**Prenatal influences on bone health in children**

**Abstract**

Introduction

Optimising bone health might reduce the burden of both fractures in childhood and fragility fractures in later life. A number of maternal dietary and non-dietary factors have been identified that might influence offspring bone health and represent targets for intervention.

Areas Covered

This article will outline the accrual of bone mineral throughout the lifecourse and how observational and intervention studies have shown that maternal diet, in particular maternal calcium and 25-hydroxyvitamin D [25(OH)D] status, and lifestyle are associated with offspring bone mineralisation. Studies examining the effects of maternal micronutrient supplementation on offspring bone mineral density (BMD) will also be discussed.

Expert Opinion

There is a wealth of observational evidence relating maternal diet to offspring BMD. However, high quality randomised controlled trials, such as the ongoing MAVIDOS study, are needed before these findings can be definitively translated into public health advice.

**Keywords**

Bone mineral density, calcium, fetal origins, osteoporosis, pregnancy, vitamin D

**Article Highlights**

* Fractures are common in childhood and later adult life and represent a significant healthcare burden.
* Birthweight has been associated with bone mineral density (BMD) in adulthood, and a number of studies have demonstrated associations between maternal dietary quality or components and lifestyle and offspring bone health.
* Observational studies relating maternal calcium status to offspring bone health are inconclusive, and the few studies of calcium supplementation have not consistently demonstrated a positive effect on offspring BMD; one study in the Gambia has shown mixed long term effects on maternal BMD and offspring growth.
* Many observational studies have identified positive relationships between maternal 25-hydroxyvitamin D status and offspring BMD. The MAVIDOS randomised controlled trial of cholecalciferol supplementation in pregnancy demonstrated that maternal vitamin D supplementation led to greater offspring neonatal BMD amongst winter born infants.

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**1.0. Introduction**

Fractures are common in childhood; approximately 30% of boys and 19% of girls will sustain at least one fracture before their 18th birthday [1]. They represent an important burden on healthcare resources [2] and are associated with pain, reduced physical function, missed education and loss of parental pay [3]. A fracture will only occur when a significant force is applied to the bone, but many studies have shown that children who sustain fractures do have lower bone mineral density (BMD) compared to non-fracturing controls [4, 5], and as such approaches to optimising BMD might reduce childhood fracture incidence. Perhaps even more importantly there is increasingly recognition that improvements to bone health in early life could reduce the burden of osteoporosis and fragility fractures during adulthood. Osteoporotic fractures are associated with increased mortality [6], poorer quality of life, functional decline [7] and are a significant cause of healthcare spending [8]. As such, new strategies to improve bone health are much needed. There are increasing data which would support targeting early life skeletal development as an approach to reducing this burden, which will be discussed in this narrative review. Additional literature was ascertained through PubMed database searches. Articles were chosen on the basis of relevance, being full-text and in the English language. No constraints were put on date of publication. Where appropriate, reference lists were checked for additional publications.

**2.0. Bone development in early life**

Development of the skeleton begins at approximately 8 to 12 weeks gestation, but the principal period of bone mineralisation is during the third trimester. During this time, bone mineral accretion is primarily determined by fetal plasma calcium ion (Ca2+) concentration. The active transport of Ca2+ from the maternal circulation to the fetus by the placenta results in a greater plasma Ca2+ concentration in the fetus compared to the mother [9]. During pregnancy, maternal intestinal calcium absorption increases as a result of changes to calcitropic hormones to meet the demand of the fetus for calcium, and as a result maternal Ca2+ remains within the normal adult range throughout pregnancy [10, 11]. Impairments to maternal calcium absorption and/or metabolism or limited availability of substrates required for bone mineralisation during pregnancy are therefore likely to impact negatively on bone mineralisation of offspring during *in utero* life.

At birth, an infant weighing 3.0-3.5 kg will have approximately 66 g of bone mineral, representing around 2% of body weight [12]. During childhood and adolescence, the skeleton grows in both length and width as a result of constant modelling and remodelling and bone mineral is accrued, resulting in an increase in bone mass (the composite of bone mineral content and bone size) (Figure 1) [13]. Although final height is reached shortly after the end of puberty, bone mineral accrual continues into the 3rd decade, with peak bone mass (PBM) being reached in the mid to late 20s. Median whole body bone mineral content (BMC) at PBM is approximately 2.7 kg for a male and 2.1 kg for a female [14]. Thereafter, bone mass declines, with an acceleration in the rate of bone loss after the menopause in women. Whilst PBM is in part genetically determined, external factors that modify an individual’s ability to achieve their genetic potential might accelerate the onset of osteoporosis. Indeed, mathematical modelling has shown that a 10% increase in PBM (given the inevitable subsequent bone loss) will delay the onset of osteoporosis by 13 years [15], and supports the notion that osteoporotic fracture could be prevented by interventions in early life to increase bone mineral accrual.

**3.0. Developmental origins of health and disease**

Development plasticity is the ability of a single genotype to give rise to multiple different phenotypes and allows organisms to adapt to the prevailing environmental conditions during critical periods of development. Typically, this confers an evolutionary advantage, but a mismatch between the expected postnatal environment and that to which the fetus has been adapted might be detrimental to survival and future health. This is known as the developmental origins of health and disease (DoHAD) hypothesis and was first proposed in relation to cardiovascular disease following the observation by Barker and colleagues of a close geographical relationship between infant mortality rates and standardised mortality from cardiovascular disease 65 years later [16]. However, over recent years, the DoHAD hypothesis has increasingly been thought to extend to other non-communicable diseases including skeletal development and osteoporosis. Indeed, birth weight has been positively associated with bone mass in both young and late adulthood. Baird *et al* performed a meta-analysis of six studies and found that for every 1 kg increase in birth weight, adult lumbar spine BMC increased by 1.49 g (95% CI 0.77, 2.21 g), and hip BMC by 1.41 g (95% CI 0.91, 1.91 g) [17]. Extrapolation of these data would suggest a 12% increase in hip fracture risk for every 1 kg reduction in birth weight. Similarly, a study in adults aged 60-64 years reported that birth weight was positively associated with radial strength strain index (SSI) [18], a measure of bone strength that is negatively associated with fracture risk [19]. Although such relationships might suggest a direct pleotropic effect of multiple genes on both infant size and adult bone mass, there is also increasing recognition that the early fetal environment influences both birth weight and bone mass. Many of these environment factors, as will be discussed in the following paragraphs, are modifiable, and therefore could be used in preventative strategies to improve bone health in childhood and later life.

**4.0. Maternal diet during pregnancy and offspring bone development**

The Princess Anne Hospital (PAH) Study was one of the earliest mother-offspring cohort studies to examine the relationships between maternal diet and lifestyle in pregnancy and offspring bone development. In this study, the diet of 198 pregnant women from Southampton, UK, was assessed between 15 and 32 weeks gestation using a food frequency questionnaire (FFQ). A dietary score was calculated from this to quantify the consistency of the dietary intake with recommendations for a healthy diet; a diet containing more fruit, vegetables, wholemeal bread rice and pasta, yoghurt and cereals and less processed meat, chipped or roast potatoes, sugar, crisps and soft drinks was labelled as prudent. A positive association was observed between a more prudent diet and offspring whole body and lumbar spine BMC and BMD measured by dual energy X-ray absorptiometry (DXA) at 9 years of age [20]. These findings are supported by data from the Danish National Birth Cohort, which showed a highly Westernised diet, which included high intake of meat, potatoes and white bread but low intake of vegetables, fruit and cereals, during pregnancy was associated with a higher risk of offspring forearm fracture during childhood when compared to mothers in the lowest quintile of Western diet [21]. However, other maternal dietary patterns, including a prudent diet score, were not associated with offspring forearm fracture risk.

Several observational studies have also examined the relationships between individual dietary components, including macro- and micro-nutrients in pregnancy and offspring bone mass. For example, using the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, maternal fibre, protein, milk sugar, magnesium, phosphorus, zinc, iron, niacin and vitamin C intakes assessed by FFQ at 32 weeks gestation were all positively associated with offspring whole body less head areal BMD (aBMD) at 9 years of age in univariate analysis. However, in multivariate analysis, only the association with magnesium intake remained statistically significant, with approximately 1% difference in aBMD between offspring of mothers in the highest and lowest tertiles of intake [22]. In the same cohort of children, a 0.6% difference in whole body aBMD was identified between children who did and did not sustain fractures [4]. The observed effect size could therefore be clinically relevant if demonstrated in an intervention study.

In the Southampton Women’s Survey (SWS) positive associations have been demonstrated between maternal serum n-3 polyunsaturated fatty acids (PUFA) concentrations in late pregnancy and offspring bone mass at 4 years of age [23], and between maternal serum vitamin A status and offspring whole body BMC at birth, which are consistent with those observed between vitamin A intake and hip fracture in adults [24]. Petersen et al using the Danish National Birth Cohort also identified a significant relationship between maternal artificial sweetener intake and offspring forearm fracture [21], but the effects of these on BMC and bone structure have not been investigated. Whilst many of these dietary factors have the potential to be modified during pregnancy to improve offspring bone health, there is a lack of evidence from intervention studies at the current time to demonstrate this.

**5.0. Maternal calcium and vitamin D and offspring bone development**

Given the importance of calcium and vitamin D to bone mineralisation, it is unsurprising that the relationships between maternal calcium intake and/or vitamin D status and offspring BMD have been the most frequently studied.

**5.1. Calcium**

Calcium is obtained from dietary sources, including dairy products, green leafy vegetables, soya and small bony fish. As discussed previously, alterations to maternal calcium metabolism occurs during pregnancy to meet the demands for fetal mineral accretion, but inadequate calcium intake I is common during pregnancy. The Institute of Medicine recommend a daily intake of 1000-1300 mg calcium per day during pregnancy [25], but even in developed countries where there is likely ready access to calcium-rich foods, this is often not achieved [26, 27].

**5.1.1 Observational trials of maternal calcium intake and offspring bone mineralisation**

Observational mother-cohort studies assessing the relationships between maternal calcium or milk intake and offspring bone mineralisation over the last three decades have found largely inconsistent results, although varying methodologies have been used, with markedly diverse cohort demographics between studies and differing co-variates chosen to be included in the multivariate analyses (Table 1) [22, 26, 27, 28, 29, 30]. Nonetheless a number of clinical trials of calcium supplementation have been carried out.

**5.1.2 Clinical trials of calcium supplementation during pregnancy**

The earliest intervention study to investigate the effects of maternal calcium supplementation on offspring BMD was carried out in India in 1978 [31]. 87 pregnant women were recruited from poorer socio-economic backgrounds, where calcium intake is traditionally low. They received 300 mg/day calcium, 600 mg/day calcium or placebo, starting from between 18-22 weeks of gestation and continued until delivery. Both supplemental calcium doses were associated with significant increases in ulna, radius, tibia and fibula radiographic bone densities, as measured by comparison of bone density to aluminium, when compared to mothers who did not receive calcium supplementation. There was no significant difference between the two doses of calcium. However, this study was limited by a small sample size and now outdated methods of assessing bone mineralisation. Studies using more advanced assessment of bone density have not replicated these findings.

A more recent study set in Memphis, Tennessee, investigated the effects of maternal calcium supplementation in a developed country, but where calcium intake is also typically low [32]. 256 mothers were recruited and randomly allocated to either 2000 mg/day calcium or placebo from 22 weeks gestation until delivery. No significant difference in offspring whole body or lumbar spine BMD measured using DXA was observed between the intervention group and the control group. Interestingly, maternal dietary calcium intake was assessed by 24 hour recall of dietary intake at randomisation and in the third trimester and a significant difference in whole body BMC was observed between the two intervention groups when comparing only mothers in the lowest quintile of calcium intake (<600 mg/day). It is therefore possible that calcium supplementation would be beneficial in women with the lowest levels of habitual calcium intake. In contrast to these findings, a randomised double blind placebo-controlled trial set in Gambia, a country where calcium intake is typically very low, found maternal supplementation with 1500 mg/day calcium from 20 weeks gestation did not result in a significant difference in offspring whole body BMD measured by DXA at 2, 13 and 52 weeks of age [33]. Moreover, potential long-term detrimental effects for both the maternal and offspring skeleton have been identified in long-term follow-up studies; women randomised to receive calcium supplementation had lower BMC and BMD than the placebo group when assessed 3 months after completion of breastfeeding, which was still evident at 5 years after study completion [34]. In the offspring, maternal calcium supplementation altered the growth trajectories, but with differing effects in males and females: at 8-12 years, females born to mothers who received calcium supplementation during pregnancy were shorter and had less fat mass than the placebo group. In contrast, males of supplemented mothers were taller with greater fat mass than those in the placebo group [35]. These effects appear to be mediated by a sexually-dimorphic alteration in the Growth Hormone (GH) - Insulin-like Growth Factor (IGF)-1 axis [36]. The authors predict that calcium supplementation has resulted in sex-specific changes in the timing of puberty, advancing it in males and delaying it in females, although pubertal stage was not directly assessed [35]. Interestingly, these findings are similar to the results of a one year trial of calcium supplementation in pre-pubertal children in the Gambia, in which supplementation resulted in an early pubertal growth spurt and subsequently shorter final height in boys, but not in girls [37]. Importantly, in this chronically calcium-deficient population, maternal calcium supplementation might not be beneficial to offspring bone health in later life, as an earlier puberty in males is likely to result in shorter final height, and later onset of puberty and menarche in females have been associated with increased fracture risk in adulthood [38]. Long-term follow-up of these cohorts is needed.

**5.2. Vitamin D**

There is also an increasing volume of published literature reporting on the relationships between maternal vitamin D status and offspring bone health both at birth and into later childhood and early adulthood. Vitamin D can be derived from the diet, either as cholecalciferol (vitamin D3) from animal sources (e.g. dairy product, eggs, oily fish) or ergocalciferol (vitamin D2) from plant-based sources (e.g. mushrooms). However, the majority is formed endogenously by the action of ultraviolet B (UVB) to convert 7-dehyrdocholesterol to pre-vitamin D3 within the skin. The main circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D], which acts as a reservoir for conversion to the active metabolite 1, 25-dihydroxyvitamin D. Serum 25(OH)D is currently used as the best biomarker of vitamin D status. Serum 25(OH)D typically displays a seasonal pattern in populations at northern latitude due to the dependence on UVB for synthesis. This is also observed in pregnant populations [39] and low levels of 25(OH)D in pregnancy are common.

**5.2.1 Observational studies of maternal vitamin D status and offspring bone mineralisation**

The seasonal variation in 25(OH)D has been used as a proxy measurement for maternal 25(OH)D status in a number of studies. Indeed, an observational study in Korea found that infants born in winter months had both lower umbilical cord levels of 25(OH)D and whole body BMC compared to those born in summer months [40]. Observational mother-offspring studies in Canada and Norway identified that low maternal 25(OH)D either measured in maternal serum during pregnancy or umbilical cord blood is associated with lower offspring BMC relative to weight [41, 42], whereas in a cohort in the Gambia no significant relationships were observed between maternal 25(OH)D during pregnancy and offspring whole body BMC at birth or in the first year of life [43]. In contrast to the other studies, no mother in the Gambian cohort had a 25(OH)D < 50 nmol/l. Poorer skeletal mineralisation might therefore only occur in fetuses born to mothers with the lowest levels of 25(OH)D.

The associations between maternal vitamin D and offspring bone health have been observed to extend throughout childhood and beyond into adulthood. Using the PAH cohort, Javaid *et al* found that maternal serum 25(OH)D measured in late pregnancy (median 34 weeks gestation) was positively associated with offspring whole body and lumbar spine bone mineralisation at 9 years of age [44]. These findings were later replicated in the Southampton Women’s Survey (SWS), a much larger cohort from the same geographical area at 6-7 years of age (Figure 2) [45], and at 20 years of age in an Australian mother-offspring study, in which a maternal 25(OH)D < 50 nmol/l at 18 weeks gestation was associated with a 2.7% and 1.7% reduction in while body BMC and BMD, respectively, compared to offspring born to mothers above this level [46]. In the SWS, a positive association between maternal 25(OH)D in late pregnancy and offspring muscle strength at 4 years of age has also been identified [47] and, given the importance of muscle strength and loading to bone mineral accrual, this is also noteworthy. In contrast, findings from the large ALSPAC mother-offspring cohort do not support these findings. Initially, Sayer *et al* reported a positive relationship between estimated maternal UVB exposure during late pregnancy and offspring whole body less head BMC, bone area and aBMD at 9.9 years of age [48], but subsequent analysis in a subset of the cohort for whom maternal serum 25(OH)D measurement was available during pregnancy did not reveal any significant associations between maternal 25(OH)D and offspring bone mineralisation. The authors suggest that collinearity between the estimated UVB during pregnancy and age at assessment of the offspring confounded the relationships observed in the initial study [49]. Furthermore, Garcia *et al* reported an inverse relationship between maternal 25(OH)D concentration and bone mass at 6 years of age in the Generation R cohort from the Netherlands [50], but the statistical models included both season and ambient sunshine in the month prior to blood sampling, which as the primary determinants of the exposure and not expected to be related to the outcome, may have influenced this finding [51]. Additionally, neonatal 25(OH)D status was also not associated with childhood fracture risk in a large Danish cohort study [52].

5.2.2 Clinical trials of vitamin D supplementation in pregnancy

Whilst the findings of observational studies demonstrate that prenatal vitamin D supplementation might be of benefit to offspring bone health in the neonatal period but with some inconsistency in later childhood, there is a clear need for intervention studies to confirm this before any public health advice can be made. The largest of these is the Maternal Vitamin D Osteoporosis Study (MAVIDOS), a double-blinded randomised placebo-controlled multicentre trial of cholecalciferol supplementation in pregnancy, of which the primary outcome was neonatal bone mass measured using DXA [53, 54]. 1134 women from three research centres in the UK (Southampton, Oxford and Sheffield) with a 25(OH)D between 25-100 nmol/l at 12 weeks gestation were randomised to 1000 IU/day cholecalciferol or placebo from 14 weeks gestation until delivery. DXA assessment of infant whole body bone mass was performed within the first 14 days after birth. There was a significant increase in maternal 25(OH)D status in late pregnancy in the women who received cholecalciferol supplementation, but overall there were no significant differences in offspring whole body BMC, bone area or aBMD between the two treatment groups, but a significant interaction was observed between season of birth and maternal randomisation group (Figure 3). Indeed, in winter born infants, whole body BMC and BMD were approximately 9% and 5% higher, respectively, in the cholecalciferol group compared to the placebo group [53]. Such an effect size is considerably larger than those observed between children with and without fractures [4] and hence this is likely to be clinically relevant if the effect persists into later childhood and beyond. Follow-up of this cohort with DXA and peripheral quantitative computed tomography (pQCT) at 4 years of age and high resolution peripheral quantitative computed tomography (HR-pQCT) at 6-8 years of age is ongoing and will determine whether these effects do persist. This study has also identified a number of maternal factors that are associated with the response to vitamin D supplementation, including maternal weight gain and single nucleotide polymorphisms (SNPs) in genes related to vitamin D metabolism [55, 56]. The interaction between these factors and supplementation might also be important to bone mineralisation and should be considered in future work.

There are currently no intervention studies to supplement both vitamin D and calcium concurrently to determine the effects on bone mineralisation. However, given the requirement for both micronutrients in bone mineral accrual, further research should be directed towards this. Indeed, Chan *et al* conducted a study in pregnant adolescents with randomisation to one of three groups: placebo, orange juice fortified with 1200 mg/day calcium or dairy product containing 1200 mg/day calcium. Birth weight and infant whole body Ca measured by DXA were significantly higher in the dairy product group compared to the placebo or orange juice group [57]. Both maternal calcium intake and serum 25(OH)D were higher in the dairy group suggesting that repletion of both nutrients might be required to affect offspring bone mineralisation. However, in one clinical trial combined calcium (500-600 mg/day) and vitamin D supplementation (200 IU/day) may increase the risk of preterm birth despite reducing the risk of maternal pre-eclampsia, potentially limiting the feasibility of it as a clinical intervention to improve bone health [58].

**6.0. Non-dietary influences on offspring bone development**

The detrimental effect of maternal smoking on placental function and intrauterine growth retardation is well documented, but the relationships between maternal smoking and offspring bone health are less conclusive. At birth, infants born to mothers who smoked had reductions in whole body BMC and bone area in the PAH study and SWS, but these relationships were no longer significant after adjustment for infant size, suggesting that smoking affects overall skeletal size rather than mineralisation [59, 60]. In contrast, maternal smoking is associated with higher measures of BMC, BMD and bone area in later childhood and adolescence, but this may be mediated by the greater body weight and higher incidence of obesity observed in offspring of smoking mothers [61, 62, 63]. Two studies reporting on the association between maternal smoking and offspring fracture rate have reported conflicting results. Parviainen *et al* reported a 1.83 increased risk of fracture before the age of 7 years in children born to mothers who smoked in Northern Finland [64], whereas Jones *et al* did not find that maternal smoking increased the risk of offspring fracture before the age of 16 years in an Australian birth cohort [65].

In the SWS a number of other maternal traits have been associated with offspring bone mineralisation: both maternal height and triceps skinfold thickness (a measure of adiposity), were positively associated with offspring BMC and BA in the neonatal period, whereas maternal walking speed, used as a marker of physical activity, was negatively associated with neonatal BMC and BA [59]. Assessment of these relationships outside of the neonatal period or in other cohorts has not been reported.

**7.0. Mechanisms**

Our understanding of the mechanisms underlying the observed associations between the early environment and future bone health is evolving. It is possible that nutrients may have a direct effect on bone mineralisation, for example, maternal 25(OH)D concentration is associated with both umbilical cord calcium concentration, and expression of a calcium transporter in the placenta is positively associated with neonatal bone mass [66]. However, one of the key mechanisms believed to mediate the interaction between the *in utero* environment and clinical characteristics is epigenetics. It is recognised that genes can be expressed differently in different cells and tissues according to function and need, and in experimental studies alterations to offspring phenotype and gene expression can occur in response to environmental cues and maternal diet [67]. Epigenetic modifications, including DNA methylation and histone modification, are stable heritable changes, which can influence gene transcription but do not affect the DNA sequence. Differences in DNA methylation has been associated with childhood bone mass [68, 69], and alterations of the *in utero* environment, including maternal smoking [70] and cadmium exposure [71], have been associated with such DNA methylation. Although Suderman et al did not identify any associations between maternal 25(OH)D at either 18 or 28 weeks gestation and offspring genome-wide methylation patterns in cord blood in an observational cohort study [72], in the MAVIDOS trial, cholecalciferol supplementation resulted in reduced methylation in specific regions near to the retinoid-X-receptor-alpha (*RXRA*) promoter in umbilical cord DNA [73]. Methylation at these loci have previously been associated with bone mass [68], and in the more recent study, addition of 25(OH)D to placental cells led to a decrease in *RXRA* methylation, consistent with the findings from the clinical study [73]. Future work is needed to establish the relationships between these methylation changes and bone health through interventional studies.

**8.0. Conclusion**

Novel strategies to improve bone health are much needed and the role of early developmental influences on later bone health should also be considered in future approaches. There is a wealth of data relating maternal diet and lifestyle characteristics to offspring bone development, although much of this is observational. Their findings are not uniformly consistent, but differences in cohort demographics, the co-variants chosen in analyses and methods for assessing dietary factors do limit the direct comparison of these studies. Therefore, high quality interventional studies are required, and indeed the ongoing MAVIDOS study will provide the basis for further evidence to support antenatal vitamin D supplementation as a possible approach to fracture and osteoporosis prevention.

**9.0. Expert Opinion**

Early interventions, for example during gestation, might represent a useful future strategy to improve offspring bone health. There is an ever-growing body of observational work, as has been presented in this review, but reverse causality and the effects of unknown confounders means the findings cannot be directly translated to public health advice. These studies are however important to guide future high quality interventional studies, which are, without a doubt, much needed. Any such study needs to be appropriately powered as it is likely that the few pre-existing studies of calcium supplementation in pregnancy have been inadequately powered to detect a small change in offspring bone mineralisation. The inclusion of over 1000 women in the MAVIDOS study has demonstrated both the acceptability of such an intervention study to women during pregnancy and the feasibility of large randomised controlled trials during pregnancy.

In the UK, the department of health (DH) currently recommends 400 IU/day vitamin D for all pregnant women to prevent biochemically low serum 25(OH)D levels and offspring hypocalcaemia. The MAVIDOS study has suggested benefits of 1000 IU/day for skeletal mineralisation in winter born infants; indeed the current Public Health England guidance incorporates a seasonal consideration. However, in order to be sure of the longer term benefits, it is important that the findings of the MAVIDOS trial are replicated in other studies, and that persisting benefits into later childhood are demonstrated. A separate trial of pregnancy vitamin D supplementation is underway (SPRING)[74] and follow-up of the MAVIDOS offspring at 6-8 years is ongoing.

The translation of these findings into a coherent public health policy is something which requires careful evaluation with considerations including the dose, given that this was 1000 IU/day MAVIDOS compared with, for example the 400 IU/day currently recommended in the UK, and whether a seasonal component should be incorporated. A blanket recommendation for a single dose which is the same across populations, time and health states is the most simple message, but then will effectively over-treat some and under-treat others. A message which varies the recommendation by circumstances such as season is more complicated and may risk reduced uptake, but may also more appropriately target the intervention.

Future research also needs to carefully consider the timing of any intervention. To date, the published intervention studies in this area have started supplementation with either calcium or vitamin D once a pregnancy has been confirmed. However, both skeletal development and epigenetic changes might predate this, and therefore a critical window to alter development might have been missed. As such, pre-conception intervention studies are necessary, particularly as many pregnancies are not recognised until well into the first trimester, meaning that changes to health behaviour, such as taking vitamin D supplementation, might not be undertaken until substantial fetal development has taken place.

There are a number of interventional studies in progress, namely the MAVIDOS and SPRING trials, which are aiming to assess the effects of vitamin D supplementation or maternal dietary manipulation on offspring bone health. Additionally the children born into these studies are likely to be followed up at regular intervals throughout childhood and into adolescence and young adulthood, and therefore future work over the next 5 years and beyond will be able to demonstrate whether any effects observed in the neonatal period persist into later childhood. Other tools, including high resolution peripheral quantitative computed tomography (HR-pQCT), which cannot be used in infancy, will also allow for more detailed assessment of bone structure and health later on during the lifecourse of study participants.

**Figure Legends**

Figure 1

Bone mass accrual throughout the lifecourse. Reproduced with permission from Harvey et al, JBMR 2014 [13]

Figure 2

Offspring whole body less head bone area, bone mineral content and areal bone mineral density at age 6 years by maternal 25-hydroxyvitamin D status in late pregnancy. [45] Shown as mean ±95 % confidence interval. Reproduced with permission from Moon et al, Osteoporosis International, 2015 [45]

Figure 3

Neonatal whole-body bone mineral content (A), bone area (B), and bone mineral density (C) by maternal randomisation to 1000 IU/day cholecalciferol or placebo and season of birth. Shown as mean and 95% CI. Winter is December to February, spring is March to May, summer is June to August, and autumn is September to November. BMC=bone mineral content. BMD=bone mineral density. Reproduced with permission from Cooper et al, Lancet Diabetes & Endocrinology 2016 [53]

Table 1

Observational studies reporting associations between maternal calcium intake and offspring bone development

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study** | **Country of study** | **N** | **Age of children at follow up** | **Maternal predictor** | **Gestation at assessment** | **Univariate Analysis** | | | | **Multivariate analysis** | **Reference** |
| **Whole body** | | **Lumbar spine** | |
| **BMC** | **BMD** | **BMC** | **BMD** |
| Pune Maternal Nutrition Study | India | 698 | 6y | Milk products | 18 weeks | ↑ a | ↑ c | ↑ a | ↑ a | Maternal milk intake at 28 weeks accounted for 1.1% variance in whole body BMD, 0.5% whole body BMC, 0.4% spine BMC. | [28] |
| Milk products | 28 weeks | (↑) | ↑ a | ↑ a | (↑) |
| Calcium intake | 18 weeks | ↑ c | ↑ c | ↑ a | (↑) |
| Calcium intake | 28 weeks | ↑ b | ↑ b | ↑ b | (↑) |
| Generation R study | The Netherlands | 2891 | 6y | Calcium intake | 13 weeks | ↑ c | ↑ b |  |  | Not significant | [27] |
| Avon Longitudinal Study of Parents and Children (ALSPAC) | United Kingdom | 3032 | 9y | Calcium intake | 32 weeks | ↑ a | (↑) | (↑) | (↑) | Not significant | [22] |
| Prospective cohort study of prone sleeping position and sudden infant death syndrome | Australia | 173 | 8y | Calcium intake | Last trimester |  | (↑) |  | (↑) | Not significant | [29, 30] |
| Milk intake | Last trimester |  | (↑) |  | (↑) |
| 216 | 16y | Calcium intake | Last trimester |  | (↓) |  | ↑ a | Both calcium and milk intake significantly associated with lumbar spine BMD in multivariate analyses. |
| Milk intake | Last trimester |  | (↓) |  | (↑) |
| Vitamin D in Pregnancy Cohort Study | Australia | 171 | 11y | Dairy intake | Last trimester | (↑) |  | (↑) |  | Not significant | [26] |

BMC, bone mineral content; BMD, bone mineral density; ↑, positive relationship, statistically significant (a p<0.05, b p<0.01, cp<0.001); (↑) positive relationship, not significant; (↓) negative relationship, not significant

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