

Epigenetic influences in the developmental origins of osteoporosis

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Abstract Osteoporosis is a major public health problem due to consequent fragility fractures; data from the UK suggest that up to 50% of women and 20% men aged 50 years will have an osteoporosis-related fracture in their remaining lifetime. Skeletal size and density increase from early embryogenesis through intrauterine, infant, childhood and adult life to reach a peak in the third to fourth decade. The peak bone mass achieved is a strong predictor of later osteoporosis risk. Epidemiological studies have demonstrated a positive relationship between early growth and later bone mass, both at peak and in later life, and also with reduced risk of hip fracture. Mother-offspring cohorts have allowed the elucidation of some of the specific factors in early life, such as maternal body build, lifestyle and 25(OH)-vitamin D status, which might be important. Most recently, the phenomenon of developmental plasticity, whereby a single genotype may give rise to different phenotypes depending on the prevailing environment, and the science of epigenetics have presented novel molecular mechanisms which may underlie previous observations. This review will give an overview of these latter developments in the context of the burden of osteoporosis and the wider data supporting the link between the early environment and bone health in later life.

Keywords Developmental origins · Epigenetic · Osteoporosis

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Introduction: the burden of osteoporotic fracture

Osteoporosis is a skeletal disorder characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture [1]. It is a widespread condition, often unrecognised in clinical practice, which may have devastating health consequences through its association with fragility fractures. These fractures typically occur at the hip, spine and wrist. It has been estimated that, at age 50, the remaining lifetime risk of fracture at one of these sites is 50% among women and 20% among men. Osteoporotic fracture has a huge impact economically, in addition to its effect on health. Osteoporotic fracture costs the US approximately \$17.9 billion per year, with the cost in the UK being £1.7 billion [2].

The risk of osteoporotic fracture ultimately depends on two factors: the mechanical strength of bone and the forces applied to it. Bone mass (a composite measure including contributions from bone size and from its volumetric mineral density) increases throughout childhood and early adulthood to reach a peak in early adulthood. The bone mass of an individual later in life depends upon the peak attained after skeletal growth and the subsequent rate of bone loss [3]. Peak bone mass (PBM) is a major determinant of later osteoporosis risk, accounting for half of the variance in bone mineral density (BMD) at age 70 [4]. More recent work has demonstrated that PBM is six times a more powerful predictor of age of onset of osteoporosis than rate of bone loss or age at menopause [5]. In addition, there are now data available that directly link growth rates in childhood to the risk of later hip fracture [6].

Many lines of evidence, including data from epidemiological, clinical and experimental studies, indicate that early

life events play a powerful role in influencing later susceptibility to certain chronic diseases, such as osteoporosis; however, the mechanisms initiating these responses remain unclear. Recent data have strongly suggested that epigenetic processes are responsible for tissue-specific gene expression during differentiation and may play a key role in adaptive responses to nutritional and environmental factors during foetal and neonatal life. Thus, epigenetic mechanisms may underlie the processes of developmental plasticity.

The epigenetic mechanisms will be best understood when placed in the context of the existing evidence relating early environment to later risk of osteoporosis. Therefore, this review will summarise the relationship between developmental plasticity and osteoporosis and will focus upon the possible mechanisms by which the epigenetic regulation of bone mass may occur, using the model of maternal vitamin D status and placental calcium transfer.

Developmental plasticity

Environmental influences during childhood and puberty have been shown to influence bone mineral accrual, but the relatively rapid rate of mineral gain during intrauterine and early postnatal life, coupled with the plasticity of skeletal development in utero, offers the possibility of profound interactions between the genome and early environment at this stage in the life course. There is a strong biological basis for such a model of disease pathogenesis. Experimentalists have repeatedly demonstrated that alterations to the diet of pregnant animals can produce lasting changes in the offspring's physiology and metabolism [7]. This is one example of a ubiquitous phenomenon: Developmental plasticity, that is, the ability of a single genotype to give rise to several different phenotypes, allowing the organism to adapt future generations to prevailing environmental conditions. In humans, the importance of the intrauterine environment was initially demonstrated with associations between birth weight and blood pressure, lipid levels and diabetes later in life. This phenomenon was termed 'programming' and defined as 'persisting changes in structure and function caused by adverse environmental influences at a critical stage of early development' [8, 9]. The evolutionary benefit of this capacity is that, in a changing environment, it maximises phenotypic diversity and enables the production of phenotypes that are better matched to their environment than would be possible by the production of the same phenotype in all environments.

During mammalian development, information about the environment, such as nutritional status, is transferred from the mother to her embryo or foetus either through the placenta or through lactation. This transfer of information from mother to foetus may act to limit foetal growth, in

preference to any genetic potential, and has been termed 'maternal constraint' [10]. The process by which maternal constraint occurs is poorly defined but may include limited nutrient availability and an effect on the metabolic-hormonal factors driving foetal growth. Maternal constraint acts in all pregnancies but is greater in certain situations such as short maternal stature, extreme young or old maternal age and multiple pregnancies. In addition, the effects of an unbalanced diet or excessive maternal thinness or fatness influence foetal nutrition in the absence of other disease. Beyond these mechanisms, foetal development may be further impaired by poor placental function or maternal disease, each of which can influence several points along the pathway from the mother's intake of food to the delivery of nutrients to growing foetal tissues. The 'developmental origins' hypothesis proposes that an altered long-term risk of disease is the result of adaptive responses that the foetus or infant makes to cues from the mother about her health or physical status. Thus, the association between reduced foetal growth rate, small body size at birth and later risk of disease may be interpreted as reflecting the long-term consequences of foetal adaptive response. Figure 1 summarises and provides a conceptual framework for this process.

The developmental origins of osteoporotic fracture

Epidemiological evidence that the risk of osteoporosis might be modified by the intrauterine and early postnatal environment has emerged from two groups of studies: Firstly, retrospective cohort studies in which bone mineral measurements are undertaken and in which fracture risk is ascertained among adults whose detailed birth and/or childhood records have been preserved; and secondly, mother-offspring cohorts relating the nutrition, body build and lifestyle of pregnant women to the bone mass of their offspring.

Birth weight, growth in infancy and adult bone mass

The association between weight in infancy and adult bone mass was shown in a cohort study of men and women aged 60–75 years who were born and still lived in Hertfordshire, England [11, 12]. These studies showed highly significant relationships between weight at 1 year and adult bone area at the spine and hip ($p < 0.005$); the relationships with bone mineral content (BMC) at these two sites were weaker but remained statistically significant ($p < 0.02$). The relationships also remained after adjustment for known genetic markers of osteoporosis risk, such as polymorphisms in the gene for the vitamin D receptor (VDR) [13], and after adjustment for lifestyle characteristics in adulthood that might have influenced bone mass (physical activity, dietary calcium intake, cigarette smoking and alcohol consumption). These findings

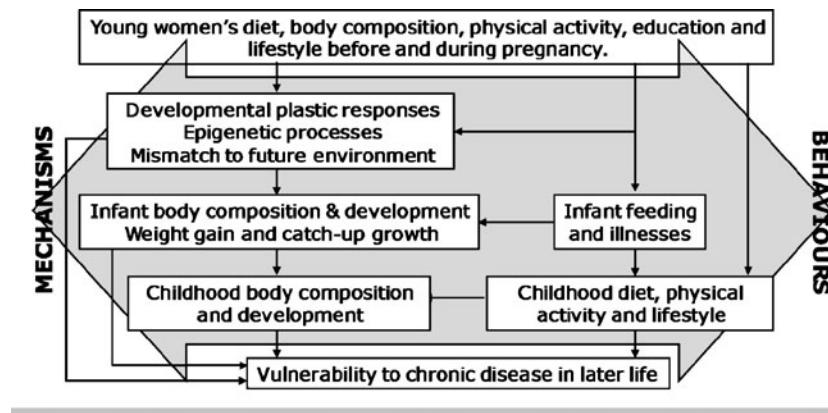


Fig. 1 A conceptual framework for the developmental origins of adult disease hypothesis. The ‘developmental origins’ hypothesis proposes that an altered long-term risk of disease is the result of adaptive responses that the foetus or infant makes to cues from the mother

about her health or physical status. The association between reduced foetal growth rate, small body size at birth and later risk of disease may be interpreted as reflecting the long-term consequences of foetal adaptive response

confirm previous observations in studies performed in the US, Australia, Sweden and the Netherlands [14].

Bone density, geometry and strength

Hip structure analysis in the Hertfordshire Cohort Study has demonstrated that poor growth in utero and during the first year of life is associated with disproportion of the proximal neck of femur in later life (narrower neck but preserved axis length), with a corresponding reduction in mechanical strength of the region, over and above that attributable to reduced BMC per se [15]. In addition, the use of peripheral computed tomography, within the same cohort, has demonstrated strong associations between birth weight, weight at 1 year and each of bone width, length, area, fracture load and strength–strain index at the tibia in both men and women, with less marked associations in a similar direction for the proximal radius [16]. These data add to those from hip structure analysis: thus, poor growth in utero and during the first year of postnatal life is associated with alterations in bone architecture, cortical size and geometry, in addition to a deficit in densitometrically measured BMC, resulting in compromised bone strength and increased fracture risk in later life. These studies also complement data from the Helsinki Cohort Study, which directly links growth rates in utero and during childhood with the risk of hip fracture. Three independent determinants of hip fracture risk were observed including tall maternal stature, shortness at birth and low rate of childhood growth [6].

Maternal nutrition, body composition and neonatal skeletal development

Studies in mother–offspring cohorts have shown that body composition and lifestyle of mothers during pregnancy are

related to the bone mass of their offspring at birth and in childhood. These influences on skeletal growth and mineralisation were in part determined by the umbilical venous concentrations of insulin-like growth factor 1 (IGF-1) and leptin [17]. In addition, further studies have confirmed that independent predictors of greater neonatal whole-body bone area and BMC include greater maternal birth weight, height, parity, fat stores (triceps skinfold thickness) and lower physical activity in late pregnancy. Maternal smoking was statistically significantly (and independently) associated with lower neonatal bone mass. These relationships were observed in both male and female offspring [18].

In another study using dual-emission X-ray absorptiometry (DXA) to assess the body composition of 198 children at the age of 9 years, reduced maternal height, lower preconception maternal weight, reduced maternal fat stores during late pregnancy, a history of maternal smoking during pregnancy and lower maternal social class were all associated with reduced whole-body BMC of the child at the age of 9 years [19]. In addition, lower ionised calcium concentration in umbilical venous serum also predicted reduced childhood bone mass. Around 31% of the mothers had insufficient and 18% had deficient circulating concentrations of 25(OH)-vitamin D during late pregnancy (11–20 and <11 µg/l, respectively). Lower concentrations of serum 25(OH)-vitamin D in mothers during late pregnancy were associated with reduced whole-body and lumbar spine BMC in children at the age of 9 years. Maternal vitamin D status was also statistically significantly associated with childhood bone area and areal BMD. Adjunctive evidence supporting a role for maternal vitamin D status was obtained in the Southampton Women’s Survey where maternal vitamin D concentrations again correlated with neonatal bone mass [20]. These findings suggested that vitamin D supplementation of

pregnant women, especially during winter months, could lead to long-lasting reductions in the risk of osteoporotic fracture in the offspring.

Further evidence that maternal calcium homeostasis might play a role in the trajectory of intrauterine and early postnatal skeletal development emerged from a mother–offspring cohort study in Pune, India which confirmed that children of mothers who had a higher frequency of intake of calcium-rich foods during pregnancy had higher total and lumbar spine BMC and areal BMD, independent of parental size and DXA measurements [21]. Circulating maternal 25(OH)-vitamin D concentrations in this cohort were relatively high and were not associated with childhood skeletal measures. Thus, in populations in nutritional transition where maternal sunlight exposure is sufficient to maintain adequate vitamin D status, the availability of calcium becomes a more critical determinant of foetal and childhood bone mineral accrual.

In most studies, maternal diet has been considered in terms of intake of specific nutrients, such as calcium and vitamin D. However, these nutrients comprise parts of broader dietary patterns and one recent study has explored maternal diet in more detail in relation to skeletal health in the offspring and showed that a high maternal prudent diet score (high intakes of fruit and vegetables, wholemeal bread, rice and pasta, yoghurt and breakfast cereals and low intakes of chips and roast potatoes, sugar, white bread, processed meat, crisps, tinned vegetables and soft drinks) was found to be associated with greater bone size and areal BMD in the offspring [22]. The observed effect was independent of social class, education, maternal height, maternal smoking status and late pregnancy vitamin D levels as well as childhood height, weight and exercise.

There is limited ecological information regarding the influence of maternal nutrition on fracture risk and further research is needed in this field. Variations in hip fracture rates have previously been described globally, across continents and also nationally. The 10-year probability of hip fracture in women aged 50 appears to vary widely worldwide and is highest in Scandinavian countries (Sweden, Denmark and Norway; with rates of up to 28.5%). Low rates have been described in Korea, Venezuela and Chile, with the lowest risk in Turkey [23]. This global difference may result primarily from differences in ethnicity. Within Europe, there appears to be a north–south gradient of fracture risk, with higher incidence in northern countries (e.g., Finland) compared to southern Mediterranean nations [24]. This may be a consequence of reduced sunlight exposure in northern territories and consequent lower vitamin D levels, combined with an increased risk of falls. Within nations, differences in fracture rates have again been observed and appear to be driven, in part, by deprivation and urbanisation. Thus, UK

hip fracture rates are low in parts of East Anglia and other rural areas, compared with rates in urban areas [24]; locations with the highest fracture rates have been observed to have had the highest rates of infant mortality in previous generations [24], this link yielding some of the earliest evidence to support the notion that an adverse environment in early life might predispose to an increased risk of fracture in older age.

Childhood nutrition and physical activity

There is evidence that the trajectory of skeletal growth may be modified, at least temporarily, by environmental factors such as nutrition and physical activity in childhood. Several, but not all, studies have indicated a positive relationship between dietary calcium intake and bone mineral accrual [25–34]. However, in one recent meta-analysis of intervention studies, an increased calcium intake only appeared to have a positive effect on BMC in those children who had the lowest intakes at baseline, suggestive of a threshold effect [25]. Individual intervention studies have suggested at least short-term benefits from additional calcium supplementation, with some evidence of more persisting (3 years) improvements for those supplements derived from milk [32, 33]. There is some limited evidence that childhood calcium intake may also influence adult bone density and fracture risk. In a study of 3,251 White women aged over 20 years, BMC was significantly greater in those aged 20–49 who consumed more than one serving per day of milk during childhood (recalled from memory) than those who did not [35]. Amongst those aged over 50, low milk intake during childhood was associated with an increased risk of osteoporotic fracture. Studies of weight-bearing physical activity interventions consistently demonstrate positive effects on bone mineral accrual in both children and adolescents [34]. Additionally, habitual physical activity in free-living children has been shown to correlate positively with bone size and density at the hip [36, 37]. Although there is some evidence that individuals who had taken high levels of weight-bearing physical activity in childhood may have increased BMD as adults [38], the relationship between habitual childhood physical activity and adult BMD remains to be elucidated.

Animal models for the developmental origins of osteoporosis

Animal models for the developmental origins of osteoporosis have been established. In the first such model, the feeding of a low-protein diet (LPD) to pregnant rats produced offspring which exhibited a reduction in bone area and BMC, with altered growth plate morphology in adulthood [39, 40]. This study also examined whether maternal protein restriction affected the proliferation and

differentiation of bone marrow stromal cells [41]. The results suggested that normal proliferation and differentiation were suppressed in offspring from mothers on LPD as assessed by alkaline phosphatase positive fibroblast colony formation at 4 and 8 weeks. In a further study, dams were given a LPD during pregnancy and 135 offspring were studied at different ages. Serum alkaline phosphatase concentrations reached peak levels earlier, and serum IGF-1 and 25(OH)-vitamin D levels were lower in the offspring of protein-restricted (PR) dams, confirming the important role of the nutritional environment during intrauterine development [39]. Using micro-CT on samples of bone removed in late adulthood (75 weeks), it was observed that the offspring from LPD dams had femoral heads with thinner, less dense trabeculae; mechanical testing showed these samples to be structurally weaker [39].

These data further support the need for a programme of interventional research aimed at improving general dietary quality among women before conception and during pregnancy, in addition to studies that evaluate the targeting of specific micronutrients such as vitamin D and calcium.

Epigenetic mechanisms

There is increasing evidence that epigenetic mechanisms are central to the process by which early environmental exposure affects development in later life. Epigenetics refers to changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA sequence and is integral in determining when and where genes are expressed. Epigenetic changes are stable and heritable and may last through multiple generations [42]. The two most studied forms of epigenetic marking are DNA methylation and histone modification, although most studies have focused on methylation. DNA methylation involves the addition of a methyl group to cytosine residues at the carbon-5 position of CpG dinucleotides. DNA methylation is generally associated with gene repression, either by decreased binding of transcription factors or by attracting methyl-CpG-binding proteins that act as transcriptional repressors [43, 44]. There is usually an inverse relationship between the extent of DNA methylation of regulatory CpGs and gene expression. Histone modification refers to post-translational modification of histone tails. Histones are small proteins involved in the packaging of DNA into chromatin, and if the way that DNA is wrapped around the histones changes, gene expression can also change. Histone modification can occur either by methylation or acetylation. These two types of epigenetic modification are mechanistically linked and work together to affect chromatin packaging, which in turn determines which gene or gene set is transcribed. The

enzymes controlling these processes have recently been identified and include DNA methyltransferases (Dnmt) [45].

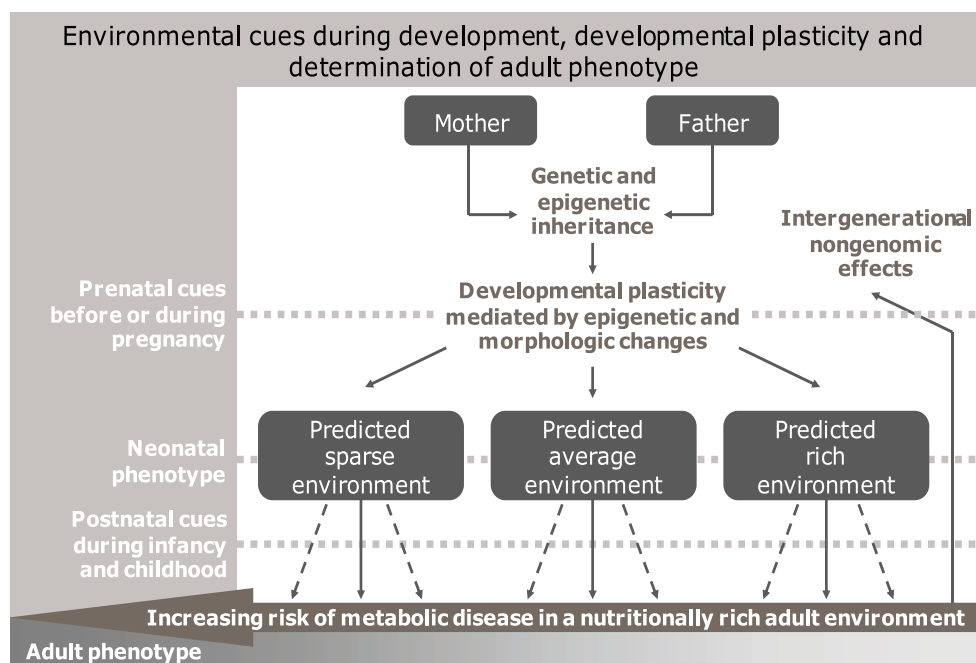
DNA methylation patterns differ through the phases of development. After conception, and with the exception of imprinted genes, gamete methylation patterns are erased during early blastocyst formation. During the implantation stage, methylation patterns become established via de novo methylation by the activities of Dnmt3a and Dnmt3b. Patterns of DNA methylation are maintained through mitosis by Dnmt1 activity [46]. In adulthood, there are variations in the amount and pattern of methylation depending upon cell and tissue type. During embryonic and foetal development, maternal or environmental factors can disrupt these patterns of DNA methylation; examples of this process have been shown in animal models and will be discussed in further detail in this review. This dysregulation of developmental programming via abnormal DNA methylation may permit specific genes to undergo inappropriate expression during adult life, resulting in disease development [45]. Emerging evidence strongly suggests that these epigenetic mechanisms underlie the processes of developmental plasticity (Fig. 2).

Experimental data from animal models

Numerous studies in animals involving prenatal nutrient imbalance have provided important information regarding the biologic basis for developmental plasticity. For example, the embryos of pregnant rats fed a LPD during the pre-implantation period of pregnancy showed altered development in multiple organ systems [47]. In addition, if the pregnancies progressed to term, then the offspring had reduced birth weight, relatively increased postnatal growth and adult-onset hypertension. Further studies have shown that the administration of glucocorticoids to pregnant rats at specific points during gestation can cause hypertension and insulin resistance in the offspring in later life and can also lead to increased sensitivity to postnatal stress [48–50]. Postnatal stress in rat models has been shown to induce neurodevelopmental changes in the rat pups and this leads to excessive responses to stress in later life. These changes may be mediated in part by effects on glucocorticoid receptor (GR) gene expression in the brains of the offspring [51].

Further exploratory work in this area has shown that maternal dietary protein restriction in rats leads specifically to a decrease in the methylation status of GR (Fig. 3) and peroxisomal proliferator-activated receptor α (PPAR α) in the liver of the offspring after weaning [52]. These genes are of particular interest because alterations in their expression are associated with disturbances in cardiovascular and metabolic control in animals and humans. The hypomethylation of the GR and PPAR persisted after weaning, when direct influence of the maternal dietary restriction had ceased, suggesting stable modification to the

Fig. 2 Epigenetic mechanisms in the process of developmental plasticity. Developmental plasticity is the ability of a single genotype to give rise to several different phenotypes, allowing the organism to adapt future generations to prevailing environmental conditions



epigenetic regulation of the expression of these transcription factors. These induced epigenetic changes have also been demonstrated to pass between generations. Eighty-day-old male grand-offspring of rats exposed to maternal PR diet during gestation had hypomethylation of GR and PPAR α promoter regions compared to controls, even though their dams received adequate nutrition throughout pregnancy [53]. In addition, supplementation of the restricted diet with folic acid has been shown to prevent hypomethylation of GR and PPAR α and the associated

increase in the expression of GR and PPAR α . This observation may reflect the impaired supply of folic acid from the mother and suggests that, despite the apparent stability of methylation changes, alterations in DNA methylation induced by maternal diet can be prevented, raising the possibility of therapeutic strategies to prevent or reduce the effects of environmental insults in early life. Other studies have demonstrated similar epigenetic changes in p53 in the kidney and angiotensin II type 1b receptor in the adrenal gland [54, 55].

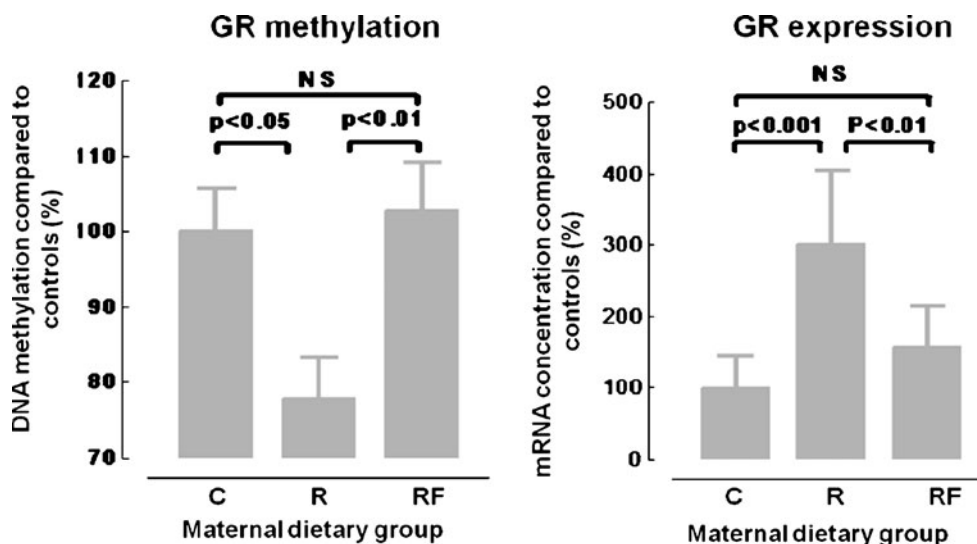


Fig. 3 Dietary protein restriction in pregnant rats and levels of GR methylation in the offspring (figure based on numeric data from Lillycrop et al. [52]). Dams were fed a control protein (C; 180 g/kg protein plus 1 mg/kg folic acid), restricted protein (R; 90 g/kg casein plus 1 mg/kg folic acid) or restricted protein plus 5 mg/kg folic acid

(RF) diet throughout pregnancy. GR gene methylation was 22.8% lower and expression 200% higher in PR pups compared to control pups. Extra folate supplementation prevented these changes. These results suggest that prenatal nutrition can induce persistent, gene-specific epigenetic changes that alter mRNA expression

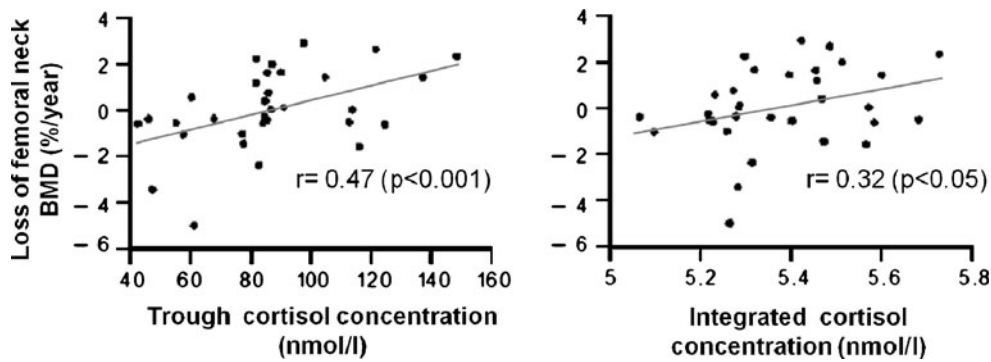


Fig. 4 Indices of adult circulating cortisol and bone loss at femoral neck (reproduced with permission from Dennison et al. [61]). Relation between characteristics of cortisol concentration and femoral neck bone loss rate in 22 men, aged 61–72 years, undergoing replicate bone density measurements over a 4-year period. There was a statistically

significant positive association between the trough cortisol concentration and bone loss rate at the femoral neck ($r=0.47$; $p<0.001$) over the 4-year follow-up period. This suggests that the endogenous cortisol profile of healthy elderly men is a determinant of their rate of involutional bone loss

These studies show that the effects of maternal nutrition and behaviour appear to target the promoter regions of specific genes rather than being associated with a global change in DNA methylation. This observation provides important clues for further work to explore epigenetic mechanisms in humans.

Epigenetics in human disease

Epigenetic mechanisms, including DNA methylation and histone modification, are now well established in the development and progression of a variety of cancer types including prostate, lymphoma, head and neck, breast and ovarian cancer [56]. Data in other human diseases are limited, particularly in relation to developmental plasticity. The first example of an association between a periconceptional exposure and DNA methylation in humans was shown in Dutch subjects prenatally exposed to famine during the

Dutch Hunger Winter in 1944–1945. Exposed subjects showed persistent epigenetic differences in a variety of genes compared to their unexposed, same sex siblings [57]. The IGF-2 gene was hypomethylated, whereas interleukin-10, leptin, ATP-binding cassette A1 and maternally expressed 3 (me3) genes were hypermethylated. IGF-2 is known to be a key factor in human growth and development. This study further supports the importance of investigating how early epigenetic modification of gene expression may influence long-term health and disease.

Epigenetic regulation of placental calcium transfer

The key nutrients likely to influence foetal bone development include calcium and vitamin D, and therefore, this axis provides a model for investigating the epigenetic regulation of bone mass. The human foetus requires a total of 30 g of calcium for bone development, most of which is

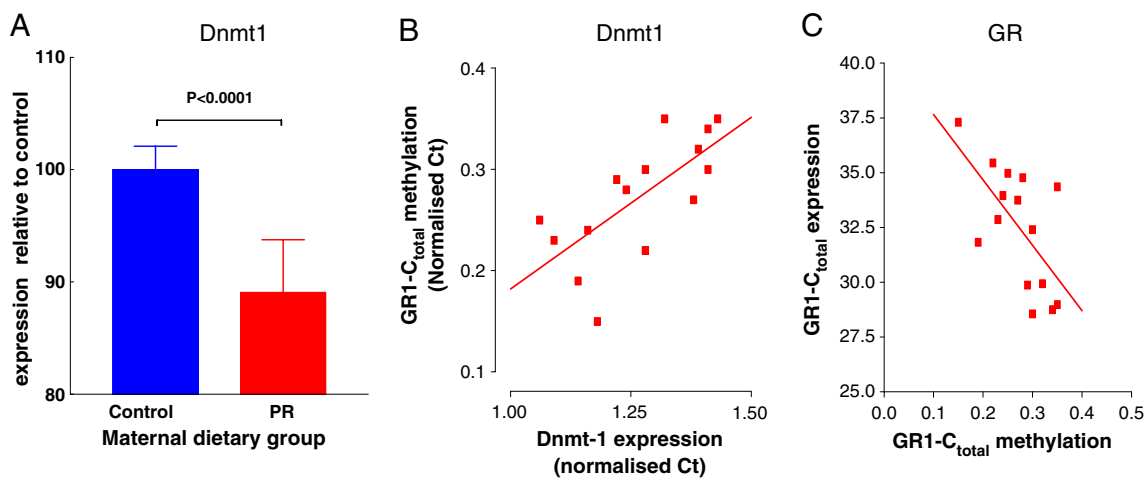


Fig. 5 An epigenetic pathway demonstrated in animal and human models. **a** Rat dietary protein restriction (PR) leads to altered Dnmt1 (DNA methyltransferase-1) expression. **b** Dnmt1 expression in human umbilical cord predicts GR1-C_{total} (human glucocorticoid receptor 1-C_{total}

promoter) methylation. **c** Finally GR1-C_{total} methylation status in human umbilical cord predicts GR (glucocorticoid receptor) expression. (Figure adapted with permission from Lillycrop KA, et al. [52])

acquired during the third trimester via active transport across the placenta, resulting in greater calcium concentration in the foetus than maternal plasma [58]. Foetal calcium needs are primarily met by increased maternal intestinal calcium absorption during pregnancy, and therefore, very low maternal calcium intakes may be a risk for lower bone mass in neonates. In addition, the importance of maternal vitamin D status has been highlighted earlier in this review. The mechanism underlying the association between maternal vitamin D, umbilical cord calcium concentration and offspring bone mass is unclear but is an area of ongoing research. 1,25(OH)-vitamin D (the active form) mediates its effects by first binding to the VDR, then by binding to the retinoic acid receptor (RXR) forming a heterodimer. This heterodimer then acts upon vitamin D response elements in target genes, initiating gene transcription by either up-regulating or down-regulating gene products. Vitamin D response elements are DNA sequences found in the promoter region of vitamin D-regulated genes [59]. Calcium transporters containing vitamin D response elements are, therefore, of particular interest.

One study has demonstrated that the expression of a placental calcium transporter (PMCA3) gene predicts neonatal whole-body BMC [60]. Modified expression of the genes encoding placental calcium transporters, by epigenetic regulation, might represent the means whereby maternal vitamin D status could influence bone mineral accrual in the neonate. Since the effects of maternal nutrition and behaviour seem to target the promoter region of specific genes rather than being associated with global changes in DNA methylation, investigating CpGs located within the promoter region of these genes, particularly those within or located near to vitamin D response elements, may provide further clues regarding the epigenetic regulation of bone mass. In addition, if validated, these epigenetic markers might provide risk assessment tools with which to target early lifestyle interventions to individuals at greatest future risk.

Hypothalamic–pituitary–adrenal axis—a putative model of epigenetic regulation

Maternal stress is known to influence the developing hypothalamic–pituitary–adrenal (HPA) axis in the foetus. Thus, epidemiological studies have demonstrated an inverse association between birth weight and fasting plasma cortisol. Indices of the circulating cortisol profile in adult life have also been shown to influence bone density and rates of bone loss (Fig. 4) [61].

As previously discussed in this review, animal studies have confirmed that protein restriction during mid and late pregnancy is associated with reduced methylation of key CpG-rich islands in the promoter region of the gene for the

GR, and this results in elevated GR expression and features of hypercortisolism [51]. Further work in rats, and subsequent replication of the work in human umbilical cords, has shown that induction in the offspring of altered epigenetic regulation of the hepatic GR promoter may be due to reduced Dnmt1 expression (Fig. 5) [61]. Previous work has shown that patterns of DNA methylation are maintained through mitosis by Dnmt1 activity, and in addition, the phenotype of an embryo can be modified by manipulation of Dnmt1 expression, hence the pattern of DNA methylation [46, 63]. This epigenetic modulation of the HPA axis represents a second mechanism for transduction between a poor maternal environment and impaired bone mineral accrual in the offspring.

Conclusions

Osteoporosis is a major cause of morbidity and mortality through its association with age-related fractures. Evidence is growing that PBM is an important contributor to bone strength during later life. Many factors influence the accumulation of bone mineral during childhood and adolescence, including heredity, gender, diet, physical activity, endocrine status, and sporadic risk factors such as cigarette smoking. In addition to these modifiable factors during childhood, evidence has also accrued that fracture risk might be programmed during intrauterine life. Epigenetic processes are important mechanisms that underpin developmental plasticity. Environmental factors including maternal stress and nutritional state are known to affect the long-term epigenetic state of a number of genes during embryonic and foetal development, although the exact mechanisms by which environmental influences are transmitted to the embryo are unclear. If validated, these epigenetic markers may be used to provide risk assessment tools and develop novel public health interventions with which to identify and target individuals at greatest future risk.

Conflicts of interest None.

References

1. Harvey N, Dennison E, Cooper C (2008) Epidemiology of osteoporotic fracture. In: Favus MJ (ed) Primer on the metabolic bone diseases and disorders of mineral metabolism, 7th edn. ASMBR, Washington, pp 198–203
2. Department of Health (1994) Advisory group on osteoporosis. Department of Health, London
3. Cooper C, Westlake S, Harvey N et al (2006) Review: developmental origins of osteoporotic fracture. *Osteoporosis Int* 17:337–347
4. Hui SL, Slemenda CW, Johnston CC Jr (1990) The contribution of bone loss to postmenopausal osteoporosis. *Osteoporosis Int* 1(1):30–34

5. Hernandez CJ, Beaupre GS, Carter DR (2003) A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis Int* 14(10):843–847
6. Cooper C, Eriksson JG, Forsen T et al (2001) Maternal height, childhood growth and risk of hip fracture later in life: a longitudinal study. *Osteoporosis Int* 12:623–629
7. Bateson P (2001) Fetal experience and good adult disease. *Int J Epidemiol* 30:928–934
8. Barker DJ (1990) The fetal and infant origins of adult disease. *BMJ* 301(6761):1111
9. Barker DJ (1995) The fetal and infant origins of disease. *Eur J Clin Invest* 25(7):457–463
10. Gluckman PD, Hanson MA, Cooper C et al (2008) Effect of in utero and early-life conditions on adult health and disease. *N Eng J Med* 359(1):61–73
11. Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D (1997) Growth in infancy and bone mass in later life. *Ann Rheum Dis* 56:17–21
12. Dennison EM, Syddall HE, Sayer AA, Gilbody HJ, Cooper C (2005) Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study. *Pediatr Res* 57(4):582–586
13. Keen R, Egger P, Fall C, Major P, Lanchbury J, Spector TD, Cooper C (1997) Polymorphisms of the vitamin D receptor, infant growth and adult bone mass. *Calcif Tiss Int* 60:233–235
14. Cooper C, Harvey N, Javaid K, Hanson M, Dennison E (2008) Growth and bone development. *Nestle Nutr Workshop Ser Pediatr Program* 61:53–68
15. Javaid MK, Lekamwasam S, Clark J, Dennison EM, Syddall HE, Loveridge N, Reeve J, Beck TJ, Cooper C (2006) Infant growth influences proximal femoral geometry in adulthood. *J Bone Miner Res* 21:508–512
16. Oliver H, Jameson KA, Sayer AA, Cooper C, Dennison EM (2007) Growth in early life predicts bone strength in late adulthood: the Hertfordshire Cohort Study. *Bone* 41:400–405
17. Javaid MK, Godfrey KM, Taylor P, Robinson SM, Crozier SR, Dennison EM, Robinson JS, Breier BR, Arden NK, Cooper C (2005) Umbilical cord leptin predicts neonatal bone mass. *Calcif Tissue Int* 76:341–347
18. Harvey NC, Poole JR, Javaid MK, Dennison EM, Robinson S, Inskip HM, Godfrey KM, Cooper C, Sayer AA (2007) Parental determinants of neonatal body composition. *J Clin Endocrinol Metab* 92:523–526
19. Javaid MK, Crozier SR, Harvey NC (2006) Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* 367(9504):36–43
20. Harvey NC, Javaid MK, Poole JR et al (2008) Paternal skeletal size predicts intrauterine bone mineral accrual. *J Clin Endocr Metab* 93(5):1676–1681
21. Ganpule A, Yajnik CS, Fall CH, Rao S, Fisher DJ, Kanade A, Cooper C, Naik S, Joshi N, Lubree H, Deshpande V, Joglekar C (2006) Bone mass in Indian children. Relationships to maternal nutritional status and diet during pregnancy: the Pune Maternal Nutrition Study. *J Clin Endocrinol Metab* 91:2994–3001
22. Cole ZA, Gale CR, Javaid MK, Robinson SM, Law CM, Boucher BJ, Crozier SR, Godfrey KM, Dennison EM, Cooper C (2009) Maternal dietary patterns during pregnancy and childhood bone mass: a longitudinal study. *J Bone Min Res* 24(4):663–668
23. Kanis JA, Johnell O, De Laet C et al (2002) International variations in hip fracture probabilities: implications for risk assessment. *JBMR* 17:1237–1244
24. Cooper C (1993) Epidemiology and public health impact of osteoporosis. *Balliere's Clin Rheumatol* 7:459–477
25. Huncharek M, Muscat J, Kupelnick B (2008) Impact of dairy products and dietary calcium on bone-mineral content in children: results of a meta-analysis. *Bone* 43:312–321
26. Winzenberg T, Shaw K, Fryer J, Jones G (2006) Effects of calcium supplementation on bone density in healthy children: meta-analysis of randomised controlled trials. *BMJ* 333:775
27. Du XQ et al (2002) Milk consumption and bone mineral content in Chinese adolescent girls. *Bone* 30:521–528, JID: 8504048
28. Rozen GS et al (2001) Calcium intake and bone mass development among Israeli adolescent girls. *J Am Coll Nutr* 20:219–224, JID: 8215879
29. Black RE, Williams SM, Jones IE, Goulding A (2002) Children who avoid drinking cow milk have low dietary calcium intakes and poor bone health. *Am J Clin Nutr* 76:675–680, JID: 0376027
30. Goulding A et al (2004) Children who avoid drinking cow's milk are at increased risk for prepubertal bone fractures. *J Am Diet Assoc* 104:250–253
31. Barr SI, Petit MA, Vigna YM, Prior JC (2001) Eating attitudes and habitual calcium intake in peripubertal girls are associated with initial bone mineral content and its change over 2 years. *J Bone Miner Res* 16:940–947, JID: 8610640
32. Bonjour JP et al (1997) Calcium-enriched foods and bone mass growth in prepubertal girls: a randomized, double-blind, placebo-controlled trial. *J Clin Invest* 99:1287–1294, JID: 7802877
33. Bonjour JP, Chevalley T, Ammann P, Slosman D, Rizzoli R (2001) Gain in bone mineral mass in prepubertal girls 3.5 years after discontinuation of calcium supplementation: a follow-up study. *Lancet* 358:1208–1212, JID: 2985213R
34. French SA, Fulkerson JA, Story M (2000) Increasing weight-bearing physical activity and calcium intake for bone mass growth in children and adolescents: a review of interventional trials. *Preventive Med* 31:722–731
35. Kalwarf HJ, Khoury JC, Lanpear BP (2003) Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. *Am J Clin Nutr* 77:257–265
36. Harvey NC, Cole ZA, Crozier SR, Kim M, Ntani G, Goodfellow L, Robinson SM, Inskip HM, Godfrey KM, Dennison EM, Wareham N, Ekelund U, Cooper C, The SWS Study Group (2011) Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. *Osteoporos Int* (in press)
37. Janz KF et al (2001) Physical activity and bone measures in young children: the Iowa bone development study. *Pediatrics* 107:1387–1393, JID: 0376422
38. Bass S, Pearce G, Bradney M, Hendrich E, Delmas PD, Harding A et al (1998) Exercise before puberty may confer residual benefits in bone density in adulthood: studies in active prepubertal and retired female gymnasts. *J Bone Miner Res* 13(3):500–507 (JID: 8610640)
39. Lanham SA, Roberts C, Perry MJ, Cooper C, Oreffo RO (2008) Intrauterine programming of bone. Part 2: alteration of skeletal structure. *Osteoporos Int* 19:157–167
40. Lanham SA, Roberts C, Cooper C, Oreffo RO (2008) Intrauterine programming of bone. Part 1: alteration of the osteogenic environment. *Osteoporos Int* 19:147–156
41. Oreffo RO, Lashbrooke B, Roach HI, Clarke NM, Cooper C (2003) Maternal protein deficiency affects mesenchymal stem cell activity in the developing offspring. *Bone* 33:100–107
42. Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic environmental signals. *Nature Genetics* 33(Suppl):245–254
43. Gicquel C, El-Osta A, Le Bouc Y (2008) Epigenetic regulation of fetal programming. *Best Practise and Research Clinical Endocrinology & Metabolism* 22(1):1–16

44. Gluckman PD, Hanson MA, Beedle AS (2007) Non-genomic transgenerational inheritance of disease risk. *BioEssays* 29:145–154
45. Tang W, Ho S (2007) Epigenetic reprogramming and imprinting in origins of disease. *Rev Endocr Metab Disord* 8:173–182
46. Bird A (2001) DNA methylation patterns and epigenetic memory. *Genes Dev* 16:6–21
47. Kwong WY, Wild AE, Roberts P et al (2000) Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 127:4195–4202
48. Levitt NS, Lindsay RS, Holmes MC et al (1996) Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* 64:412–418
49. Nyirenda MJ, Lindsay RS, Kenyon CJ et al (1998) Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* 101:2174–2181
50. Welberg LAM, Seckl JR, Holmes MC (2001) Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience* 04:71–79
51. Weaver ICG, Cervoni N, Champagne FA et al (2004) Epigenetic programming by maternal behaviour. *Nat Neurosci* 7:847–854
52. Lillycrop KA, Phillips ES, Jackson AA et al (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* 135:1382–1386
53. Burdge GC, Slater-Jefferies J, Torrens C, Phillips ES, Hanson MA, Lillycrop KA (2007) Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. *Br J Nutr* 97:437–439
54. Pham TD, MacLennan NK, Chiu CT et al (2003) Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full-term IUGR rat kidney. *Am J Physiol Regul Integr Comp Physiol* 285:R962–R970
55. Bogdarina I, Welham S, King PJ et al (2007) Epigenetic modification of the renin–angiotensin system in the fetal programming of hypertension. *Circ Res* 100:520–526
56. Grønbaek K, Hother C, Jones PA (2007) Epigenetic changes in cancer. *APMIS* 115(10):1039–1059
57. Heijmans BT, Elmar WT, Stein Ad (2008) Persistent epigenetic differences associated with prenatal exposure to famine in humans. *PNAS* 105(44):17046–17049
58. Namgung R, Tsang RC (2003) Bone in the pregnant mother and newborn at birth. *Clin Chim Acta* 333:1–11
59. Kimball S, El-Hajj Fuleihan G, Vieth R (2008) Vitamin D: a growing perspective. *Critical Reviews in Clinical Laboratory Sciences* 45(4):339–414
60. Martin R, Harvey NC, Crozier SR et al (2007) Placental calcium transporter (PMCA3) gene expression predicts intrauterine bone mineral accrual. *Bone*; 40:1203–1208
61. Dennison E, Hindmarsh P, Fall C et al (1999) Profiles of endogenous circulating cortisol and bone mineral density in healthy elderly men. *J Clin Endocrinol Metab* 84(9):3058–3063
62. Lillycrop KA, Slater-Jefferies JL, Hanson MA et al (2007) Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* 97(6):1064–1073
63. Biniszkiwicz D, Gribnau J, Ramsahoye B et al (2002) Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. *Mol Cell Biol* 22:2124–2135