DNA methylation and the social gradient of osteoporotic fracture: A conceptual model
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Highlights:

- Social disadvantage increases stress throughout the lifespan, and engenders a proinflammatory epigenetic signature.
- A heightened inflammatory state increases osteoporotic fracture risk in disadvantaged groups that are chronically stressed.
- Future clinical utility of this model may include addressing causative environmental pathways that are greater for disadvantaged individuals.
- An epigenetic evidence-base may strengthen the importance of lifestyle modification and stress reduction programs.
Abstract

Introduction: Although there is a documented social gradient for osteoporosis, the underlying mechanism(s) for that gradient remain unknown. We propose a conceptual model based upon the allostatic load theory, to suggest how DNA methylation (DNAm) might underpin the social gradient in osteoporosis and fracture. We hypothesise that social disadvantage is associated with priming of inflammatory pathways mediated by epigenetic modification that leads to an enhanced state of inflammatory reactivity and oxidative stress, and thus places socially disadvantaged individuals at greater risk of osteoporotic fracture.

Methods/Results: Based on a review of the literature, we present a conceptual model in which social disadvantage increases stress throughout the lifespan, and engenders a proinflammatory epigenetic signature, leading to a heightened inflammatory state that increases risk for osteoporotic fracture in disadvantaged groups that are chronically stressed.

Conclusions: Our model proposes that, in addition to the direct biological effects exerted on bone by factors such as physical activity and nutrition, the recognised socially patterned risk factors for osteoporosis also act via epigenetic-mediated dysregulation of inflammation. DNAm is a dynamic modulator of gene expression with considerable relevance to the field of osteoporosis. Elucidating the extent to which this epigenetic mechanism transduces the psycho-social environment to increase the risk of osteoporotic fracture may yield novel entry points for intervention that can be used to reduce individual and population-wide risks for osteoporotic fracture. Specifically, an epigenetic evidence-base may strengthen the importance of lifestyle modification and stress reduction programs, and help to reduce health inequities across social groups.

Key words: DNA methylation; osteoporosis; social gradient; fracture; epigenetic; life course

Mini abstract:

Our conceptual model proposes how DNA methylation might underpin the social gradient in osteoporotic fracture. We suggest that social disadvantage is associated with priming of inflammatory signalling pathways, which is mediated by epigenetic modifications, leading to a chronically heightened inflammatory state that places disadvantaged individuals at greater risk of osteoporosis.
Introduction

Osteoporosis is a common skeletal disease that is characterised by microarchitectural deterioration of the bone matrix and depletion of bone mineral density (BMD), with a subsequent increase in risk for fragility fracture [1]. Following a hip fracture, there are many financial, personal and psychosocial outcomes: one in five individuals die within the first year, while 60% of individuals who survive a hip fracture still require assistance to walk one year later, and 33% are totally dependent or are admitted to a nursing home [2, 3]. Osteoporotic fractures accounted for more Disability Adjusted Life Years (DALYs) lost than cancers, with the exception of lung cancer [4]. To advance understanding of the aetiology and pathogenesis of osteoporosis, there are now large-scale efforts to identify genes associated with fracture risk via genome-wide association studies (GWAS) of BMD [5-8]. The maintenance of BMD is not static, rather osteoblast and osteoclast differentiation processes are highly organized and driven by modifications in gene expression patterns throughout the life course [9]. Indeed, it is argued that the risk of developing osteoporosis occurs over the life course, with potential mechanisms involving epigenetic processes [10]. Epigenetics is the study of alterations in gene expression potential that are not caused by changes in DNA sequence [11]. These processes include DNA methylation (DNAm), histone modification and non-coding RNA (ncRNA) activity [12], with much interest recently directed toward the modulation of epigenetic pathways via DNAm [9]. A seminal review by Delgado-Calle et al [9] argued that DNAm plays a role in the onset and progression of musculoskeletal disorders including osteoporosis; a role similarly reported in other non-communicable diseases such as obesity [13], cancer [14], cardiovascular [15] and metabolic diseases [12, 13]. Furthermore, recent data have suggested that patients with fractures have differentially methylated genes that are related to skeletal development [16]. A role for DNAm in osteoporosis onset is biologically plausible; DNAm not only influences gene expression, but also plays a role in establishing a bone cell phenotype. Furthermore, DNAm is involved in the regulation of osteogenic differentiation of mesenchymal cells [17], and epigenetic mechanisms (one of which is DNAm) are important for osteoclast differentiation [18].

There is emerging interest in the role that social factors may play in influencing DNAm. For instance, in a study of 92 Canadian adults (aged 24-45 years; 62% female) socioeconomic position (SEP) during early life, defined as parental occupation (manual vs. non-manual),
showed associations with DNA methylation (DNAm) in later life [19]. Similar associations were observed in a larger study of 239 adults (aged 35-64 years; 51% female) from the United Kingdom (UK), where SEP during adulthood was defined as residing in an affluent vs. deprived area [20]. In a study of 89 women (aged 38-46 years) from the United States of America (USA) low income at birth, being raised in a single parent family, and lower adult educational attainment were all associated with higher DNAm profiles in adulthood [21]. Those observations are supported by evidence from a rhesus macaque model, which showed that dominance-rank, an indicator of social hierarchy and thus a proxy for SEP, was associated with differences in levels of chronic stress and the subsequent modulation of physiological responses and DNAm profiles [22]. The available studies indicate plasticity in molecular responses in stress response pathways might be related to the influence of SEP on the epigenetic pathway/s. However, conflicting data also exist: a study of 85 women from the USA found no association between SEP (an aggregate measure derived from maternal and paternal education, occupation and income at birth and at 7 years of age), and DNAm [23]. Nevertheless, an association between the allostatic load (the cumulative dysregulation of biological systems [24]) and SEP has been reported in some [25], although not all [26], reviews.

The public health importance of the social gradient of osteoporosis is underscored by increased attention in recent years [27-30]. Yet, the underlying mechanism for that gradient remains uncertain. The epigenetic signature is influenced by a multitude of environmental factors across the lifespan, and the epigenome appears to function as a vital conduit that transduces exposures into phenotypic expression and disease risk [15, 31]. We suggest that understanding this mechanism with respect to specific social determinants may identify various entry points for interventions in order to reduce the prevalence of osteoporosis and, consequently, reduce the social gradient of osteoporotic fracture [27]. This paper proposes a conceptual model, based on the challenge posed by social disadvantage to achieving allostasis (the maintenance of stability, or homeostasis, through change [24]), and the ‘three-hit theory’ of the allostatic load model as identified by McEwen et al [32], and later expanded upon by Daskalakis et al [33], whereby genetic predisposition provides the first ‘hit’ to allostasis, the early life environment provides the second, and later life environment provides the third. Our proposed model posits why socially disadvantaged individuals may be at greater predisposition for increased risk of osteoporotic fracture compared to their more
advantaged counterparts, and explores the modulation across the life course of the epigenetic signature. We argue that epigenetic mechanisms such as DNAm are highly influenced by SEP, and that social determinants are dynamic modulators of gene expression with relevance to the field of osteoporosis.

**Why might DNA methylation explain the social gradient in osteoporosis?**

*In utero*
In addition to non-modifiable genetic predisposition (including sex and ethnicity [34]; see Fig., Box 1), mechanisms have been proposed to explain epigenetic influences that occur *in utero* and which impact adult bone health later in life.

Recent research has demonstrated that foetal under-nutrition, indicated by low birth weight, is associated not only with adverse childhood outcomes such as stunting and reduced cognitive function, but also with increased morbidity in adult life from osteoporosis, sarcopenia, type 2 diabetes mellitus, metabolic syndrome and coronary heart disease [35]. These findings have been extensively replicated by other groups and are known to be independent of adult environmental risk factors for these disorders. These long term associations form the basis for the Developmental Origins of Health and Disease (DOHaD) concept [36]; this proposes that when the foetus or infant is exposed to adverse environmental influences, its metabolism becomes altered in a lasting way, resulting in increased vulnerability to later disease. There is a strong biological basis for such a model of disease pathogenesis. Experimentalists have repeatedly demonstrated that maternal stress and nutritional alteration during pregnancy can produce lasting changes in the offspring's physiology and metabolism [37]. This is one example of a ubiquitous phenomenon (phenotypic or developmental plasticity), which enables one genotype to give rise to a range of different physiological or morphological states in response to different prevailing environmental conditions during development. 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. Disease risk in later life is increased when the adaptive response made during earlier life is inappropriate for the later environment actually encountered.
Furthermore, there is an important role played by vitamin D levels and calcium on bone; the associations between maternal vitamin D levels and bone mineralisation in the fetus are influenced by placental calcium transporters that influence vitamin D receptors [11, 38, 39]. In the offspring, the hypothalamic-pituitary-adrenal (HPA) axis, which is a key component of the neuroendocrine response to stress, suppresses the immune system and is influenced by prenatal maternal stress [38]; this process has been hypothesised to modulate stress-responses which interact with immune system functions, and some studies have investigated this with regards to depression [40], schizophrenia [41], metabolic disease [13] and cardiovascular disease [15]. Importantly, chronic stress up-regulates immune function and inflammatory responses in a way that is epigenetically determined although it is presently unclear whether this is mediated by the HPA axis, the sympathetic nervous system or both. Specific to bone, Goodfellow et al suggest plausibility for an increased expression of glucocorticoid receptors to result in an increased sensitivity of osteoblasts to cortisol, which might influence a reduction in BMD during the life course [42].
**BOX 1**
Non-modifiable genetic predisposition
- Sex
- Ethnicity

**BOX 2**
Stressors (cumulative)
- Adversity in utero, during early life, adolescence and adulthood
- Lifestyle and stress
- Environmental/geographical: climate, trace elements, sun exposure, regional toxins

**BOX 3**
Response to stressors
- Behavioural
- Psychological
- HPA axis
- Inflammatory

**BOX 4**
Heightened inflammatory state
- Oxidative stress
- Variation in peripheral blood monocyte function
- Comorbid conditions

**BOX 5**
Osteoporotic fracture risk

**Socioeconomic Position**
Lifestyle behaviours, and clinical risk factors (ie, smoking, physical activity, alcohol, diet)

**Fig:** Conceptual model showing the influential role played by socioeconomic position (SEP) in epigenetic pathways to increased risk for osteoporotic fracture; adaptation of a conceptual model proposed by Saban et al with regards to social disparities in cardiovascular health [15]
Although there are limited data specifically pertaining to associations between social determinants, DNAm and osteoporotic fracture risk in adults, each of the examples highlighted above (placental calcium transporters and alterations to neuroendocrine-immune network set-points) is likely to be influenced by SEP (Fig., Box 2). For instance, studies that have investigated associations between social factors measured \textit{in utero} or early life and the outcome of DNAm have primarily employed the DOHaD paradigm [43, 44]. During pregnancy, there is cross-national and cross-cultural evidence that women of lower SEP are more likely to experience chronic stress than their more advantaged pregnant counterparts [45]; it may be no coincidence that preterm birth occurs more often with social disadvantage [45]. Following the identification of a link between low birth weight and coronary heart disease in adult life [46], the Barker Hypothesis became a significant development in the DOHaD field, whereby it was suggested that “…adverse environmental influences \textit{in utero} and during infancy, associated with poorer living standards, directly increased susceptibility to the disease [in adult life]”[47]. The introduction of this theory paved the way for further investigation into the role of socioeconomic factors during early life to determine the subsequent disease sequelae across the lifespan.

There are many factors at the environmental- and individual-levels specific to osteoporosis that heighten inflammatory responses and thus disease susceptibility that may be mediated by epigenetic mechanisms. One of them is vitamin D deficiency, which has been associated with lower SEP [48]; furthermore, there are reported associations between maternal vitamin D deficiency and reduced long bone growth in utero [49], and reduced bone mineral acquisition in the offspring and the risk of fracture in adulthood [50]. That association was also observed in a study of ~1.23 million adults (56% female) that investigated season of birth as a proxy measure of maternal vitamin D status with hip fracture later in life [51]. Furthermore, retinoid-X receptor-alpha (RXRA) plays an essential role in the actions of vitamin D (1,25\([\text{OH}]_2\)), and childhood bone mineral content (BMC) has been associated with the methylation status of the RXRA promoter at birth [52]. Similarly, endothelial nitric oxide synthase (eNOS) has an important role in bone metabolism, and associations have been observed between the eNOS promoter extracted from umbilical cords and BMC in the offspring nine years later [53]. In line with this, studies on the Dutch famine and DNAm, specifically in the insulin-like growth factor (IGF) region, show that maternal diet quality
influences the growth of the offspring [54, 55]. Data from a number of animal models have also shown nutrition during pregnancy consistently produces lasting changes to the physiology and metabolism of offspring [38, 56]. Therefore, regardless of the specific epigenetic changes occurring (including effects on placental calcium transporters and modulation of glucocorticoid receptor levels) the environment in utero seems highly likely to play a key role in adult bone health.

Taken in context, there is growing evidence that epigenetic mechanisms (DNAm, histone modification and non-coding RNAs) are responsible for tissue specific gene expression during differentiation and that these mechanisms underlie the processes of developmental plasticity. Furthermore, interventions during life pre-conception and pregnancy may reverse epigenetic and phenotypic changes induced by an unbalanced maternal diet and thereby alter later risk of disease.

**Infancy and childhood**

Bone health is clearly influenced by genetic predisposition and in utero programming [57]; however, the epigenetic signature can be modulated by the environment over the lifespan [15, 31], especially during the critical growth periods of early life such as infancy and childhood [11, 58]. Evidence that osteoporosis risk is modified during development emerges from adult and mother-offspring cohort studies [59], and the link between early development and adult osteoporosis risk was investigated in a subsequent systematic review [11, 60]. More detailed skeletal phenotyping in such cohort studies demonstrated that the poor intrauterine environment, marked by low birth weight, was also associated with altered femoral geometry [61], compromised bone microarchitecture [62] and reduced bone strength [63]. Finally, analyses suggested that an elevated risk of hip fracture in the offspring was associated with poor maternal stature, small body size at birth and impaired childhood growth [42]. Contemporaneous mother-offspring cohort studies demonstrated specific parental influences (maternal body build, smoking, physical activity and nutrition) on infant and childhood body composition [64]. Among nutritional influences, maternal vitamin D status was a strong determinant of childhood bone and muscle mass, independently of postnatal nutrition and physical activity [65].
Longitudinal data show that epigenetic variation of DNA methylation (DNAm) follows stressor events [66, 67]; experiences of childhood adversity, including forms of trauma such as abuse and neglect, will increase the secretion of stress hormones and subsequently influence the risk for chronic disease [68, 69], indeed sustained high levels of cortisol may have direct effects on bone mass. However, Carpenter et al. have shown that in adults where no psychopathology has been diagnosed, childhood maltreatment was associated with a lowered the secretion of cortisol in response to stress, and thus a diminished HPA axis response [70]; those data are indicative of the normal adaptive response of the neuroendocrine system to a chronic stressor. Given the intimate relationship between the neuroendocrine stress response and the immune system, detuning of the HPA axis may lead to increased levels of proinflammatory cytokines (such as IL6) and thereby a predisposition to bone loss [71]. Furthermore, development during early life stages is also influenced by maternal lifestyles, for instance smoking, diet and alcohol consumption, and by environmental exposures, for instance lead exposure [72]. However, development during infancy and childhood is also influenced by direct factors such as nutritional intake and physical activity of the child (Fig., Box 2). These behaviours are strongly linked to the parental attention given to health behaviours of the child [73]. Parental understanding of preventive health behaviours is linked to health literacy [74], which itself has an inverse association with SEP [75]. Furthermore, the lifestyle-related factors that contribute to the accrual of peak bone mass, including dietary calcium [76] and physical activity [77, 78] are influenced by social determinants; SEP is consistently observed to have an inverse association with a multitude of less healthy lifestyle behaviours that tend to co-aggregate [79, 80]. In addition to an increased disposition to osteoporotic fracture associated with lifestyle behaviours, data have shown associations between smaller birth weight and lower adult bone mass [81]. Additionally, social disadvantage during infancy and childhood has been associated with differences in adult gene expression patterns [71, 82, 83], specifically heightened inflammatory responses.

Taken in context, these data suggest that the cumulative stressors and their subsequent impact on DNA methylation are highly associated with adversity during infancy and childhood and also with exposures to stress and environmental and lifestyle behaviours: all of which are influenced by SEP. This has been eluded to previously by Holroyd et al in discussing the developmental plasticity when affected by a ‘sparse’, ‘average’, or ‘rich’ environment [38].
Adolescence

Peak bone mass is a determinant of osteoporosis [1]. Similar to what has been observed during earlier phases of life, the achievement of peak bone mass during adolescence is highly influenced by SEP-related issues. Those SEP issues may include environmental, social or geographical exposures, and the more individual-level factors associated with SEP including alcohol consumption and/or smoking, overall nutritional intake [84], dietary calcium [85] and physical activity [86]. Bone remodelling is influenced by the changing needs associated with each of these lifestyle behaviours, although the relative contribution of each to bone status has not been fully quantified. However, associations between physical activity and bone health are becoming increasingly understood [87]; for instance the influential role played high impact or weight-bearing physical activities and the resultant positive influence on bone mass [77]. Importantly, and by 20 years of age, approximately 90% of total adult bone mass has been accrued [88].

Pubertal changes that occur during adolescence are significant stimuli for changes in skeletal mass; oestradiol and testosterone are key factors that influence bone physiology. Although oestradiol increases BMD, it also modulates bone remodelling by suppressing bone turnover at the endocortical surface; this leads to a subsequent increase in cortical thickness [89]. However, there are many environmental influences during adolescence that influence mineral accrual in bone [38] and will therefore influence the modulation process of bone remodelling (Fig., Box 2). Social status is related to testosterone in males: in primates, dominant males have higher testosterone [90]. Similarly in human models of social dominance, higher testosterone is linked to social success [91], thereby supporting a plausible link to bone accrual and loss pathways.

Responses to stressors, including psychological, behavioural, the HPA axis and immunological responses, strongly influence DNAm, and these responses are determined by the cumulative nature of adversity over the lifespan (Fig., Boxes 2 and 3). Following physiological responses to chronic stress, immune dysfunction is heightened, although it should be noted that not all immune responses to stressors are dysfunctional. Nevertheless, an altered immune system that results in allostatic overload has been associated with many diseases including psychiatric disorders [92], cardiovascular disease [15], obesity, diabetes
and osteoporosis amongst others. Furthermore, the sympathetic nervous system, in response to stressors, may increase proinflammatory cytokines via the actions of adrenalin and/or noradrenalin [93]. Of the proinflammatory cytokines, tumor necrosis factor-alpha (TNFa), interleukin 1 (IL-1) and IL-6 are interrelated and implicated in changes to bone [94]: IL-6 also stimulates RANK ligand expression, which is an essential factor for osteoclastogenesis and has a demonstrated role in controlling immune responses [95, 96]. As previously highlighted, a heightened inflammatory state lays the foundation for subsequent triggering of events, which transduces vulnerability and risk into actual osteoporotic fracture.

Links have been made between lower levels of vitamin D and a heightened inflammatory state [97], with metabolism [98], and with the epigenome [99]. It has even been speculated that vitamin D deficiency may be a biological determinant or marker of increased health disparities [100]. In addition to lower levels of vitamin D, individuals that are predisposed to a heightened inflammatory state are more likely to have lower BMD and an increased risk of falls, especially in older age [97]. Whilst some data suggest that it is during adolescence when the prevalence of low vitamin D status is greatest [101], indicative perhaps of a modal peak in fracture rates at this age, vitamin D deficiency is most common in the elderly populations [102].

**Adulthood**

Correlated with our ageing populations is an increase in the prevalence of age-related osteoporosis. However, variations in the amount and pattern of methylation exist in adulthood, which are dependent upon cell and tissue type [38]: this has been imprinted on the adult through their own epigenetic processes and those of their parents. By the time adulthood has been reached, bone will have been remodelled several times, with approximately 10% of the skeleton renewed each year [103], and peak bone mass will have been reached in the third decade of life [104].

A heightened inflammatory state that increases the vulnerability of adults to have BMD in the osteoporotic range (a T-score of ≤2.5 standard deviations below the young adult mean) is influenced by current lifestyle behaviours and exposures, and by cumulative adversity that increases the risk of fracture as well as many other comorbid conditions (Fig., Boxes 4 and
5). The dysregulation of developmental programming via abnormal DNAm may permit specific genes to undergo detrimental expression during adult life, resulting in sequelae related to the development of chronic disease [38, 105].

Finally, DNAm appears to contribute to variation in peripheral blood monocytes function, thereby influencing the phenotypic and disease-susceptibility variation with a subsequent increased risk for comorbid conditions [106] (Fig., Box 4). Although sensitivity to the social environment is supported by data pertaining to the link between neural, endocrine and immune function [93], there are currently limited data specifically investigating the complex associations between social determinants, DNAm content and osteoporosis risk in humans.
Table 1
Proposed epigenetic effects at different stages of life, having an effect on bone metabolism and osteoporosis.

<table>
<thead>
<tr>
<th>Cumulative stressors across the life-course</th>
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<tr>
<td><strong>SEP-related environmental factors</strong></td>
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<td><strong>In utero</strong></td>
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<td><strong>Infancy and childhood</strong></td>
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<td><strong>Adolescence</strong></td>
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<td><strong>Adulthood</strong></td>
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<td>Stress</td>
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<td>Nutrition</td>
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<td>Vitamin D</td>
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<td>Genetic predisposition</td>
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<td>Altered metabolism; Placental calcium</td>
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<td>sympathetic nervous system; increased</td>
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<td>expression of glucocorticoid receptors;</td>
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<td>methylation of RXRA promoter; alteration</td>
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<td>Vitamin D status</td>
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<td>Secretion of stress hormones and altered</td>
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<td>Oestradiol and testosterone effects on</td>
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**Abbreviations:** BMD = bone mineral density; eNOS = endothelial nitric oxide synthase; HPA = hypothalamic-pituitary-adrenal axis; IL = interleukin; SEP = socioeconomic position; RXRA = retinoid-X receptor-alpha; TNFα = tumor necrosis factor alpha.
Future clinical utility:

The utility of this model includes the targeting of those most at risk for developing osteoporosis, firstly by addressing causative environmental pathways that are greater for disadvantaged individuals, and secondly, in the longer term, potentially intervening with compounds that function as demethylating agents if these can be engineered with sufficient target specificity. Approval for therapeutic use of demethylating agents has been provided in respect to some types of cancer in humans, however tolerability, safety and efficacy barriers to their use in other disorders remain high [107]. Furthermore, current demethylating agents indiscriminately demethylate the genome, thus affecting a broad cross-section of genes. This may have important implications for diseases such as osteoporosis that have slower progression compared to cancers, as there may be a greater likelihood of negative side effects. Understanding the role played by DNAm in osteoporosis not only has much potential to enhance lifestyle interventions, but also the development of novel osteoporotic drug treatment [9]. Furthermore, knowledge of these associations also has the potential to reduce the social gradient of osteoporotic fracture by enhancing the current evidence base regarding why interventions should be targeted toward socially disadvantaged individuals as the most at risk group. This model also has potential to reduce the social gradient of many other chronic, non-communicable diseases via health and/or social policy. For instance, policies regarding built environments may support access to better food choices, appropriate levels of sunlight exposure, and green spaces for recreational activities. Substantiating and refining this model to achieve a mature model that has clinical utility will require a focused research effort across many stakeholders in the bone research community.

Strengths and limitations:

We propose a conceptual model that posits biological underpinnings to explain the observed social gradient of osteoporotic fracture. DNAm occurs to optimise overall organismal fitness for the current environment: our conceptual model focuses upon parameters of SEP that will plausibly have negative influences on the ability to counteract inflammation. Furthermore, this model argues the biological plausibility for associations between SEP and osteoporotic fracture risk. Our identification of critical points for intervention throughout the life course aligns with the “three-hit theory” of vulnerability to inflammation that is associated with the
allostatic load model as identified by Daskalakis et al [33]. Our model builds upon the “three-hit theory” however, by identifying that, specific to osteoporotic fracture risk, adolescence (the prime period for achievement of peak bone mass) is perhaps a fourth critical stage of influence. Furthermore, our model identifies subgroups of the population that can be targeted for intervention to reduce the impact of insults to DNAm during these four critical stages of life.

This conceptual model also has some limitations. First, there are no data to date that have investigated associations between DNAm, SEP and osteoporotic fracture risk, and thus there is limited direct evidence regarding epigenetic-mediated dysregulation of inflammatory pathways influencing the social gradient of osteoporosis. However, the literature regarding associations between DNAm and osteoporotic fracture provide support for our model, furthermore, there are ongoing studies in this area of enquiry that may provide evidence to either support, refute, or revise the proposed model presented here. Those data are imperative in order to build an evidence base. We did not attempt to provide an exhaustive review of associations between DNAm and osteoporotic fracture risk, which others have done [9, 11, 108]; rather, our review of existing literature was to inform our proposed conceptual model regarding the social gradient of osteoporosis and fracture. Although our model supports data suggesting that environmental factors including maternal stress and nutrition affect the long-term epigenetic state of a number of genes during embryonic and foetal development, we do not speculate as to the exact mechanisms by which environmental influences are transmitted in utero [38]. Furthermore, we acknowledge that the in utero environment may affect offspring phenotype by epigenetic or direct influences, hence the difficulties in distinguishing ‘intergenerational’ from ‘transgenerational’ transmission [109]. Finally, it is plausible that the direct effects of alcohol, smoking, physical inactivity and diet on osteoporotic fracture risk may be greater than those resulting from changes in DNAm, especially for individuals of lower SEP; nevertheless, the relative contributions of DNAm and direct effects of lifestyle behaviours on osteoporosis are yet to be determined empirically.

**Conclusion:**

Taking the emerging evidence-base in context, cumulative stressors, responses to stressors, a heightened inflammatory state and subsequent increase in osteoporotic fracture risk are all
influenced by SEP. Whilst factors such as physical activity and nutrition exert direct biological effects on bone, and are associated with DNA methylation (DNAm) status, the recognised social gradient of risk factors for osteoporosis also act via epigenetic-mediated dysregulation of inflammation. More specifically, relationships between DNA methylation and osteoporotic fracture risk are influenced by social determinants at various stages throughout the life span, as has been suggested for other chronic non-communicable diseases. Based upon that literature, we have proposed a conceptual model that posits biological plausibility for a key mechanistic role played by DNA methylation in the social gradient in osteoporotic fracture. Empirical data is needed to determine the driver/s of the SEP-related stress effects on bone, for instance determining whether a blunting of the HPA axis is increased with accumulated disadvantage over the life course, resulting in a consequent dysregulation of the immune system. Nevertheless, our model identifies various entry points for interventions in order to reduce the prevalence of osteoporosis and importantly also reduce the social gradient of osteoporotic fracture. Furthermore, this model provides a foundation on which to build the evidence-base in this field of enquiry, and has potential to inform novel treatments with which to target those at most risk of osteoporotic fracture. This model may also indicate that lifestyle modifications for all individuals of lower SEP may be appropriate in the first instance in order to reduce osteoporotic fracture risk; however, the modification of some factors such as alcohol consumption or smoking may have greater influence on DNA methylation than nutritional modification. What is clear is that there remains much more to understand regarding DNA methylation and its contribution to explaining the social gradient of osteoporosis.

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Conflict of interest:

Sharon Brennan-Olsen, Richard Page, Michael Berk, José Riancho, William Leslie, Scott Wilson, Karen Saban, Linda Janusek, Julie Pasco, Jason Hodge, Shae Quirk, Natalie Hyde, Sarah Hosking, and Lana Williams declare that they have no conflict of interest.
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