THE EPIGENOME AT THE CROSSROAD BETWEEN SOCIAL FACTORS, INFLAMMATION AND OSTEOPOROSIS RISK

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ABSTRACT

Both genetic and environmental factors are involved in the pathogenesis of osteoporosis and other skeletal disorders. Epidemiological studies have revealed an influence of a variety of social factors, including socio-economic status (SES) on the risk of osteoporosis. The mechanisms involved are complex and still incompletely elucidated. Nevertheless, a variety of clinical risk factors known to influence skeletal homeostasis have been reported as being socially patterned, including nutrition, exercise, and other lifestyles, amongst others. These factors may impact the skeleton through a variety of mechanisms. Among them, there is increasing evidence for a role of DNA methylation and other epigenetic mechanisms. Indeed, several studies of human cohorts and experimental models showed that social deprivation is associated with changes in the methylation pattern of a number of genes, including some involved in stress and inflammatory responses. The influence of socioeconomic factors may be important not only during postnatal life, but also in utero, and may be transmitted to future generations by its direct effect on peripheral and target tissues and perhaps through epigenetic inheritance. Although the exact relevance of these pathways in humans has not been fully elucidated yet, they bring attention to the influences of social factors on the skeletal health of the individuals and their descendants. Therefore, they also bring forward our responsibility for both present and future generations.

KEYWORDS: EPIGENETICS, DNA METHYLATION, STRESS, OSTEOPOROSIS, SOCIOECONOMIC FACTORS, HEALTH STATUS DISPARITIES, INFLAMMATION

A number of epidemiological studies suggest that social determinants influence skeletal health. Social determinants include factors such as lower income and/or educational attainment, or lower skilled occupations (highly associated with income and education), and these have been associated with an increased prevalence of osteoporosis and osteoporotic fractures (1–3). Furthermore, lifecourse analyses have reported that social disadvantage whilst in utero or at birth is associated with an increased risk of osteoporosis during later life (4,5), thus suggesting that, in addition to non-modifiable genetic predisposition, the social environment influences the skeleton at all stages of life. Indeed, the Developmental Origins of Health and Disease (DOHaD) theory (6) provides much evidence for adverse environmental conditions increasing vulnerability to disease. Another social determinant that has strong associations with social disadvantage is lower health literacy (7). Health literacy is defined as one's ability and capacity to seek, access, understand and implement health-related information, and is beginning to attract much attention for its relevance in the osteoporosis field, especially with regards to preventive lifestyle behaviours (8) .

The data indicate that the relationship between socioeconomic factors and the skeleton is complex and non-linear, but in general are consistent with an increased osteoporosis risk in population groups with lower socioeconomic status (SES) (9–11). Furthermore, it is suggested that many clinical and lifestyle risk factors for osteoporosis are socially patterned, and thus individuals of lower SES are likely to have a disproportionately greater risk. However, osteoporosis risk may be exacerbated by more than direct biological insults on bone. We recently proposed a conceptual model suggesting that the biological mechanisms involved are multiple and likely result from the interaction between genetic and acquired factors and may be mediated, among others, by differences in the epigenetic marks (Figure 1).

The genome is generally constant throughout all body cells and stable from conception until death. However, changes in DNA sequence in somatic cells occasionally appear and, if they escape the control mechanisms, may lead to the development of diseases such as cancer. Sequence differences in the coding or regulatory regions of DNA have obvious consequences on gene activity. However, DNA sequence is not the only factor modulating gene transcription. In fact, epigenetic mechanisms are able to regulate gene activity in a stable manner, without implying changes in DNA sequence, and are potentially heritable through cell divisions. Among them, chromatin conformation, histone marks, noncoding RNAs and DNA methylation have received greatest attention.

The DNA double helix in chromosomes is tightly packed, bound to histone proteins, forming the nucleosomes, where DNA is wrapped around a core formed by four histones. The conformation of the chromatin contributes to modulate gene expression. When chromatin has a loose conformation, DNA is more accessible to the transcription machinery, thus allowing gene transcription. On the other hand, the condensed chromatin tends to be associated with repressed genes. Histone tails experiment a variety of post-translational modifications, such as methylation and acetylation, among others. These changes are also specifically associated with gene activity. For example, the methylation of lysines at position 9 or 27 of histone 3 bound to promoter regions is associated with gene repression, whereas the methylation of lysines at position 4 and the acetylation tend to associate with active gene transcription (12–14).

Non-coding RNAs are divided into several groups according to their length. Long noncoding RNAs (lncRNAs) are longer than 200 nucleotides and modulate gene activity at the transcriptional and post-transcriptional level (15). Among small non-coding RNAS, mature microRNAS (miRNAs) are about 20-nucleotide length molecules that bind to mRNAs bearing specific sequences. The binding of miRNAs to their target mRNAs block the translation of the mRNAs and, in some cases, induces the degradation of the mRNA. By doing so, miRNAs inhibit the synthesis of the proteins encoded by their target genes (16) .

Many cytosines in DNA are methylated, particularly those that are followed by a guanine (forming CpG dinucleotides). During cell divisions, a family of enzymes called DNA methyl-transferases are responsible for maintaining the pattern of methylation in DNA of mother cells into the DNA of daughter cells. Thus, DNA

methylation signatures are conserved through mitosis. To some extent, methylation patterns may also be maintained through meiosis into different generation of individuals, but this is less constant.

The functions of DNA methylation have not been completely elucidated, but vary depending on the genomic regions. The methylation of intergenic regions and gene bodies likely provides stability to DNA, maintains repetitive and transposable elements silenced, and reduces the transcription of pseudogenes and transcriptional noise. However, the methylation of cytosines in gene-regulatory regions contributes, along other epigenetic mechanisms, to modulate gene expression, adapting it to the changing environmental conditions (17,18). CpGs in gene bodies tend to be heavily methylated in active genes, which may help to avoid the initiation of transcription at alternative sites. Many gene promoters contain regions particularly rich in CpGs, the so-called "CpG islands". In general, the methylation of those regions is associated with gene repression, whereas when those regions remain unmethylated the genes are actively expressed (19,20). However, this is by no means a universal rule. Recent studies have delineated a more complex picture. On the one hand, the inverse correlation between promoter DNA methylation and gene expression is not constant; on the other hand, the methylation of distant regulatory regions appears to have an important role in the modulation of gene expression (21). In fact, Ziller et al reported that almost 22% of CpGs show methylation differences across various human cell types and those differentially methylated CpGs tend to be located in enhancers and other regulatory elements (22).

Whereas the genome is constant throughout life, the epigenome, and particularly DNA methylation, changes along lifetime and across tissues. Thus, the DNA methylation pattern of a given cell depends of genetic influences (this is, DNA sequence), environmental influences, stochastic variation and some tissue-specific factors. The relative importance of these factors likely varies across genes and genomic regions, but some studies suggested that genetic factors (ie, DNA sequence variation) explain about 20% of the variation in DNA methylation, with differences across genomic regions (23)(24). Thus, epigenome-mediated interactions between genetic and environmental factors likely play a major role in the pathogenesis of common disorders, such as hypertension, obesity, diabetes, or osteoporosis. In particular, it has been hypothesized that early life experiences, determined by socioeconomic position and other factors, may induce stress reactions and other responses that modify DNA methylation patterns and consequently influence bone mass and the risk of osteoporosis in later life (10) . We will provide here an overview of the evidence for this hypothesis.

SOCIOECONOMIC STATUS INFLUENCES THE EPIGENOME (AND INFLAMMATION BIOMARKERS)

SES may influence the epigenome both during early life, including pre and postnatal growth periods, as well as during middle age and older adulthood. Factors associated with SES and potentially influencing the epigenome are varied and include: nutrition, exposure to environmental toxics, smoking and other lifestyle behaviors, and stress and other psychological factors (25–27). However, measures of SES are heterogeneous, being measured, for instance, at the individual, neighborhood or wider geographic area, and by use of single parameters or aggregate indexes, in addition to other approaches, such as social stratification or life-course methodologies. Despite the high heterogeneity in this field of enquiry, SES is receiving increased attention in the field of epigenetics in recent years.

The importance of the prenatal environment on the epigenome has been highlighted by a number of cohort studies relating the environmental factors before birth with epigenetic marks later in life. For example, Tobi et al explored DNA methylation in adults who were conceived around the Dutch famine during the Second World War. Those individuals showed several differentially methylated regions, which preferentially occur at regulatory regions, and map to genes enriched for differential expression during early development and metabolism (28,29). Differences in the methylation of some genes have also been reported in individuals from Bangladesh exposed to famine during gestation (30). The first 10 weeks after

conception may represent the critical period in which mothers nutrition has a greater influence on the methylation of the offspring (31).

Studies in rats and other experimental animals have shown that the methylation patterns of genes involved in the stress response, including the glucocorticoid receptor (Nr3c1), Bdnf, Avp, Crh, Crhr2, and Gad1, can be modified by psychosocial factors, such as maternal care during the first week of life. These changes result into differences in the behavioral and endocrine responses to stress in adulthood (32). Indeed, evidence from a rhesus macaque model 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 subsequent modulation of physiological responses (33). A few human data are in line with this concept. For example, the methylation and expression of the human NR3C1 is altered in the hippocampus of victims of suicide exposed to child abuse (34). Early life experiences, such as lower socioeconomic position in childhood or parental stress during adolescence, have also been associated with specific DNA methylation profiles in adult peripheral blood or epithelial cells (reviewed in (35)).

McGuinness et al reported an association between global DNA methylation in peripheral blood cells and SES among individuals of the pSoBid cohort in Scotland. They found a lower DNA methylation in the most socio-economically deprived individuals, as well as in manual workers, in comparison with non-manual workers (36). Although the investigators did not explore the methylation of specific genes, they found an inverse correlation between global DNA methylation and the levels of the inflammatory biomarkers IL-6 and fibrinogen. Thus, lower SES was associated with lower DNA methylation and higher levels of inflammation biomarkers. In line with this concept, Stringhini et al found that Italian subjects with low SES showed lower degrees of methylation at several inflammation-related genes, such as NFATC1, IL-1 and GPR132 (37).

Needham et al extracted nucleic acids from the monocyte fraction of the peripheral blood of large group of American individuals aged 55-94 years and explored the association of the methylation of 18 genes related to inflammation and stress responses with SES and gene expression. They found that low childhood SES was associated with DNA methylation of 4 out of 7 stress-related genes (AVP, BDNF, FKBP5, and OXTR) and 3 out of 11 inflammation-related genes (CCL1, CD1D, and F84 out of 7 stress-related genes. Similarly, methylation was associated with adult SES in 2 out of 7 stress-related genes (AVP and SLC6A4) and 5 out of 11 inflammation-related genes (CD1D, F8, KLRG1, NLRP12, and TLR3). However, in general low SES was associated with higher DNA methylation and it was inversely associated with gene expression (38). Thus, although this study supports the concept that SES influences the methylation of stress/inflammation genes, it does not support the hypothesis of higher activity of these genes in individuals of low SES.

Many studies have shown an association of psychological factors with DNA methylation patterns. For instance, Kim et al recently studied a group of men with a mean age of 73 years and found that psychological distress (measured by anxiety, depression and hostility) were associated with the methylation of several genes related to stress/inflammatory responses (ICAM-1, TLR2, iNOS, glucocorticoids receptor, γ-interferon or IL-6) (39). In line with this concept, Bam et al identified specific changes in the methylation of genes involved in immune system pathways in war veterans with post-traumatic stress disorder, a condition known to be associated with chronic low grade inflammation (40).

Nutritional habits are clearly associated with the socioeconomic position of the families and may influence the epigenome. For instance, energy balance and some vitamins may modulate the methylation of a number of genes. Thus, in obese adults, Bollati et al reported an association between dietary vitamin and fat intake with the methylation of the gene encoding the pro-inflammatory cytokine tumor necrosis factor (TNF) (41). Kok et al also showed that vitamin B12 and folic acid supplementation in older adults induced measurable changes in the methylation of some genes (42).

These studies must be interpreted with some caution. Due to obvious feasibility reasons, they were carried out with blood, but the methylation levels in blood cells (leucocytes) may not reflect the methylation levels of other more biologically relevant tissues. Also, in several studies, authors did not consider differences in leucocyte counts as a potential confounding factor.

In general, and despite key deficiencies in this nascent field of enquiry being the heterogeneous measures of SES employed by different studies, the most extended hypothesis assumes that SES influences DNA methylation and this, in turn, influences gene expression. However, some intriguing data suggest that relationships in the opposite direction might also take place. For instance, in a study with the African cichlid fish, in which social rank dictates reproductive access, Lenkov et al showed that changes in DNA methylation induced pharmacologically were associated with ascents or descents in the social rank (43). There is no proof for a similar phenomenon of a direct role of DNA methylation in human society. However, DNA methylation has been associated with the risk of a number of disorders (including neurological and psychiatric diseases), as well as with emotional and learning abilities, which may indirectly influence social behavior and social status (44)(45)(23).

In previous paragraphs we have mentioned that changes in epigenomic marks, and specifically in DNA methylation, influence the transcription of genes involved in stress and inflammatory responses and, in consequence, modulate the intensity and duration of those responses. This has been recently illustrated by a study showing that the methylation of 58 CpG is associated with the levels of C-reactive protein and several cardiometabolic phenotypes (46). However, it is likely that interactions in the opposite direction also occur. Indeed, experimental and some epidemiological data suggest that cellular stress may induce changes in the epigenome (reviewed in (47)). Mitochondrial dysfunction and enhanced activation of cyclooxygenase and lipoxygenase driven by inflammation are common causes of increased oxidative stress. This has been shown to result in chromatin changes, including changes in the methylation pattern of DNA, that in turn may affect tissue function and the ability of cells to divide in an ordered manner (48).

Most studies about the influence of SES on epigenomic signatures have focused on DNA methylation. There are very few data about the potential role of social factors on other epigenetic marks, such as histone modifications or non-coding RNAs. In a small study of 28 patients with esophageal carcinoma, Stanitz et al did not found differences in miRNA expression between patients living in urban and rural areas. However, they reported that the expression of two miRNAs (miR-143 and miR-203) was reduced in the low-social group (49).

EPIGENOMIC MARKS INFLUENCE BONE REMODELLING

A proper activity of skeletal cells, and particularly osteoblasts, is critical for bone development and the accrual of bone mass during the uterine and postnatal growth periods. Likewise, an adequate balance between the bone formation activity of osteoblasts and the bone resorption activity of osteoclasts is required to maintain bone mass in adulthood. DNA methylation and other epigenetic mechanisms play a critical role in the differentiation and activity of bone cells (reviewed in (50–53)).

Some data also suggest that DNA methylation may influence bone mass and the risk of osteoporosis. Jintaridth et al reported an association between hypomethylation of Alu elements in leukocytes with postmenopausal osteoporosis (54). However, the relevance of blood cells as proxies for skeletal epigenomic studies is unclear.

An epigenome-wide study of DNA methylation in femoral bone samples showed differences in the methylation signatures of bones from patients with osteoporotic fractures and controls, particularly in some genes related to skeletal development (55).

However, in these studies it is difficult to establish the direction of the associations and inverse causality cannot be excluded (ie, differences in methylation marks are not the cause, but the consequence of the disease). Prospective studies analyzing epigenomic marks in healthy individuals who are later followed prospectively would be ideal to work out this question. However, large, well-designed, prospective, epigenome-wide studies are not available yet. Nevertheless, some preliminary data are in favor of the influence of early-established epigenome patterns on bone mass. Nitric oxide (NO) is produced from arginine by a family of NO synthases (NOS). The muscle-relaxant and vasodilatory activity of NO are among its best-known effects. However NO has widespread effects, including bone anabolic effects, and it may contribute to transduce the influence of mechanical loads on bone cells (56)(57). Harvey et al reported that the methylation level of the endothelial-type NOS in umbilical cord obtained at birth was associated with bone mass at age of 9 years (58). This group later reported an association between methylation of the retinoid-X receptor and bone mineral content in childhood (59).

Many miRNAs have been reported to participate in skeletal homeostasis, including processes such as osteoblast and osteoclast differentiation (51,60,61). However, a link between bone phenotypes and SES mediated by non-coding RNAs have not been demonstrated yet.

INFLAMMATION AND STRESS RESPONSES INFLUENCE BONE HOMEOSTASIS

Stress is a physiological response that serves as a mechanism of mediation linking any given stressor to its target-organ effect. Psychosocial stressors do not directly cause a stress response; rather they need to work out through cognitive appraisal mechanisms. On the other hand, tissue lesions and other biogenic stressors elicit a stress response independently of the affective-cognitive processing (62).

The stress response is initiated by activation of limbic and cortical areas that in turn modulate hypothalamic centers and the descending autonomic pathways, including both the sympathetic and parasympathetic systems. These descending pathways modulate the activity of a variety of peripheral tissues and organs. The activation of the sympathetic system enhances the release of catecholamines by the adrenal glands, which amplifies and prolong the adrenergic response (63,64).

Other endocrine changes also take part of the stress response, including increased secretion of growth hormone and ADH, and complex changes in the metabolism of thyroid hormones. Most important, the release of CRF in the hypothalamus is enhanced; it stimulates the secretion of adrenocorticotropic hormone (ACTH) by the pituitary, which in turn stimulates the release of glucocorticoids in the adrenal cortex.

The effects of the stress response include mobilization of energy stores and heightened vigilance. They also exert a negative feedback control that helps to finish the response when the stressor is under control. The timely activation and deactivation of stress response systems thus allow an organism to successfully manage a threat and return to normal function. An abnormal, or pathological, stress response may represent an inability to activate or deactivate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in failure to manage a potentially life-threatening stressor, or such things as prolonged exposure to glucocorticoids. This in turn may have long-term consequences for behavior, memory and vulnerability to mental illness (65).

Stress response may also affect skeletal tissues. After the paper by Napal et al showing that acute stress induced a prompt inhibition of the secretion of osteocalcin by osteoblasts (66), many studies have confirmed that stress and inflammation have marked effects on skeletal homeostasis. In general, they tend to inhibit osteoblast activity and frequently enhance osteoclast-mediated resorption. Hence, a decrease in bone mass is the long-term expected consequence of persistent stress and/or inflammation (Figure 2).

The mechanisms involved have not been completely elucidated, but they likely include physical, humoral and neural factors. Inflammatory disorders have a negative impact on body function. The activation of the HPA axis is a hallmark of the stress response. It results in increased secretion of glucocorticoid hormones. Glucocorticoids have profound effects on bone cells. High concentrations of glucocorticoids promote osteoblast and osteocyte apoptosis and consequently inhibit bone formation. They also tend to transiently increase PTH secretion and enhance osteoclast survival, thus increasing bone resorption. The effects of glucocorticoids on other tissues may also secondarily affect the skeleton. Thus, excess of glucocorticoids tend to decrease the levels of sex hormones and have a catabolic effect on muscle tissue. The resulting sarcopenia, or muscle wasting, also negatively impacts skeletal homeostasis (67).

On the other hand, several inflammatory mediators also have a negative impact on skeletal homeostasis. Thus, several pro-inflammatory cytokines, such as IL-1, IL-6 or TNF, enhance bone resorption, by direct or indirect mechanisms (68)(69,70). They stimulate the release of RANKL and decrease osteoprotegerina production by several cell types present in the bone microenvironment, which, in turn, promote the differentiation of osteoclast precursors (Figure 2). At the same time, some of those cytokines tend to inhibit bone formation, acting either directly on cells of the osteoblastic lineage or increasing the production of DKK1 and other inhibitors of the bone anabolic Wnt ligands (71).

Besides the humoral-mediated effects, inflammatory diseases may result in decreased physical activity, and reduced mechanical loading of the skeleton, which has a negative impact on bone mass.

PERSPECTIVES AND CONCLUSIONS

Osteoporosis is a complex disorder resulting from the interaction of genetic and environmental factors. Despite the burgeoning evidence-base regarding the influence of SES and other social factors on the risk of osteoporosis, the mechanisms involved are likely multiple and still incompletely elucidated. Nevertheless, a variety of clinical risk factors known to influence skeletal homeostasis have been reported as being socially patterned, including nutrition, exercise and other lifestyle behaviors, and psychological factors, amongst others. These factors, in turn, may also impact the skeleton through a variety of mechanisms. Among them, there is increasing evidence for a role of DNA methylation and other epigenetic mechanisms (Figure 3). Indeed, several studies of human cohorts and experimental models showed that social deprivation is associated with changes in the methylation pattern of DNA in a number of genes, including some involved in stress and inflammatory responses.

Several lines of evidence suggest that adverse experiences in early life, such as those that frequently occur in population groups in the lowest socioeconomic strata (including maternal stress, anxiety and depression during pregnancy, maternal lifetime history of depression, insecure mother–child bonds and increased psychosocial risk), may permanently affect the ability of the body to mount a normal stress response, resulting in increased activation of the HPA axis after stressful situations (65). Therefore, the influence of socioeconomic factors may be important not only during early postnatal, adolescence and adult life, but also in utero and may be transmitted to future generations by its direct effect on peripheral and target tissues and perhaps through epigenetic inheritance. The extent to which methylation signatures is transmitted transgenerationally is unclear. As mentioned above, many studies suggest that in utero experiences have consequences in adulthood. There is also some evidence suggesting that those experiences may influence the phenotype of the descendants. For example, studies of the Overkalix cohort in Sweden have shown an association between the food supply of the grandmothers and the cardiovascular mortality of the grandchildren (72). However, a definitive confirmation of such transgenerational inheritance of non-imprinted genes has not been obtained in humans yet

Although much more research is needed in this field, including the analysis of epigenomic marks in skeletal tissues, globally, already available studies represent the biological underpinning for the role of social factors in the skeletal health of present individuals and their descendants. Understanding that the epigenetic signature is influenced by a multitude of environmental factors across the lifespan is imperative to efforts aimed at reducing the burden and prevalence of osteoporosis. Furthermore, elucidation of the mechanistic crossroads between the epigenome and social determinants may support the identification of various entry points for interventions in order to reduce the social gradient of osteoporosis. Furthermore, the recognition of these epigenome-mediated sociobiological interactions bring forward our responsibility for both present and future generations.

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ABBREVIATIONS

ACTH: adrenocorticotropic hormone
AVP (ADH): arginine vasopressin (antidiuretic hormone)
BDNF: brain derived neurotrophic factor
CRH: corticotropin releasing hormone
CRHR2: corticotropin releasing hormone receptor 2
DNMT: DNA methyl-transferase
F8: coagulation factor VIII
FKBP5: FK506 binding protein 5
GAD: glutamate decarboxylase
GPR132: G protein-coupled receptor 132
ICAM-1: intercellular adhesion molecule 1
IL: interleukin
KLRG1: killer cell lectin like receptor G1
NFATC1: nuclear factor of activated T-cells 1
NLRP12: NLR family pyrin domain containing 12
NOS: nitric oxide synthase
Nr3c1: nuclear receptor subfamily 3 group C member 1
OXTR: oxytocin receptor
RANKL: Receptor activator of nuclear factor kappa-B ligand (TNFSF11)
SES: socio-economic status
SLC6A4: solute carrier family 6 member 4
TLR: toll like receptor
TNF: tumor necrosis factor

FIGURE LEGENDS

Figure 1. A conceptual model of the relationship between socioeconomic factors and fracture risk. Reproduced from Bone with permission (10).

Figure 2. Influence of stress and inflammatory responses on skeletal homeostasis.

Figure 3. Multiple pathways and mechanisms involved in the interaction between social factors and the skeleton. The potential role of epigenomic mechanisms is emphasized. Dashed lines represent relations that have not been confirmed yet. Dotted red lines represent "backward" pathways by which biological factors may influence socioeconomic status.

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Figure

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Figure 1

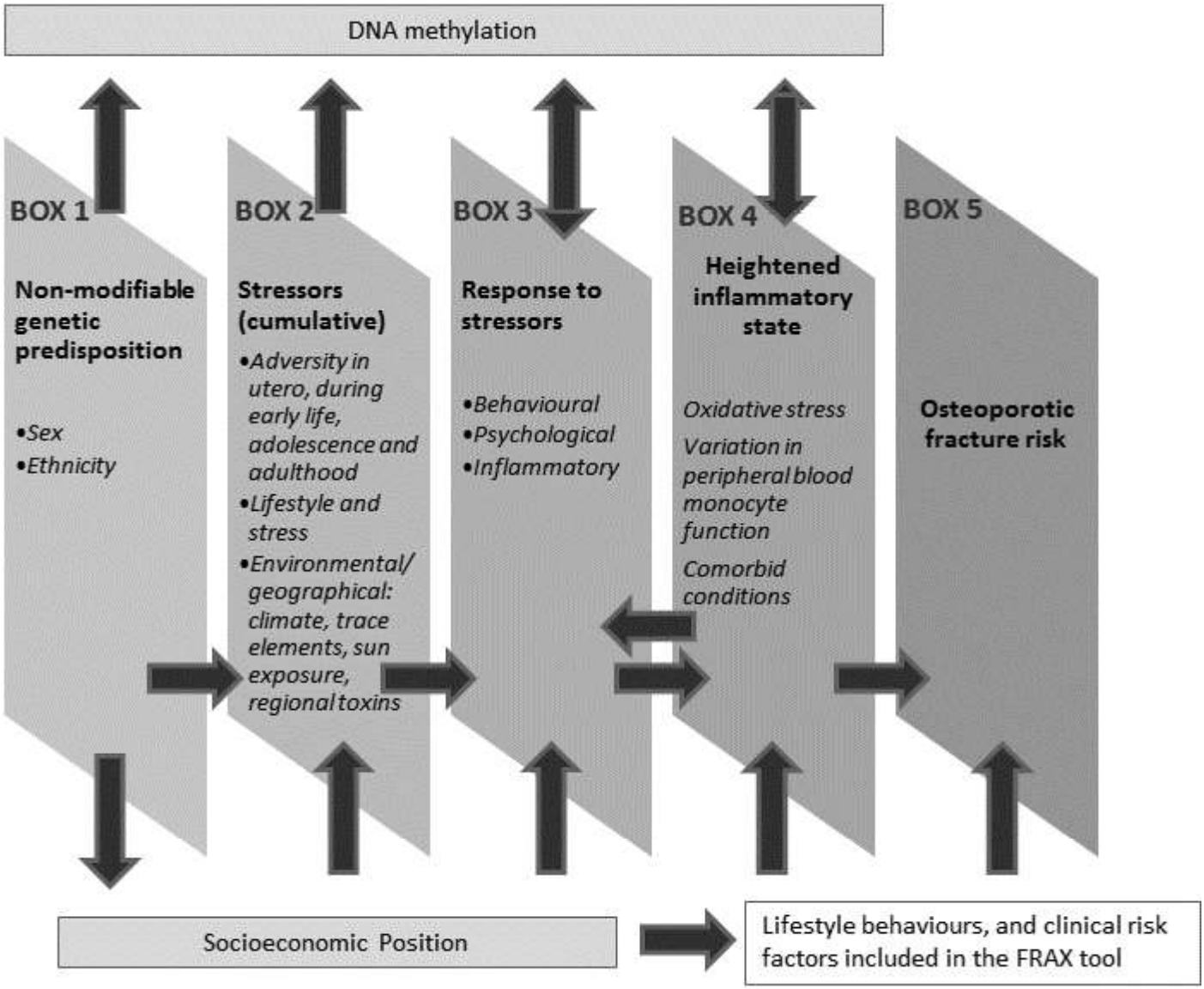


Figure 2

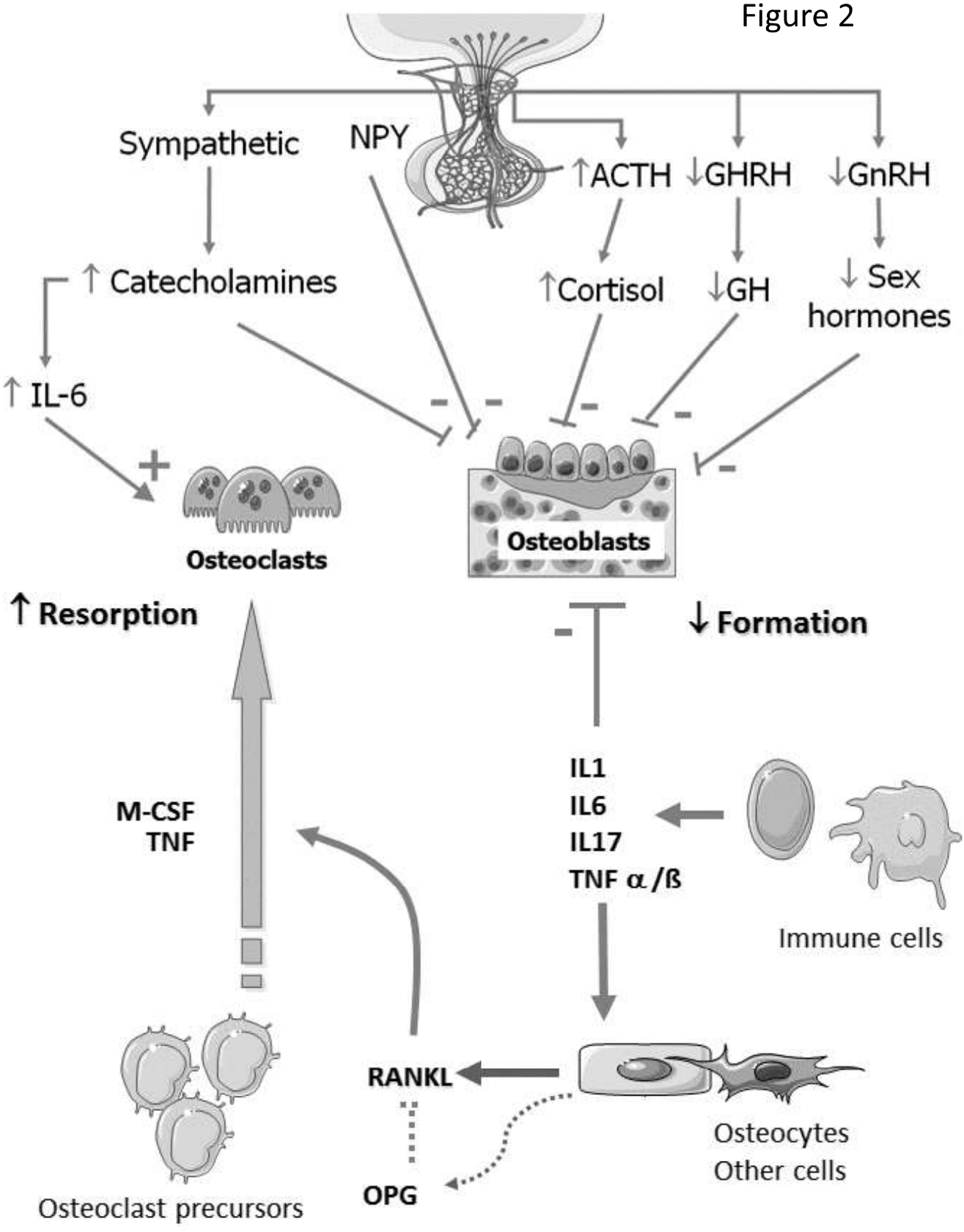
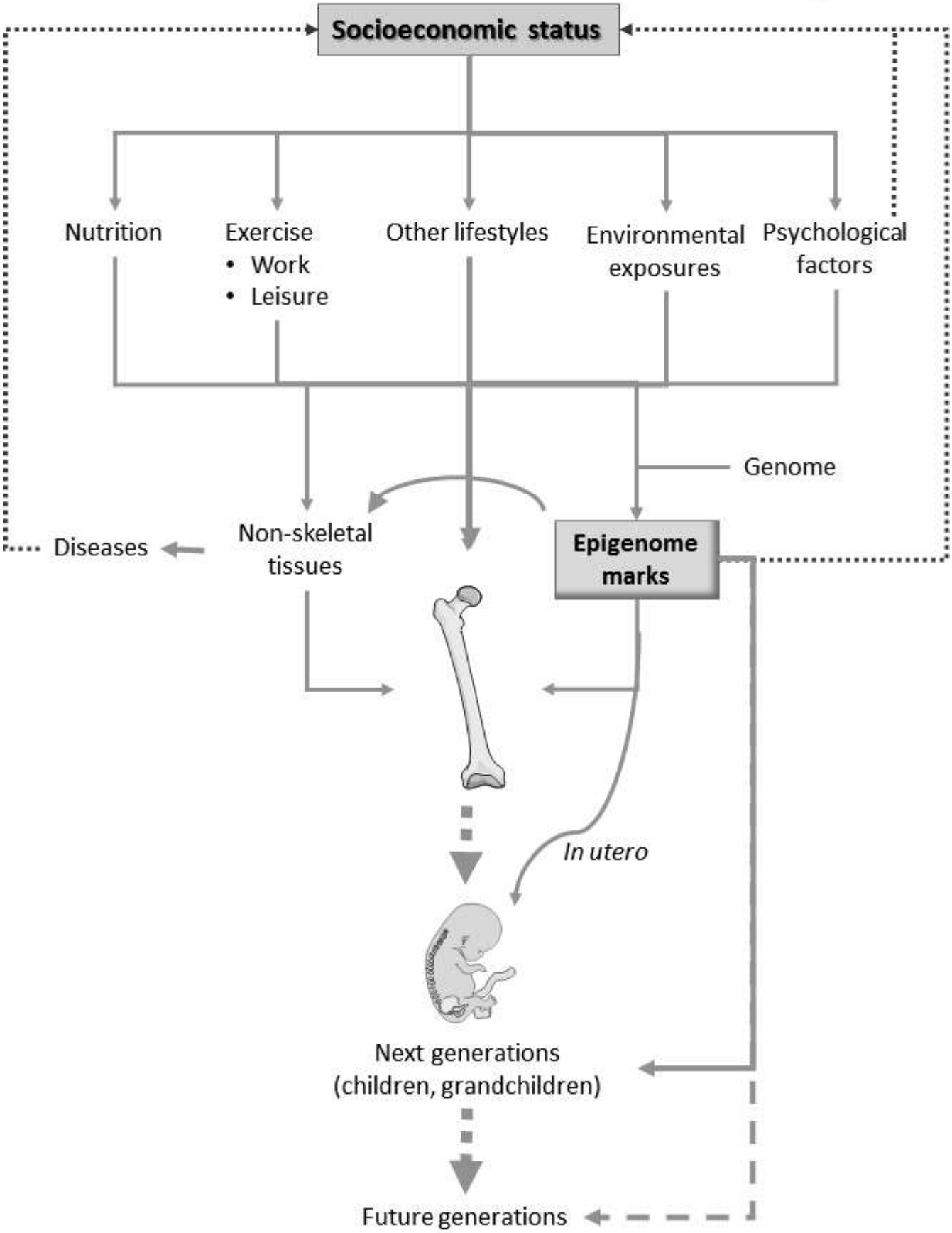

Sympathetic
NPY
↑ACTH
↓GHRH
↓GnRH
↑ Catecholamines
↑Cortisol
↓GH
↓ Sex hormones
↑ IL-6
Osteoclasts
Osteoblasts
↑ Resorption
↓ Formation
IL1
IL6
IL17
TNF α /ß
M-CSF
TNF
Immune cells
RANKL
OPG
Osteocytes
Other cells
Osteoclast precursors

Figure 3


Socioeconomic status
Nutrition
Exercise
• Work
• Leisure
Other lifestyles
Environmental exposures
Psychological factors
Genome
Diseases
Non-skeletal tissues
Epigenome marks
In utero
Next generations
(children, grandchildren)
Future generations