Epidemiological studies of osteoporosis in children

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Recent epidemiological studies have shed new light on the contribution of underlying skeletal fragility to the occurrence of fractures in childhood. In addition, there have been important advances in our understanding of the factors that influence the development of skeletal traits in childhood, such as bone mass and bone size, which determine fracture risk in later life. These factors operating in childhood include potentially modifiable influences, such as physical activity and body composition, as well as prenatal/early life exposures, such as maternal vitamin D status in pregnancy. In addition, constitutional factors play an important role in skeletal development, particularly puberty and sex steroids. Several genetic polymorphisms have also been found to affect skeletal development; although exerting relatively small effects alone, emerging evidence suggests that genetic influences play an important role in childhood by altering the skeletal response to factors such as physical activity and puberty.

Osteoporosis is a disease characterized by low bone mass, leading to enhanced bone fragility and an increased risk of fractures, although there is no widely accepted definition of osteoporosis in children. The increase in bone fragility in osteoporosis is thought to reflect a range of parameters that affect bone mass, which together comprise 'bone quality'. These include overall bone size and shape, cortical and trabecular microarchitecture and the material properties of bone tissue. Recent evidence that bone mass is inversely related to fracture risk in childhood suggests that skeletal fragility and osteoporosis contribute to fracture risk in children as well as adults. In addition, to the extent that skeletal traits that affect bone mass and skeletal fragility in childhood persist into later life, studying how these traits are influenced in childhood may shed light on the pathogenesis of osteoporotic fractures in adults. In this article, we discuss selected epidemiological studies of the determinants of fracture risk and bone mass in childhood. In particular, we have focused on recent findings gained from studying the Avon Longitudinal Study of Parents and Children (ALSPAC), a unique birth cohort of predominantly Caucasian children born in southwest England between 1991 and 1992.

Keywords: bone mass, dual energy x-ray absorptiometry, fat mass, fractures, physical activity, puberty, sex steroids, vitamin D

Fracture incidence in childhood

Fractures in children are common. Between 1.2% [1] and 3.6% [2] of children fracture a bone each year, and the lifetime risk of sustaining a fracture in childhood is 42–64% for boys and 27–40% for girls [1,3]. There is a peak in fracture risk at approximately 14 years of age in boys and 11 years in girls [4]. There is also evidence that the rate of childhood fractures has been increasing with time [3], although it may now be plateauing [5]. For example, the fracture rate in the 1970s was almost double that in the 1950s [3]. The most common fracture in children is fracture of the distal radius, which accounts for 25% [3] to 43% [1] of all fractures. Fractures of the upper limb account for approximately 65% of all childhood fractures [6].

Measurement of bone mass in childhood

For children, the most common technique used for measuring bone mass is dual energy x-ray absorptiometry (DXA), because it is widely available, results in a modest dose of radiation and is relatively inexpensive. DXA machines produce values for bone mineral content (BMC) and bone area (BA), then calculate the so-called bone mineral density (BMD) by dividing the BMC value by BA. This is not a true density but a 2D measurement that can be affected by the subject's size. Simply measuring BMC or BMD in children means that any association studies are likely to be confounded by body size. Similarly, tracking BMC or BMD in individuals through childhood needs to be interpreted alongside the rapid changes in skeletal size that occur throughout growth and puberty.

This dependency of bone mass on body size has important implications in clinical interpretation of DXA measurements in children, since comparison of an individual's result with an age-matched reference population is misleading in cases of

abnormal growth. Different strategies have been developed to overcome this limitation, such as comparison of an individual child's Z scores for BMC, height and lean mass [7]. In the context of epidemiological studies, an equivalent approach is to adjust BMC results for parameters related to body size, based on relationships between these variables observed in the dataset as a whole.

In our studies based on ALSPAC, we adjusted BMC for height, weight and BA by linear regression, in an attempt to derive an estimate for volumetric BMD (vBMD), which is independent of bone size. DXA-derived estimates of vBMD obtained in this way primarily reflect a combination of cortical thickness and trabecular bone volume. The relative size of these contributions differs at different sites, with vBMD at the spine predominantly reflecting trabecular bone volume, and sites such as the femoral neck mainly reflecting cortical thickness. However, cortical and trabecular compartments contribute to vBMD at many sites.

More precise delineation of the relative contributions of cortical and trabecular bone can be achieved by utilizing peripheral quantitative computed tomography (pQCT), in which the skeleton is analyzed in cross-section, enabling cortical thickness and trabecular bone volume to be analyzed separately. Other techniques previously used to evaluate bone mass accrual in childhood include radiogrammetry, in which cortical thickness is measured on radiographs obtained from sites such as metacarpal bones.

Determinants of fractures in children

Bone density & size

Recently, we reported our prospective study of the relationship between DXA measurements and fracture risk in childhood, based on the ALSPAC birth cohort. For each standard deviation (SD) decrease in estimated vBMD, the risk of fracture over the following 2 years approximately doubled in children aged 10–12 years [8]. This agrees with previous findings based on case-control studies; a meta-analysis of ten previous case control studies [9] combined with the results from the ALSPAC cohort gives a standardized mean difference of -0.26 (95% confidence interval -0.40 to -0.12), suggesting that children with fractures have a lower bone mass than those without (**Table 1**).

An intriguing observation was that whereas total body BA was similar in children with and without fractures, following adjustment for height and weight, fracture risk was seen to increase by approximately 50% for each SD decrease in BA [8]. Equivalent findings were recently reported in a smaller cross-sectional study [10]. Taken together, these results suggested that growing children with bones that have not yet 'caught-up' with gains in their soft-tissue (fat and lean) mass have an increased fracture risk. This imbalance may be more marked in obese children who gain relatively more soft-tissue mass, in light of the finding that girls with forearm fractures have a higher body weight, but a reduced cross sectional area of the distal radius measured by pQCT [11]. As well as contributing to an individual's risk of fracture, this transient imbalance may in part explain the peak of fracture risk around 14 years of age in boys and 11 years in girls.

Weight

It is well recognized that in adults obesity is protective for postmenopausal bone fractures [12]. However, in children there is contradictory evidence for the association between obesity and fracture risk [8,13,14]. This may be partly due to the use of body weight or BMI as a proxy for obesity, since these measurements are an ill-defined combination of lean mass, fat mass and height. However, even when fat mass has been measured directly in children by DXA, the results from different populations are not consistent. Again, this may be because obesity is not important on its own, but instead it is whether the bone size or density is appropriate for the body habitus of the child, which is important for determining fracture risk (**Table 1**) [8].

Physical activity

Studies investigating the association between physical activity and fracture risk in childhood commonly use television viewing as an inverse indicator of physical activity [15], assuming more TV viewing means less physical activity. However, this assumption may be flawed as watching TV may replace other sedentary activities, such as reading, rather than replacing physical activity [16]. Interestingly, light physical activity appears to reduce fracture risk in children [15], presumably by increasing bone density, whereas sports participation increases fracture risk in children [17,18], probably through increased exposure to injuries (**Table 1**).

Determinants of bone mass in children

Table 2 provides a summary of the determinants of bone mass in children. An important point that needs to be born in mind is that bone mass as measured by DXA is strongly dependent on

Table 1. Risk factors for fracture risk in childhood for which there is some evidence available in the literature.

Nonmodifiable risk factors	Risk factors with composite genetic and environmental causes	Potentially modifiable risk factors
Individual risk factors	***Individual risk factors***	***Individual risk factors***
• Age	• Bone density	• Diet and nutrition
• Gender	• Bone size	– Calcium intake
• Genetic determinants	• Pubertal stage	– Carbonated beverage
– *COL1A2*	• Obesity	• Physical activity
• Time of year	• Psychological attributes	• Drug treatments
	– Risk-taking behavior	– Oral corticosteroids
	– Attention deficit hyperactivity disorder	***Family risk factors***
	• Balance	• Socio-economic status
		• Home safety and environment
		Mechanistic risk factors
		• Trauma level
		• Landing surface
		• Injury type
		– Falls
		– Road traffic injuries

COL1A2*: Collagen Type I gene coding for the second* α *peptide.*

bone size, and that the residual variation in bone mass after adjusting for bone size, which represents vBMD, is relatively small. Therefore, the strongest influences on DXA-based measures of bone mass in childhood are generally on bone size rather than vBMD. The implications of this relationship with respect to peak bone mass and fracture risk in later life are uncertain; since bone size is inversely related to fracture risk in adulthood, understanding those determinants of bone mass in childhood that act via skeletal size is clearly relevant to the pathogenesis of fracture risk in later life. However, the relatively weak estimates of vBMD derived from DXA scans may underestimate influences on parameters such as cortical thickness, which also make a major contribution to fracture risk in the elderly, justifying the use of other methods such as pQCT as discussed above.

Prenatal & early life determinants of childhood bone mass

Several maternal dietary factors have been found to influence childhood bone mass, although in well-nourished populations this makes a relatively small contribution (statistical correlation [R2] value of approximately 0.5%) [19]. For example, in the Southampton Women's Survey, maternal vitamin D status, as reflected by 25-hydroxyvitamin D3 levels, was found to be associated with bone mass of the child as measured at 9 years of age [20]. In ALSPAC, we examined the relationship between maternal diet as assessed by food frequency questionnaire, and bone mass of the child at age 9 years. Maternal magnesium and potassium intake were found to have positive, albeit relatively weak, effects on total body bone mass as measured by DXA, mediated by effects on height and weight, respectively [19]. In addition, maternal folate intake appeared to affect trabecular bone volume of the spine of the child, as reflected by estimated spinal vBMD, independent of height or weight. A similar association between maternal folate intake and spinal BMD in childhood was recently reported in a population of rural Indian children [21].

Taken together, these findings suggest that maternal factors such as vitamin D and folate intake may program skeletal development of the fetus, leading to long-lasting effects on the bone phenotype of the offspring. Alternatively, to the extent that maternal and childhood dietary intakes are related, these findings may reflect an effect of dietary intake in childhood on skeletal development. That these dietary constituents can affect skeletal development during childhood is supported by observations that genetic influences on serum levels in childhood exert similar phenotypic effects to those of maternal diet described above. For example, a polymorphism in the methylene tetrahydrofolate reductase (*MTHFR*) gene responsible for regulating serum folate levels has been found to affect

Table 2. Determinants of bone mass in childhood for which there is some evidence available in the literature.

Nonmodifiable determinants	Early-life determinants	Potentially modifiable determinants
• Age • Gender • Genetic determinants – Vitamin D receptor – Estrogen-receptor α • Ethnicity • Parental size • Pubertal stage • Hormonal/endocrinal influences – Parathyroid hormone – IGF-I – Leptin	• Intra-uterine – Maternal nutrition – Maternal smoking – Maternal activity – Maternal illness – Season of birth – Gestational age – Birth weight/length • Early post-natal life – Breast feeding or formula – Vitamin D status	• Diet and nutrition in later childhood – Calcium intake – Carbonated beverages • Socio–economic status • Body composition – Fat mass • Physical activity • Sunlight exposure • Drugs and medications – Steroids – Warfarin – Anticonvulsants • Illnesses involving malnutrition, inflammation or paralysis

spinal BMD in young adult men [22], with a similar association seen in 9-year old children from ALSPAC [Unpublished data].

Other maternal factors have also been found to affect bone mass of the offspring, such as smoking and higher levels of physical activity in pregnancy [23]. In terms of early life determinants of childhood bone mass, several studies have investigated the role of diet. For example, large interventional studies compared breast-fed babies with babies randomized to various formula feeds, but no association was found with childhood bone mass in well-nourished populations [24,25]. Vitamin D supplementation of early life feeding regimes may increase BMD in 3-month-old children, but long-term follow-up studies suggest that any increase has disappeared by 10 years of age [26]. By contrast, in another study, vitamin D supplementation during the first year of life in breast-fed infants was found to be associated with increased BMD in later childhood as assessed in 7–9-year-old girls [27].

Postnatal determinants of childhood bone mass

Relationships between body composition & skeletal growth

Our studies of the ALSPAC cohort have also revealed important influences of body composition on skeletal growth in childhood. In addition to affecting the acquisition of peak bone mass, as discussed above, the relationship between body composition and skeletal growth may also affect fracture risk in childhood. The role of body composition in bone accrual in childhood is illustrated by the results of our study of the relationship between social position and skeletal development. We found that social position, as measured by level of maternal education, is positively related to bone mass acquisition in childhood as a consequence of an enhanced gain in height (i.e., longitudinal growth). However, this influence was counteracted by the tendency for increased fat deposition in those from a lower social position to increase BA, presumably reflecting the stimulation of appositional (i.e., outwards, radial) bone growth [28].

It is well recognized that body composition variables are strongly related to bone mass, which is thought to reflect a major influence of lean, as opposed to fat, mass. Consistent with this view, our analyses in ALSPAC revealed that the relationship between fat mass and bone size/bone mass is partly mediated by associations with lean mass [29]. However, after adjusting for both lean mass and height, a relatively strong positive association persisted between fat mass and bone size/bone mass, which was evident in both cross-sectional and longitudinal analyses. Interestingly, this positive association was present for upper as well as lower limb fat mass, suggesting the role of a common endocrine factor involved in regulating fat mass and skeletal growth, rather than that of increased mechanical strain due to greater weight. Possible candidates include leptin and IGF-1, both of which were found to be associated with bone mass in a subgroup of ALSPAC [Unpublished data].

Physical activity

As well as interventional studies that suggest that exercise causes, albeit transient, gains in bone mass in childhood, several epidemiological studies have

analyzed the relationship between habitual levels of physical activity and skeletal development in childhood, based on questionnaire data. In ALSPAC, we were able to investigate relationships between DXA-based measures of bone mass and habitual levels of physical activity as assessed by accelerometers attached to the waist during waking hours for 7 days. In an analysis based on 4467 11-year-olds, those children whose participation in activity equivalent to that associated with brisk walking was in the upper quartile had a lower-limb bone mass 0.60 SDs greater than those whose activity levels were in the lower quartile [30]. Further analysis suggested that this increase in bone mass largely reflected a gain in bone size. Since height was unaffected, we interpreted these findings as indicating stimulation by physical activity of appositional bone growth.

Interestingly, these relatively strong effects of physical activity on skeletal development were only observed after adjusting our results for body composition variables. Unadjusted analyses revealed that the influence of physical activity on skeletal development in children is strongly modified by associated changes in body composition, which, as discussed above, appears to be a major influence on appositional bone growth. For example, greater physical activity was associated with a gain in lean mass, which tended to increase bone size. By contrast, greater physical activity acted to decrease fat mass, thereby reducing bone size. We are in the process of confirming these relationships based on more robust pQCT-based measures of cortical bone geometry. Other research is planned to examine whether relationships between physical activity and skeletal development are influenced by genetic polymorphisms, in light of the recent finding from the GOOD cohort of young adult men, which suggests that a val158met polymorphism of the catechol-*O*-methyltransferase gene modulates the association between physical activity and BMD as assessed by DXA and pQCT [31].

Sex steroids & puberty

Puberty is associated with a gain in bone mass of over 50%, and therefore represents a critical time in terms of skeletal development and attainment of peak bone mass. Sex hormones, which exert a variety of important effects on skeletal development, are thought to play an important role in this process. For example, Wang *et al.* recently reported that the gain in cortical thickness in late pubertal girls, as assessed by serial pQCT scans of the tibial shaft, was related to serum estradiol levels [32]. Consequently, several epidemiological studies have examined whether determinants of sex steroid exposure influence bone mass accrual in childhood. For example, in the GOOD cohort, age of puberty was found to be inversely related to BMD [33]. In the same cohort, the G allele of the Val80 single nucleotide polymorphism of the *CYP19* aromatase gene was reported to be associated with a greater cortical bone size as assessed by pQCT, which was thought to be mediated by increased levels of serum testosterone [34].

In ALSPAC, we investigated possible interactions between gains in bone mass during puberty and polymorphisms in the *ESR1* gene encoding estrogen receptor-α. Our results suggested that the gain in spinal vBMD in late pubertal girls is increased twofold according to the presence of two closely linked intron 1 polymorphisms, whereas the gain in total body vBMD was reduced by 50% according to the presence of an intron 4 polymorphism [35]. Although the functional significance of these intronic polymorphisms is currently unclear, we speculate that they affect functional regulation of the *ESR1* gene, leading to differences in gene expression. When considered in conjunction with results of the study by Lorentzon *et al.* above, these findings suggest that the influence of puberty on skeletal development is modified by genetic factors that affect either the synthesis of active sex steroid or the level of sex steroid receptor expression. It seems likely that genetic variation in other components of sex steroid signaling will also be found to influence the skeletal response to puberty, such as polymorphisms that affect expression of transcription factors and other downstream molecular mechanisms involved in sex-steroid-dependent gene transcription.

Conclusion

Recent epidemiological studies based on ALSPAC and other cohorts of children and young adults have helped to improve our understanding of the determinants of bone mass acquisition in childhood. Some of these influences, such as the relationship between fat mass and periosteal bone growth, may play a role in the pathogenesis of fractures in childhood. By virtue of the important contribution of peak bone mass to fracture risk in the elderly, factors that influence bone mass acquisition in childhood may also affect the risk of sustaining an osteoporotic fracture in later life. Consequently, an important justification for further research in

this area is to identify influences on skeletal development that are potentially modifiable, providing the basis for population-based interventions intended to optimize peak bone mass accrual. Possible examples include maternal dietary intake of vitamin D and folate, and childhood levels of physical activity. Following the identification of genetic factors that influence the relationship between skeletal development and factors such as exercise exposure, it may be possible to identify those at risk of impaired peak bone mass acquisition, in whom these interventions should be targeted.

Future perspective

One of the challenges for future research into the determinants of bone mass acquisition in childhood is to develop interventions such as exercise programs that can be shown to have sustained benefits, leading to an enhancement of peak bone mass in adulthood. Epidemiological studies are likely to play an important role in this process, by helping to identify new mechanisms and determinants of bone mass development, and by refining methods for identifying high-risk populations in whom these interventions need to be targeted. Perhaps the most important advances are likely to occur in studies intended to identify genetic factors that determine an individual's sensitivity to exposures such as diet and physical activity.

To date, relatively few genetic determinants of bone mass acquisition in childhood have been identified, but this situation is likely to change rapidly over the next 5 to 10 years, owing to advances in methodology and technology. For example, it has only been appreciated relatively recently that in order to perform sufficiently well-powered studies providing robust results, investigations into the genetic determinants of traits such as peak bone mass need to be based on large cohorts of several thousand individuals, and to be replicated across a range of populations. In addition, technological advances in high-throughput genotyping have led to a reappraisal of the biological basis for genetic variability. For example, recent genome-wide analyses have revealed that polymorphisms that are most strongly related to phenotypic traits are frequently located in noncoding areas well away from genes, possibly reflecting an important and hitherto unrecognized contribution to genetic variability of miRNAs, which function as repressors in all known animal and plant genomes [36].

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Executive summary

Determinants of fractures in children

- Fracture risk in childhood is inversely related to estimates of volumetric bone mineral density as measured by dual energy x-ray absorptiometry.
- The risk of fractures in children is also increased in those whose skeletal size is relatively small in comparison with their height and weight.

Determinants of bone mass in children

- Bone mass in childhood has been associated with maternal factors, such as intake of vitamin D in pregnancy.
- Bone mass in childhood is also related to factors operating during childhood itself, such as fat mass, physical activity and age of onset of puberty.
- Genetic factors have been identified that appear to influence the response of individual children to factors such as physical activity and puberty.

Conclusion

- Improved understanding of the determinants of peak bone mass in childhood may lead to opportunities for developing interventions designed to optimize this process, and thereby reduce the risk of osteoporotic fractures in later life.
- Identification of genetic factors that affect the response of individual children may provide the basis for finding high-risk individuals in whom these interventions should be targeted.

Future perspective

- Understanding of the interactions between environmental and genetic influences on bone mass acquisition in childhood is likely to improve in the foreseeable future, owing to greater availability of large child cohorts, which provide sufficient statistical power, and improved understanding of the genetic determinants of this process.

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