Early Life Factors in the Pathogenesis of Osteoporosis

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Osteoporosis is a major public health burden through associated fragility fractures. Bone mass, a composite of bone size and volumetric density, increases through early life and childhood to a peak in early adulthood. The peak bone mass attained is a strong predictor of future risk of osteoporosis. Evidence is accruing that environmental factors in utero and in early infancy may permanently modify the postnatal pattern of skeletal growth to peak and thus influence risk of osteoporosis in later life. This article describes the latest data in this exciting area of research, including novel epigenetic and translation work, which should help to elucidate the underlying mechanisms and give rise to potential public health interventions to reduce the burden of osteoporotic fracture in future generations.

Introduction

Osteoporosis is a global public health and financial burden and is a major cause of morbidity and mortality through associated fragility fractures. With current global trends toward an aging population, the burden is set to increase over the next 50 years. In addition to targeting those most at risk for osteoporotic fracture, population-based public health interventions are needed to improve bone health in general. In the past 20 years, it has become apparent that factors in early life (eg, maternal nutrition, lifestyle, and body build) may have persisting influences on postnatal skeletal development in offspring. Because peak bone mass appears to be a major determinant of osteoporosis risk in later life, much work has focused on exploring the link between growth in utero and postnatal bone health. Recent work has increased our understanding of the possible environmental influences and the mechanisms that underlie these associations. This article focuses on the latest developments in the field, mainly over the past 2 years.

Adult Cohorts With Birth Records

Building on a series of studies based in cohorts of adult men and women, which demonstrated that early size predicts skeletal size rather than volumetric density, Oliver et al. [1••] used peripheral quantitative computed tomography (pQCT) to measure bone strength in 313 men and 318 women 65 to 73 years of age for whom birth records were available as part of the Hertfordshire Cohort Study, United Kingdom. Lifestyle factors were evaluated by questionnaire, anthropometric measurements, and pQCT examination of the radius and tibia (XCT 200, Stratec Medizintechnick GmbH, Pforzheim, Germany). Birthweight and conditional weight at 1 year were strongly related to radial and tibial length in both sexes ($P < 0.001$) and to measures of bone strength (fracture load X, fracture load Y, polar strength strain index) at both the radius and tibia. These relationships were robust to adjustment for age, body mass index (BMI), social class, cigarette and alcohol consumption, physical activity, dietary calcium intake, hormone replacement therapy use, and menopausal status in women. Additional support for the notion that intrauterine and early infant growth patterns may influence adult bone geometry, and thus strength, comes from Javaid et al. [2], who examined the associations between weight at 1 year and adult (in the sixth to seventh decade) femoral neck length, width, and cross-sectional moment of inertia (a measure of bending strength) in the same cohort. They found that there were statistically significant positive correlations between weight at 1 year and femoral neck width and strength. These relationships were independent of adult weight and hip bone mineral content (BMC), suggesting that this is a true effect on shape rather than mineralization. These recent studies confirm the connection between the early environment and later bone size and shape; both factors are critical in determining fracture risk. In contrast,

volumetric density appears to be more dependent on contemporary factors such as loading and body build. Thus, maternal influences likely modify the trajectory of skeletal growth in offspring, more than the accrual of volumetric density within this overall envelope.

Mother–Offspring Cohorts

Maternal vitamin D status and dietary patterns

Recent work in mother–offspring cohorts has revealed avenues for further mechanistic studies and potential public health interventions. Javaid et al. [3••] studied the offspring of women recruited to a pregnancy cohort in Princess Anne Hospital, Southampton, United Kingdom. This longitudinal study involved 198 children born to Caucasian women in 1991 to 1992. The body build, nutrition, and vitamin D status of their mothers had been characterized during pregnancy, and the children were followed up at 9 years of age. Maternal vitamin D insufficiency (31% were < 20 ng/mL) or deficiency (18% were < 10 ng/mL) was common during late pregnancy and was associated with reduced whole-body and lumbar-spine BMC ($r = 0.21$, $P = 0.009$ and $r = 0.17$, $P = 0.03$, respectively) in the offspring at 9 years. These associations were found to be mediated in part through concentrations of umbilical cord venous calcium. Similar results were observed in neonates from the Southampton Women's Survey, a large mother–offspring cohort in Southampton, United Kingdom [4]. Sun exposure during pregnancy was a major determinant of maternal 25(OH)-vitamin D level in the mothers in the Princess Anne cohort 9-year follow-up study and also correlated with offspring BMC in childhood. Consistent with these results, Sayers and Tobias [5••] observed similar relationships in another mother–offspring cohort: the ALSPAC (Avon Longitudinal Study of Parents and Children), United Kingdom. This recent study included 6955 9-year-old children. Ambient maternal ultraviolet B (UVB) exposure during pregnancy was found to be positively related to whole-body BMC, bone area, and areal bone mineral density (BMD) in the children. Thus, a 1-SD increase in UVB was associated with a 9.6-g increase in offspring whole-body BMC at age 9 years. The positive association for bone area in particular was independent of height and lean mass, suggesting an association between maternal sun exposure in pregnancy and offspring childhood skeletal size, independent of linear growth.

Previous work in ALSPAC demonstrated the importance of other maternal dietary factors in determining childhood bone mass [6]. Thus, maternal magnesium intake at 32 weeks' gestation predicted whole-body BMC at 9 years in the offspring, but this association and a similar association between potassium intake and spinal BMC did not persist after adjusting for childhood height. However, the relationship between maternal folate intake and offspring spine BMC was robust to adjustment for child's height and weight. These data raise the question of whether the relationships seen relate to particular nutrients or are simply markers of more general dietary patterns. Cole et al. [7] recently explored this issue in the Princess Anne cohort. Those children who were born to mothers who had a healthy, "prudent" pattern of diet in pregnancy (characterized by greater consumption of fruits, vegetables, and whole-meal bread), had a greater whole-body bone size and BMD at 9 years old. These relationships were independent of a range of confounding factors, including vitamin D status of the mother in late pregnancy. Thus, the general pattern of dietary intake during pregnancy may be an important determinant of intrauterine bone mineral accrual, independent of vitamin D status. An intervention study based on changing general lifestyle and dietary factors before and during pregnancy is currently underway in Southampton, United Kingdom (The Southampton Initiative for Health).

Placental calcium transport

The study by Javaid et al. [3••] suggested the mother's 25(OH)-vitamin D might be acting via placental calcium transport. Martin et al. [8] investigated relationships between mRNA expression of active placental calcium transporters (plasma membrane calcium transporters [PMCA] 1–4) and bone mass at birth. In this study of neonates from the Southampton Women's Survey, levels of placental PMCA3 mRNA expression were positively correlated with neonatal whole-body neonatal bone area ($r = 0.28$, $P = 0.02$) and whole-body BMC ($r = 0.25$, $P = 0.04$) as determined by dual X-ray absorptiometry (DXA) scanning within 2 weeks of birth. Although numbers were too small to allow detection of any relationships with maternal 25(OH)-vitamin D levels, these data suggest one possible mechanism that could link mother's vitamin D levels to offspring bone development. Additionally, these studies give rise to the idea that supplementing mothers with vitamin D in pregnancy might be a potential public health strategy to improve offspring bone mineral accrual. UK health recommendations in this area have historically been conflicting, and current guidance lacks a robust evidence base. Work by Gale et al. [9] in the Princess Anne cohort explored the safety of maternal vitamin D supplementation by examining the relationships between high levels of maternal circulating 25(OH)-vitamin D in pregnancy and several offspring outcomes at 9 years old. These children underwent measurements of body build, cardiac function by echocardiogram, IQ testing, and questionnaire assessment of health in addition to DXA measurements of bone mass. There were no relationships between high levels of maternal 25(OH)-vitamin D and any of the non-bone outcomes other than weak positive relationships with atopic eczema in infancy and asthma at 9 years. Thus, children whose mothers were in the highest quartile of 25(OH)-vitamin D concentrations in pregnancy had a slightly increased risk of eczema on examination at 9 months and asthma at age 9 years compared with children whose mothers were in the lowest

part of the distribution. Although these associations are weak and not supported by data from the Southampton Women's Survey, they do suggest that supplementation might be best aimed at bringing women out of vitamin D insufficiency and not at achieving supranormal levels. A trial of vitamin D supplements in pregnant women to optimize neonatal bone mass that is under way in Southampton (MAVIDOS [Maternal Vitamin D Osteoporosis Study]) should help to clarify this issue.

Other maternal and paternal influences on neonatal bone mass

The Southampton Women's Survey has allowed exploration of other influences on offspring growth. Data from 448 mother–offspring pairs from the study were analyzed [10•]. Taller women and those with higher parity had offspring with increased birth weight, fat, and lean mass ($P < 0.05$). Mothers who were taller, were of greater parity, had greater fat stores, or walked more slowly also had offspring with greater proportionate body fat at birth (all $P < 0.05$). A weaker trend was found toward lower percentage fat and greater percentage lean in the offspring of mothers who smoked during pregnancy.

The influence of paternal bone mineral was explored in a subset of 278 pregnancies (142 male, 136 female neonates), in which the fathers and offspring underwent DXA assessment of bone mass [4]. After adjusting the paternal DXA indices for father's age and the neonatal DXA indices for baby's gestational age and age at DXA scan, highly significant positive associations were found between the baby's whole-body bone area, BMC, BMD, and the corresponding indices in the father ($r = 0.25$, $r = 0.32$, $r = 0.17$, respectively) among female infants. These relationships were independent of maternal height and fat stores. There appeared to be no relationship between paternal DXA indices and those of male infants, suggesting a possible gender-specific mechanism.

Animal Models

Great advances in our understanding of possible underlying mechanisms have come from animal studies. One animal model that has proved particularly useful in the investigation of intrauterine influences is that of rat protein restriction. In this paradigm, female rats are fed normal or low-protein diets during pregnancy, and offspring are studied. In the first such model, feeding a low-protein diet to pregnant rats produced offspring that exhibited a reduction in bone area and BMC, with altered growth plate morphology in adulthood [11]. The same group also examined whether maternal protein restriction affected the proliferation and differentiation of bone marrow stromal cells [12]. The results suggested that normal proliferation and differentiation were suppressed in offspring from mothers on low-protein diets as assessed by fibroblast colony formation at 4 and 8 weeks. In a larger study, dams were given a low-protein diet during pregnancy, and 135 offspring were studied at different ages. Serum alkaline phosphatase concentrations reached peak levels earlier and serum insulin-like growth factor-1 and 25(OH)-vitamin D levels were lower in the offspring of protein-restricted dams, confirming the important role of the nutritional environment during intrauterine development [13••]. Using micro-CT on samples of bone removed in late adulthood (75 weeks), Lanham et al. [14••] observed that offspring from low protein–diet dams had femoral heads with thinner, less dense trabeculae; mechanical testing showed these samples to be structurally weaker.

Consistent with these results, Fetoui et al. [15] focused on the effects of protein-restricted diets in late pregnancy and early postnatal periods on the offspring. Undernourished pups, compared to the control group, showed adverse outcomes as demonstrated by several factors, including femur length (-47%; $P < 0.001$) and bone calcium (-67%; $P < 0.001$) and phosphorus (-46%; $P < 0.001$) contents. Using a different approach, Snow and Keiver [16] investigated the influence of maternal ethanol intake on offspring bone formation. In this Canadian study, 37 rats were divided into three groups. The first was fed a liquid diet of 36% ethanol-derived calories, the second a calorifically matched diet without ethanol, and the third an unlimited liquid control diet. The diets were imposed for 3 weeks before breeding and during 3 weeks of pregnancy; at day 21 of gestation, fetal tibiae were analyzed. Maternal ethanol intake led to a significant decrease in fetal tibial length ($P < 0.001$), reflecting the shorter diaphysis in this group. Epiphyseal histologic organization was disrupted, whereas epiphyseal length was unaffected. Interestingly, prenatal ethanol exposure not only augmented the length of the hypertrophic zone, but decreased the length of the resting zone. These data would suggest that ethanol affects bone development at multiple stages.

Developmental plasticity and epigenetic mechanisms

These animal studies give examples of a ubiquitous phenomenon (phenotypic or developmental plasticity), which enables one genotype to give rise to a range of diverse physiologic or morphologic states in response to different prevailing environmental conditions during development [17]. Its essence lies in the critical period during which a system is plastic and sensitive to the environment; in later development, this plasticity is lost, resulting in a fixed functional capacity. The evolutionary benefit of the phenomenon is that in a changing environment, it maximizes phenotypic diversity and enables the production of phenotypes that are better matched to their environment than would be possible with the production of the same phenotype in all environments. There is good evidence that this occurs in the natural world. For example, coat thickness in meadow vole pups depends on ambient sun exposure to the mother at the time of conception. Water fleas conceived in the presence of a particular predator are born with a

protective "helmet." The problem arises when the expected conditions do not materialize. The summer coat of the meadow vole would be too thin to sustain life in a sudden cold snap, and daphnia born with protective helmet are at a reproductive disadvantage in the absence of the predator.

Recent work has revealed novel mechanisms by which the environment may influence gene expression in a graded fashion. These "epigenetic" mechanisms [18] and their relationship to potential explanatory mechanisms relating early environment to later health and disease are well described in a recent review by Gluckman et al. [19•]. DNA methylation and chromatin histone acetylation are two such processes shown to be involved in regulating gene expression. It is currently early in terms of investigative work directly exploring bone outcomes, but the following gives examples of epigenetic mechanisms of the sort that are highly likely to be relevant to the relationships between early environmental factors and later bone health. Lillycrop et al. [20] recently demonstrated different levels of promoter methylation for the peroxisome proliferator-activated receptor (*PPAR*-α) gene in offspring of rats fed a protein-restricted maternal diet with low (PR) or higher (PRF) amounts of folic acid, as compared to a control group. Thus, there was decreased mean PPAR-α promoter methylation due to specific reductions at CpG dinucleotides in the PR group ($P < 0.05$). These data are novel, as increased methylation of individual CpG dinucleotides in juvenile rats ($P < 0.05$) was demonstrated only in rats from the PFR group. In another study, the same group investigated how altered epigenetic regulation of the hepatic glucocorticoid receptor (GR) 1(10) promoter is induced in the offspring [21•]. Rats were fed a control or protein-restricted diet throughout pregnancy and chow during lactation. Offspring were killed at postnatal day 34 ($n = 5$ per maternal dietary group). Methylation-sensitive polymerase chain reaction (PCR) showed that GR 1(10) promoter methylation was 33% lower ($P < 0.001$) and GR expression was 84% higher ($P < 0.05$) in the PR offspring. Reverse transcription-PCR showed that DNA methyltransferase-1 (Dnmt1) expression was 17% lower ($P < 0.05$) in PR offspring, whereas Dnmt3a/b and methyl-binding domain protein-2 expression was not altered. In human umbilical cord ($n = 15$), a twofold difference was found between the highest and lowest level of glucocorticoid receptor 1-C_{Total} (GR1-C_{Total}) promoter methylation. Dnmt1 expression but not Dnmt3a expression predicted 49% ($P = 0.003$) of the variation in GR1-C_{Total} promoter methylation. These findings suggest that induction in the offspring of altered epigenetic regulation of the hepatic GR 1(10) promoter, and thus metabolic phenotype, may be due to reduced Dnmt1 expression. These exciting data suggest that epigenetic mechanisms are likely to be relevant and pave the way for further work relating epigenetic changes to bone development.

Conclusions

Osteoporosis is a major public health issue because of associated fragility fractures. Peak bone mass, reached in adulthood, is an important determinant of risk for osteoporosis in later life. Work in the last few years has confirmed previous observations that growth in early life is associated with adult BMC, as well as geometric and strength-related indices. The maternal determinants of offspring bone mass and body composition have been elucidated. Thus, maternal lifestyle, body build, and vitamin D status have been shown to influence intrauterine bone mineral accrual. More general patterns of maternal diet in pregnancy have also now been shown to modify offspring bone mass, suggesting that programs aimed at improving general lifestyle and dietary patterns in pregnant women, in addition to nutrient-specific interventions such as supplementation with vitamin D, are warranted. Finally, new discoveries of the mechanisms of epigenetic modification of gene expression, which allow adaptation of gene expression during development to produce phenotypes suitable for the expected environment, are helping to elucidate these processes at the molecular level. It is hoped that interventional studies under way will lead to potential novel public health strategies to optimize bone mineral accrual, from the start of the life course, to reduce the risk of osteoporotic fractures in future generations.

Disclosure

No potential conflicts of interest relevant to this article were reported.

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