Maternal nutrition and bone health in the offspring

Osteoporosis is a major public health issue as well as a considerable socioeconomic burden owing to its association with fragility fractures. Bone mass (a composite of bone size and mineral density) increases through life, from conception to a peak in early adulthood. The magnitude of this peak bone mass is a major determinant of osteoporosis risk in later life. Over the last couple of decades, evidence has accrued that factors *in utero* and in early life may have persisting influences on later health and disease. Thus, low birthweight is associated with reduced bone density at peak and in older age, and poor infant growth predicts increased risk of hip fracture in later life. Maternal lifestyle, physical activity, body build and, in particular, vitamin D status, appear to be important determinants of intrauterine bone mineral accrual, with a persisting negative influence of maternal vitamin D insufficiency demonstrated at 9 years of age in the offspring. This review examines the impact of maternal nutrition on bone development in offspring and on the later risk of osteoporosis, and suggests that these observations may pave the way for novel population-based public health strategies to reduce the burden of osteoporotic fracture in future generations.

KEYWORDS: fetal origins ■ nutrition ■ osteoporosis ■ pregnancy ■ programming ■ vitamin D

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Osteoporosis definition & epidemiology

Osteoporosis is a systemic disorder comprising both low bone mass and loss of the normal bone microarchitecture, resulting in increased bone fragility and susceptibility to fractures [1]. The WHO have issued a clinical definition of osteoporosis based on measurements of bone mineral density (BMD) by dual x-ray absorptiometry (DXA) as a T score of less than -2.5 [2]. This definition is now used internationally to identify those at risk of osteoporotic fracture, and forms an integral part of treatment algorithms for both the primary and secondary prevention of osteoporotic fracture.

According to data from the WHO, 1.7 million hip fractures occurred worldwide in 1990, and this is projected to increase to 6.3 million per year by 2050 [201]. In the UK, an estimated 330,000 patients are admitted to hospital with fractures and approximately a quarter of these are hip fractures [3]. With the aging population in the UK, hip fractures have been increasing by 2% per year from 1999 to 2006 [202]. If this trend continues, the incidence is projected to increase from approximately 70,000 per year at the present time to 101,000 in 2020 [202].

In the UK alone, the annual cost of osteoporosis is estimated to be GB£1.7 billion, mostly due to hospitalization as a result of hip fractures [4]. It is estimated that the cost of care for fragility fractures could increase to GB£2.2 billion by 2020 [5]. In addition to the costs of hospitalization for fractures, especially those of the hip, substantial morbidity and mortality is also associated with these fractures. A total of 10% of patients admitted to hospital with a hip fracture die within 1 month [6]. This rises to a third by 1 year [6]. Even for those who survive, many will experience ongoing hip pain or difficulty walking, with only approximately half of the patients returning to their previous levels of functioning [6]. Between 10 and 20% of patients will move into either residential or nursing homes following a hip fracture [6].

These data clearly illustrate that osteoporosis is a significant public health issue. By focusing on maternal nutrition as an essential determinant of peak bone mass (PBM), this review will demonstrate the importance of devising novel public health strategies to maximize attainment of PBM and reduce osteoporosis in future generations.

Peak bone mass

Definition of peak bone mass

Peak bone mass is defined as the maximum total skeletal mass accrued at the end of skeletal development [7]. Bone mass increases through fetal life, infancy and childhood to young adulthood, mainly owing to increasing bone size due to linear growth. The attainment of PBM is

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known to be gender- and site-specific, but the available evidence does not allow definite conclusions to be drawn. Follow-up data of a previously characterized cohort of women from Bath (UK), who were initially studied at the age of 21 years [8] and then followed up at the age of 31 years, demonstrated that the women continued to accrue bone mineral into their 30s, both at the lumbar spine and, to a lesser extent, at the hip (authors' unpublished data). This is consistent with a previous US study measuring forearm bone mineral content (BMC) by single-photon absorptiometry as well as more recent DXA-based studies measuring BMD at the hip, lumbar spine and in some cases radius, which demonstrate ongoing increases in bone mass into the third and even fourth decades [9,10–15]. A recent Swedish cohort study examining the attainment of PBM in men between the ages of 18 and 20 years concluded that PBM had been reached in the lumbar spine and hip in this age group, but not at the radius or tibia [16]. Other groups, however, have determined the age of attainment of PBM to be at, or shortly after, the end of longitudinal growth, with estimates ranging from late adolescence to the late 20s [11–13,17–20]. Some anatomical studies even report loss of trabecular bone as early as the start of the third decade [21,22].

Since DXA gives a two-dimensional representation of a three-dimensional object, most of these studies are limited by an inability to distinguish change in bone size from change in volumetric mineralization. Indeed, radiological studies using computed tomography have demonstrated that females have a smaller vertebral cross-sectional area than males throughout life, even after correcting for body size [23,24].

Importance of peak bone mass

Following attainment of PBM, an individual's bone mass at any given point in time is determined by both the PBM achieved during growth and the rate of subsequent bone loss. Studies directly relating PBM to risk of fracture have not yet been performed, but a recent mathematical modeling study carried out by Hernandez *et al.* (Figure 1) suggested that PBM was a six times more powerful predictor of the age of osteoporosis development than either age at menopause or the rate of subsequent age-related bone loss [25]. A previous study showed that at 70 years of age, PBM accounted for 50% of the variation in BMD [26]. These observations strongly suggest that factors that influence the magnitude of PBM are likely to have significant effects on an individual's later risk of developing osteoporosis.

Genetic determinants of peak bone mass

Inheritance studies suggest that approximately 50–80% of the variance in PBM is determined by genotype [27,28]. These widely varying estimates may reflect the different genetic make-up of different populations and different study methodologies. Twin and family studies suggest that the inherited component of peak BMD is polygenic, and that environmental factors appear to be the major determinants of bone loss [29]. Thus, the variance in rate of bone loss explained by heredity is much lower, with postmenopausal bone mass being predicted much less strongly than that before the menopause [29]. In addition, in terms of fracture, which is the most important outcome, there is little increased concordance in monozygotic compared with dizygotic twins in both men and women [30]. Indeed in one study, the intrapair difference in BMD between twins was greater for mono- than di-zygotic twins, suggesting that environmental influences *in utero*, such as differential placentation, may have persisting effects [31].

Much work has focused on several putative candidate genes: the vitamin D receptor (*VDR*), collagen 1α1 (*COL1A1*) and *IGF-1*. There is now evidence concerning *IL-6* [32], *TGF-β1* [33] and LDL-receptor related protein 5 (*LRP-5*) [34]. However, most studies have found small effects, and often with conflicting results. Polymorphisms of the *VDR* have been studied most and have been shown to explain a small proportion of the variance of BMD in most studies [35]. Since many of the *VDR* polymorphisms studied appear to be nonfunctional, it may be that there is linkage with another allele that is actually responsible for the functional change. Type I collagen is an important constituent of bone matrix, and polymorphisms of the Sp1 binding site of *COL1A1* have been shown to be associated with BMD and fracture risk in several [36], but not all studies [37].

None of these studies have demonstrated that any of these genes account for more than a small proportion of variance in bone mass. Thus, it seems likely that the genetic component of BMD is determined by a multitude of different genes. In addition, nonfunctional mutations and genetic variation between populations further complicate the issue.

It is unlikely that the genome and environment always act independently on the skeleton; in fact, there is increasing evidence that an interaction occurs between them; for example, between

birthweight and the *VDR* gene [38], calcium intake and the *VDR* gene [39], and calcium intake and the *COL1A1* gene [40].

Environmental determinants of peak bone mass

Environmental factors known to influence bone mass include diet (particularly calcium and vitamin D intake), levels of physical activity, smoking and alcohol consumption. A small number of studies, mainly observational or retrospective in design, have suggested the benefits of increased calcium intake and weight-bearing exercise on bone mineral accrual during childhood and adolescence [41–45]. Prospective trials involving interventions to increase either calcium intake [46–53] or levels of weight-bearing activity have supported these findings [41,54–60]. It is unclear whether these improvements in bone mass during growth are maintained into adulthood as there is a lack of large prospective studies in this situation, but current evidence suggests that calcium intake from milk is likely to have longer-term effects than elemental supplements [61]. Similarly, both cross-sectional [62,63] and prospective intervention studies in premenopausal women have suggested that calcium supplementation may promote the maintenance of bone mass, and may even prevent or delay bone loss, almost until the time of the menopause [64–69].

Early life influences & developmental plasticity

In the natural world there are numerous examples of developmental plasticity – that is, the ability of a single genotype to give rise to several different phenotypes. This allows developing organisms to adapt to the prevailing environmental conditions. For example, if times are hard, maternal undernutrition may act as a signal to the developing fetus, leading to an altered pattern of gene expression in a way that is appropriate to the environment that is likely to be encountered at birth. An example is the water flea *Daphnia*; if the mother is exposed to traces of a predator, the young are born with a protective 'helmet' [70]. The problem arises when the developing organism is then exposed to a mismatch between the expected and actual environment; the protective helmet of the water flea actually reduces reproductive competitiveness in the absence of the predator. Nutritional abundance in an organism whose genotype is expecting nutritional constraint may lead to disease.

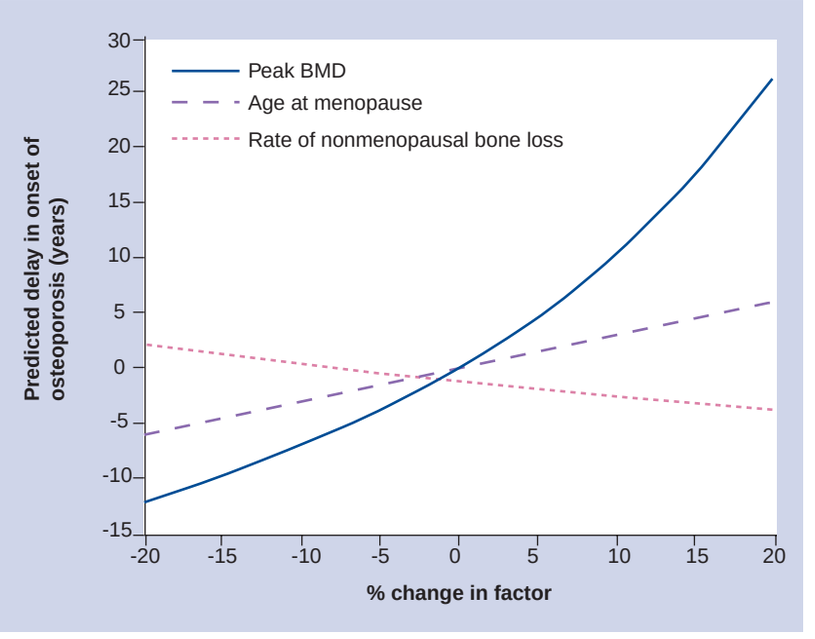

Figure 1. Change in peak bone mineral density, age at menopause and rate of nonmenopausal bone loss and predicted delay in onset of osteoporosis. The delay in the predicted development of osteoporosis in response to a percentage change in peak BMD, age at menopause or the rate of nonmenopausal bone loss is shown. A small percentage change in peak BMD can generate a much larger delay in the development of osteoporosis than a similar change in the age at menopause or the rate of nonmenopausal bone loss.
BMD: Bone mineral density.

Evidence has been accruing that for human diseases, such as osteoporosis, that show a dramatic increase in prevalence with age and that heredity can only partly explain, there is an interaction between the genome and the environment in the expression of the disease. Thus, given a particular genotype, the environmental factors that a subject is exposed to in early life are a critical determinant of later health and disease. This phenomenon is known as 'programming' and is defined as 'persisting changes in structure and function caused by environmental stimuli acting at critical periods during early development' [71]. There is no doubt that the skeleton can be permanently changed by an adverse early environment – rickets is a very visible example. The data supporting this hypothesis will be reviewed in this article. These include epidemiological studies of BMD and fracture in cohorts where birth details are known, physiological studies, exploration of maternal determinants of childhood growth and studies of potential underlying mechanisms using animal models.

Epidemiological studies

Several large, well-characterized birth cohorts exist including individuals from Bath, Sheffield and Hertfordshire in the UK, from which

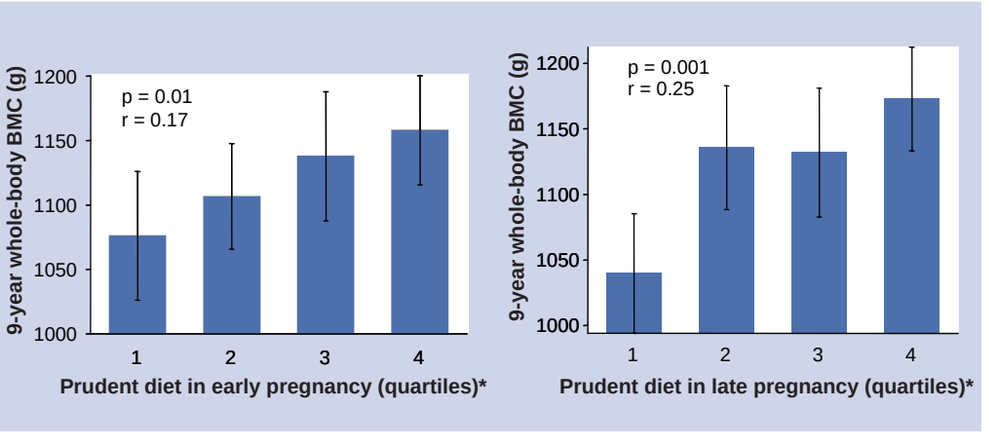

Figure 2. Maternal prudent diet score and offspring whole-body bone mineral area and content at age 9 years. *Values are mean (95% CI).
BMC: Bone mineral content.
Redrawn from [92].

epidemiological data have been derived over a number of years [44,72–73]. These individuals had detailed records completed by local midwives at birth and throughout infancy, including a wide range of demographic variables. The Bath cohort was composed of women in their 20s who underwent assessments of height, weight and bone mass by DXA, and completed a questionnaire to assess a variety of factors known to influence bone mass [44]. The other two cohorts included men and women in their 60s and 70s who had similar measurements made [72,73]. Studies in these cohorts showed that low birthweight and weight at age 1 year predicted lower BMC in later life, even after adjusting for factors known to influence bone mass. There appeared to be a stronger correlation for BMC than BMD. This may reflect different influences affecting overall size of the skeletal envelope and volumetric mineral density [44]. Thus, the skeletal growth trajectory appears to be partly set early in life, whereas volumetric mineralization may be more influenced by contemporary factors such as nutrition and loading.

Childhood growth & subsequent risk of hip fracture

Clinically, the most important consequence of osteoporosis is fracture. The correlation between growth in childhood and risk of hip fracture in later life was demonstrated in a longitudinal study in Helsinki, Finland [74]. Data were collected on a total of 7086 men and women born between 1924 and 1933. Body size at birth had been recorded and an average of ten measurements of height and weight had been taken throughout childhood. Incidence of first hip fracture was identified using the national hospital discharge register. After adjusting for age and sex, two major determinants of hip fracture in later life were identified: tall maternal height ($p < 0.001$) and a low rate of childhood growth from age 7 to 15 years (height: $p = 0.006$; weight: $p = 0.01$). The effects of maternal height and slow childhood growth were statistically independent of each other and remained after adjusting for socio–economic status. In addition, fracture subjects were shorter at birth but of average height by 7 years of age. Further work in a second Finnish cohort demonstrated a relationship between poor growth in infancy and increased risk of hip fracture in later life [75], with a 6.4-fold increase in risk for those subjects in the lowest quartile of weight gain between 1 and 12 years of age. These findings are interesting as they suggest several paths to increased fracture risk. Thus, a low rate of childhood growth, both early and late in childhood, could lead to poorer mineralization of bone tissue and/or decreased bone width, and thus, lower bending strength. This is supported by evidence from the Hertfordshire cohort that demonstrated reduced infant growth to be associated with a narrower femoral neck in older age [76]. Greater maternal height may act via a longer femoral neck or faster catch-up growth, particularly in those children who were smaller at birth and of average size by 7 years of age, whose skeletal growth may have been pushed beyond its capacity for mineralization. This concept is supported by the observation that fractures in children occur most frequently in early puberty, when linear growth velocity is high and ahead of volumetric mineralization [77].

Maternal nutrition

These epidemiological data have led investigators to examine the specific early-life factors that may have persisting influences on postnatal skeletal development. There is now a growing body of evidence, from both human and animal mother–offspring studies, that maternal nutrition is of vital importance in optimizing skeletal growth in the offspring.

Animal models

Data from a number of animal models support the hypothesis that undernutrition during pregnancy results in a poorer outcome for the offspring. This was first demonstrated by Widdowson, who found that maternal undernutrition in pigs led to intrauterine growth retardation and that the offspring remained small throughout life [78]. Her subsequent work in rats revealed that there is a specific time period during which undernutrition can bring about these changes in size; after this period detrimental effects on growth are no longer apparent [79].

More recent work on the effects of maternal undernutrition has been carried out in a rat maternal protein-deficiency model. Using this model, Mehta *et al.* demonstrated that the offspring of protein-restricted mothers had significantly reduced bone area and BMC, but not areal or volumetric BMD, compared with the offspring of mothers fed a normal-protein diet [80]. Not only were histomorphometric bone parameters altered in the offspring of protein-restricted mothers, their growth plates were also found to be abnormally wide [80]. Since then, interest has focused primarily on trying to elucidate the mechanisms responsible for these observed differences. A similar rat maternal protein-deficiency model was used, in which rat dams were fed either a diet with 8% (low) or 16% (normal) casein from conception to the end of pregnancy [81]. The offspring themselves were then fed a normal diet until harvesting. At 8 weeks, significantly fewer colony-forming units fibroblastic (CFU-Fs) and alkaline phosphatase (ALP)-positive CFU-Fs were present, and ALP activity was significantly lower in the low-protein group. At 12 weeks, there was no significant difference in the number of CFU-Fs, and ALP activity was significantly increased in the low-protein group. This suggests that maternal protein restriction during pregnancy results in changes in skeletal development in the offspring through a delay in the normal proliferation and differentiation of mesenchymal stem cells, but this initial delay is then followed by a period of 'catch-up' growth [81]. Further work in this same model, published earlier this year, demonstrated that maternal protein restriction during pregnancy also altered the osteogenic environment of the offspring, with ALP activity peaking earlier, osteocalcin levels being higher and IGF-1 and 25-OH vitamin D levels being significantly lower than in the normal-protein group [82]. These changes persisted into adulthood. Furthermore, analysis using microcomputed tomography showed that female offspring from mothers in the low-protein group had thinner, less dense trabeculae at the femoral head, and closer packed trabeculae at the femoral neck than the mothers fed a normal diet [83]. By contrast, thicker, denser trabeculae were found in the vertebrae, and denser cortical bone was found in the midshaft of the tibia. On mechanical testing, the femoral necks and vertebrae were stronger whilst the femoral heads and tibiae were weaker [83]. This suggests that maternal undernutrition during pregnancy results in significant changes in both the structural and mechanical properties of the offspring's skeleton and these effects vary at different sites.

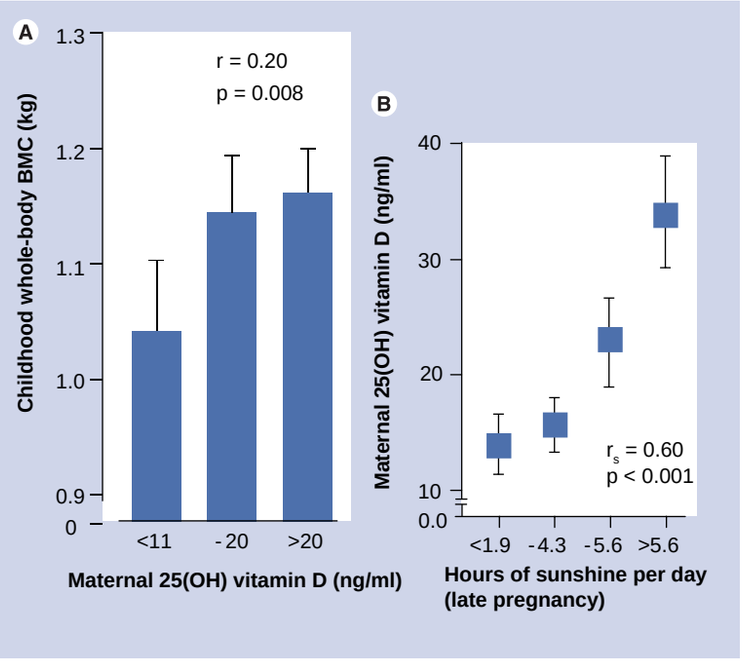

Figure 3. Maternal vitamin D and childhood bone mass.
(A) Maternal 25(OH) vitamin D status in late pregnancy and offspring whole-body BMC at 9 years of age. **(B)** Ambient daily sunshine and maternal 25(OH) vitamin D status in late pregnancy.
BMC: Bone mineral content
Adapted from [93].

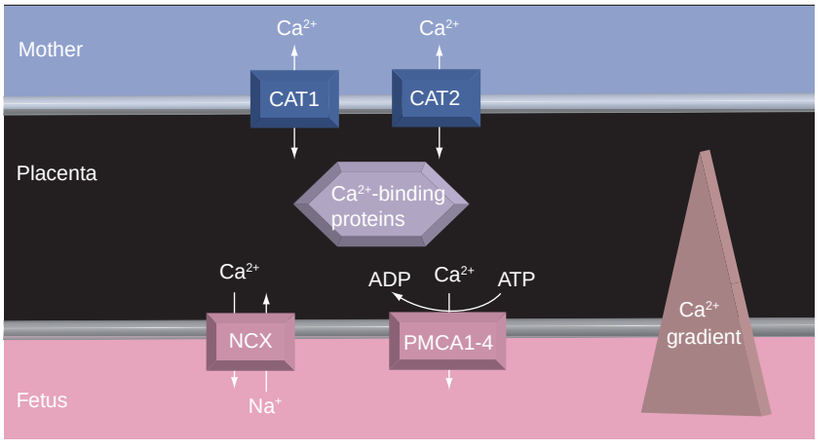

Figure 4. Schematic of placental calcium transport. Facilitated calcium transporters allow Ca^{2+} ions to cross from the maternal circulation into the cytosol, where carrier proteins transport them to the fetal side. Here, active transporters (NCX) and PMCA proteins move Ca^{2+} to the fetal circulation, resulting in a positive concentration gradient from mother to fetus.
NCX: Sodium/calcium exchanger; PMCA: Plasma membrane calcium ATPase.
Redrawn from [96].

Epidemiological data

Parental influences on neonatal bone mass

Complementing the accumulating evidence from animal models that modulation of the intrauterine environment can influence skeletal development, there have been several longitudinal studies in humans investigating parental influences on intrauterine skeletal growth. The Princess Anne Maternity Hospital Group is one such cohort. This includes women living in Southampton (UK) who had detailed anthropometric data collected during their pregnancies. A total of 145 infants born at term to women in the study had their weight, length and placental weight recorded and underwent DXA scanning [84]. Maternal smoking, low maternal fat stores, frequent and very vigorous exercise in the third trimester, and low maternal birthweight all emerged as predictors of lower neonatal whole-body BMC [84]. These associations may be partly mediated by alterations in fetal nutrient supply through effects on the placenta, in contrast with the likely genetic effect of paternal birthweight and height, which were also positively related to offspring BMC.

More recently, parents and neonates were recruited from another cohort, the Southampton Women's Survey (SWS). This is a unique group of women, recruited before pregnancy and aged 20–34 years at enrolment, who were assessed in detail before and during pregnancy, and their children were subsequently followed up. In a subset, 278 neonates and their fathers underwent DXA scanning within 2 weeks of birth [85]. Analysis of these data demonstrated highly significant positive associations between neonatal whole-body bone area ($r = 0.25$; $p = 0.003$), BMC ($r = 0.32$; $p = 0.0002$) and BMD ($r = 0.17$; $p = 0.046$) and the corresponding values in the fathers, but only for female infants [85]. These correlations remained significant after adjusting for maternal height and fat stores. This provides further supportive evidence for the hypothesis that paternal skeletal size predicts neonatal skeletal size independently of the mother's body build. The fact that this correlation only reached statistical significance for female offspring raises the possibility that there is a degree of gender specificity in some of the influences modulating intrauterine bone mineral accrual.

Another cohort of individuals from the Princess Anne Maternity Hospital Group – similarly characterized for maternal nutrition, body composition and lifestyle during pregnancy – were followed up when the children reached 9 years of age [86]. The children underwent DXA scanning as well as measurements of height and weight. Lower maternal height, lower maternal weight prior to conception, maternal smoking, lower maternal fat stores and lower socio–economic status were all associated with lower whole-body BMC in the children at 9 years of age [86]. This provides supportive evidence that these maternal factors not only influence neonatal bone mass, but also influence bone mass later in childhood.

Further work in these mother–offspring cohorts has demonstrated strong, positive associations between umbilical venous IGF-1 concentration and neonatal whole-body BMC, whole-body lean mass and whole-body fat mass [87]. The authors suggest that these data indicate a crucial role for circulating levels of IGF-1 in the growth of the fetal skeleton, more so than its mineralization [87]. However, levels of umbilical venous IGF-1 do not appear to be the mechanism through which the maternal factors known to influence neonatal bone mass exert their effects. In the same cohort there were strong, positive associations between umbilical venous leptin concentration and neonatal whole-body BMC, estimated volumetric BMD and whole-body fat mass, independent of the influences of serum IGF-1 levels [88]. Therefore, umbilical venous leptin concentration is a predictor of both the size and volumetric bone density of the fetal skeleton, and also provides a plausible mechanism through which maternal fat stores can influence neonatal bone mass [88].

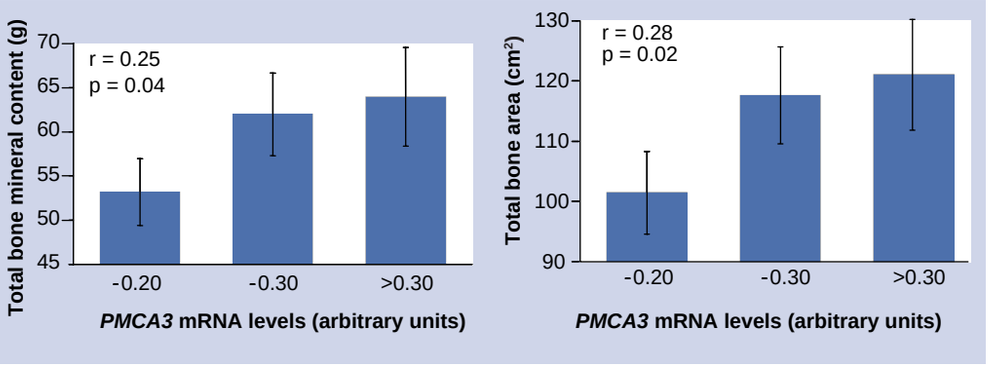

Figure 5. Expression of placental *PMCA3* gene and offspring whole-body bone area and mineral content at birth. PMCA: Plasma membrane calcium ATPase.
Redrawn from [96].

Maternal micronutrient status & neonatal bone mass

Once the importance of maternal nutrition and lifestyle factors in determining bone mass in the offspring became apparent, interest moved towards trying to establish which nutrients were the most important. A longitudinal study investigating the association between maternal diet in the third trimester and bone mass in the children at 8 years of age was carried out in Tasmania between 1988 and 1996 [89]. A total of 177 male and female children were included and underwent DXA scanning. In these children, femoral neck, lumbar spine and total body BMD were found to be positively correlated with magnesium and phosphorus density of the maternal diet; lumbar spine BMD was also positively correlated with potassium density of the maternal diet, but negatively associated with dietary fat density. In addition, total body BMD was positively associated with protein density of the maternal diet and negatively associated with fat density. Overall, this study provided multiple associations between maternal diet during pregnancy and bone mass of the children at 8 years of age, but was unable to indicate which nutrients were most important [89].

The Avon Longitudinal Study of Parents and Children (ALSPAC) assessed the relationship between maternal diet during pregnancy using a food frequency questionnaire, and childhood bone mass at 9 years of age as measured by DXA [90]. Significant associations were demonstrated between maternal magnesium intake and total body BMC and BMD in the children, maternal potassium intake and spinal BMC and BMD, and maternal folate intake and spinal BMC [90]. After adjusting for the height and weight of the children, only the relationship with maternal folate levels persisted. However, this provides further corroboration that maternal diet during pregnancy is one of the crucial determinants of skeletal development during infancy and childhood.

A recent longitudinal study investigating the association between maternal nutritional status and diet during pregnancy and childhood bone mass included 797 pregnant women living in rural India [91]. A total of 698 of these children were followed up with DXA at age 6 years. Children whose mothers had a higher intake of calcium-rich foods during pregnancy were found to have higher total and spinal BMC and BMD. Similarly, those children whose mothers had higher folate intake at 28 weeks gestation were found to have higher total and spinal BMD [91]. This suggests that maternal calcium and folate intake during pregnancy influence childhood bone mass, although a causal relationship cannot be inferred purely from this observational study.

Subsequently, our group studied the influence of maternal diet during pregnancy on bone mass at 9 years of age in a sample of 215 children from one of the Southampton (UK) mother–offspring cohorts [92]. Instead of focusing on individual nutrients, this study examined whether the pattern of foods consumed during pregnancy had an impact on bone mass in the offspring. Both a validated food frequency questionnaire and a dietary scoring system were used to assess maternal nutritional status and diet during pregnancy [92]. A higher or 'more prudent' diet score was given for a diet including greater proportions of fruit, vegetables and high-fiber foods. Children born to mothers with higher 'prudent' diet scores in late pregnancy had significantly higher whole-body BMC, BMD and bone area at 9 years of age (Figure 2). This association remained significant even after adjusting for other known maternal influences on neonatal bone

mass including: maternal birthweight, height, smoking, arm circumference (as a measure of maternal fat stores at 32 weeks), socio–economic status and vitamin D status. Overall, these data suggest that a more 'prudent' maternal diet during pregnancy, which corresponds with current recommendations for a healthy, balanced diet, is associated with increased bone mass at 9 years of age. It appears that it may be the pattern of foods consumed during pregnancy that is important in influencing bone mass in the offspring, rather than individual nutrients, although further studies are needed to investigate this hypothesis further. A key question is whether these patterns directly influence intrauterine bone mineral accrual or whether they are a marker of some other factor related to fetal bone development. Certainly, there is very little conclusive evidence relating bone mass to intake of fiber, fruit or vegetables in adults.

Maternal vitamin D status & neonatal bone mass

One particular nutrient, vitamin D, has received considerable attention with regard to the determination of offspring bone mass. The effects of maternal vitamin D status during pregnancy on childhood bone mass were studied in 198 children from a Southampton birth cohort, which has previously been extensively characterized [93]. A total of 31% of the mothers included in the study were found to have insufficient levels of circulating 25(OH) vitamin D (11–20 µg/l), whilst a further 18% were vitamin-D-deficient (<11 µg/l). The children born to mothers with lower 25(OH) vitamin D levels during late pregnancy were found to have lower whole-body BMC, bone area and areal BMD at 9 years of age [93]. Although causality cannot be imputed for certain as these data are observational the results were independent of maternal social class, lifestyle and dietary factors and childhood diet and physical activity; this suggests that the associations were not the result of an inherited environment. Similar results were found at birth for neonates in the SWS [85]. In addition, umbilical, venous, ionized calcium concentration also emerged as a significant predictor of whole-body BMC at 9 years of age, with lower ionized serum-calcium levels predicting lower childhood bone mass [93]. This was partially explained by maternal vitamin D status. Both ambient UVB levels during late pregnancy and use of vitamin D supplements were significantly correlated with maternal 25(OH) vitamin D status (Figure 3). This correlation between plasma ionized-calcium concentration, maternal vitamin D status and bone mass in the offspring at 9 years of age, together with the fact that plasma ionized-calcium concentrations are likely to reflect the extent of placental calcium transfer, highlights the importance of maintenance of the materno–fetal calcium gradient in determining the future trajectory of skeletal growth [94]. The authors hypothesized that maternal vitamin D insufficiency during pregnancy might mediate its effects on bone mineral accrual through impairment of placental calcium transport, for example by parathyroid-hormone-related peptide (PTHrP) [93]. Evidence from animal models supports a vital role for both parathyroid hormone (PTH) and PTHrP in fetal calcium homeostasis [95]. In transgenic mice, PTH and PTHrP have been shown to interact, increasing the size of the trabecular envelope, whilst at the same time reducing the cortical envelope [95]. It has been suggested that fetal calcium deficiency may result in stimulation of PTH/PTHrP activity, which in turn could decrease the size of the cortical envelope. If this change was then tracked into childhood and later life, it might alter the trajectory of skeletal growth. The recent finding by our group that expression of an active placental calcium transporter (PMCA3) is an independent predictor of neonatal whole-body BMC provides another possible mechanism through which maternal vitamin D status can influence bone mass accrual in offspring [96]. Figure 4 shows a schematic of placental transport mechanisms and Figure 5 demonstrates the positive associations between levels of *PMCA3* expression, and whole-bone area and mineral content in the offspring at birth.

Based on the data reviewed above and given the high levels of vitamin D insufficiency and deficiency amongst pregnant women, it would seem sensible to consider vitamin D supplementation in this group, in order to improve bone mass in their offspring. Although there are some short-term studies of vitamin D supplementation during pregnancy with resultant increases in circulating levels of calcium and vitamin D in neonates, the data have failed to demonstrate an accompanying increase in either fetal weight or length [97]. This is consistent with our observational study described above [95]. These trials have varied widely in terms of participants, dosage, method of administration of vitamin D and outcome parameters. Thus, dosage varies from 200 IU/day vitamin D taken orally, to a 600,000 IU/day bolus intramuscular injection. Bone mass has not been adequately addressed

as an end point, and systematic reviews of the effects of calcium and vitamin D supplementation on various parameters of bone health yield little helpful information on supplementation in this group, owing to the small numbers of studies available [98]. However, they do suggest that such supplementation is not associated with an increased risk of adverse effects, although, with the data currently available, they were unable to address potentially harmful longer-term effects. In view of this paucity of data, a multicenter, randomized, double-blind, placebo-controlled trial of vitamin D supplementation in women with low levels of vitamin D in early pregnancy is currently being undertaken in Southampton (Maternal Vitamin D Osteoporosis Study [MAVIDOS]). FIGURE 6 outlines the pilot phase of the study, and this work may help to inform public health policy regarding vitamin D supplementation in pregnant women.

Epigenetics & developmental plasticity

Developmental plasticity involves interaction between genes and the environment. Thus, environmental influences, such as maternal vitamin D status, might influence expression of placental calcium transporters, resulting in alterations in fetal skeletal development. There is growing evidence that epigenetic phenomena may be critical in these processes. Epigenetic mechanisms allow modification of gene expression without any change in the fundamental genotype. Examples include coordinated changes in cytidine–guanosine (CpG) nucleotides in the promoter regions of specific genes, changes in chromatin structure through histone acetylation and methylation, and post-transcriptional control by micro-RNA [99]. After fertilization, there is widespread epigenetic reprogramming and challenges during early embryogenesis may result, for example, in promoter methylation and thus alter gene expression [100]. The resulting changes are usually stable through mitotic cell divisions, and so will continue through a life-course. Many examples of epigenetic modification have been demonstrated in animals, and the concept provides a very plausible mechanism for long-term changes resulting from early gene–environment interactions.

Conclusion

Osteoporosis is a major public health issue and its importance is continuing to increase owing to the aging population. It is now recognized

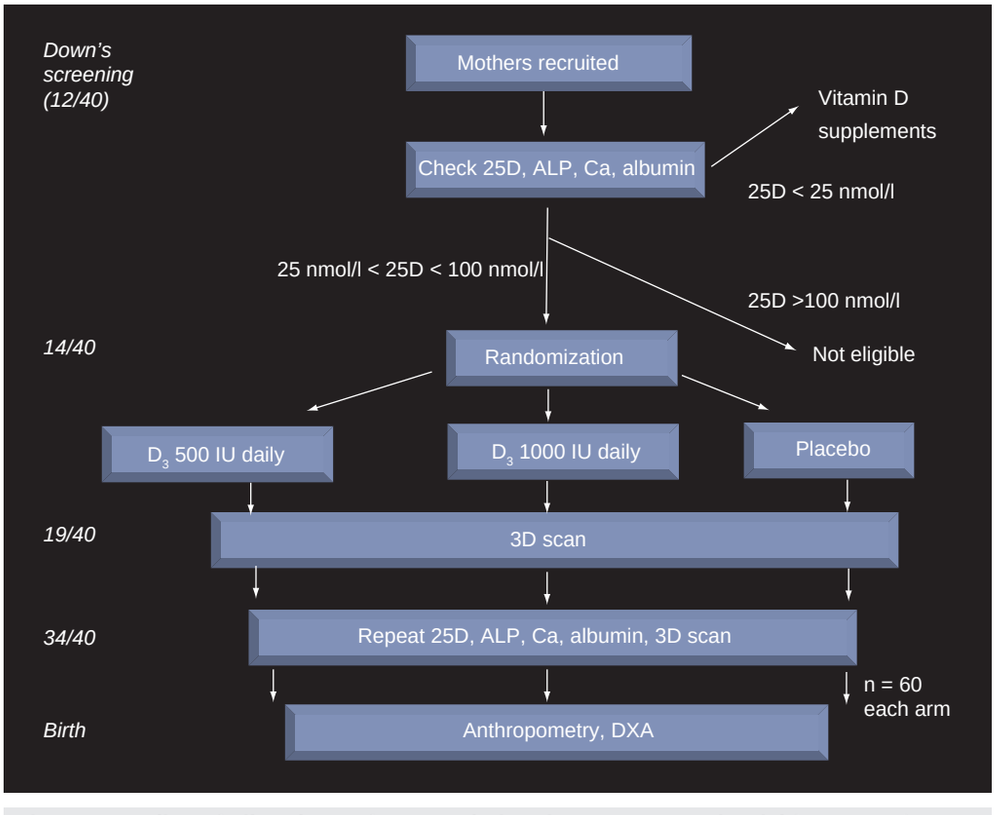

Figure 6. Outline of pilot phase of Maternal Vitamin D Osteoporosis Trial (MAVIDOS).
ALP: Alkaline phosphatase; DXA: Dual x-ray absorptiometry.

that PBM is a major determinant of future risk of osteoporosis, and hence of fracture. PBM is influenced by both genetic and environmental factors, and is a predictor of the risk of developing osteoporosis and fractures. The phenomenon of developmental plasticity provides a mechanism for the interaction of genotype and environment in determining phenotype, and animal studies support hypotheses made in human disease. Mother–offspring cohorts have demonstrated the importance of maternal lifestyle, body build and particularly vitamin D status, in determining fetal bone mineral accrual. The father's genetic influence is also important, and the available evidence really supports the notion that osteoporosis prevention should now be considered at every stage of the life course, from before conception to old age.

Future perspective

Over the next 5–10 years, work will focus on understanding the underlying mechanisms of the epidemiological observations: the SWS will allow further exploration of the underlying influences on bone mineral accrual, particularly of true volumetric density assessed by peripheral quantitative computed tomography at 6 and 8 years of age. Secondly, it will be important to translate these findings into public health benefits. Thus, the link between low maternal vitamin D status and reduced offspring bone mass is being tested in a randomized, controlled trial in Southampton. Women will be randomized to receive either daily oral vitamin D or placebo from 14 weeks until delivery, and bone mass will be measured in the babies by DXA. A program of health advice and support for women of childbearing age is also being examined. By following this approach with novel public health interventions, some being focused on maternal nutrition in particular, we hope that the burden of fragility fracture may be reduced in future generations.

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Executive summary

Osteoporosis definition & epidemiology

- Osteoporosis is a major socio–economic burden owing to its attendant morbidity and mortality, predominantly due to increases in the occurrence of fractures.
- A total of 1.7 million hip fractures occurred worldwide in 1990 and this is projected to increase to 6.3 million per year by 2050.
- In the UK alone, the annual cost of osteoporosis is estimated to be GB£1.7 billion and could increase to GB£2.2 billion by 2020.

Peak bone mass

- Peak bone mass (PBM) is a major determinant of osteoporosis risk in older age.
- PBM is determined by genetic and environmental factors operating at every stage of growth from conception.

Early life influences & developmental plasticity

- Birthweight is associated with lower PBM and bone mass in later life.
- Poor early growth predicts an increased risk of hip fracture in older adulthood.
- Developmental plasticity describes the phenomenon by which a single genotype may give rise to different phenotypes, depending on environmental factors.

Maternal diet

- Mother–offspring studies demonstrate the influence of maternal lifestyle, diet, body build, physical activity and vitamin D status on intrauterine bone mineral accrual.
- Animal models suggest that maternal undernutrition during pregnancy results in significant changes in both the structural and mechanical properties of the skeleton in their offspring.

Epigenetics

- Epigenetic phenomena, such as DNA methylation and chromatin–histone acetylation, provide possible mechanisms to explain observed associations between early environmental influences and later health and disease.

Conclusion

- Evidence suggests that osteoporosis prevention should now adopt a life-course approach, beginning before conception. Thus, novel public health strategies, such as vitamin D supplementation in pregnancy, and programs of health advice and support, may help reduce the burden of osteoporotic fracture in future generations.

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