any influence of maternal diet on bone development of the child by studying associations between maternal diet in the last trimester as assessed by food-frequency questionnaire and DXA measures at age 9 y. Several dietary constituents were shown to be related to DXA measures such as bone mass. For example, magnesium intake in pregnancy was the strongest dietary determinant of TBLH BMC and BMD at age 9 y, but this association was lost after adjustment for height, which suggested that any influence of maternal magnesium intake on bone development of the child was mediated by effects on longitudinal growth (11).

Because \( r^2 \) values for dietary factors combined in relation to bone outcomes at age 9 y were \( \approx 0.5\% \), maternal diet would only appear to account for a relatively small proportion of the population variance in bone development during childhood. Nonetheless, the identification of associations between intakes of specific dietary constituents in pregnancy and subsequent bone development may be helpful in terms of understanding the mechanisms involved in the programming of bone development. For example, an association was seen between maternal dietary intake of folate and BMD and aBMC at the spine subregion, which may point to a role of epigenetic changes in the programming of skeletal development because folate status was likely to influence the availability of methyl donors for methylation during gestation, which is a key mechanism in epigenetic gene silencing. In light of in vitro findings that vitamin D regulates the transcription of key DNA methyltransferase enzymes (12), a similar pathway might have also contributed to the vitamin D–dependent programming effects discussed.

Because maternal folate intake was shown to be related to spinal aBMC, which was fully adjusted for body size, this association may represent a true influence on volumetric BMD rather than a result of a co-association with body size. Consistent with this interpretation, similar results were shown after adjustment for height and weight. Furthermore, maternal dietary intake of folate was shown to be unrelated to indexes of body composition in ALSPAC, which excluded another potential mechanism of confounding (13). On the other hand, no association was observed between the use of folate supplements in pregnancy and DXA variables as measured at age 9 y, which raised the possibility that the maternal folate intake was related to other confounding factors. However, the association between maternal dietary intake of folate and aBMC was unaffected by adjustment for a wide range of other variables including sex, age of scan, pubertal status, and social class factors.

Mendelian randomization is thought to play a useful role in examining the contribution of confounding to results from association studies (14). We applied this method to the study of associations between maternal folate intake and subsequent bone development on the basis of the MTHFR C677T polymorphism, which is associated with circulating folate concentrations (15). Although the maternal MTHFR genotype should provide a marker of maternal folate status, theoretically, this could also affect bone development through the co-association with the child MTHFR genotype. Our observation that the MTHFR genotype of the child was related to spinal BMD and aBMC at age 9 y was consistent with such a pathway (16). Therefore, to conduct these analyses, it was necessary to examine associations between the maternal MTHFR genotype and DXA measures of the child after adjustment for genotype of the child, the results of which are shown in Figure 1. As shown, our results were consistent with a small independent influence of the maternal MTHFR genotype on spinal BMD, particularly in boys, which provides some evidence of a causal relation between maternal folate status and the subsequent bone development of the child.

**CONCLUSIONS**

In conclusion, studies in ALSPAC provide evidence that bone development of the child is related to the in utero environment as assessed by a range of measures that included birth weight, background ultraviolet B exposure during the third trimester of pregnancy, and maternal magnesium and folate intake. Several of these influences were explained, either partly or completely, by shared relations with bone and body size. The in utero environment also exerted some influence on bone development
independent of growth, which suggests an effect on volumetric density. For example, folate intake was positively related to spinal BMD and aBMC, whereas birth weight was inversely related to TBLH BMD and aBMC. In terms of the mechanisms by which these early life factors influence bone development, background ultraviolet B exposure as a proxy for maternal vitamin D and maternal folate status could potentially act by altering the methylation status of key target genes, which justifies further work into the influence of epigenetic changes in utero on subsequent skeletal development.

We are extremely grateful to all families who took part in this study, the midwives for their help in recruiting families, and the whole ALSPAC team, which included interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

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REFERENCES