Bone Health

A Reflection of the Social Mosaic

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To all those whose health is affected
by social inequity and inequality

Foreword

Human beings are a combination of three major dimensions: biological, psychological and social. Interactions between these dimensions and the environment determine health and disease. Musculoskeletal conditions are not an exception. In the particular case of osteoporosis, our bones are constantly affected by biological factors that increase or decrease bone quality based on biological changes associated with ageing, hormones and interactions between multiple factors including nutrition, disease and medications. Interestingly, bone is also affected by psychological factors from multiple directions. Connections between the autonomic system and bone cells have been reported, which explain why high levels of stress or depression could have an impact on bone quality. Depression is associated with social isolation and sedentarism, which induce bone loss. From the social perspective, the editors of this book have been prolific authors in this area demonstrating strong associations between socio-economic status and bone health.

Being passionate on medieval studies myself, I found the first part of this book astounding and highly informative. Our modern societies are now used to easy access to multiple resources, which was not the case in medieval times when people were exposed to major nutritional limitations, conflict, epidemics and a short life span. It is therefore expected that these major differences between ancient and modern societies would have an impact on bone quality and their predisposition to osteoporosis fractures. I must admit that my only experience with ancient bone was acquired during my visits to natural history museums where I was astonished observing the bones of the dinosaurs. This book opened my eyes to new knowledge on the relevance of analysing ancient human samples and on the extremely valuable information that those bones could provide to modern science as a "message from the past."

This book elegantly moves from ancient to modern societies without losing its continuity. While highlighting the importance of the evidence obtained from medieval societies, the second part of the book describes contemporary evidence on bone health in a diversity of social and cultural groups. This is not an easy task indeed. Our modern societies are becoming extremely diverse and multicultural. Migrations have facilitated interculturalism and biological mixing, which have had a major impact on genetics, social interactions, diet, education and other factors that regulate bone health. Therefore, it is not surprising that the major contrasts between ancient and modern societies, nicely summarised in this book, serve as a demonstration of how the human being responds to change and evolution not only from the biological but also from social and psychological dimensions.

Longevity and easier access to resources (including communications) are probably the most important determinants of the changes observed in modern societies. All societies, developing and developed, experienced longer life span, which comes associated with growing societal demands and higher prevalence of musculoskeletal diseases, including osteoporosis. The chapter dedicated to differences in bone health and fracture risk between countries nicely illustrates the differences and similarities between developing and developed societies and the strategies that have been implemented to improve bone health in their communities.

Osteoporosis is a major challenge to public health worldwide. Early diagnosis, preventive measures and osteoporosis treatments have demonstrated to be effective in preventing fractures and reducing fracture-related adverse outcomes. However, there is major gap in the identification and treatment of osteoporosis worldwide. The causes of this gap still remain unclear. Furthermore, adherence to osteoporosis treatment is very low. Aiming to provide some lights on the causes of this gap and also of poor adherence to treatment, this book approaches the social gradient as an important determinant of this situation. Going beyond the diagnosis point, the authors propose practical and feasible ways to solve this issues that could be cost-effective at any public health system in the world.

Finally, as a bone biologist, I could not finish without highlighting the importance and relevance of the third part of this book. Epigenetics has been associated with multiple human diseases, but only recently, and thanks to the fantastic research performed by the authors, epigenetics has been considered as an important determinant of bone health. In particular, I like the concept proposed in the book that epigenetics is situated at the crossroad between genetic and environmental determinants of disease, thus having an impact on the three dimensions of human beings. Our genes and their expression start since conception but are clearly determined by our parents' genomes. From there, we start a complex process of interactions that could sometimes modify our genes (positively or negative) in a way that could end up as health, longevity or disease. This book summarises all those changes that happen to our genes from the maternal components to the role of social and environmental factors, which end up regulating the way our bones develop, grow and then decline.

In summary, I invite the reader to enjoy this book as much as I did. This excellent book is a real-time travelling experience that transports us back to medieval times and then brings us back to the busy and complicated times of modern societies. If after reading this book you have a solid idea of the roles of social aspect of human lifestyle and its impact on our skeletal health from past to present, then the authors have fulfilled their objectives.

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Preface

The concept for this volume was born at the 2017 World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases in Florence, Italy, where we presented an interdisciplinary symposium exploring the effect of socio-economic status (SES) on bone health. Despite each of us being an expert in different disciplines (i.e. biological anthropology, social epidemiology and genetic epidemiology), we quickly realised that, collectively, we can present uniting lines of evidence for the extent to which human social mosaic is reflected in bone quality and quantity. We hope that this volume will be of interest to researchers, students and the general public curious about musculoskeletal disorders. Our main goal here is to emphasise the socio-economic aspect of human lifestyle and its impact on skeletal health from recent past to the present.

In any discipline investigating musculoskeletal health and disease, it is becoming increasingly clear that a combination of social and biological factors is at play. Here, we argue that the human skeleton should indeed be considered a reflection of our social lives. Globally and temporally, the modern world sees increased longevity, yet our socio-economic and lifestyle factors are more complex than ever before. Here, we employ medieval, contemporary and epigenetic evidence that unites social, biological and epigenetic explanations for skeletal disorders. This evidence clearly illustrates that in addition to direct biological changes in bone health that take place in response to nutrition and physical activity, osteoporosis risk factors that are socially patterned also act through epigenetic mechanisms. The risk of bone fragility becomes increased as epigenetic mechanisms transduce the psychosocial environment. This nexus of social and biological bone health factors ought to be incorporated into future research informing treatment of skeletal disorders. As a result of this volume, we encourage a broader understanding of bone health at individual and population level. We hope that novel entry points for skeletal health intervention can be identified. Consequently, the social inequities observed in fracture risk may become reduced.

In this volume, we first collate biological anthropology evidence demonstrating differences in bone health across human groups derived from distinct social strata in the European Middle Ages, paying key attention to archaeological English societies (Part 1). We focus on this time period as there is well-recorded historical and archaeological support for feudalism dividing medieval human societies into distinct classes. Second, we put forward a conceptual model for the social gradient of

osteoporosis and fracture risk. The model synthesises relationships between cumulative life course stressors and their corresponding biological mechanisms. Social epidemiology research is presented, identifying social and ethnic inequalities in bone health (Part 2). Third, we provide an overview and discuss how human psychosocial environment may be transduced by epigenetic-mediated (dis)regulation of inflammation (Part III). Biological plausibility for a key mechanistic role underpinning the social gradient of osteoporosis is suggested.

Part 1 mainly focuses on medieval English human societies that were subject to stark social status stratification. Modern research efforts into human bone health primarily rely on biomedical and animal model experimental and epidemiological data. However, these can be greatly complemented by data from human skeletal remains that derive from historical and archaeological contexts, contributing perspectives on bone quality and quantity in a range of economic and social ancient groups. This section of the volume extracts historical evidence for medieval lifestyles within an SES framework, introducing parallels from biological anthropology research for modern studies of skeletal health (Chap. 1). Evidence for an effect of SES on bone quality and quantity is extracted and summarised from published literature reporting bone data for a range of medieval European samples (Chap. 2). New data investigating medieval English children's experience of SES are supplied, focusing on dental histology as it offers insights into childhood physiological health disruptions captured by developing deciduous teeth (Chap. 2). Using new analyses of published data, a preliminary model for "catch up" in bone quality and quantity in high SES medieval samples theorising how and if the human skeleton accounts for childhood ill health is proposed (Chap. 3). Given the importance of the radius as a bone frequently affected by Colles fractures, new data reporting medieval human radius cortical bone remodelling are reported, inviting comparisons to radius biology in the living (Chap. 3). Overall, these three chapters demonstrate that medieval skeletons of low and high SES are drastically different in their experiences of events of physiological stress disruption in childhood and developed adult bone density. These findings provide medieval human support for the effect of SES on skeletal health.

Part 2 focuses on contemporary evidence for the effect of SES on bone health. Generally, though few exceptions have been noted, there is an inverse relationship between social disadvantage and experience of chronic disease. This is true for osteoporosis. The growing body of evidence demonstrating a social gradient of osteoporosis indicates that there are vast disparities in bone quality and resulting fracture risk across the life course. These appear to be strongly related to the many diverse and interwoven aspects of human social disadvantage. It is difficult to fully explain these disparities using clinical or lifestyle risk factors alone. As research into these disparities continues, we are yet to unravel the underlying mechanisms for this social gradient. However, modelling the complex relationships between biological mechanisms and cumulative stressors across the life course can help elucidate the responses to these stressors within a social framework. Therefore, a conceptual model built on social disadvantage, allostasis and the "three-hit theory" is presented in Part II in this volume. It is based on two key

components: (a) social disadvantage posing a challenge to individuals achieving allostasis and (b) genetic predisposition providing the first "hit" to allostasis with the early ontogenetic environment providing the second and later adulthood environment providing the third—encompassing a "three-hit theory" allostatic load model. Based on that model, it is suggested that besides direct bone biological responses to factors such as lifestyle and nutrition, social patterns in osteoporosis risk factors may also act epigenetically. These possibly act on a disease pathway via epigenetic mechanisms transducing the psychosocial environment. Consequently, the risk of osteoporosis and fracture is increased. The chapters outlining this evidence explore bone quality in socially and ethnically diverse groups investigating downstream and upstream determinants across the life course (Chap. 4). Differences in fracture risk between countries, within countries and between social and ethnic groups are further discussed (Chap. 5). The social gradient of preventive testing and treatment adherence in those with osteoporosis is explored in the last chapter in Part 2 in this volume (Chap. 6).

Part 3 focuses on the epigenetic evidence for the effect of SES on bone health. It is known that gene activity can be modulated through epigenetic mechanisms in stable manner. This can take place independently of DNA sequence changes and is transmitted via cell divisions. In particular, DNA methylation has received substantial attention as one of the epigenetic modifications. A family of DNA methyltransferases can methylate cytosines, which are followed by a guanine. Gene transcription is influenced by the extent of methylation in promoter and other regulatory genomic regions. The epigenome is cell- and tissue- specific. It is different from the genome and varies with environmental (and other) factors throughout a life span. It is, therefore, plausible to turn to epigenetics to help, at least in part, explain relationships between genetics and acquired aspects of the pathogenesis in a series of disorders. This includes osteoporosis. Multiple studies that range from experimental animal models to social epidemiology have indicated that SES is related to patterns of DNA methylation. It is difficult to tease out specific and single factors driving this association, and these are certainly complex. They include nutrition, lifestyle habits, environmental constraints and pressures, as well psychological conditioning. Of particular interest to our volume are the adverse nutritional and psychological experiences during the early life. These impose long-term effects in the methylation patterns of genes that include glucocorticoids and cytokines as they are involved in stress and inflammatory responses. The activity of bone cells is modulated by these factors as they induce an uncoupling of bone tissue resorption and deposition. Thus, it can be speculated that an association between SES and osteoporosis is related to epigenetic changes. The three chapters discussing and outlining this epigenetic line of evidence for bone health offer a helpful overview of the complexities associated with epigenetic function and discuss their relevance to bone fragility. The crossroads between genetic and environmental determinants of disease are summarised first (Chap. 7). The influence of maternal and social factors during intrauterine life is discussed second (Chap. 8). The final chapter in this part of the book discussed postnatal social factors explained in the context of the epigenome and the skeleton (Chap. 9).

We end the volume with concluding remarks (Chap. 10) highlighting the urgent need for consideration of human social and economic factors in researching bone health. Humans are variable in their lifestyles, which in some cases is not a choice but a result of our social and economic environment. By shedding light on the complex social mosaic, we can formulate deeper explanations for patterns in musculoskeletal disorders in the modern world. We hope that this volume inspires the readership to consider the many ways in which we can improve bone health in the present, predict bone fragility risk factors in the future, by learning from the past.

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Abbreviations

aBMD	Areal BMD
ACSO	Australian Classification of Standard Occupations
AD	Anno Domini
ADHD	Attention deficit hyperactivity disorder
ADLs	Activities of daily living
BER	Base excision repair
BET	Bromodomain and extra-terminal domain
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMU	Basic multicellular unit
BP	Before present
CE	Common era
CEO	Chief executive officer
CHARLS	China Health and Retirement Longitudinal Study
CLOCK	Circadian locomotor output cycles kaput
CT	X-ray computed tomography
Ct.At	Cortical area
Ct.Wi	Cortical width/thickness
DISH	Diffuse idiopathic skeletal hyperostosis
DNAm	DNA methylation
DNMTs	DNA methyl-transferases
DOHaD	Developmental Origins of Health and Disease
DSR	Daily enamel secretion rate
DXA	Dual X-ray absorptiometry
H.Ar	Haversian canal area
H.Dm	Haversian canal diameter
HATs	Histone acetyltransferase enzymes
HDAC(s)	Histone deacetylase(s)
HELIX	Human Early Life Exposome Study
HFD	High-fat diet
HMTs	Histone methyltransferases
HRpQCT	High resolution peripheral quantitative computed tomography
IAP	Intracisternal A-particle

JPOS	Japanese Population-based Osteoporosis Study
LADs	Lamina-associated domains
LC-MS	Liquid chromatography-mass spectrometry
LEH	Linear enamel hypoplasia
lncRNAs	Long non-coding RNAs
MA	Middle Ages
meQTLs	Methylation quantitative trait loci
MESA	Multi-ethnic Study of Atherosclerosis
MetS	Metabolic syndrome
miRNAs	MicroRNAs
mRNA	Messenger RNA
MrOS	Osteoporotic Fractures in Men Study
MSCs	Mesenchymal stem cells
N.On	Intact osteon density
N.On.Fg	Fragmentary osteon density
ncRNAs	Non-coding RNAs
NHANES	National Health and Nutrition Examination Survey
NORA	National Osteoporosis Risk Assessment
On.Ar	Osteon area
On.Dm	Osteon diameter
OPD	Osteon population density
OR	Odds ratio
pQCT	Peripheral quantitative computed tomography
PRD	Protein-restricted diet
pri-miRNAs	Primary miRNA transcripts
RISC	RNA-induced silencing complex
RLGS	Restriction landmark genomic scanning
ROI	Region of interest
SD	Standard deviation
SDoH	Social Determinants of Health
SES	Socio-economic status
SFI	Skeletal frailty index
SNP	Single nucleotide polymorphism
SWAN	Study of Women's Health Across the Nation
TADs	Topologically associating domains
TET	Ten-eleven translocation
TSS	Transcription start sites
UV(B)	Ultraviolet B light
vBMD	Volumetric bone mineral density
WHO	World Health Organization

Part I

Medieval Perspective

1 Medieval English Social Inequality and Bone Health: What Lessons are There to be Learnt for the Living?

Justyna J. Miszkiewicz

1.1 Introduction

With social and economic systems determining the structure and function of human groups come health repercussions that affect our longevity and quality of life [1, 2]. Consequently, skeletal disorders in the modern world are becoming increasingly problematic as populations experience ageing-driven changes in bone metabolism [3–5]. Coupled with often poor quality nutrition and largely sedentary lifestyles, skeletal adaptation in contemporary populations is facing more challenges than ever before [3, 6–9]. As biological anthropologists attempt to understand the interwoven biological and cultural relationships influencing the ways in which we grow and adapt to our environments [10], the medical realm tackles bone fragility and fracture risk at a patient and society level [11]. Human lifestyle as a research theme appears to unite bio-anthropological and medical queries into bone health.

Our daily activities and diet choices are often a result of socio-economical positioning which determines access to resources and health care [12, 13]. Gender, age, and ethnic background further add to bone health inequality in the living [14–16]. This social mosaic can be difficult to tease apart in contemporary, demographically complex, populations as current epidemiological research uses varying definitions and measures of occupation and physical activity [17, 18]. Reconstructing lifestyle is not any easier to tackle in human history, but past societies characterised by distinct socio-economic status (SES) stratification systems can be identified. Members of these societies would have held well-defined roles that usually carried through the entirety of an individual's lifespan. Because social inequality paints a dismaying picture of human existence, highlighting its effects on bone health can contribute

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evidence upon which preventative, or at least educational, measures informing future generations can be built.

It is difficult to fully understand modern bone disorders that may have social determinants (e.g. osteopenia, osteoporosis, osteoarthritis [19, 20]) without first looking into ancient human bones and lifestyles [21]. Bio-anthropological studies of past human remains have indeed revealed that bone loss and osteoporosis were found in antiquity [22], afflicting populations that ranged from Roman Britain [23], medieval England and Norway [24], early Bronze Age Austria [25], and Twelfth Dynasty Egypt [26] to name a few (also see [27] for review of studies from the 1960s to 1990s and Chap. 2 in this volume for further discussion) . Medieval Europe ruled by feudalism is evidence for exceptional levels of social inequality, understanding of which has the potential to expand our current views on bone loss and maintenance in modern social contexts [28]. The goal of this chapter is to provide an overview of high and low status medieval English lifestyles, collated from historical records, to illustrate social inequality and its implications for skeletal health as understood from research in bioarchaeology (the study of archaeological human remains; also see Chaps. 2 and 3 in this volume) and modern epidemiology.

1.2 The Medieval Social Mosaic and Human Skeletal Remains

The Middle Ages (MA) are divided into early (fifth to tenth centuries AD, hereafter "centuries"), and high and late (eleventh to sixteenth centuries) period. Human skeletal remains representing the latter have received extensive attention in bioarchaeology [29]. In order to appreciate osteological evidence from that period (Chaps. 2 and 3), the medieval background and lifestyles need to be first summarised. In brief, political and economic developments, famine and epidemic events underlined the daily lives in medieval England, all against the backdrop of population expansion [30]. Urbanisation and prosperity, increasing between the eleventh and fourteenth centuries, were interrupted by the Black Death pandemic in the mid-fourteenth century [30, 31]. The fourteenth century saw famine, heavy rains, and harsh winters reducing crop production, though market trade and industrial growth in towns continued expanding [32–34]. Between the eleventh and thirteenth centuries, most of the English land was rural [35], and as economy was largely focused on farming [36, 37], commercial industry and merchant trade thrived in towns [37]. Increasing urbanisation brought about poor quality and sanitation living conditions that intensified the need for hospitalisation [30]. The Black Death pandemic arrived from Asia in AD 1347 [38], spread rapidly between trading ports due to poor sanitation and overcrowding, killing an estimated one third of the European population [38, 39]. Major changes in economy and social stratification followed the pandemic as the class system was interrupted by death and disease [38–40]. By the fifteenth century, medieval England developed organised politics and economy that included basics of royal administration, bureaucracy, a judicial system, and an initial foundation for a parliament [41].

Medieval England was mostly an aristocratic (upper class) and (lower class) peasant, villein, and serfdom society, which meant that only a small proportion of the population held wealth and power [42]. Society classification, feudalism and Church (as an insitution) driven, into high and low SES groups, gave land owners and others from privileged backgrounds power over the lower classes [32, 42–46]. Throughout medieval history, the high SES individuals would have encompassed a variety of groups, including the elite, noblemen, aristocracy, barons, knights (sometimes referred to as "middle" class), clergy, lords, bishops, and kings [30]. Most of their income derived from tenancies and tax collected from the low SES peasants [35, 36, 42, 47]. The clergy, bishops, monks, canons, priests, nuns, and other monastery members were supported through donations from the society [46]. The economy of the MA was almost entirely dependent on the peasant and serf workforce cultivating land and serving the upper classes [42]. The low SES groups had barely any rights until the Peasant's Revolt in 1381 AD [35]. The uprising erupted following the Black Death as it killed regardless of social status and thus led to increasing tension between extravagant lords and working peasants, inflation, and corruption at higher levels of power [35, 40, 48].

This stark social classification determined medieval lifestyles and occupations typical of each SES group. These are summarised in Fig. 1.1 as based on extractions from historical textual evidence. It is currently well established that, except for genetic underpinning, bone metabolism is a highly complex process that responds to, and is influenced by, a series of different internal and external factors that include disease, hormones, diet, and biomechanical load [49]. Therefore, modern bone physiology paradigms, particularly in relation to mechanically induced modelling and remodelling, can be used in a comparative framework explaining skeletal variation with medieval SES lifestyles to elucidate examples or uncover temporal changes in bone structure [28]. Indeed, comparisons between modern and medieval bone samples can be of great use when investigating the fragile external phenotype in living people. For example, a 2007 study evaluating proximal femur anatomy in $n = 118$ medieval and $n = 67$ modern adults found that the human femoral neck axis has become elongated with a smaller and more oval-shaped cross section over the past several hundreds of years [50]. Identifying this phenotypic trait may explain frequent fall-induced stress fractures in the femoral neck in the living.

The implications of medieval SES for bone health need to be understood from a multi-faceted theoretical perspective. The "Developmental Origins of Health and Disease" hypothesis [51] driven by Barker [52] proposes that early life stressors can underlie adult development, leading to chronic conditions and affecting survival. The study of ancient human remains has been no stranger to this concept [53, 54]. Extensive research by Steckel and Rose [55, 56] analysing human skeletal data from the Western Hemisphere archaeological skeletons covered $n = 12{,}520$ individuals and 65 locations over 4500 BC to twentieth century, supporting the effect of early childhood stress on adult mortality. Using markers that include disturbance to dental development, lesions associated with anaemia, and stature as an indicator of attained adult height, this project showed that stressful childhood environmental contexts elevate the risk of dying younger. Stemming from this large scholarly endeavour

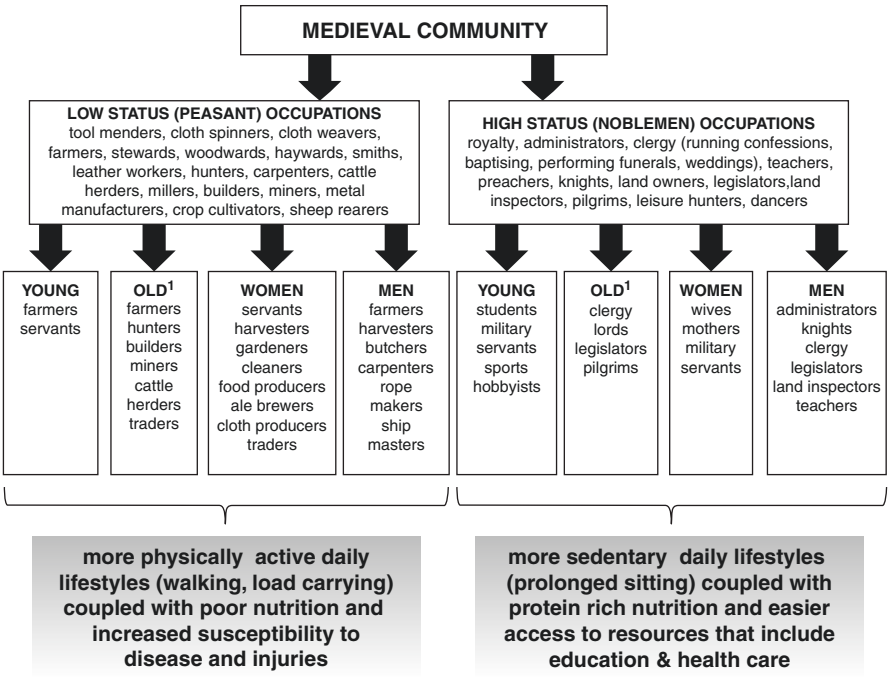

Fig. 1.1 This diagram summarises medieval occupations as per socio-economic standing, age, and gender. The individual activities were extracted from written historical records where specific daily occupations are named (see references in-text in this chapter). The division here is made strictly into low and high SES, though medieval "middle" class was also recognised (e.g. knights). [1]"Old" refers to age category consisting of both "middle-aged" (in their 30s and 40s) and "elderly" (50 years old and above) individuals. Historical evidence [48] indicates that a "middle-aged" category per se was not recognised, and older generations were considered over 30–35 years of age [48]

and turning attention to Europe, further effort reviewing osteological, historical, and archaeological evidence for temporal change in past health has been collated in a 2019 volume titled *The Backbone of Europe: Health, Diet, Work and Violence over Two Millennia* [57]. By incorporating data from medieval human remains into a broader context, amongst multiple findings, social inequality driving diet and workload was presented from ancient skeletal remains. Relating medieval English lifestyles to bone health in the present volume is complicated by the varying degree of definitions used to describe "bone health" in the clinical and bio-anthropological realm. Some scholars refer specifically to bone fragility, structure, strength, physiology, fracture risk, as well as conditions (e.g. osteopenia, osteoporosis). Not all these are possible to infer from medieval samples due to preservation and methodological problems [27]. Nevertheless, bioarchaeologists work within a framework of "(1) environmental constraints; (2) cultural systems; and (3) host resistance" [58, p. 6] when interpreting skeletal data. These are important to bear in mind when attempting to place skeletal changes on ancient skeletons into socio-economic

contexts. The complexity of medieval social and economic structure is also difficult to reconstruct from history and archaeology alone, so this chapter provides a summarising and largely introductory synthesis and inferences (see Chap. 2).

1.3 Medieval Social Status Occupations, Bone Health, and Modern Epidemiological Parallels

Individuals of high SES enjoyed a rich lifestyle within the medieval community. Their privileged status facilitated access to protein-rich meats, accompanied by a selection of wines and ales, but also determined mostly sedentary occupations [59] (Fig. 1.1). Secular noblemen and landlords were preoccupied with wealth distribution and overseeing their properties and servants, attending land hearings, and participating in political discussions and land inspections [42, 59–61]. Daily duties of the clergy involved performing funerals, masses, weddings, and also assisting the sick and less privileged in hospitals [62]. Substantial bioarchaeological evidence exists reporting tens of human leprosy-inflicted skeletons recovered from segregated medieval cemeteries [63] and a range of skeletal pathologies associated with reduced mortality in individuals from church graveyards designated for the lower classes [64]. For example, $n = 38$ leper skeletons dating to the eleventh to twelfth centuries uncovered at the St. Mary Magdalen Cemetery in Winchester were buried amongst pilgrim burials indicating contact between the two status groups [63]. The most intense physical exertion for high to middle SES groups would have arisen as part of leisure activities that included hunting, dancing, feasting [65], horse riding, and military training by knights [22, 66, 67]. Prolonged periods of walking would have stimulated lower limb mechanical load for the clergy participating in pilgrimages [68].

Low SES status was associated with diet that included meals based on pottage (see Chap. 2) and required the undertaking of manual labour for (land)lords [34, 69]. Further class stratification within peasants meant that some occupations were deemed of higher rank than others. For example, farmers, stewards, woodwards, and haywards were generally of high standing [34], though skilled craftsmen such as smiths, leatherworkers, carpenters, and millers were also desirable [30]. Rural and urban occupations were also different, with the former allowing some peasants to own land, though most had to pay rent to landowners. Rural serfs had no freedom, worked 6 days a week [36] outdoors through all seasons except winter [70] which is when indoor activities such as tool mending, cloth making, and food grinding were done [33, 47]. Ploughing, hay mowing, swineherding, cowherding, sheepherding, and gooseherding were daily rural peasant occupations [35]. Urban peasants drove stock to local markets and carried materials to building sites and worked in textile, leather, wood, and metal manufacture [35, 59]. Groups of low SES engaged in the most hazardous activities that often led to injuries [71]. Farming and building would have been associated with falls from ladders, lofts, and horses. For example, rural skeletons from the tenth to twelfth centuries Raunds in Northamptonshire had a much higher prevalence of long bone fracture trauma when compared to urban

skeletons [71]. With restricted leisure time, peasants played dice, board games, bowls, water-tiling, and ice-skating whenever they could [65].

Age and gender further determined SES-specific labour and daily lifeways in the MA. Limited accounts of medieval youth indicate that individuals under the age of 30 were all referred to as "young" [72]. Bio-anthropologists usually classify ancient skeletons as "young adult" when age-at-death estimates range between 20 and 35 years old, with "middle-aged" adults falling into 35 to 50 years old [73]. Therefore, as much as the analysis of "young" medieval skeletons in relation to modern cohorts is complicated by varying age definitions, we can speculate that medieval peak bone mass would have been reached by the "young" in their third ontogenetic decade [74]. The attainment of peak bone mass is a crucial determinant of the adult bone "bank", and SES factors can indeed drive its tempo and timing [75]. It is well understood today that except for heritability, peak bone mass attainment can be influenced by fracture experiences, nutrition, hormonal factors, and exercise [75] – these often relate to social opportunity. Indeed, medieval bone histomorphometric indicators of bone remodelling in the young have been previously found to differ between low and high SES groups at the eleventh to sixteenth centuries St. Gregory's Priory in Canterbury [28]. The degree of physical regime differences was certainly great amongst the medieval young. As per a twelfth century account written by an early secular clerk William Fitz Stephen, as much as all SES groups found leisure time to play ball games in the field and ice-skate on frozen Thames in London [76], younger men of higher SES would have been largely sedentary spending time in monasteries or organised education, with only military service facilitating the highest levels of physical activity [66, 76]. Young sons of low SES helped in the field, whereas girls assisted at home with a variety of tasks such as wool spinning [33]. Young peasants often began serving at teenage years, which is when they were sent out to work outside of their home [65, 77–80]. One extensive bioarchaeological analysis [81] reported $n = 4940$ juvenile and adolescent skeletal remains spanning 151 medieval English sites to show increased prevalence of spinal and joint disease in urban contexts, as well as high rates of injuries and trauma accruing before 18 years of age.

Adult men of low SES almost always undertook the more physically demanding labour including ploughing, seeding, weeding, and harvesting [33]. Adult women assisted in fields at harvest [33]. In towns, husbands ran butcheries, whereas wives tended to vegetables in farmyards, cleaned cottages, prepared cheese and butter, brewed ale, collected nuts and wood, milked cows, fed livestock, and fetched water and washed clothes in local rives [33, 77, 78]. Outside food production, women also made nets, shoes, and purses and bound books, weaved silk, and embroidered cloth [79]. Aristocratic females mainly stayed at home as wives and mothers, and ruled over serfs [33, 78]. There is some evidence, however, that some women of high to middle SES sporadically engaged in military training [33].

The outlined medieval English SES behaviours highlight key inequality issues in access to care, education, nutrition (see Chap. 2), and biomechanical stimulation. A 2003 analysis [81] of a young English female skeleton showing pectus carinatum (chest deformity), recovered from a fifteenth century high status burial in Ripon

Cathedral, North Yorkshire, suggested that upper classes had a close support system. Her sternum deformity was likely due to a congenital developmental problem, yet [82] she likely received extensive care from her close group attempting to treat her deformity. Social support system and care within a SES framework have also been shown to correlate with hip fracture risk in postmenopausal women in the modern 1960s–1980s Sweden [83]. In this study [83], a comparison of $n = 1327$ hip fracture cases and $n = 3262$ female controls showed that being employed within higher tertile of income was associated with a lower risk of hip fracture. Therefore, modern risk factors for osteoporotic fractures appear to include employment, household type, and income.

One of key reasons why sedentary and active occupations are important to consider when discussing bone quality and quantity in the past is mechanical load and resulting strain stimulating bone remodelling [84]. The largely sedentary lifestyles of medieval individuals of high SES were confirmed using adult cortical bone histomorphometry at the eleventh to sixteenth century St. Gregory's Priory, Canterbury [28], with lower SES individuals showing bone histological geometric properties in line with mechanically induced bone adaptation [84]. However, their bone density reconstructed from secondary osteon population density (OPD) appeared to be compromised when compared to the high SES counterparts – likely a reflection of their poor health and frequent illness. This medieval occupation and bone link is mirrored in modern populations. A 1998 study [85] of 416 patients whose hip fractures were treated between 1990 and 1991 at Westmead Hospital in Sydney, Australia, determined that SES-specific occupations had a strong effect on the risk of fracture occurring later in life. Examining employment these patients had engaged in between the ages of 20 and 50, the study [85] found that females in sedentary occupations at the age of 50 were more likely to develop a hip fracture. Higher SES of the longest held occupation led to a lower fracture risk. This study reported a clear relationship between age, sex, and SES occupation, highlighting the need for inclusion of one's social background when designing individual exercise plans that improve and maintain bone health. Using The Australian Classification of Standard Occupations (ACSO), the study [85] determined that, for example, a clerk or an accountant can be classified as sedentary, a housekeeper would be exposed to "moderate" weight bearing, but a blacksmith and a butcher are considered largely "standing" (also see Table 1 on p. 429 in [85] for more occupation gradient changes). We see a similar pattern in medieval England [28] where lower SES occupations include butchery in urban settings and farming in rural land, whereas high SES activities are mostly sedentary. The former associate with quicker secondary osteon infilling in leg bones responding to increased mechanical load and resulting strain [86] but within an otherwise a compromised bone quantity due to developmental health disruptions.

Finally, as access to organised education in medieval England was reserved for the upper classes, we cannot underestimate it as a factor contributing to skeletal health inequality as noted for eleventh to sixteenth centuries Canterbury [28], also mirrored in modern populations. A study [87] evaluating osteoporosis and fracture risk across different levels of education in 6160 postmenopausal Italian females

demonstrated that the most educated women had the lowest risk of developing osteoporosis. Years of formal education were incorporated into models investigating the occurrence of chronic conditions as related to diet and physical activity. A gold standard densitometric assessment through dual-energy X-ray absorptiometry (DXA) of bone yielded data that showed a strong inverse correlation between osteoporosis prevalence and education level. It differed by almost 10% with women from lower and higher education backgrounds with 27.8% and 18.3% osteoporosis prevalence, respectively. Up to 9 years of schooling was shown to be a strong predictor for a significant reduction in osteoporosis risk.

What we can certainly take out from this study (and others mentioned here) is that the SES background we find ourselves in has the potential to manifest itself through bone quantity and quality, either in later life or earlier. As we learn from bioarchaeology, it was the same in medieval England as much as the MA may seem temporally distant. When discussing adult human bone strength and quality in the past and present, there are two key aspects that unite the two time periods. First, we have first three decades of our lives to build our bone "bank" and attain peak bone mass [74, 75]. Nutrition, physical activity, and developmental homeostasis regulate this crucial period of skeletal growth as bone tissue is modelled in length, width, and strength [88]. For most medieval and living people, these factors are determined socio-economically. Second, menopause-driven reduction in oestrogen and associated bone loss in females was a problem for medieval women and continues to affect living women [21, 89]. Their bone fragility and related fracture risk appear to be in association with occupation and physical activity during reproductive and menopause transitional life phases, which were and still are socio-economically determined. Geopolitical socio-economic inequality in the modern world continues to underlie variation in access to education, nutritious diet, and health care. Using the medieval society as a model, we learn that bone health suffers from stark disparities in wealth and workforce distribution.

1.4 Conclusion

Social status was a key determinant of nutrition, health, disease, and general lifestyle throughout the MA [30, 32, 69]. Individuals of high SES had easier access to a nutrient-rich diet and led a privileged quality of life which resulted in greater wellbeing [29, 90]. Peasants of lower SES predominantly consumed basic grain-based diets and led a more distressed lifestyle ruled by the upper classes [32, 36, 91, 92]. They would have likely developed poorer immunity and higher frailty, being impacted by infectious disease and malnutrition to a much higher extent that those from socially advantaged backgrounds [93, 94]. The occupations and activities both classes engaged in were vastly different, determining their musculoskeletal health adaptation, with the lower SES groups experiencing regular and more intense physical strain. However, high SES individuals were not always shielded from degenerative and metabolic disease [95] and infections carried by the Black Death [87] (further explored in Chaps. 2 and 3 in this volume). It is clear that these

SES-determined lifestyle differences in behaviour, diet, health, and pathogen load left a mark on medieval human bone health [28, 96, 97]. One key lesson we can learn from the study of medieval human skeletal remains is that social inequality in modern societies ought to be taken into consideration when researching bone fragility in the living.

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2 Skeletal Health in Medieval Societies: Insights from Ancient Bone Collagen Stable Isotopes and Dental Histology

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2.1 Introduction

Traditionally, the study of human skeletal remains from ancient backgrounds (bioarchaeology) has examined skeletal size and shape in relation to written historical records or made interpretations based upon the archaeological record in order to contextualise the biology of once-living people [1–3]. The unique and irreplaceable value of ancient skeletal remains means that more invasive or destructive methods of analysis are undertaken less often [4]. However, recent advancements in microscopic, biochemical, and biomolecular techniques increasingly open up new possibilities for reconstructing human skeletal growth and physiology at the cell level from small amounts of tissue, thus greatly minimising the destruction to skeletal remains [5]. In this chapter, we provide selected examples of bioarchaeological studies that use a range of technical approaches to examine the effect of medieval socio-economic status (SES) on skeletal health, disease, and adult bone development (e.g. [6]). This chapter focuses on medieval bone tissue, but we also extend analyses to medieval human dental remains in the latter part of the chapter. We present both hard tissue types because teeth are often the only part of a skeleton that survives without damage due to their highly mineralised outer enamel coating. Furthermore, some aspects of lifestyle in the past leave a record in adult teeth that cannot otherwise be inferred from adult bone (e.g. weaning from permanent teeth). In other cases, analyses of both teeth and bone can be combined to gain novel insights into past human skeletal growth (also see Chap. 3) [7, 8].

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Studying the effects of medieval SES on skeletal growth is certainly complex. It is difficult to extract the exact individual behaviours or components of past daily lifestyles and subsequently match them accurately to a skeletal manifestation [9]. However, skeletal indicators of lifestyle can sometimes emerge when related to social strata as inferred from burial location (e.g. the layout and cemetery structure) [10], archaeological material culture (e.g. grave goods) deposited within and excavated from a grave [11], and/or written historical archival or other documentation describing medieval lives and lifestyles [12] (also see Chap. 3 for a specific example). These types of data are considered secondary evidence in bioarchaeology [13]. An overview of medieval SES lifestyles is presented in Chap. 1 in this volume. Here, we focus on the influence of medieval diet, physical activity or occupation, and disease and health on bone. We present a short analysis of stable isotope data recovered from medieval English femoral bone indicating higher dietary protein consumption in a group of upper class individuals when compared to a lower class group from the same archaeological site (also see Chap. 3). Finally, we assess evidence of stress retained in teeth against textual evidence of weaning age in medieval England.

2.2 Medieval Human Bone

A key component to a successful bioarchaeological analysis is the understanding of bone biology, growth, structure, and variation principles [2]. Paradigms forming this knowledge are usually borrowed from current experimental skeletal biology literature on animal models and human bone tissue studied in clinical and biomedical contexts [14]. The most direct surviving biological evidence for a medieval individual is their skeleton, gross examination of which can provide insights into anatomical variation with sex, age, diet, disease, physical activity, environmental constraints, and pressures [15]. These skeletal data form primary evidence in bioarchaeology [13]. However, macroscopic analyses alone give us a limited insight into the complex bone physiological processes experienced by a once-living bone tissue [5, 9, 12]. It is well established that bone is dynamic and undergoes modelling and remodelling throughout our entire lifespan [16, 17], both of which require a series of inter- and intra-playing external and internal stimuli that include mechanical loading, endocrine, mineral, and vitamin homeostasis [17]. Based on this, bioarchaeologists can target macro- and microscopic bone structures to collect a series of data further evaluated within and supported by secondary evidence that is non-biological.

Multiple studies have demonstrated that individuals of lower medieval SES would have been at a health and disease disadvantage with their bones suffering from abnormalities (either as a primary infection or secondary result of another soft tissue condition [13]) and developing lower quality and quantity in adulthood [6, 12] (see Chap. 3). However, interpretations like these are inherently complex. In order to arrive at the above conclusions, one must make a conscious and informed study design decision of segregating medieval humans into well-defined and strictly

distinct SES groups. This would mean assigning a single social stratum a series of lifestyle factors assuming no overlap in the type or frequency of these with another layer of the medieval society concerned. A further complication may stem from SES behaviours that originate from cultural practices determining lifestyles in one European location vs. another. For example, medieval monks living in cloisters and priories would have held high social standing, which facilitated access to a wide variety of good-quality foods, but also encouraged unhealthy habits of overeating and alcohol consumption [18]. Coupled with overwhelmingly sedentary daily activities, the high SES would have meant that not all medieval clergymen carried healthy bones [12]. On the other hand, the practice of pilgrimages and fasting elsewhere in Europe [19, 20] would introduce further confounding sociobiological factors when interpreting bone data within a high SES context, as we understand bone can have a range of responses to increased mobility and suppressed hunger [21, 22]. To complicate this further, evidence exists that even fasting monks found ways to smuggle domesticated animals into their cloisters in thirteenth-century France [20], whereas lower-ranking laymen in eleventh- to twelfth-century Poland consumed more meat than a low medieval SES would suggest otherwise [23]. The social mosaic of medieval lifestyles means that evidence for the effect of SES on bone health needs to be population specific.

A bone condition of major relevance to clinical medicine research and practice is osteoporosis [24]. Increased risk of development and prevalence of osteoporosis have been noted in modern populations that originate from socially disadvantaged backgrounds (see Part 2 in this volume) [25, 26]. Its associated fracture experience due to reduced bone mass is of complex aetiology that includes ranging levels of physical activity, biological sex, ageing, dietary regimes, and hereditary predisposition [27]. Though largely considered a recent disease as a result of modern lifestyles, bioarchaeological evidence exists that medieval populations in Europe also suffered from this condition, e.g. [28–32]., or at least had increased SES-specific skeletal frailty.

A 1996 [28] study examining $n = 83$ male and $n = 71$ female peasant skeletons recovered from a church and associated churchyard at medieval (eleventh to sixteenth centuries) Wharram Percy (North Yorkshire, England) demonstrated a drastic loss of age-dependent cortical bone in females. Using metacarpal radiogrammetry to measure cortical bone thickness, it was shown that bone loss in these medieval English peasant females was at an almost exactly same level when compared to modern Finnish women in a menopause 50+ years of age category [28]. Healed trabecular bone fractures of the axial skeleton were also reported, providing evidence that age- and sex-related bone loss afflicted this medieval English group of lower SES. In 2015, [29] $n = 55$ high and low SES medieval (eighth to thirteenth and seventeenth centuries) Italian skeletons recovered from San Michele's Church in Trino Vercellese (Piedmont) were examined for lumbar vertebrae and femur cortical bone and bone mineral density (BMD) using X-ray computed tomography (CT) and dual X-ray absorptiometry (DXA). No age or sex differences in bone quantity were noted in the sample, but lifestyles in the low SES group correlated, counter-intuitively, with increased BMD [29]. However, these results matched contextual

data demonstrating that the rural low SES population consumed more calcium, was exposed to more daylight, and undertook more physically demanding occupations when compared to the high SES group. In medieval (eleventh to sixteenth centuries) Trondheim in Norway, proximal femora of $n = 63$ males and $n = 65$ females recovered from a churchyard of St. Olav's Church were examined for BMD using DXA and the prevalence of osteoporosis-related fractures [30]. These data were later compared to the aforementioned Wharram Percy rural population, noting similar levels of BMD but higher frequencies of osteoporotic fractures in the medieval Norwegian women who lived in cold and built environments. Despite comparative modern human BMD data indicating abnormal levels in Norwegian populations when compared to the English [33], the medieval analysis [30] confirmed it being a recent phenomenon due to the two ancient groups having equal BMD. Using a combination of gross anatomical and X-ray imaging methods, a study of a fourteenth to seventeenth century female from medieval Coimbra in Portugal [31] reported a unique case of osteoporosis-related extracapsular fracture of the proximal femur. The medieval female skeleton aged >50 years derived from the Santa Clara-a-Velha Monastery and likely belonged to an elderly nun whose privileged background extended her life expectancy increasing the risk of development of osteoporosis. Historical evidence for Italian nuns spending prolonged periods of time indoors with limited sun exposure inhibited vitamin D absorption [31].

Other skeletal abnormalities reported for medieval skeletons also appear to be SES specific. For example, a modified skeletal frailty index (SFI) analysis which seeks to assess skeletal phenotypic characteristics associated with sarcopenia and osteopenia was evaluated in a medieval English sample of monastic and non-monastic groups in London [32]. The high SES groups were represented by a collection of skeletal remains from the Merton Priory (1117–1538 CE) and Bermondsey Abbey (1066–1540 CE), whereas the lower SES groups came from lay community in Guildhall Yard (1140–1350 CE), Spital Square (1200–1500 CE), St. Mary Graces (1350–1538 CE), and St. Benet Sherehog (1250–1666 CE) [32]. An analysis of a series of skeletal conditions that ranged from dental developmental disturbances, osteoarthritis, bone infection to rickets or osteomalacia (see table 3 in [32] for further details of methods), on a maximum sample of $n = 517$, revealed that monastic individuals aggregated higher SF indices, showing somewhat increased skeletal frailty indicating that high SES did not shield medieval people from physiological upsets.

A strongly high SES correlated condition of diffuse idiopathic skeletal hyperostosis (DISH) has been noted several times in human remains recovered from monastic sites (see [18]). However, not all cases of medieval DISH have made direct links to monastic SES lifestyles due to limited archaeological and historical evidence [34]. The condition of DISH (Forestier's disease) manifests through ossification of spine ligaments and other joint areas, is of unclear aetiology but has been consistently associated with diabetes and sedentary lifestyles, and has older male preponderance [35]. A 2011 study [18] discussed evidence for DISH recorded in multiple high SES medieval English sites associated with monk burials, including the Merton Priory and Wells Cathedral (thirteenth to sixteenth centuries) and the Royal Mint

(1350 AD) in London. A high prevalence of older monastic males diagnosed with DISH (total of 5 out of 28) at Wells Cathedral, and 6 out of 52 at the Royal Mint, was presented [18]. A case study of a 25–35-year-old male recovered from S. Angelo Abbey in Montescaglioso (Italy), dated 1100 and 1400 AD, also reported a convincing DISH diagnosis, though limited historical and written data were available to argue for a monastic SES lifestyle as the site included secular burials as well. Social status differences in the prevalence of DISH were also noted in various high SES medieval male skeletons from Lithuania when compared to lower SES urban and rural skeletons in the same region [36]. The authors in [18] demonstrated statistically that the incidence of DISH could not have been due to chance alone, arguing that high SES lifestyles at monasteries underlined its development. Overall, most studies of DISH in the medieval period place focus on diet as a core aetiological factor. We will return to high SES medieval diets later in this chapter. Certainly, a sedentary lifestyle, coupled with diabetes or obesity, may have led to the development of DISH due to high protein intake as a result of meat overconsumption. Indeed, as noted in [18], the average calorie intake by monks from Westminster Abbey (London) would have well exceeded the modern daily energy recommendation, with estimates of 6207 calories ingested daily in the medieval period (5291 during Advent and 4870 during Lent) [37].

Further examples of medieval SES lifestyle effects on bone health include poor adult skeletal phenotype characteristics in low SES groups [38], increased adult cortical bone loss associated with cranial and axial skeleton osteogenetic indicators of developmental stress [6], and adult femur cortical bone remodelling differences between low and high SES groups [12]. A 2011 [38] study measuring skeletal size and shape in $n = 20$ females and $n = 32$ male from medieval (eighth to thirteenth centuries) Trino Vercellese in Italy reported high SES males to achieve greater adult body mass and stature estimates when compared to low SES individuals. Despite their tall stature, the high SES males also had relatively short lower limbs, which shed light on the biocultural plasticity of intrapopulational variation on adult body size underlined by social background. A 2001 study [6] of Polish samples from high SES medieval Cedynia (twelfth to fourteenth centuries) and low SES Słaboszewo cemetery (fourteenth to seventeenth centuries) correlated skeletal disruptions in early ontogeny from cranial and axial markers of stress with cortical bone loss estimates from metacarpal bone. Using $n = 150$ males and $n = 69$ females of high SES and $n = 85$ males and $n = 60$ females of low SES, the study demonstrated that individuals characterised by shorter height of skull base and vertebral canal also had decreased bone quantity in their metacarpals. This pattern was particularly apparent in the low SES group. Finally, a 2016 [12] study investigating low and high SES groups from medieval (eleventh to sixteenth centuries) Canterbury in England explored bone remodelling variation with SES lifestyles. Using femur bone histology, it was shown that $n = 40$ individuals buried in a high SES Priory developed higher osteon population density (OPD) when compared to lower ($n = 410$) class of poor and sick individuals from a neighbouring cemetery. However, through geometric properties of their bone histology, bone adaptation to more strenuous physical activity in the lower SES category was also observed. It was speculated that these

differences were due to a mosaic of social and biological factors originating from medieval English lifestyles, including protein- and meat-rich diet in the high SES skeletons (also see Chap. 3). However, the authors (two of us) were not able to support their dietary argument with primary data, which we report for the first time in Sect. 2.3 in this chapter.

The above examples of medieval studies clearly reflect the mosaic of factors affecting bone health in relation to SES status. We selected a range of publications that describe the many different ways in which medieval skeletons reflected their social environments.

2.3 High-Protein Diet and High SES in Medieval English Human Bone: Insights from Bone Collagen Stable Isotopes

A 2016 [12] medieval bone histology study suggested that diet could play a role in explaining femoral remodelling differences observed between medieval English upper and lower class individuals recovered from eleventh to sixteenth century St. Gregory's Priory and associated cemetery in Canterbury, England. The higher SES individuals had access to a diet rich in meat, fats, and dairy and had significantly higher OPD values (mean = 20.5/mm^2, SD = 3.9) in comparison to the lower class group (mean = 18.6/mm^2, SD = 3.2), whose daily diet would have been largely grain based with little variation and almost no meat or dairy [12]. It was suggested that these differences could have been related to lifestyle characteristics, such as biomechanical stress arising from physical activity and high levels of manual labour in the lower class group, or due to the historically recorded dietary components, which were vastly different between the classes [12]. Low SES medieval people would have fed on vegetables, cereals, and pottage—thick stew made from oats, peas, and beans [39–41]. With respect to the high SES Priory individuals, there is written evidence of one visiting scholar being offended by the amount and variety of foods presented to Canterbury monks with numerous meat dishes (including fish, capons, chickens, ducks, geese, pheasants, pigeons, and swan) all accompanied by ale, wine, and beer [37, 42]. The above bone histology results, and secondary evidence, have not yet been supported using a diet-specific technique, such as stable isotope analysis. Here, we use new δ^{15}N isotope data, an established archaeological measure of dietary protein in ancient humans [43], reconstructed from bone collagen in a sample of individuals studied in [12], to test whether potential higher protein intake indeed can be attributed to higher SES individuals, but not those of lower SES in medieval Canterbury.

2.3.1 Brief Background

Bone remodelling data preserved in archaeological human remains allow us to reconstruct bone adaptation to low and high SES medieval lifestyles. Frequently,

researchers will attribute variation in bone structure or morphology to lifestyle factors such as diet or restricted, or excessive, access to varied or nutritional food types [12, 18, 44]. Osteon population density has been defined as a count of whole and fragmented secondary osteons within a given region of interest and is an established measure of bone remodelling rates [45]. Variation in OPD may be indicative of modifications in bone structure due to biomechanical loading, diet, health, or general lifestyle [5].

δ^{15}N isotope studies of palaeo-diet are widely used in bioarchaeology [43, 46]. An individual's isotopic composition from bone collagen reflects their dietary protein over numerous years before their death. There are several types of isotopes that are useful for estimating elements of ancient diet and behaviour, but δ^{15}N values in particular can identify dietary protein potentially relating to animal products like meat, seafood, and dairy [46]. δ^{15}N isotope values are commonly used in conjunction with carbon isotope (δ^{13}C) values to provide a C:N ratio, often used to indicate the inclusion of additional components in a diet such as terrestrial or marine foods. Usually, environmental baseline data are needed to facilitate this type of analysis. For example, stable isotope data have recently been used to infer SES diets in medieval Tomar (Portugal) [47] and early medieval (ninth to eleventh centuries) Bohemia (Czech Republic) [48].

2.3.2 Materials and Methods

We studied $n = 22$ femoral bone samples from the medieval St. Gregory's Priory and cemetery collection held and widely researched at the University of Kent (also see Chap. 3).[1] Fifteen samples represented high SES Priory, and $n = 7$ samples were extracted from the low SES cemetery individuals. Baseline data for medieval Canterbury are unavailable, so we use δ^{15}N isotope values to compare between individuals from the same geographic and temporal location but of two distinct SES groups of historically recorded dietary differences. Sex and age at death of the individuals were estimated using methodological standards [49] based on the morphological appearance of the pelvis and skull, and postcranial joint surface morphology, described in [12]. Both females ($n = 6$) and males ($n = 15$) are represented in the data, consisting of young adults (20–35 years) ($n = 2$), middle-aged adult (35–50 years) ($n = 15$), and old adults (50+ years) ($n = 4$), with one individual of unknown sex and age. The δ^{15}N data were obtained from samples taken adjacent to the sampling location for the earlier histological analysis [12]. Collagen sampling followed Longin [50], Brown et al. [51], and Richards and Hedges [52] and samples

[1] Appropriate ethical guidelines and codes of practice for the analysis of human skeletal remains from archaeological contexts were followed, including the code of ethics of the American Association of Physical Anthropologists (2003), the British Association of Biological Anthropology and Osteoarchaeology code of practice, and Mays S, Elders J, Humphrey L, White W, Marshall P. Science and the dead: guidelines for the destructive sampling of archaeological human remains for scientific analysis. Advisory Panel on the Archaeology of Burials in Britain, English Heritage; 2013.

Table 2.1 Descriptive δ^{15}N (‰) data and published OPD data [12] sub-divided by SES, sex, and age at death groupings in the medieval Canterbury sample

Sub-group	Variable	*N*	Min.	Max.	Mean	SD.
Entire sample	δ^{15}N (‰)	22	10.8	14.0	12.5	0.71
	OPD (mm^2)		14.42	30.36	20.86	4.61
Priory (high SES)	$\underline{\delta}^{15}$N (‰)	15	11.6	14.0	12.7	0.64
	OPD (mm^2)		14.84	27.23	20.78	4.36
Cemetery (low SES)	δ^{15}N (‰)	7	10.8	12.4	11.9	0.57
	OPD (mm^2)		14.42	30.36	21.05	5.48
Females	δ^{15}N (‰)	6	11.6	13.0	12.7	0.57
	OPD (mm^2)		14.88	19.20	17.94	1.61
Males	δ^{15}N (‰)	15	10.8	14.0	12.3	0.73
	OPD (mm^2)		14.42	30.36	21.64	4.92
Young adults	δ^{15}N (‰)	2	11.6	11.9	11.8	0.25
	OPD (mm^2)		19.20	27.23	23.21	5.68
Middle adults	δ^{15}N (‰)	15	10.8	14.0	12.5	0.75
	OPD (mm^2)		14.42	30.36	20.31	4.68
Old adults	δ^{15}N (‰)	3	11.8	12.9	12.5	0.49
	OPD (mm^2)		16.41	26.34	20.28	4.31

Sex and age at death were estimated following standard anthropological procedures [49]

were combusted into CO_2 and N_2 in an isotope-ratio mass spectrometer. The resulting δ^{15}N data were compared between high and low SES using a non-parametric Mann-Whitney *U* test.

2.3.3 Results

The high SES Priory individuals had a significantly higher average δ^{15}N (mean = 12.8‰, SD = 0.753) when compared to the lower SES individuals from the cemetery (mean = 11.7‰, SD = 0.932) ($p = 0.002$) (Table 2.1). These differences in δ^{15}N are consistent with OPD values presented previously for this sample [12].

2.3.4 Discussion and Conclusions

Our δ^{15}N results are consistent with the 2016 [12] suggestion that higher OPD may have been driven by increased protein consumption at the Priory site. The combined results of these histological and isotope studies suggest it is likely that dietary protein intake plays a role in femoral bone microstructure, as inferred using OPD, which is an established marker of bone health and remodelling and an accepted proxy for studying bone density [45]. In the clinical realm, it has been suggested that high protein consumption, when combined with regular calcium or dairy ingestion, corresponds with healthier bone [53]. Without environmental baseline data for this region, we cannot identify sources of protein in medieval Canterbury. Instead,

they may only be used comparatively, and only within this population, but can still accurately represent varying amounts of dietary protein between individuals of the same temporal period and geographical location. Having a high protein diet is beneficial for bone health, while excessive protein is detrimental, but the point at which this shift occurs remains debated [54–56]. The results agree with textual records implying high protein consumption at the Priory [37, 42]. Similar outcomes were found in ninth to eleventh century Bohemia (Czech Republic), where elite ducal families fed on highly varied diets, whereas lower class groups ate largely millet-based foods [48]. This preliminary analysis invites future research testing stable isotope variation at the Canterbury site, accounting for limitations in our study which included the effects of physical strain on the posterior femur, small and uneven demographic selection, and limited age controls. We provide some insight into bone health during the late medieval period in Britain from two SES groups of significantly different levels of dietary protein.

2.4 Stress, Weaning, and SES in Medieval Canterbury: Insights from Dental Histology

Human tooth enamel is formed by secretory ameloblasts. These cells commence enamel secretion early in the second trimester in deciduous central incisors, and this stage of enamel formation is typically complete in permanent third molars by 10 or 11 years of age. Unlike human bone, enamel does not remodel and retains a permanent record of its own development. As such, teeth can be used to address some questions about childhood lifestyles in past human societies [57].

2.4.1 Brief Background

Weaning age can provide insights into the diet, health, and social structure of present and past human populations [58–61]. Weaning typically involves a period of dietary transition that includes both maternal milk and additional foods ("mixed-feeding") followed by cessation of suckling [62]. Mixed-feeding can sometimes lead to a period of physiological stress. While it may provide the rapidly growing infant with micronutrients as well as other foods that are higher in protein and calories than provided by maternal milk alone, it can also produce a dilemma [63, 64]. The growing requirement for supplemental foods brings increased risk of illness through food-borne pathogens and intestinal parasites at the same time that immunological support provided by maternal milk is reduced [65–69].

Disruptions to secretory ameloblasts can lead to enamel defects that become visible as dark bands at magnification under polarised light in longitudinal thin sections [70–73]. These microscopic accentuated markings—also known as Wilson bands [74]—can lead to abnormally structured enamel crystals and rods that can be less mineralised than surrounding enamel [75]. The markings are signs of non-specific age-related episodes of physiological stress that develop in response to

several causes. One of these causes is the physiological stress that can sometimes occur during weaning [62], which has been related to accentuated markings in primates [76–79]. Based upon these types of correlations, studies have inferred dietary weaning age in archaeological samples of modern humans [72, 80].

Whether weaning stressors varied with social inequality in the medieval period in England has not been determined. Neither is it know if weaning age was determined by status, though contemporary textual accounts indicate that it might have commenced earlier in poorer sectors of urban and rural society [81, 82]. This short study assesses the frequency of accentuated markings in thin sections of teeth from young children (birth to 2 years of age) recovered from St Gregory's Priory and cemetery in Canterbury. Results are related to medieval textual evidence for weaning. Comparisons are undertaken between high and low SES children.

2.4.2 Materials and Methods

Deciduous second (dM2) and permanent first molars (M1) were selected from $n = 43$ skeletons recovered from St Gregory's Priory ($n = 10$) and the associated cemetery ($n = 33$)[2]. These molars were selected because the period over which the enamel formed represented the period between birth and 12 months of age ($n = 43$: dM2 + M1) and between 1 and 2 years of age ($n = 14$: just M1). Birth was identified from the neonatal line.

Thin sections for dM2 and M1 were reused from studies of enamel development and thickness [83, 84]. Each section was examined under a high-powered microscope (Olympus BX53) at a magnification of 10–40× using polarised light. Images were captured with a digital microscope camera (Olympus DP74) and analysed using Olympus cellSens software. The distance between the neonatal line in cuspal enamel and the next accentuated line was measured along the long axis of a prism. Either cross striations were counted along the prism, or the distance was divided by a local mean daily enamel secretion rate (DSR) to estimate the number of days elapsed between birth and the accentuated marking. The procedure was repeated on subsequent markings to establish a chronology of stress events. The time elapsed between accentuated markings in lateral enamel was estimated from prism lengths divided by DSRs [85]. Accentuated markings were distinguished from Retzius lines by their broader width, extended length, and atypical rod structure [86]. The daily occurrence of accentuated markings was recalculated into prevalence in monthly intervals [72, 87].

2.4.3 Results

There were two peaks in the prevalence of accentuated markings (Table 2.2, Fig. 2.1a). When all of the children were considered together, markings gradually

[2] See footnote 1.

Table 2.2 Prevalence of dental accentuated markings in children from medieval Canterbury ([1]month, [2]count, [3]prevalence)

Birth to 1 year (n = 43)			1–2 years (n = 14)		
M^1	C^2	P^3	M^1	C^2	P^3
1	5	11.62	13	4	28.57
2	10	23.25	14	7	50.00
3	12	27.90	15	7	50.00
4	14	32.55	16	6	42.85
5	17	39.53	17	3	21.42
6	19	44.18	18	2	14.28
7	19	44.18	19	2	14.28
8	16	37.20	20	1	7.14
9	13	30.23	21	1	7.14
10	12	27.90	22	2	14.28
11	12	27.90	23	1	7.14
12	7	16.27	24	1	7.14

increased from birth, peaking between the fifth and seventh month, followed by a gradual reduction through the remainder of the first year. A second sharper peak occurred between months 15 and 16 that included 53% of the children. When high- and low-status children were separated into two groups and compared, there was higher prevalence of markings for the high-status children between 2 and 8 months of age (Fig. 2.1b).

2.4.4 Discussion and Conclusions

The peak period of stress in the year after birth did not coincide with mortality for this sample of children. This period of stress occurred when the children were aged between 5 and 7 months. Yet, all of the children survived their first year as their crown enamel had formed, which takes about 13 months in dM2 and at least two and half years in M1. Thus, the trajectory of accentuated markings during the first year does not suggest a biased sample of "sickly" children.

The trajectory of accentuated markings in the year after birth may reflect the infant immune response to mixed-feeding. Even though the infant immune system has developed to approximately half the level seen among adults by around 6 months of age [88], it is still an immature system. As such, the introduction of new foods during mixed-feeding produces a period of intense immunological activity as an array of new antigens initiate a 'first response' of T and B cells [68]. For example, first consumption of wheat can stimulate celiac disease (diarrhoea, weight loss, stomach pains) which occurs when the immune system responds abnormally to the protein gluten. The disease has been related to deciduous enamel defects and structural changes to enamel rods [89]. While this disorder would likely have been limited to a small proportion of infants, foods can also introduce pathogens [62], and this would be compounded if food preparation or feeding methods were unsanitary

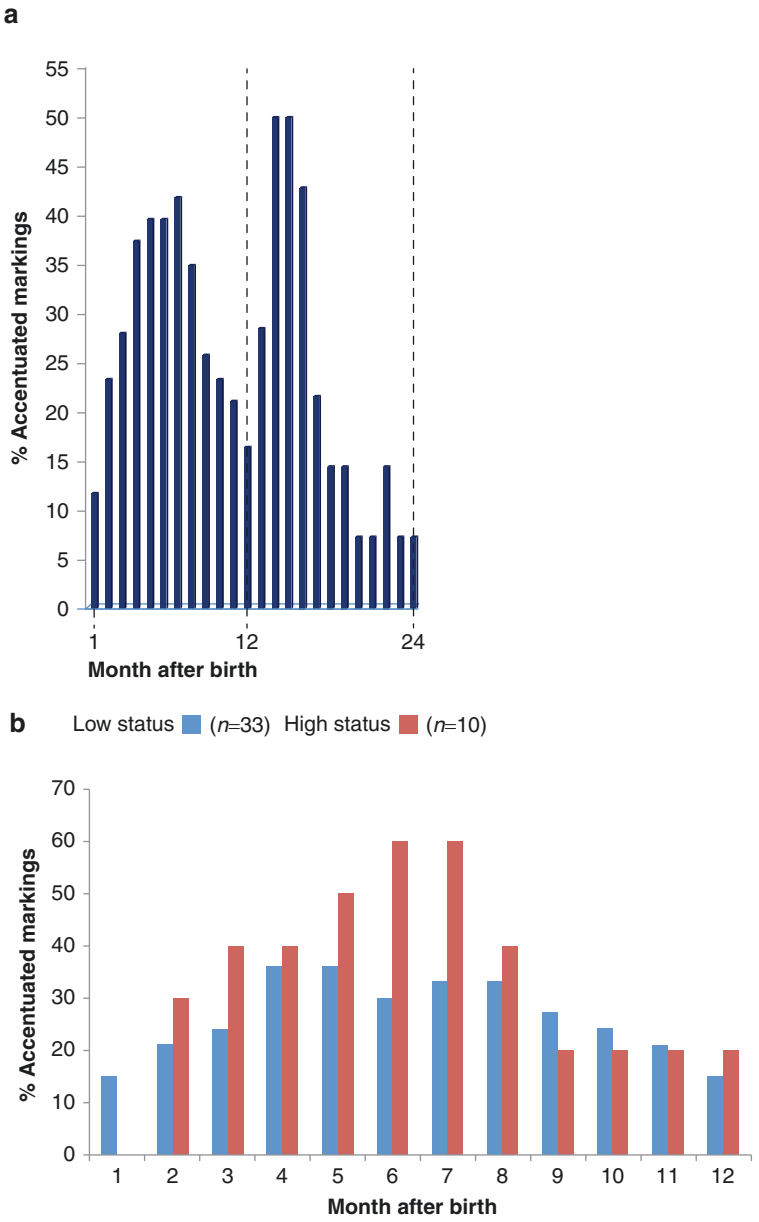

Fig. 2.1 Accentuated markings (**a**) for children aged 1 and 2 years of age and (**b**) for high and low status children in the year after birth

[81]. Rags soaked in milk for a child to suck [90], as well as the increasingly unsanitary conditions in the growing towns of medieval England [13], could have promoted the "weaning illness" described by medieval physicians [91]. If this idea is placed alongside the trajectory of accentuated markings in the first year (Fig. 2.1), then the peak prevalence between 5 and 7 months of age may signify the period when the majority of infants had commenced mixed-feeding. That infants might have started mixed-feeding at this age is supported by writers from the period [91] and also by the presence of microwear in children from the age of 1 year [92].

The prevalence of stress markings differed between high- and low-status infants (see Fig. 2.1b). The higher prevalence of markings for high-status children between 2 and 8 months of age suggests they may have experienced a period of greater stress relative to the lower-status children. This difference between the groups may however simply relate to differences in sample sizes. Furthermore, the trajectories of accentuated markings throughout the first year were broadly similar for both groups, with the highest prevalence of markings occurring between the age of 4 and 8 months for high-status children and between the age of 5 and 7 months for those of lower status. These findings do not suggest that mixed-feeding age varied greatly between socio-economic groups [81, 82], though it may have led to a period of relatively greater stress for the higher-status children. These data lend support to the idea that the relationship between socio-economic status and aspects of diet for children in medieval England might not be as clear as it is for adults [92].

A sharp increase in markings occurred around 14–15 months of age, which differs to the more gradual rise and fall in markings seen during the year after birth. If the peak period of stress in the second year marks the end of mixed-feeding when breast milk was finally removed [62], leading to the "weaning illness" [81] and increased markings [72], then the dietary change was a rapid one. Thus, the two episodes of relatively greater stress in the first and second year after birth may relate to an immature immune system and mixed-feeding, followed by the removal of breast milk, respectively. The reduction in accentuated markings between the two peaks may signify the strengthened immunity of the child, as the period of the "first response" to some antigens and pathogens passes. The presence of microwear in children age between 1 and 2 years of age suggests that mixed-feeding, at least, had commenced for some children by the start of their first year [92]. If breast-feeding was completely removed from the diet of the Canterbury children at or around the start of the second year after birth, then this would lie within the lower end of the age range recommended for weaning in texts from the period [91]. It would also lie within the lowermost end of the weaning age range indicated by isotopic studies at contemporary Wharram Percy in the north, where breast-feeding ceased between 1 and 2 years of age [93]. However, the presence of microwear in the youngest of infants at Canterbury, aged 1–1.5 years, indicates that weaning might have occurred slightly earlier compared to children from Fishergate House in the north, where breast-feeding continued until 1.5 years of age [94].

2.5 Conclusions

This chapter reviewed published bioarchaeological evidence for the effect of SES on medieval skeletons retrieved from a range of European archaeological sites. We showed that bone from both low and high SES individuals reflected their social positioning, with poorer and greater adult bone quality, respectively. However, we also emphasised the complexity of SES and skeletal health interpretations, whereby high SES groups in some medieval locations led unhealthy lifestyles that included overeating and alcohol consumption and potentially associated development of bone metabolic disorders. We also reported two new analyses investigating SES-specific adult diet and childhood stress and weaning age in a medieval population from Canterbury, UK. We firstly showed that bone collagen stable isotope signature in higher SES adults from an eleventh to sixteenth century Priory implied increased protein in diet when compared to a low SES group from an adjacent cemetery. This supports the disparity between low and high SES groups in access to balanced nutrition. Secondly, we reconstructed episodes of childhood stress related to SES and weaning age from medieval children's teeth at the same archaeological site. We reported that the prevalence of histological stress markings differed between high and low SES infants, with high SES children between 2 and 8 months old likely experiencing greater stress. This analysis further supported the relationship between SES and skeletal health but demonstrated that it is not as straightforward as it is for adults.

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Ancient Human Bone Microstructure Case Studies from Medieval England

3

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3.1 Introduction

The reconstruction of human health in past populations can be successfully undertaken by analysing their surviving skeletal remains that derive from archaeological and historical contexts [1]. Outside of standard gross anatomical examination techniques that include recording the morphology and morphometry of different skeletal parts (see Chap. 2), mapping bone histological variation can contribute a more in-depth understanding of skeletal metabolic activity in past humans [2, 3]. While ancient samples cannot be studied using dynamic or experimental bone histology, multiple studies have shown that, preservation permitting, static histology can successfully yield data about cortical bone density and its geometric properties [2–5]. Many different research themes of past human bone histology have been covered to date, including ageing [6], sex-specific division of labour [7], lifestyle [8] and osteoporosis [9]. However, little research has been done using samples that derive from

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distinct socio-economic status (SES) groupings. In 2016 [3], two of our team reported the first ever large sample-based femoral bone microstructural patterns comparing low and high SES medieval English groups of adults (also see Chap. 2). We showed that low SES skeletons of adult males and females developed poorer bone health when compared to higher SES individuals. In an earlier 2012 study [10], Miszkiewicz reported differences between the low and high SES groups in their dental health and associated longevity. It was inferred that more prevalent childhood physiological disruption events and higher mortality may have characterised lower SES medieval individuals. As bone and dental enamel tissues respond to external and internal biological and environmental factors [11–14], an analysis of their association can elucidate how and if adult bone quality and quantity accounts for early ontogenetic developmental disturbances. This is the aim of the first part of this chapter.

In the second part of this chapter, we report on the relationships between cortical bone dimensions and histomorphometry of the medieval human radius. Bone histomorphometry can be influenced by age [6], sex [7], lifestyle [8] and pathology [9], but the potential links between bone size and histomorphometry have received less attention in the literature. Previous studies have reported correlations between the size of femoral cortical bone and the size and frequency of osteons [15]. The aim of the second part of this chapter is to examine the potential link between cortical dimensions of the medieval human radius and the underlying histology.

In both cases, we use samples[1] taken from a large skeletal St. Gregory's Priory and associated cemetery collection dated to eleventh to sixteenth centuries, curated at the University of Kent (Canterbury, UK) [16, 17]. This is a well-preserved collection of medieval human skeletons that has been studied over the past few decades revealing their general health and disease [10, 16, 18], as well as, more recently, bone histological variation during ontogeny [4], basics of adult bone biology and biomechanics [15, 19], lifestyles [3] and human dental and bone biorhythms [20, 21]. The basic biological affinities of each individual in the collection are estimated following standard anthropological methods that determine biological sex and age-at-death based on a series of pelvic, cranial, dental and postcranial anatomical characteristics [22].

As supported by archaeological and historical records outlined below, there is evidence that this site was split into two distinct regions of burial used for low (i.e., peasants buried in the associated cemetery) and high SES (i.e., the wealthy and clergy members of the society buried in the Priory) [23, 24]. Three key lines of evidence for this SES divide can be identified. Firstly, the basic demographic distribution of the recovered skeletons indicates that the Priory was designated both for

[1] Appropriate ethical guidelines and codes of practice for the analysis of human skeletal remains from archaeological contexts were followed, including the code of ethics of the American Association of Physical Anthropologists (2003), the British Association of Biological Anthropology and Osteoarchaeology code of practice and Mays S, Elders J, Humphrey L, White W, Marshall P. Science and the dead: guidelines for the destructive sampling of archaeological human remains for scientific analysis. Advisory Panel on the Archaeology of Burials in Britain, English Heritage; 2013.

monastic and secular individuals, with the latter securing high SES burial through donations [17, 24]. An excavation of 21 subadults and a number of female skeletons at the Priory, alongside an overwhelming presence of male skeletons [24], confirms a combined ecclesiastical and secular high SES community. Several excavated graves were located inside the Priory, with male skeletons unearthed in the Priory's resonance chambers and the chapter house [17]. Initial archaeological reports [24] hinted the cemetery skeletons represented a population with high mortality at a young age, which was subsequently confirmed [10] with lower average age-at-death estimates in the cemetery (39.8 years) when compared to the Priory (44.1 years). Secondly, oral health assessment indicated high prevalence of dental caries in the Priory samples [17] suggesting an increased carbohydrate consumption, also associated with medieval cloisters in other parts of the UK [25]. Thirdly, elaborate grave goods uncovered in the Priory point to high social standing of those buried in the Priory. For example, one adult male was excavated with a chalice and a gold-embroidered garment, with Hicks and Hicks [17] speculating this may have been the skeleton of Prior Alured (1146–1470 AD). The difference in lifestyles and daily occupations between low and high SES in the medieval English period is well established [26] (also see Chaps. 1 and 2 in this volume).

3.2 Medieval Individuals from High SES Backgrounds Achieve Higher Bone Density Despite Experiences of Childhood Developmental Disruption

Based on prior observations, in past and living populations, that low SES has an adverse effect on multiple human (bone) health variables (see Chap. 1 for theoretical background and Parts 2 and 3 in this volume) [3, 10, 27, 28], we hypothesised that being born into, and growing up in a high medieval SES context, should have a positive effect on adult bone density. We test this hypothesis by studying proxy variables for adult bone density from the human femur and dental indicators of childhood non-specific physiological disruption in $n = 17$ adult individuals from medieval Canterbury for whom these matched data are available.

3.2.1 Brief Background

Linear enamel hypoplasia (LEH) is a dental condition that manifests as a decrease in enamel density on the external surfaces of the teeth [29] (Fig. 3.1). It is thought that it occurs following a disruption to the formation of enamel during childhood while permanent teeth are developing [30]. Because enamel formation is a tightly controlled process, a temporary reduction in enamel thickness and shortened enamel prisms are a consequence of systemic or localised disturbance to its development [31]. It is impossible to reconstruct the exact aetiological factors underlying the formation of LEH in ancient human samples; thus LEH can be used as a permeant representation of non-specific childhood physiological "stress" [32]. This "stress" can take many forms of interpretation depending on

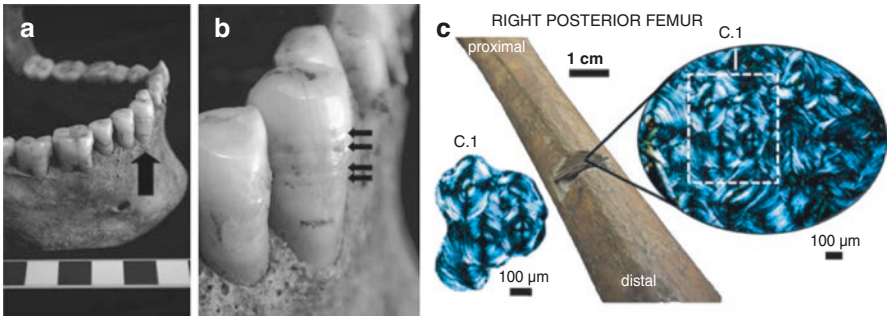

Fig. 3.1 Mandibular anterior dentition (**a**, **b**) and right posterior femur (**c**) from the Canterbury collection. Arrows in images (**a**, **b**) mark linear enamel hypoplastic lines expressed on the labial dental surface of a canine. These indicate several episodes of enamel development disruption during childhood. Image C shows the histology sampling location previously reported in [3, 18, 19] and associated histological appearance under linearly polarised light. The C.1 image gives an example of secondary osteons showing intact and fragmentary remnants of bone remodelling. Images (**a**, **b**) are reprinted with permissions from John Wiley and Sons [10]

the population context, but there is evidence that LEH can form in response to malnutrition [33], systemic illness and pathogens [11], weaning [34] and consumption of toxins [35]. The aetiology of LEH and some causal links made to lifestyle factors in anthropological research remain debated [35–38], but it is clear that studying LEH in adults indicates developmental disturbance recovery with the sufferer surviving a period or series of physiological disruption events [38]. As per the 2012 LEH study in medieval Canterbury [10], low SES adults show an average of 17.6 LEH lines when compared to 7.9 LEH lines in the high SES group. Not only do these data support the SES divide in this population, they also demonstrate that high SES individuals were not shielded from childhood physiological stress.

To assess the relationship between childhood developmental issues and adult bone health, we turn to secondary osteon population density (OPD) collected from femoral samples in this medieval population. As the bone is a living tissue that remodels through the processes of targeted and nontargeted remodelling [39], we can reconstruct basic bone density information by recording secondary products of bone remodelling from cortical bone histology [40]. The basic multicellular unit (BMU) of coupled osteoblasts and osteoclasts remodels bone by resorbing and depositing new tissue, either during an equilibrium or bone balance phase or in response to mechanical load, diet and disease [12]. As BMUs dig cortical "tunnels," they leave behind osteon structures composed of lamellae with a central Haversian canal that delivers blood supply to bone tissue (Fig. 3.1). The sum of osteons per area of examined section is a representation of the accumulated osteons and is informative of bone remodelling rates experience at a given bone site [40].

Table 3.1 Descriptive linear enamel hypoplasia (LEH) [10] and osteon population density [3] data subdivided by SES, sex and age-at-death groupings in the whole sample

SES	Sex	Age-at-death	Dental and bone data	*N*	Min.	Max.	Mean	SD
Low (most disadvantaged)	Female	Young	LEH	1	2.00	2.00	2.00	–
			OPD	1	17.86	17.86	17.86	–
		Middle-aged	LEH	1	6.00	6.00	6.00	–
			OPD	1	15.41	15.41	15.41	–
	Male	Middle-aged	LEH	8	6.00	32.00	16.75	10.14
			OPD	8	14.62	22.84	17.91	2.43
High	Female	Young	LEH	1	11.00	11.00	11.00	–
			OPD	1	26.79	26.79	26.79	–
		Middle-aged	LEH	2	4.00	6.00	5.00	1.41
			OPD	2	17.63	18.86	18.25	0.87
	Female	Middle-aged	LEH	4	2.00	30.00	12.75	12.04
			OPD	4	14.73	26.34	21.45	4.88
			Total *n*	17				

Sex and age-at-death were estimated following standard anthropological procedures [22]

3.2.2 Sample and Methods

We were able to match and extract previously collected and published LEH and OPD data for a set of $n = 10$ low SES (cemetery) and $n = 7$ high SES (Priory) adults [3, 10] (Table 3.1). The raw LEH data [10] derive from unworn anterior maxillary and mandibular permanent incisors and canines and represent total frequencies. These were collected using the "field method" approach, which scans labial tooth surfaces for macroscopic presence of LEH [41]. The OPD data are from subperiosteal posterior cortical mid-shaft femur regions collected from thin sections as reported in [3, 15, 19]. The OPD values were calculated as the sum of intact osteon (N.On) and fragmentary osteon (N.On.Fg) densities (Fig. 3.1) per section area (2.24 mm^2) from a maximum of six regions of interest (ROIs). Given the limited sample size, we correlated individual LEH and OPD data points using non-parametric Spearman's Rho tests.

3.2.3 Results

A highly significant, strongly positive correlation was found between OPD and LEH in the high SES group only (Rho = 0.929, $p = 0.003$, $n = 7$) (Fig. 3.2). When considering the low SES group, no relationship as such was identified (Rho = 0.227, $p = 0.528$, $n = 10$). For the former correlation, it was clear that there was an associated increase in OPD alongside LEH (Fig. 3.2), with individuals of more dental disturbances also showing higher bone density. We pooled sexes within each SES

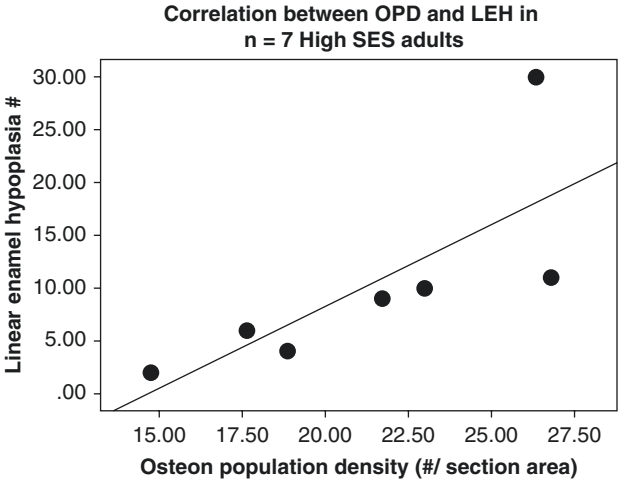

Fig. 3.2 A correlation between total linear enamel hypoplasia (LEH) frequencies of the anterior dentition and associated osteon population density (OPD) in seven individuals deriving from a high medieval SES group ($r = 0.929$, $p = 0.003$)

group for the above analyses due to the small sample size. Once males of both high and low SES were examined ($n = 14$), there was no effect of sex on LEH (Rho = 0.248, $p = 0.393$).

3.2.4 Discussion and Conclusions

These preliminary results demonstrate that an increase in LEH is associated with an increase in OPD in individuals from high SES backgrounds. This may indicate that developmental disturbances that arise from physiological health issues in high SES children are accounted for by developing a robust or "stronger" skeleton in adulthood. The lack of significant relationships between LEH and OPD in the low SES group suggests that malnutrition and/or general physiological ill health leave a mark on the adult skeleton in the form of poor bone health. Our data support previous studies utilising this human sample where significant differences in mortality [10] and bone remodelling were observed [3], with the low SES individuals living less long and presenting with bone loss in adulthood. We propose a preliminary skeletal growth model within a medieval Canterbury SES context, whereby advantageous SES backgrounds may facilitate healthy bone accrual later in ontogeny (see further theory in Chap. 1).

Considering medieval historical records, skeletons recovered from the Priory would have included members of the clergy and lay wealthy noblemen who would have had access to good-quality nutrition, undertaken less physically demanding occupations and led lifestyles within more secure environments [26, 42]. On the contrary, individuals buried in the associated cemetery represent the sick and poor

locals of medieval Canterbury [43], who would have fed on simple and less nutritious diets, undertaken more strenuous occupations and not been shielded from daily stress and insecurities [44]. Of course, human behaviour is complex, and so it is plausible that a mosaic of dietary and lifestyle factors played a role in skeletal growth within each SES group. For example, Polish peasants (eleventh and twelfth century Giecz) are reported to have consumed an animal meat-rich diet [45] challenging the idea of simple dietary regimes in lower medieval European classes. The multiple LEH events recorded in the Priory samples in our study agree with previous research where higher SES groups are affected by developmental disturbances [46]. As much as our results support the notion that higher SES skeletons grow higher density bone in adulthood, they cannot strictly imply that people of privileged backgrounds were shielded of infections or other disease. One must consider poor sanitation and hygiene, overcrowding and limited health care in the medieval past, which would have made the spread of infections and epidemics easier [47].

Bone biology principles suggest that our results may demonstrate an example of a skeletal growth catch up in the high SES group. Developing bone adapts to periods of growth disruption and has a well-functioning recovery system in place [48, 49]. However, where children develop in contexts of poor prenatal care and nutrition, early weaning, and high loads of pathogens, skeletal growth is compromised, and height can become stunted [50]. This indicates that the early conditioning of bone quality and quantity in our low SES group did not lead to bone density catch up in adulthood. We acknowledge the difficulty in extrapolating individual lifestyle factors underlying our results, alongside limitations that include genetically and mechanically driven bone functional adaptation [12], LEH accrual rate and small sample size. However, these preliminary results invite future research investigating our hypothesis using medieval juvenile skeletal data.

3.3 Histomorphometry of Cortical Bone in the Human Radius

Many intrinsic and extrinsic factors can influence cortical bone histomorphometry including age, sex, body weight, biomechanical loading, economic and nutritional status, as well as pathology [3, 51–58]. This study examines the relationship between cortical bone dimensions and the histomorphometry of the human radius—the bone that is commonly injured in modern osteoporotic patients [59]. Dimensions from the entire cross-section of seven adult radii were assessed against osteon population density and the size of osteons and Haversian canals to reveal insights into medieval bone growth.

3.3.1 Brief Background

Few studies have evaluated the histomorphometry of the entire cross-section of a bone. Gocha and Agnew [60] examined OPD across the mid-shaft of the femur. Not

only did the authors find a difference between the periosteal, mid-cortex and endosteal thirds, they also discovered a lack of consistency in OPD distribution [60]. Different patterns were observed across almost every ROI possibly in relation to levels and types of strain. Other studies have demonstrated relationships between femoral cortical size and histomorphometric variation [3, 19]. Two of our team [15] measured cortical thickness (Ct.Wi) of the mid-shaft of the femur and revealed a significant positive correlation between Ct.Wi and OPD. A significant negative correlation was found between Ct.Wi and osteon area (On.Ar), Haversian canal area (H.Ar) and Haversian canal diameter (H.Dm) [15]. This indicated that changes in bone remodelling and the resulting secondary osteons may be linked to femoral cortical width and robusticity [15]. The aim here is to assess the entire cross-section of the mid-shaft of the radius—by dividing it into 32 ROIs—to determine if there is a structural relationship between cortical bone size and histomorphometric variation in the mid-shaft of the human radius. These relationships will be sought in the periosteal, mid-cortex and endosteal regions.

3.3.2 Sample and Methods

The bone sections were from $n = 7$ age-matched individuals deriving from the aforementioned medieval eleventh to sixteenth centuries Canterbury collection [2]. Age-at-death and sex were estimated following standard methods [22]. Only one cross-section was incomplete, with the posterior and posterolateral sections missing, removing a potential eight ROIs. However, the rest of the section was intact and clear under the microscope meaning that 24 ROIs were still usable, as such this fragmented sample was included. This meant that while samples were obtained from $n = 7$ individuals, statistical tests varied in sample size when subdividing by area of the cross-section ($n = 54$) (Table 3.2); examining all ROIs using Ct.Ar had a much larger sample size ($n = 222$), which varied only slightly when examining Ct.Wi ($n = 216$) as average width was not measured for the entire cross-sections, only smaller ROIs.

The samples came from individuals of the same age range (25–35 years old, $n = 4$ females, $n = 3$ males) ensuring that age, which has a much more significant influence on bone histomorphometry than sex [54], was accounted for in analyses. These bone samples had been prepared for a previous study [61] using standard histological methods. Thick sections were removed from the mid-shaft of the radius, embedded in resin and using a precision saw were further reduced to 0.3 ± 0.1 cm. The sections were then mounted onto glass slides and further grounded to a final thickness of 50–100 μm and then polished to ensure that the histological features were clearly visible under the microscope [4, 15, 19, 61].

Images of the whole cross-sectional area of each radius were captured at 10× magnification using a BX53 Olympus microscope and associated camera. The background of each image was removed using Affinity Photo software (v 1.6.5) to ensure that measurements focused only on the dimensions of the radius. The image was compared to the original slide and the radius from which it had been taken to

Table 3.2 Descriptive statistics values of which represent mean and SD for averages of variables measured as subdivided into areas of the radius cross-section

	Octants	Endosteal sections	Mid-cortex sections	Periosteal sections
Variable measured	(n = 54)			
Cortical area (Ct.Ar)	11.104 (4.532)	2.602 (1.007)	3.631 (1.476)	4.902 (2.149)
Cortical width (Ct. Wi)	3.187 (1.174)	1.062 (0.391)	1.062 (0.391)	1.062 (0.391)
Intact osteon density (N.On)	10.501 (2.113)	7.220 (2.662)	10.740 (2.699)	12.129 (3.039)
Fragmentary osteon density (N.On.Fg)	2.680 (1.213)	2.364 (1.501)	3.056 (1.626)	2.585 (1.384)
Osteon population density (OPD)	13.181 (2.913)	9.583 (3.644)	13.796 (3.778)	14.714 (3.837)
Osteon area (On.Ar)	36969.934 (7661.622)	38595.49 (13283.449)	38154.881 (10062.025)	34597.839 (11331.311)
Osteon diameter (On.Dm)	186.35 (20.463)	192.072 (34.44)	190.826 (25.452)	177.622 (29.869)
Canal area (H.Ar)	2937.160 (864.934)	3565.179 (2011.747)	3137.497 (1122.386)	2417.98 (834.573)
Canal diameter (H. Dm)	51.237 (7.843)	55.85 (16.467)	53.472 (10.081)	46.82 (8.39)

ensure that directional labels were correctly assigned. The image was then rotated so that the medial and lateral regions were situated horizontally. This made it easier to methodically number each image, allowing the ROIs of each section to correspond uniformly.

The moment-macro plugin (v 1.3) was used in ImageJ (v1.46) software to find and mark the exact centroid of the section [60]. An eight prong spoke axis was then overlaid onto the image using Affinity Photo software, with the marked centroid providing the central point. This divided the section into octants—medial, posteromedial, posterior, posterolateral, lateral, anterolateral, anterior and anteromedial. The eight regions were subdivided into three areas using the cellSens Standard software. These three represented the periosteal, mid-cortex and endosteal regions of each octant (Fig. 3.3). This was done by measuring the length of the bone along each spoke prong and dividing it into three. The same was done through the middle of the octant to ensure a more even split for the area of the thirds. The measurement through the centre of the octant also provided the measurement for the width of each ROI. This method was based on that implemented by Gocha and Agnew [60] in their evaluation of OPD through the cross-section of the femoral mid-shaft. Other projects have also used a similar methodology for cross-section division [62–66].

3.3.2.1 Image Analysis

Using cellSens Standard software, the total area and medullary area were measured first. The cortical area was calculated by subtracting the medullary area from the

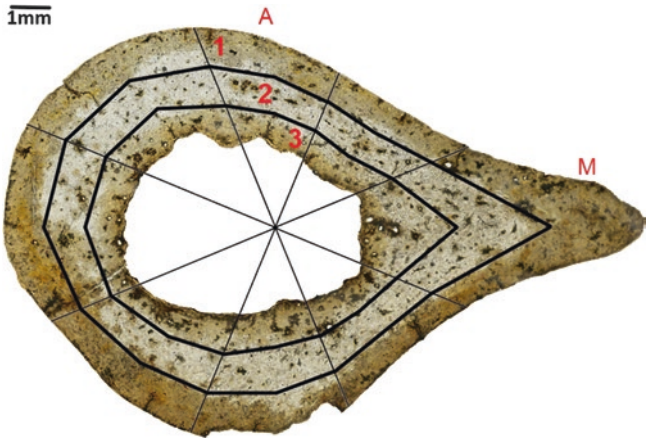

Fig. 3.3 The entire cross-section of a radius (A, anterior; M, medial) divided into sections, showing octants, and further subdivision into (1) periosteal, (2) mid-cortex and (3) endosteal regions

total area. The area and perimeter of each ROI was measured, including each octant and the three sections within. Second, the intact secondary osteons and osteon fragments were marked and counted to ascertain the OPD of each ROI. An osteon was considered a fragment if more than 10% of the Haversian canal had been remodelled by the development of a subsequent secondary osteon, as is consistent with previous studies [4, 67].

Next, the following variables were measured or calculated:

1. OPD, number of intact secondary osteons and fragmented secondary osteons as defined by the above criteria divided by area of ROI
2. On.Ar, average osteon area measured in μm^2 using the measurements taken of all viable osteons within the ROI, excluding those with unclear cement lines or irregular shape
3. H.Ar, average Haversian canal area measured in μm^2 using the measurements taken of all viable canals within the ROI, excluding those with unclear outlines or irregular shape
4. On.Dm, average osteon diameter measured in μm^2 by measuring the shortest diameter from edge to edge of all viable osteons within the ROI (Fig. 3.3)
5. H.Dm, average Haversian canal diameter measured in μm^2 by measuring the shortest diameter from edge to edge of all viable canals within the ROI

All abbreviations used follow the standard nomenclature as reported by Dempster et al. [68].

3.3.2.2 Statistical Analysis

A total of 90 linear regressions were performed. This allowed for the relationship between the dimensions of the cortical bone and every histomorphometric variable

Table 3.3 Results of linear regressions of different radius bone cross-section segments evaluating relationships between a series of histological and cortical measurements

Variables	Region of interest	*N*	Intercept	Slope	*R*	*P*	Residual
Ct.Ar and OPD	All sections	222	1.081	0.009	0.023	0.729	99.951
	Octants	**54**	**1.373**	**−0.260**	**−0.527**	**0.000**	**72.315**
	Endosteal	**54**	**1.066**	**−0.287**	**−0.318**	**0.019**	**89.912**
	Mid-cortex	**54**	**1.273**	**−0.280**	**−0.484**	**0.000**	**76.563**
	Periosteal	**54**	**1.321**	**−0.258**	**−0.457**	**0.001**	**79.098**
Ct.Wi and OPD	All sections	216	1.094	−0.066	−0.123	0.071	98.481
	Octants	**54**	**1.263**	**−0.319**	**−0.553**	**0.000**	**69.451**
	Endosteal	54	0.956	−0.160	0.168	0.225	97.193
	Mid-cortex	**54**	**1.126**	**−0.334**	**−0.494**	**0.000**	**75.521**
	Periosteal	**54**	**1.153**	**−0.424**	**−0.583**	**0.000**	**66.015**
Ct.Ar and On.Ar	All sections	222	4.546	0.003	0.008	0.907	100
	Octants	**54**	**4.415**	**0.143**	**0.300**	**0.027**	**91.026**
	Endosteal	54	4.509	0.132	0.139	0.318	98.131
	Mid-cortex	54	4.526	0.079	0.126	0.366	98.390
	Periosteal	**54**	**4.365**	**0.236**	**0.343**	**0.011**	**88.235**
Ct.Wi and On.Ar	All sections	**216**	**4.541**	**0.089**	**0.182**	**0.007**	**96.702**
	Octants	**54**	**4.459**	**0.211**	**0.379**	**0.005**	**85.641**
	Endosteal	54	4.560	0.190	0.188	0.173	96.495
	Mid-cortex	54	4.567	0.137	0.187	0.177	96.486
	Periosteal	**54**	**4.518**	**0.303**	**0.342**	**0.011**	**88.337**
Ct.Ar and On. Dm	All sections	222	2.265	−0.001	−0.004	0.955	100
	Octants	**54**	**2.182**	**0.085**	**0.328**	**0.016**	**88.696**
	Endosteal	54	2.236	0.106	0.217	0.115	95.266
	Mid-cortex	54	2.261	0.030	0.094	0.498	99.432
	Periosteal	**54**	**2.161**	**0.128**	**0.357**	**0.008**	**87.313**
Ct.Wi and On. Dm	All sections	222	2.262	0.034	0.131	0.054	98.291
	Octants	**54**	**2.226**	**0.088**	**0.293**	**0.032**	**91.304**
	Endosteal	54	2.276	0.092	0.178	0.197	96.746
	Mid-cortex	54	2.277	0.028	0.075	0.590	99.432
	Periosteal	**54**	**2.244**	**0.139**	**0.301**	**0.027**	**91.045**

Significant results ($p < 0.005$) are shown in bold

measured to be assessed using all ROIs grouped together and also subdivided to show the possible significance for each variable when examining the octants in isolation and each third (endosteal, mid-cortex and periosteal) separate from the other ROIs. Pearson's correlations were performed to identify correlation between the OPD and any other histomorphometric variables. This allowed further investigation into any potential relationships identified in the linear regressions by determining if they would be reflected in the histomorphometric variation and not just their relationship to the cortical bone. The level of significance for all analyses was set at $p \leq 0.05$.

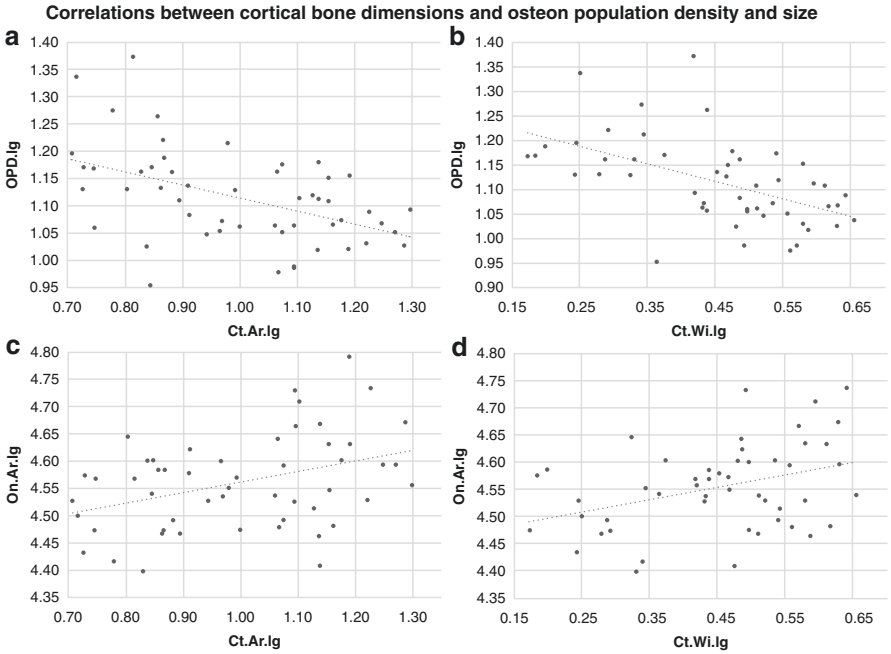

Fig. 3.4 Correlations of variables when evaluating the octants—(**a**) shows the negative correlation between osteon population density (OPD) and cortical area Ct.Ar, (**b**) shows the negative correlation between OPD and Ct.Wi, (**c**) shows the positive correlation between osteon area (On.Ar) and Ct.Ar and (**d**) shows the positive correlation between On.Ar and cortical width (Ct.Wi)

3.3.3 Results

3.3.3.1 Linear Regressions

An increase in the size of cortical bone was linked to larger osteons but a decrease in their density (Table 3.3, Fig. 3.4). Significant negative correlations emerged between Ct.Ar and OPD in the octants ($p < 0.000$, $r = -0.527$), the endosteal sections ($p = 0.019$, $r = -0.318$), the mid-cortex sections ($p < 0.000$, $r = -0.484$) and the periosteal sections ($p = 0.001$, $r = -0.457$). Significant negative correlations also emerged between Ct.Wi and OPD in the octants ($p < 0.000$, $r = -0.553$), the mid-cortex sections ($p < 0.000$, $r = -0.494$) and the endosteal sections ($p = 0.001$, $r = -0.424$). In contrast, significant positive correlations were present between Ct.Ar and On.Ar in the octants ($p = 0.027$, $r = 0.300$) and the periosteal sections ($p = 0.011$, $r = 0.343$). Significant positive correlations occurred between Ct.Wi and On.Ar when testing all sections ($p = 0.007$, $r = 0.182$), just octants ($p = 0.005$, $r = 0.379$) and the periosteal sections ($p = 0.011$, $r = 0.342$). The size of cortical bone was also positively related to On.Dm in the octants (Ct.Ar, $p = 0.016$ and $r = 0.328$; Ct.Wi, $p = 0.032$ and $r = 0.293$) and the endosteal sections (Ct.Ar, $p = 0.008$ and $r = 0.357$; Ct.Wi, $p = 0.027$ and $r = 0.301$).

3.3.3.2 Correlations

Negative correlations were found between OPD and H.Ar ($p = 0.004$ and $r = -0.196$), OPD and H.Dm ($p = 0.019$ and $r = -0.159$) and OPD and On.Ar ($p = 0.001$ and $r = -0.215$) and between OPD and On.Dm ($p = 0.008$ and $r = -0.117$).

3.3.4 Discussion and Conclusions

Results show negative correlations between the size of cortical bone and OPD and a positive correlation between the dimensions of cortical bone and the size of osteons. The results for the radii differ to those previously presented for femur [15]. Increased biomechanical loading is typically interpreted by both increased cortical bone robusticity and decreased osteon size [55, 69]. If this understanding is applied to the radii, it suggests the thicker cortical bone relates to *decreased* biomechanical loading indicated by the *larger* osteons and the *lower* OPD.

Our findings for OPD have significance for studies that estimate age from OPD in forensic and archaeological contexts [54], as well as our current understanding of radius bone biology. Firstly, this analysis reveals insights into age-related OPD asymptote. The asymptote is a point at which new Haversian systems begin to remove evidence of existing Haversian systems [54, 57]. If it can be assumed that cortical bone develops in the radius in the expected fashion, with size increasing with heavy loading [55, 69], this could lead to the OPD asymptote being reached faster in this bone than in others, based on the relationships established here. The point is reached at different times in different bones as the average asymptote for the rib is 30/mm^2 [58, 70] but is 50/mm^2 for the femur [58, 71]. The radius may prove to be less reliable for age estimation at an earlier age as the larger osteons could cause the asymptote to be reached earlier, especially in active individuals. As such, the results of the current study suggest the radius may be a less reliable indicator of age compared to other long bones in medieval samples. As with other studies that have examined bone cross-sections, our results were not consistent in every region [61]. Only the octants as a whole and the periosteal sections showed significant correlations in all variables. Further research should focus on the impact of the cortical bone dimensions on the formation of Haversian systems and investigate the differences identified between the radius and the femur.

Secondly, this short analysis illustrates new macro- and microscopic bone growth relationships in the medieval human radius, which is a bone of importance when studying osteoporosis-related fractures in the living. Radius is the site of Colles fractures which commonly occur following falls on the forearm by people of fragile bone [59]. Distal radius fractures are routinely used as indicators that a patient may be osteoporotic [72]. A 2001 estimate suggested that annually 71,000 adults (males and females combined) develop a radius fracture in the UK [73]. While radiographic methods are the gold standard of radius assessment in these cases, our understanding of radius bone remodelling remains limited in the living. Our short study suggests that the accumulation of secondary bone tissue within the radius anatomy

occurs faster than in other bones in medieval humans. Coupled with recent modern evidence that the distal radius fracture is more prevalent in socially deprived patients (n = 4463 patients treated at the Leicester Royal Infirmary between 2007 and 2010) [74], our preliminary data invite further research into radius bone histology per SES groups to elucidate the complex cortical radius bone modelling and remodelling relationships.

3.4 Conclusion

This chapter focused on the analysis of limb bone microstructure from medieval human remains to propose a SES-related bone quality and quantity development model and demonstrate that medieval human bone samples can be used to expand our current understanding of bone biology of relevance to osteoporosis. We firstly evaluated childhood developmental disturbances recorded in the teeth against bone microstructure density in low and high SES groups from an archaeological site in eleventh to sixteenth centuries Canterbury, UK. We reported that only high SES individuals appear to develop higher adult bone density despite their experiences of development stress in the early years of their ontogeny. Using the same skeletal collection, we then used bone microstructure density in human medieval radius samples to investigate relationships between bone remodelling and entire cross-sectional area. We reported implications for modern and ancient human bone research supplying preliminary data for the human radius suggesting its bone microstructure asymptote may be reached earlier than in other skeletal elements. This second shortly illustrates that medieval human remains, preservation permitting, can make contributions to modern bone biology research.

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Part II

Contemporary Perspective

4 Bone Quality in Socially and Ethnically Diverse Groups: Downstream and Upstream Determinants Across the Life Course

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4.1 Social Determinants of Health

In 1991, Dahlgren and Whitehead presented the "rainbow model" of Social Determinants of Health [SDoH] [1] (Fig. 4.1): although now superseded, this model demonstrated in simplistic terms how social structures, networks and factors at various levels influence health outcomes of the individual. Most commonly understood are the direct biological effects of lifestyle behaviours at the individual level on bone health that are, perhaps, more likely to be within one's control. However, the broader cultural, social, political, economic and physical environments influence the potential for good health, both negative and positively, directly and indirectly; these influences are referred to as upstream social determinants [2–4]. Affordable and accessible education opportunities, civil and political rights, housing availability and affordability, sanitation, access to fresh water, social security policies, terrorism, natural disasters and immigrant and refugee processing mechanisms, amongst many others, are upstream SDoH that impact one's capacity to focus on preventive healthcare and to manage poor bone health. In context of existing health-related policies that influence availability, accessibility, quality and affordability of healthcare and preventive options, SDoH that are not traditionally considered in the realm of health nonetheless have the capacity to limit or increase opportunities and influence decisions and behaviours that are related to bone health

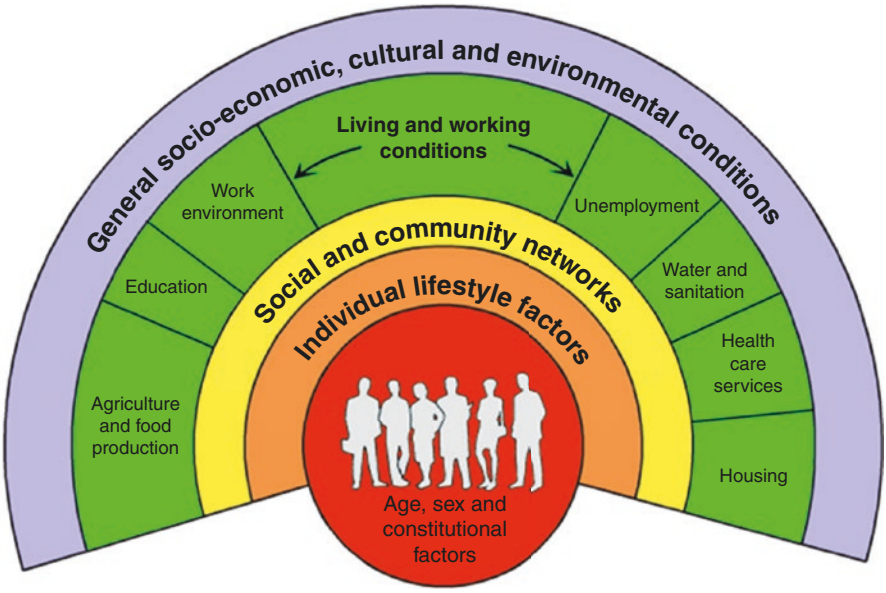

Fig. 4.1 Dahlgren and Whitehead "rainbow model" of Social Determinants of Health outcomes [1]. Reprinted from "Levelling up (Part 2): a discussion paper on European strategies for tackling social inequalities in health" (2008), with permission from the World Health Organization

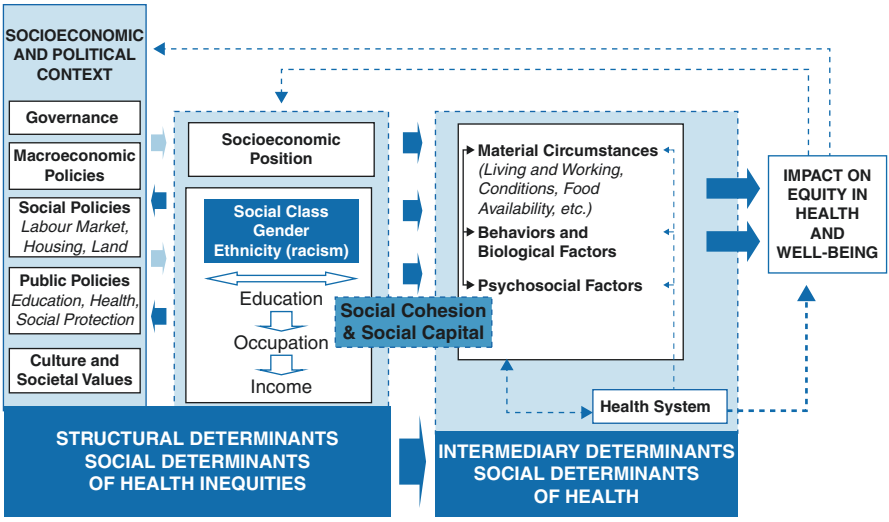

Fig. 4.2 Conceptual framework by the World Health Organization's Commission on Social Determinants of Health [6]. Reprinted from "Closing the gap in a generation: Health equity through action on the social determinants of health. Report" (2008), Commission on Social Determinants of Health, World Health Organization, with permission from the World Health Organization

[2, 3, 5]. The imperative is to understand that upstream determinants of bone health are inextricably linked; they are interdependent and interrelated. Thus, it becomes clear that an individual's capacity to achieve the highest possible level of well-being may be constrained by factors outside of their control, hence global action on the SDoH [4].

Subsequent models, such as the example from the Commission on Social Determinants of Health presented in Fig. 4.2 [6], have encompassed the multidirectional influence of social determinants at many levels. This model is inclusive of the role of biological factors, wealth and social support on which the individual can draw, health policy, societal norms, availability and access to preventive strategies and healthcare and the quality of healthcare for those in need, amongst other factors.

4.2 Education and Income

There are much data pertaining to the role on bone of downstream SDoH that support models such as these; however fewer data are available that pertain to upstream SDoH. For instance, greater educational attainment has been cross-sectionally associated with higher BMD in a study from across the world. This has previously been reported in 6160 women (aged 54.5 ± 6.4 years) from Italy [7], 569 women (60.4 ± 7.2 years) from Turkey [8], 685 women (55.0 ± 3.5 years) from China [9],

2905 US-based women (61.9 ± 0.7 years) enrolled in the NHANES III study [10] and 821 women (45.0 ± 13.6 years) from Qatar [11]. Similar cross-sectional associations have been reported across income levels. For instance, in a study of 1116 adults from Spain (age range 20–79 years), higher family head income, adjusted to Barcelona income per capita, was protective against lower BMD [12], and in an investigation of 51,327 Canadian women aged ≥50 years, significantly greater BMD was observed in those with the highest compared to the lowest mean household income quintile [13–15]. There is an obvious inextricable link between education and income, whereby greater educational attainment would increase the likelihood of higher-paid employment: this link underpins the importance of upstream SDoH on health outcomes such as affordable education and available employment options.

4.3 Ethnicity

Furthermore, many large epidemiological studies have described differences in bone health between different ethnicities [16]. For instance, data from the third National Health and Nutrition Examination Survey (NHANES III) showed that Black Americans have the highest mean femoral neck and total hip aBMD levels compared to White American men, who had the lowest levels [17]. A cross-sectional analysis of five large independent cohorts of men aged 65 years and older reported that unadjusted lumbar spine aBMD was greatest in men who were Afro-Caribbean followed by Black American, White American, Asian American and Hispanic American, whilst Korean and Chinese men had the lowest spine aBMD [18]. Femoral neck aBMD was almost 1 SD higher in Afro-Caribbean men compared to African American men whilst similar amongst White American, Hispanic American and Korean men with the lowest values in Asian American and Hong Kong Chinese men [18].

One of the largest multi-ethnic studies, the National Osteoporosis Risk Assessment (NORA) reported that Black American women had the highest aBMD and Asian Americans had the lowest [19]. Even after adjusting for body weight and other covariates, the greater aBMD in Black American women persisted [19]. A study comparing women aged over 44 years from the Gambia to White British women showed lower size-adjusted BMC in Gambian women [independent of height and weight] compared to British White women [20].

More recently, studies have shown that bone structure and microarchitecture, which are measured in 3D with peripheral quantitative computed tomography (pQCT), high-resolution pQCT (HRpQCT) and axial QCT, differ between ethnic groups. For instance, whilst the Study of Women's Health Across the Nation (SWAN) reported no differences in aBMD between Chinese American, Japanese American and White American women, authors reported that bone structure varied greatly [21]. Hip structural analyses measured with DXA showed that femoral neck cross-sectional area and section modulus were higher in Japanese American compared to White American women [21]: findings that suggest Japanese American

women had better resistance of axial compressive and bending stresses due to greater bone area which conferred a larger section modulus. Data from pQCT showed that premenopausal South Asian British women had lower cortical vBMD, BMC and thinner cortices at the radial diaphysis compared to White British women; this was independent of age, height and weight [22]. Despite this, bone strength estimated using the strength strain index [SSI] was similar, suggesting that bones of premenopausal South Asian British women may have efficiently adapted to maintain strength [22, 23]. In contrast, another study has shown that postmenopausal South Asian British women have decreased SSI and fracture load at the radius and tibia despite having thicker cortices and higher vBMD; bone cross-sectional area was smaller [24]. These two studies indicate the need to study differences in the skeleton at different stages of life and not to apply a "one-size-fits-all" approach to the study of skeletal health.

A study using HRpQCT in older women (mean age of 60 years) showed that Black American women had larger and denser bones compared to White American women and displayed better cortical microarchitecture, whilst most trabecular bone characteristics were similar between the two groups [25]. However, in a follow-up study, individual trabecular segmentation analyses revealed that Black American women had advantageous plate-like qualities and greater axial alignment of trabecular bone (known to improve bone strength for that loading situation) compared to White American women, who displayed greater rod-like trabecular structural characteristics [26]. These differences in trabecular characteristics contribute to the greater estimated bone strength seen in Black American women compared to White American women.

In the Osteoporotic Fractures in Men Study (MrOS), African American and Asian American men had thicker cortices, measured using axial QCT, than White Americans, which led to greater bone strength at the hip [27]. Similarly, greater estimated bone strength was found in British Afro-Caribbean and South Asian British men compared to White British men. Lower hip strength in the White British men was due to smaller bone size at the mid-shaft radius [28]. Black British men from the UK had higher aBMD compared to White British and South Asian British men, independent of weight and height, whilst the difference between White British and South Asian British men was attenuated by correcting body size [29]. Further analyses using pQCT revealed that the differences in vBMD were far fewer than in DXA outcomes where Black British men did not differ to White British or South Asian British men—rather, the geometry of bone differed between the groups [29].

4.4 Life Course Trajectory of Bone Accrual and Loss

The accrual of bone mass begins in utero and continues sharply until reaching peak bone mass around the third decade of life [30] (Fig. 4.3). After a consolidation period, age-related bone loss involves a gradual and progressive decline [30, 31] which is a result of a predominance of bone resorption over bone formation [32].

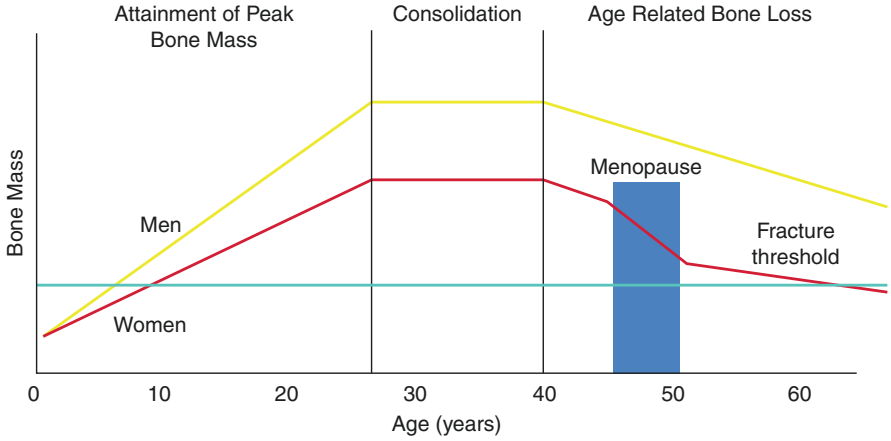

Fig. 4.3 Age-related changes in bone mass across the life course. Modified from Compston et al., Clinical Endocrinology, 1990 [31], and reprinted with permission from John Wiley and Sons, Inc

A significant reduction in bone formation is associated with increasing age for both sexes [30]; however, a steeper decline in bone loss is observed for women compared to men [31]: a factor primarily associated with low levels of oestrogens due to menopause [30, 32] (Fig. 4.3, [31]).

Whilst poor bone health is more commonly the domain of gerontologists or those interested in the skeleton of older adults, the potential for good bone health begins in utero and potentially prior to conception [31] (Fig. 4.3). It has been postulated that osteoporosis is in fact a "paediatric disease with geriatric consequences" [33]. The "Barker hypothesis", which was initially in reference to coronary heart disease, suggested that "… adverse environmental influences in utero and during infancy, associated with poor living standards, directly increased susceptibility to the disease" [34, 35]. From this came the Developmental Origins of Health and Disease [DOHaD] theory, which posited, over and above the foetal genome, a biological "programming" role played by interactions with the environment whilst in utero. Combined, the environmental, nutritional and genetic influences in utero have potential for long-term impact upon a broad range of offspring health outcomes [36]: outcomes that include bone health, particularly resulting from the effect of vitamin D status, nutrition and chronic stress of the mother [37–43] on osteoblastic invasion of the embryonic cartilaginous skeleton and thus bone development [44].

Indeed, longitudinal data suggest a detrimental influence of lower socio-economic status [SES] during childhood on bone health later in life. For instance, in 729 adults (age range 34–85 years) enrolled in the Midlife in the US Biomarker Project, childhood socio-economic advantage and adult education level were associated with higher adult lumbar spine BMD; however, current financial advantage was not [45]. Growing up in a single-parent household has also been associated with lower femoral neck BMD in adulthood [46].

As indicated earlier, two of the more common lifestyle behaviours known to influence bone health are physical activity and dietary calcium intake. These behaviours, amongst others, are well-documented as being socially patterned [47, 48], whereby less healthy lifestyle behaviours are more likely observed in those that are more socially disadvantaged, although a systematic review of global data showed no clear pattern of intake across SES, nonetheless differences between social groups existed [49]. Indeed, these lifestyle behaviours have much potential to impact bone health during the formative years, where bone mass is accruing. Given that more than 90% of peak bone mass is accrued by late adolescence [50], childhood physical activity levels prior to puberty have considerable impact on optimizing peak bone accrual and thus would contribute substantially to avoiding the onset of osteoporosis later in life [51]. However, recreational activity patterns during childhood and adolescence are substantially influenced by societal norms: in contemporary society, recreational norms are becoming increasingly screen-based and thus highly sedentary. Indeed, recent data suggest that children spend approximately 60% of their waking hours engaged in sedentary behaviours [52]. Regular physical activity during childhood is highly beneficial on bone mass accrual. For instance, data have shown that children who engaged in regular weight-bearing physical activity over 7 years had 6–8% greater cortical bone mass and bone area than their inactive peers [53–55]. Furthermore, differences in bone health of children aged 4–12 years according to the length of time engaged in moderate-vigorous activity have been observed. Those who participated in 40 min of moderate-vigorous activity per day over 6 years had a 3–5% greater cross-sectional area and femoral neck section modulus than those who participated in 10 min per day at the same intensity [56]. Other data show that children with high levels of physical activity accrue, on average, 14% more trochanteric BMC and 5% more whole-body BMC relative to peers with low levels of physical activity [57]. Importantly, longitudinal data suggest there may be a sustained effect of physical activity during early life: high levels of childhood physical activity have been positively associated with bone strength in late adolescence, even after drastic reductions in physical activity levels during puberty [58]. However, children and adolescents from socially disadvantaged environments are less likely to participate in physical activity than their more advantaged counterparts [59]: in addition to social norms, this may be potentially related to low parental SES limits capacity to pay for sporting activities [60] and/or parental role modelling of physical inactive lifestyles [61].

In addition to regular weight-bearing activity, adequate dietary calcium intake is critical for skeletal development and maintenance [51, 62–64] and provides a dynamic store to maintain the intra- and extracellular calcium pools [65]. Vitamin D is important for optimal calcium absorption and bone formation and assists in the regulation of calcium levels [66–71]. The key source of vitamin D is ascertained from sunlight exposure of the skin: notably, exposure to ultraviolet B (UVB) light. Poor diet quality is associated with social disadvantage [47, 48, 72] and has been correlated with low BMD in many populations [73–75]. Lower dietary calcium intakes have been observed in lower SES populations in many countries [76, 77], and in a small study of 289 Indian women aged 30–60 years

who resided in a large slum (and thus indicative of low SES), lower dietary calcium intake was significantly associated with lower hip BMD [78]. There are few data pertaining to dietary calcium intake across SES during childhood. Taking these data in context, it is plausible that differences in bone accrual in children exist across social groups. Where a lower peak bone mass is achieved, this presents a lower starting point from which the age-related trajectory of bone loss begins. Thus, socially disadvantaged individuals may have a disproportionately greater risk of crossing the threshold line for osteoporosis earlier compared to their more advantaged counterparts who had achieved a higher peak bone mass during the third decade of life.

4.5 Health Literacy: An Influence Across the Life Course

There is a growing body of evidence to suggest that the relationship between social disadvantage and poorer bone health may be, in part, mediated by health literacy [79–82]. Health literacy is a multidimensional concept that encompasses the broad range of abilities and supports that an individual requires to find, understand and use health information and resources to effectively manage health [83–85]. Those more likely to have lower health literacy are culturally and linguistically diverse populations, socially disadvantaged individuals as identified by lower income or educational attainment and older persons [86–89]. Lower health literacy is a barrier to effective healthcare [90] and has substantial implications for poorer bone health outcomes, for instance, adherence to treatment [91–94]. Key factors influencing bone health are regular weight-bearing activity and adequate dietary calcium intake: behavioural factors that are influenced by lower health literacy [91]. In addition, an association has been reported between low health literacy and poorer uptake of healthy lifestyle behaviours [95–97] and preventive healthcare [98] and poorer management of chronic disease [98, 99], including effective and safe use of medications [92, 100, 101]. As a consequence, individuals with low health literacy are more likely to require acute care [102] and have increased rates of premature mortality [103, 104]. Low health literacy is associated with factors that are likely to contribute to premature bone deterioration and thus disability and dependency: this would include lifestyle behaviours that contribute to chronic disease later in life, as well as poorer uptake of chronic disease management, which would result in a greater reliance on acute care. This has prompted a movement towards addressing disparities in health outcomes by delivering health information and services in ways that address health literacy needs [84, 86, 105].

This is critical for older adults, who report lower levels of health literacy and have increasingly complex healthcare needs. However, adequate health literacy is required for good bone health across the lifespan, beginning with the influence of parental role modelling and health literacy on offspring health in early life [106–108] and continuing as children and adolescents develop their own health literacy abilities and begin to make independent health-related decisions [109–111].

4.6 Conclusion

There is much potential to reduce the disproportionate risk of poor bone health experienced by socially disadvantaged and culturally diverse persons. Given that the predisposing sex and ethnicity cannot be mediated, clinical attention could be focussed towards identifying those most at risk and affording extra time to ensure effective health communications, thus ameliorating the negative effect of low health literacy. Community-based health promotion programmes are numerous and encompass an array of lifestyle modification options that will enhance bone health. However, it is imperative that efforts to reduce overall health inequities are prioritized in national health-related and multisectoral policies and strategies [112, 113, 114]. Whilst much expenditure is dedicated to the prevention of non-communicable diseases, large proportions of that investment are commonly targeted towards individual behavioural factors such as physical inactivity or poor nutritional intake. Without a focus on the wider context of health inequities such as the high cost of living and education and the low availability of employment, often referred to as the "causes of the causes" [114, 115], taking a primary focus on behaviours will have likely have little impact on reducing health inequities [112, 113].

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5 Differences in Fracture Risk Between Countries, Within Countries and Between Social and Ethnic Groups

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5.1 Introduction

Low bone mineral density (BMD), defined as a *T*-score of less than 2.5 standard deviation (SD) from the mean as measured by dual-energy X-ray absorptiometry (DXA), is considered as osteoporotic [1] and thus increases the risk of fracture [2]. Current data suggest one in two women and one in five men will suffer a fragility fracture after the age of 50 years [3–5], and having a prior fracture is a major predictor of future fracture [6]. Hip fractures lead to a substantial loss of healthy life years in older adults [7], have significant social and health implications [8, 9] and are a major source of morbidity and premature mortality [10].

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5.2 Between-Country Differences in Fracture

Globally, compared to age-matched White-American or British/European populations, a lower fracture incidence is observed in other ethnic groups (Fig. 5.1). Overall, more than a tenfold variation in age-standardized hip fracture risk exists across 63 countries, and data indicate a notable difference between Western and Eastern populations [11–14]. The highest annual age-standardized hip fracture incidences per 100,000 person-years have been reported in Northern European countries, including Denmark, Norway and Sweden (574, 563 and 539, respectively), whilst the lowest incidences were observed in Nigeria and South Africa (2 and 20, respectively) [15]. However, country-specific fracture should not be interpreted as ethnic-specific fracture rates, as there exist many within-country, between-ethnicity variations in fracture.

5.3 Within-Country, Between-Ethnicity Differences in Fracture

Within-country differences have been observed between ethnic groups in the incidence of hip fracture [16] and in outcomes postfracture including earlier mortality, longer hospital stays and reduced ambulation at discharge [16]. Data suggest that certain ethnic groups have lower areal BMD (aBMD) compared to other ethnicities which may increase the disparities in fracture between ethnic groups; however, lower aBMD does not necessarily translate into increased fracture risk.

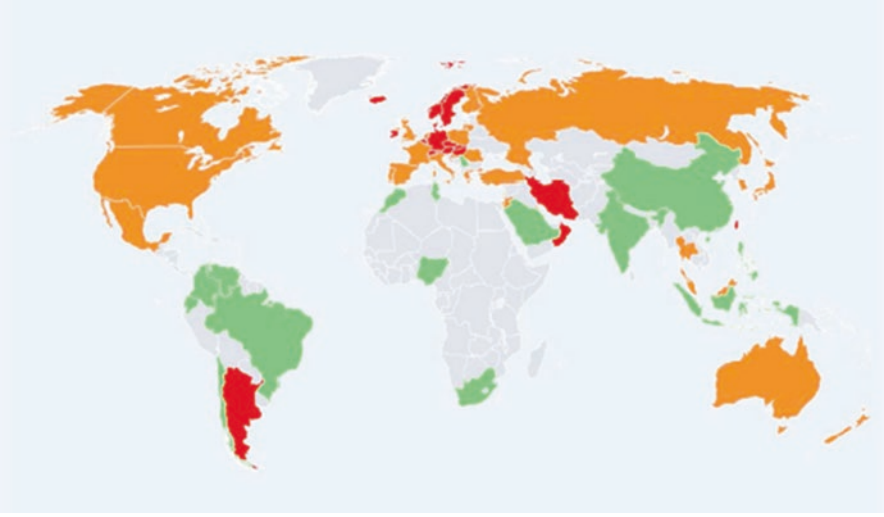

Fig. 5.1 Hip fracture rates (sexes combined) in different countries: where estimates were available, countries are colour-coded red (annual incidence >250/100,000), orange (150–250/100,000) or green (<150/100,000). Reprinted with permission from Osteoporosis International [11]

For instance, in a large pooled cohort of ~12,400 men aged 65 years and older, femoral neck aBMD was reported to be almost 1SD higher in Afro-Caribbean men compared to African American men whilst similar among White American, Hispanic American and Korean men with the lowest values in Asian American and Hong Kong Chinese men [17]. However, these patterns of differences in aBMD between ethnicities were not reflected in the patterning of non-traumatic fracture prevalence; White American men had the highest age-adjusted prevalence of fracture (17%), followed by Black American (15.1%), Hispanic American (13.7%) and Asian American (10.4%), and similar rates between Tobago Afro-Caribbean, Hong Kong Chinese and Korean [17].

Similarly, in one of the largest multi-ethnic studies, the National Osteoporosis Risk Assessment (NORA), Black American women had the highest aBMD whilst Asian Americans had the lowest [18]. However, after adjusting for body weight and other covariates, and despite lower aBMD in Asian American women, lower fracture rates compared to White American women were reported [18]. In line with this, a study comparing Gambian women aged 44 years and older to White British women showed that despite lower size-adjusted BMC in Gambian women (independent of height and weight), they had lower fracture incidence compared to British White women [19].

Taken as a whole, data suggest that differences in body-segment proportions (sitting and standing height) and in histomorphometrically measured cortical parameters contribute to ethnic differences in fracture rates [20, 21]; however, differences in bone quality and strength do not consistently parallel ethnic patterns in fracture rates [14].

5.4 Vitamin D [Sun Exposure]: Key Risk Factor for Fracture

It has been suggested that variations in latitude and environmental factors may be one explanation for variations in fracture between ethnicities and countries [14, 22]. Higher fracture incidence has been observed in latitudes further from the equator [22]. Vitamin D 25(OH)D plays an important role in skeletal homeostasis by maintaining extracellular calcium ion levels in the human body. However, within-country variations in vitamin D have also been reported between different ethnic groups [23, 24]. Taking into account that methods for measuring serum 25(OH)D levels vary widely between studies (enzyme-linked immunosorbent assay, electrochemiluminescence binding assay and liquid chromatography-mass spectroscopy) [23], ethnic differences in vitamin D metabolism are nonetheless affected by skin pigmentation, clothing, nutrition, cultural practices and lifestyle conditions: combined, these factors contribute to the heterogeneity in fracture rates observed between ethnic groups [24].

For instance, Black Americans have been shown to have lower serum 25(OH)D concentrations yet maintain similar or higher circulating 1,25(OH)D concentrations compared to White Americans [25], suggesting greater ability to maintain calcium homeostasis in states of apparent 25(OH)D deficiency. The Multi-Ethnic Study of Atherosclerosis (MESA) measured vitamin D metabolites using the gold standard, liquid chromatography-mass spectroscopy, in men and women aged 45 years and

over [26]. The findings from MESA show that mean serum 25(OH)D and 24,25(OH2)D3 concentrations were highest among White Americans, intermediate among Asian Americans and Hispanic Americans and lowest among Black Americans [26]. Furthermore, lower serum 25(OH)D was associated with significantly lower trabecular vBMD [measured with CT scans of the abdomen] at the thoracic vertebrae (T7–T10) in White Americans, with similar associations in Asian Americans; in contrast, there were no associations between serum 25(OH)D and aBMD in Black Americans or Hispanic Americans—these findings were independent of osteoporosis risk factors and body size [26].

Several studies have demonstrated low serum 25(OH)D levels in populations across the Asian continent. A study in Lucknow, India, reported mean (SD) serum 25(OH)D levels of 12.3 ± 10.9 ng/mL in healthy hospital staff (age range, 24–53 years); only one third of the study participants had serum vitamin 25(OH)D levels above 15 ng/mL [27]. Furthermore, a study measured UVB across various regions of India (north, north-east, west and south) over 12 months and showed that maximum UVB was recorded during summer (March to June) and monsoon/autumn (July to October), followed by winter (November to February)—with barely any difference between the first two seasons, suggesting that atmospheric pollution may prevent UVB rays from reaching the Earth's surface [28]. Pakistani women aged 18–36 years residing in Manchester, UK, had mean serum 25(OH)D levels of 7.9 ± 4.2 ng/mL: lower serum 25(OH)D levels were associated with lower aBMD at the total hip, femoral neck and distal radius [29]. Consistent with findings from South Asian ethnic populations, the Japanese Population-based Osteoporosis (JPOS) cohort study in women aged 50 years and over reported women who had any clinical, non-vertebral or fragility fractures over 15 years of follow-up had significantly lower lumbar spine and femoral neck aBMD and serum 25(OH)D levels compared to those who did not have any fracture [30]. A large study in postmenopausal Chinese women from the Shanghai district had median [interquartile range] serum 25(OH)D levels of 23.0 ng/mL [17.1–30.5]; there was a positive relationship between total hip aBMD and serum 25(OH)D [31].

Similar to Asia, a recent meta-analysis has shown that there is a high prevalence of vitamin D deficiency in the Middle East despite year-round sunshine [32]. A retrospective study from hospital records in men and women (mean age 46.9 ± 16.3 years) from Saudi Arabia reported mean serum 25(OH)D levels of 11.2 ± 9.6 ng/mL (aBMD or fracture data were not available) [33]. A study in Saudi adolescents aged 12–17 years reported serum 25(OH)D levels of 9.7 ± 5.8 ng/mL in those with a history of minimal trauma fracture compared to 12.2 ± 7.0 ng/mL in those with no fracture history [34]. In Palestinian women aged 45 years and over, mean serum 25(OH)D ranged from 13.6 to 14.1 ng/mL and positively associated with lumbar spine aBMD; there were no associations at the total hip or femoral neck [35].

Most countries in Africa have abundant sunshine year-round, although skin pigmentation in ethnic groups across the continent is darker and this is known to produce less vitamin D in the skin for the same exposure to UVB light compared to lighter-skinned individuals [24]. Studies from The Gambia, West Africa, over various stages of the life course (children, young women, older women, pregnant and

lactating women) report mean concentrations between 32 and 40 ng/mL [36–39]. A study of Black women from rural and urban areas in the north-west province of South Africa reported similar serum 25(OH)D levels between rural and urban women who were aged 50 years or younger [40]. However, rural Black South African women aged 50–70 years had higher serum 25(OH)D levels compared to urban women [40]. Although the total number of fractures was reported to be low in the entire cohort, the highest incidence of any fracture [hip, forearm, wrist and vertebrae] was observed in urban women [14.4%] compared to rural women [5.3%] aged 50–70 years [40]. A study from the southwest region of Cameroon in men and women aged 35–85 years reported median [IQR] serum 25(OH)D levels of 27.4 (8.4) ng/mL, with similar levels between the sexes [41].

Whilst not all countries have fracture data, within-country variations in vitamin D between ethnic groups may plausibly be explained by differences in skin pigmentation and clothing styles, nutrition, cultural practices and lifestyle conditions: combined, these factors contribute to the heterogeneity in fracture rates observed between ethnic groups [24], however, also by socially patterned lifestyle behaviours. As discussed, social disadvantage influences bone health and fracture risk, and SES differences have been observed between ethnic groups. In addition to this, plausibly contributing further to disparities in fracture risk is the high prevalence of diabetes observed in some ethnicities. Ethnicity could be considered as an ecosocial construct and as a biomedical concept: the latter suggesting it a proxy for other disease-related risk factors [42].

5.5 Diabetes: Key Risk Factor for Fracture

Diabetes, or more specifically the duration of diabetes and poor glycaemic control, significantly increases the risk of osteoporotic fracture [43–46]. Recent data estimate that 1 in 11 adults have diabetes: a global prevalence of approximately 425 million [45]. A report from 2013 that represented 130 countries showed that 382 million people had T2DM and that most of these people lived in lower- and middle-income countries with predictions of the greatest increases in prevalence in the coming 22 years [47]. A recent review identified that the clear majority of diabetes studies show an increased risk of fracture [48] potentially explained by compromised bone strength in those with diabetes [49]. Thus, it is plausible that ethnic groups who have a higher prevalence of diabetes or are more susceptible to the onset of this disease may also have a greater risk of falls and fracture.

In Australia, the prevalence of diabetes is more than three times greater among Indigenous compared to non-Indigenous Australians [50, 51]. A prospective study spanning 11 years examined diabetes incidence in Indigenous Australians from a remote community and showed that in adults aged 45–54 years, diabetes incidences were 53.2 per 1000 years in Indigenous women and 23.6 per 1000 years in Indigenous men [52]. Consistent with this, a separate study over 10 years in Australian adults aged ≥40 years reported a disproportionate increase in minimal trauma hip fracture rates in Indigenous Australians, whilst there was a decrease in

non-Indigenous Australians [53]. Although no studies have examined the associations between diabetes and fracture in Indigenous Australians, it is likely that the high prevalence of diabetes will have a greater effect on the bone and contribute to the high fracture rates in this population. A recent review of fracture rates and aetiology in Indigenous compared to non-Indigenous persons [54] identified that diabetes was independently associated with an increased likelihood of hip fracture (RR 1.48, 95%CI 1.12–1.97) in Canadian First Nations persons [55]. In addition to First Nations peoples having a higher prevalence of diabetes (compared to non-First Nations Canadians) (12.9 vs 3.1%) [55], a longer duration of T2DM was associated with increased rates of osteoporotic fracture (spine, hip and wrist) in First Nations compared to non-First Nations Canadians [55].

Data from the Women's Health Initiative from the United States of America (USA) showed that Black American women with diabetes had a higher risk of any fracture (RR 1.33, 95% CI 1.00–1.75), compared to White American women with diabetes (RR 1.18, 95% CI 1.08–1.29) [56]. Findings from the National Health and Nutrition Examination Survey (NHANES III) show that associations between fracture risk and diabetes differed significantly by ethnicity [57]. Among Black Americans and Hispanic Americans, risk was 1.9–2.4 times higher in those with diabetes than in those without diabetes even after adjusting for several confounders. In contrast, fracture risk in White Americans with diabetes was only 1.2 times higher than among those without diabetes and was not statistically significant [57].

China has one of the world's largest diabetes epidemics, which continues to rise as the prevalence was reported to be 0.67% in 1980, which has dramatically increased to 9.7% in 2010 [58], which is equivalent to 92.4 million adults with diabetes. A more recent study from China measured HbA1c levels in 170,287 participants aged 18 years or older and reported that the overall standardized prevalence of T2DM in Chinese adults was estimated to be 10.9% (95% CI, 10.4%–11.5%), with 10.2% (95% CI, 9.7%–10.7%) in women and 11.7% (95% CI, 10.9%–12.4%) in men [59]. When analysing specific ethnic groups within the Chinese population, the crude T2DM prevalence was highest in Han participants, which was 14.7% (95% CI, 14.6%–14.9%), whilst the lowest prevalence was seen in Tibetan (4.3% (95% CI, 3.5%–5.0%)) and Muslim participants (10.6% (95% CI, 9.3%–11.9%)) [59].

Following China, India is one of the epicentres of the global T2DM epidemic and has the second highest number of people with the disease in the world (~69 million individuals as of 2015) [60]. Asian Indian individuals who originate from the Indian subcontinent (India, Pakistan, Bangladesh, Sri Lanka, Afghanistan, Nepal, Bhutan and the Maldives) have a specific phenotype that predisposes them to T2DM and is characterized by high levels of intra-abdominal fat and insulin resistance despite low BMI [61]. There have been several studies from India estimating the prevalence of T2DM—there has been an increase in T2DM prevalence since the first documented reports from 1966–1975 to 2014, in both rural and urbans areas [62]. More recently, the ICMR-India Diabetes study reported that the overall prevalence of diabetes was 7.3% (95% CI 7.0–7.5); diabetes prevalence varied depending on the region, with a prevalence of 4.3% in Bihar (95% CI 3.7–5.0) to 10.0% (8.7–11.2) in Punjab, and was higher in urban areas (11.2%, 10.6–11.8) compared to rural areas (5.2%, 4.9–5.4; $p < 0.0001$) [63]. A large study from the UK in White British, Black

British and South Asian British adults aged 40–69 years showed that the greatest prevalence of diabetes was in South Asian men (17.4%) and women (12.8%), followed by Black British men (11.8%) and women (9.4%), with the lowest prevalence in White British men (5.4%) and women (3.0%) [64].

From the African continent, a study from Zambia and the Western Cape in 57,809 adults aged 18 years and over reported that the age-standardized prevalence of diabetes was 3.5% and 7.2%, respectively [65]. Analysis of pooled data from 12 sub-Saharan countries in 38,311 individuals demonstrated that 2156 individuals were diabetic (6%) and the single-country prevalence of diabetes ranged from 2% in Mozambique to 14% in the Seychelles, with a median prevalence of 5% [66]. In a study of adults aged 55–64 years, the greatest prevalence of diabetes was in those from South Africa and the Seychelles (23–30%), with the lowest rates in those from Benin and Mozambique (2–5%) [66]. Data from WHO STEPS showed that in 2007, the prevalence of diabetes was 22.5% in Niger—making it one of the highest rates in the West African region [67]. Analysis of various studies from Latin America, South Asia and South Africa—all of which are lower- and middle-income countries undergoing rapid transition—demonstrated that the highest prevalence of diabetes was in adults from South Asia (19.0%, 95% CI, 18.4–19.8), followed by the Southern Cone of Latin America (14.0%, 95% CI, 13.2–14.8) and South Africa (13.8%, 95% CI, 11.9–16.0), with the lowest rate in Peru (9.8%, 95% CI, 8.8–10.9) [68].

5.6 Disparities in Fracture Risk Across Social Groups

As discussed, differences in the bone between ethnic groups do not consistently parallel ethnic patterns in fracture rates [14]. Similarly, in a population of 1494 Caucasian Australian women stratified by quintiles of SES, the pattern of BMD [69] did not replicate the same pattern of fracture across SES in the same population [70]. The social gradient of fracture has been observed in populations across the world [70–86], yet the mechanisms are unknown. Here, the large-scale efforts to identify genes associated with fracture risk via genome-wide association studies of BMD and fracture risk [87–90] come to mind. However, the maintenance of BMD is a dynamic process, whereby osteoblast and osteoclast differentiation processes are driven by modifications in gene expression patterns throughout the life course [91]. Combined, these data raise questions concerning stressors across the life course that may influence fracture risk in specific population subgroups: factors over and above genetics, latitude and clinical risk factors. Many now argue that the risk of developing osteoporosis, and subsequent fracture risk, begins in utero, continues across the life course and involves epigenetic processes [92]. Epigenetic processes are multiple and include DNA methylation (DNAm), histone modification and non-coding RNA (ncRNA) activity [93]. Recent attention has been directed the modulation of epigenetic pathways via DNAm [91], and data have shown that differentially methylated genes that are related to skeletal development have been observed in patient with fracture compared to those without [94]. In addition to influencing gene expression, DNAm plays a role in establishing a bone cell phenotype and regulates osteogenic differentiation of mesenchymal stem cells [95].

Data suggest that social factors and related stressors play a key role in influencing DNAm. In a study of 92 young Canadian adults (24–45 years), early life SES was associated with DNAm in later life [96]. Similar associations were observed in a study of 89 women (38–46 years) from the USA where being raised in a single parent family or with low household income was associated with higher DNAm in later life [97]. A cross-sectional study of 239 adults from the UK (aged 35–64 years) showed that residing in an affluent area influenced lower DNAm than those residing in a deprived area [98]. Potential mechanisms for these associations have been posited as increased levels of chronic stress and subsequent modulation of physiological responses and DNAm profiles [99]. It is well-documented that the epigenetic signature is influenced by a multitude of environmental factors across the life course: the epigenome is a vital conduit that transduces environmental exposures into phenotypic expression and disease risk [100, 101].

Thus, in addition to non-modifiable genetic predisposition of sex and ethnicity, social disadvantage may increase the risk of osteoporotic fracture via exposure to cumulative stressors, responses to stressors and a heightened inflammatory state (Fig. 5.2). Although the well-documented biological effects on the bone of physical activity, calcium and vitamin D are themselves associated with DNAm status, the associations between DNAm and osteoporotic fracture risk are influenced by social determinants at various stages throughout the life course.

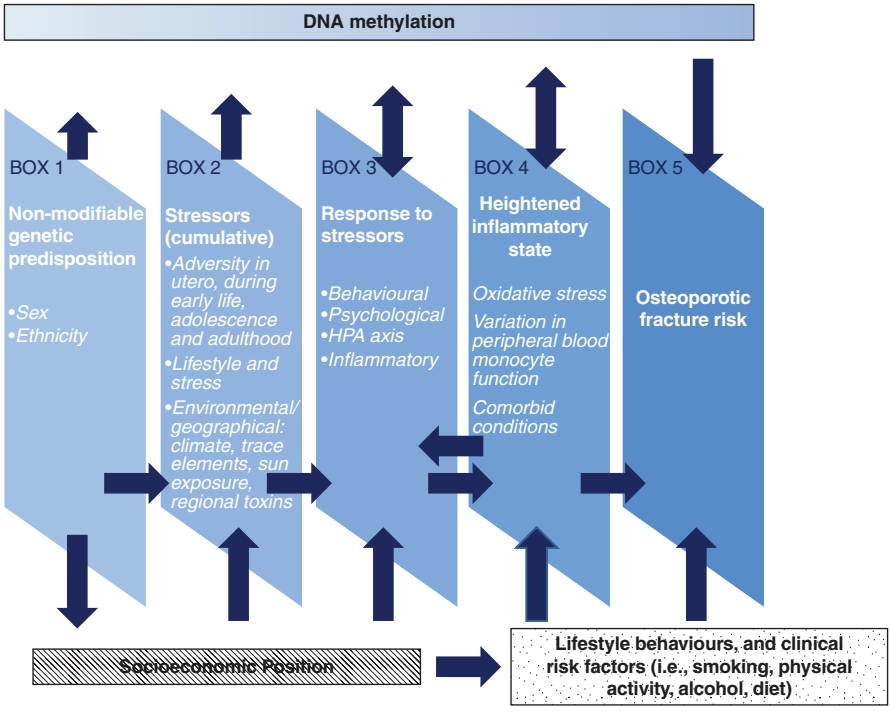

Fig. 5.2 A conceptual model of the relationship between socioeconomic factors and fracture risk. Reproduced from Bone with permission [102]

The utility of this model includes targeting those most at risk for developing osteoporosis by addressing causative environmental pathways that are greater for socially disadvantaged persons. However, the development of compounds that function as demethylating agents may be plausible, as seen for some types of cancers [103], but only if those agents can be engineered with sufficient target specificity. Substantiating and refining this model for clinical utility continues, but what remains clear is that DNAm, among other epigenetic processes, may contribute significantly to explaining the social gradient of osteoporotic fracture.

5.7 Conclusion

Disparities in fracture incidence, prevalence, rates and risk factors exist between social and ethnic groups. These differences cannot be fully explained by measures of the bone alone. In addition to genetic predisposition (sex and ethnicity), social disadvantage may increase exposure to cumulative stressors, influence responses to stressors and result in a heightened inflammatory state, thereby increasing osteoporotic fracture risk. Understanding the mechanisms that underpin the social gradient of fracture may identify various entry points for interventions to reduce the social and ethnic disparities observed in the incidence of osteoporotic fracture.

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Social Determinants of Preventive Testing and Adherence to Treatment for Osteoporosis

6

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6.1 Introduction

Commonly referred to as a 'silent disease', osteoporosis is primarily asymptomatic until a fracture occurs [1]. One in three women and one in five men aged 50 years and older will suffer an osteoporotic fracture [1–4]. Following a hip fracture, 10–20% of people will require long-term nursing care, and one in five people will die in the first 12-month post-hip fracture [5, 6]. Thus, the identification of osteoporosis prior to fracture and the provision of effective postfracture care are imperative. However, it is now established that disparities exist in screening, diagnosis and treatment of osteoporosis between sexes, social groups and ethnicities [7].

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6.2 Preventive Testing

Scanning of the axial skeleton by dual-energy X-ray absorptiometry (DXA) is currently the gold standard for determining measures of bone mineral density (BMD), which subsequently informs clinical decision-making regarding osteoporosis. Given the well-documented relationship between low socio-economic status (SES) and increased fracture [8], it could be expected that the largest benefit derived from DXA is for those of lower SES [9, 10]. This would result in an inverse association between SES and DXA [9–11]; however, this has not been observed. For instance, less uptake of DXA was observed in those of lower SES in Canada, where DXA scans for adults aged 70 years or older are fully subsidised [9] in a similar model of reimbursement to the Australian healthcare system. However, whilst some countries such as Australia may subsidise BMD testing, patients referred for densitometry may still be required to pay a gap fee. Furthermore, not all countries provide reimbursement for DXA tests or may only provide subsidisation after fracture [12]. In a study of 35,681 women (age 65–89 years) from the USA, a positive association between income and DXA utilisation was only observed for those pre-fracture [12]. In a best-evidence analysis and systematic review, it was reported that limited, but consistent, evidence existed for a positive association between DXA utilisation and income and education [10]. Clearly, out-of-pocket costs for DXA or osteoporosis therapies present a key barrier to access for individuals of lower SES.

Area of residence has been shown to play a role in the uptake of DXA, whereby women residing in urban areas are more likely to be referred for DXA than women in rural areas (RR 1.15, 95%CI 1.08–1.22), with stronger results observed for men (RR 1.46, 95%CI 1.17–1.81) [13]. However, the urban-rural disparity may not be surprising given that patients in rural areas are more likely to experience difficulties accessing services due to the concentration of DXA services in metropolitan areas, medical workforce shortage in rural areas and travel distances to specialist services, as has been reported for mammography [14, 15].

External factors influencing DXA utilisation, including cost and accessibility, interact with individual factors, such as health literacy and perceptions of fracture risk and treatment benefit. Combined, this creates inequity in the uptake of preventive testing between advantaged and disadvantaged populations: the latter group being most at risk of fracture. Health literacy, a term that describes the broad range of abilities and supports that an individual requires to manage health [16], has been identified as a potential mediator in the relationship between social disadvantage and poor health outcomes [17]. However, there exists a paucity of data concerning the role health literacy play in the relationship between social disadvantage and uptake of DXA scans. One Australian study found that among patients hospitalised for minimal trauma fracture, individuals who had previously undergone assessment for osteoporosis reported higher functional health literacy [18]. This suggests that patients with better functional health literacy, an aspect of health literacy related to reading and comprehending written health information [19], may be more likely to undergo a DXA scan. However, there are a broader range of health literacy abilities and supports beyond functional health literacy likely to play a role in the utilisation of DXA scans that have yet to be explored.

A relationship has been observed between higher functional health literacy and greater osteoporosis knowledge [20]. However, an exploratory study of the beliefs and perceptions of outpatients with osteoporosis found that even those with a good level of osteoporosis knowledge attributed their own fractures to factors such as falls and poor vision rather than bone fragility [21]. These patients may have adequate functional health literacy required to find and understand basic osteoporosis information but lack the more complex health literacy abilities necessary to apply this information to their own situation [19]. Those data indicated that many patients lacked awareness of anti-fracture treatments [21]: an unfortunate knowledge state, given that higher perceived benefit of anti-fracture treatment has been associated with increased treatment uptake and with increased uptake of DXA scans [22].

Higher levels of health literacy would enhance the ability of individuals to address their perceived or real barriers to undergoing a DXA scan [22]. For example, an individual requires health literacy abilities to understand the financial burden of undertaking a DXA scan. This may require navigating their region- or country-specific reimbursement system to understand if they are entitled to any reimbursement, how much of the cost would be reimbursed, how to seek reimbursement and how long the reimbursement process would take. However, given that lower SES is strongly associated with lower health literacy, the choice to avoid undergoing a DXA may be considered the preferred option.

Barriers to DXA uptake must also be considered in the context of ageing and multimorbidity. Older adults are more likely to have low health literacy compared to their younger counterparts [23–25]. This may relate to cognitive decline observed in older adults [26, 27] and/or the higher number of chronic conditions among older adults [28] placing additional burden on health literacy skills. Individuals managing multiple conditions are more likely to report lower health literacy [24, 25, 29]. Previous research has also identified low salience of osteoporosis among patients when compared with other long-term conditions [30]. It is possible older patients managing several chronic conditions with limited health literacy may not prioritise screening for an asymptomatic condition such as osteoporosis.

In addition to the patient level factors that influence uptake of DXA scans, healthcare providers play a crucial role in the utilisation of DXA scans not only in making the decision to refer but also adequately communicating the need for bone density assessment to their patient. Thus, the beliefs and perceptions of referrers are important in determining whether a patient receives a DXA scan. One study demonstrated increased likelihood of undergoing a DXA scan among patients of female healthcare providers [22]. A recent qualitative study found that over one third of patients with a fragility fracture described referrer barriers to DXA scan [31]. These included being told their bone density was normal based on their physical appearance or X-rays or being told their fracture was not a fragility fracture, despite an osteoporosis screening coordinator categorising it as such [31]. This suggests either a misunderstanding between the patient and healthcare provider regarding the need for a DXA scan or a lack of understanding among some GPs regarding osteoporosis assessment and characteristics of a fragility fracture. An earlier survey of GPs suggests that, despite the overwhelming majority recognising the importance of

preventing osteoporosis, many felt they lacked the necessary tools to address the issue with patients [32]. In a qualitative exploratory study, GPs identified the perceived availability of DXA scans in the local area also influence their decision to refer [33], suggesting healthcare providers may be sensitive to the barriers faced by their patients in accessing healthcare.

6.3 Non-pharmacological Treatment

The first-line treatments for low BMD are non-pharmacological interventions, primarily vitamin D and/or calcium supplementation and physical activity.

Vitamin D is important for optimal calcium absorption and bone formation and assists in the regulation of calcium levels. The key source of vitamin D is ascertained from sunlight exposure of the skin: notably, exposure to ultraviolet B [UVB] light. Sun exposure should be outdoors, as UVB transmission is unlikely to occur through normal clear windows. Vitamin D deficiency may be more likely observed in older or housebound persons [including residents of aged-care facilities], individuals with naturally darker skin, those that avoid sun exposure such as persons whose bodies are covered for cultural or religious reasons, babies of mothers that are vitamin D deficient and those that are unable to absorb or process vitamin D [34].

Calcium plays an imperative role in normal growth and maintenance of bone and is a dynamic store of intra- and extracellular calcium pools [35]. Adequate dietary calcium intake is essential to achieve peak bone mass and to reduce age-related loss of bone [36]. Different life stages require different levels of dietary calcium intake, and recommendations for daily calcium intake vary between countries; however, the recommended daily consumption of calcium [from foods] can be achieved by consuming 3–5 serves daily of calcium-rich foods. In older community-based individuals and residents of aged-care facilities, reducing falls risk is imperative, with the end-goal being to reduce both falls and fractures. It is universally recommended that a combination of vitamin D and calcium supplementation be optimised in all residents of aged-care facilities [37]. However, previous research suggests that compared to other osteoporosis treatments, calcium and vitamin D supplementation have lower adherence rates among patients with osteoporosis [38]. Patients who discontinued calcium and vitamin D supplements were more likely to identify lack of motivation as the reason [38]. Fear of side effects was the most commonly cited reason for stopping other prescribed anti-fracture medications [38].

Age-specific requirements for the type, duration, intensity and regularity of physical activity have been proposed to maximise bone health [39]. The beneficial effect of selected exercise modalities on bone health ranges from those that are highly osteogenic [basketball/netball, impact aerobics, tennis, jumping], moderately osteogenic [running/jogging, hill walking, resistance training, stair climbing], low osteogenic [leisure walking, lawn bowls and yoga/Pilates], to non-osteogenic [swimming, cycling] [39]. Whilst leisure walking is not recommended as an adequate strategy for bone health, this activity nonetheless provides overall health and fitness benefits.

Greater health literacy and higher SES have been associated with an increased uptake of preventive health behaviours, including better diet quality [29, 40] and increased physical activity levels [24, 29, 40, 41]. It has previously been suggested health literacy may be important in meeting dietary calcium requirements [42]. However, there is a need for further research regarding the role of health literacy in preventive health behaviours directly related to bone health including dietary calcium intake, vitamin D levels and osteogenic activity.

6.4 Worldwide 'Care Gap' in Osteoporosis and Fracture Treatment

Despite it being well-documented that experiencing one fracture substantially increases the risk for a subsequent fracture, large-scale studies that have investigated healthcare systems demonstrate suboptimal postfracture care. For instance, national audits in Australia [43], Canada [44], Germany [45], Italy [46], Japan [47], Korea [48], the Netherlands [49], Switzerland [50], the UK [51] and the USA [52] reported the proportion of patients with fracture that were assessed for subsequent fracture risk ranged from 5 to 65%; similarly, the proportion of patients with new fractures who received appropriate osteoporosis treatment ranged from 7 to 60%. Whilst the postfracture care gap is a worldwide phenomenon, there are data to suggest that specific population subgroups are disproportionately affected compared to others. For instance, in a large Canadian study of 11,234 major osteoporotic fractures, it was observed that, postfracture, First Nations peoples were less likely to receive a BMD test (OR 0.1, 95%CI 0.0–0.05), osteoporosis-related pharmacotherapy (OR 0.05, 95%CI 0.3–0.7) or a diagnosis of osteoporosis (OR 0.5, 95%CI 0.3–0.7), compared to non-First Nations peoples [53]. The worldwide failure to effectively treat fractures has led to an unacceptable care gap for patients, leading to a predominantly avoidable risk of subsequent fracture and increased burden for healthcare systems [54].

6.5 Postfracture Care Pathways

Given the increased likelihood of subsequent fracture and the imperative to reduce the fracture care gap, there is now much worldwide attention focused towards secondary fracture prevention. One initiative has been the development of fracture care pathways, commonly referred to as 'clinical care pathways', 'models of care', 'integrated care pathways' or 'ortho-geriatric care models'. Care pathways aim to deliver evidence-based treatment plans for patients presenting to hospital [55]. As opposed to usual fracture care, care pathways encompass a multidisciplinary team approach to fracture care, which, more commonly than not, involves an orthopaedic surgeon and a geriatrician. The three key goals of postfracture care pathways are related to the identification and treatment of osteoporosis and fracture, specifically, identify, investigate and initiate [56].

Fracture care pathways are now being implemented internationally as they have been found to be cost-effective [57] and shown to reduce the health burdens of fractures when compared to usual care. Multiple systematic reviews have aimed to determine the effectiveness of care pathways compared to usual care on a variety of outcomes. A meta-analysis of nine studies demonstrated lower odds of deep venous thrombosis (odds ratio (OR) 0.33, 95%CI 0.14–0.75), pressure ulcer (OR 0.48, 95%CI 0.30–0.75), surgical site infection (OR 0.48, 95%CI, 0.25–0.89) and urinary tract infection (OR 0.71, 95%CI 0.52–0.98) in patients managed according to care pathways compared to those receiving usual care [58]. A meta-analysis of 15 randomised controlled trials [RCTs] showed that, compared to controls, more patients in the care pathway group regained the same level of basic activities of daily living (ADLs) (29.1–46.0%) and walking ability (56.3–68.9%) 12 months after hospital discharge compared to controls [59]. A subsequent review reported similar improvements in basic ADLs [standardised mean difference (SMD 0.32, 95%CI, 0.17–0.47)] and mobility (SMD 0.32, 95%CI, 0.12–0.52) compared with usual care [60]: reviews have also reported decreased refracture rates [61] and increased treatment initiation [61], but outcomes have varied in terms of length of hospital stay [62, 63] and long-term mortality [59, 62]. In addition to biomedically orientated measures, a recent meta-analysis demonstrated that, compared to usual care, care pathways following hip fracture achieve short- and long-term improvements in patient-reported outcomes such as quality of life and physical performance [64]. The overall positive findings of these reviews suggest care pathways contribute to better outcomes after fracture.

There exists a lack of data pertaining to the uptake and impact of care pathways according to the SES of patients; this is despite the well-documented influence of social determinants on osteoporosis and fracture risk. Given this paucity of data, there is no evidence, to date, to suggest how care pathways can overcome these inequalities; however, it could be speculated that at the service-level, the communication of these pathways to patients [65] would plausibly improve their uptake and adherence across the spectrum of SES.

6.6 Adherence to Osteoporosis Treatment and Management

Low adherence to a prescribed treatment regime is a worldwide phenomenon, described by the WHO as '…a worldwide problem of striking magnitude' [54, p. 7]. As for all chronic diseases, treatment adherence plays a critical role in effective management of osteoporosis and reduces the likelihood of subsequent fracture. Patient claims data indicate that less than 50% of patients are adherent to their osteoporotic medications [66]. The consequences of low adherence include poorer outcomes and increased healthcare costs [54]. The WHO, among others, has identified that social disadvantage decreases the likelihood of treatment adherence [54]. Data also suggest a correlation between social disadvantage and lower health literacy [67], which will influence unintentional non-adherence [related to

forgetfulness, regimen complexity, physical problems] and intentional non-adherence [patient decision-making, perceived benefits] [68].

6.6.1 Patients

Several patient factors have been identified as potentially contributing to poor uptake, suboptimal adherence or discontinuation of pharmacological osteoporosis treatments. One's capacity to correctly identify osteoporosis status has previously been associated with greater uptake of anti-fracture medications [22]. Concerningly, it has been reported that 28–63% of individuals who have undergone a DXA scan are unable to correctly identify their osteoporosis status [69–71]. Another study of 3484 White and 1041 Black women from the USA who underwent DXA testing observed that White women were more likely to correctly identify their actual DXA results; these results were sustained after adjustment for income and health literacy [72]. It is apparent that adequate communication of DXA results and fracture risk is important in supporting medication uptake and adherence; however communication methods between practitioner and patient are imperative to reducing disparities in understanding health information [72]. Patient perceptions also play a role in determining effective pharmacological management of osteoporosis. Patients who perceive greater benefit to using anti-fracture medications are more likely to initiate treatment [22, 73]. Conversely, perceived side effects or fear of side effects has been identified as common motivations for discontinuing pharmacological treatment for osteoporosis [38].

Health literacy abilities may influence a number of these adherence-related, and, as previously discussed, social disadvantage and cultural diversity are strongly associated with lower health literacy. In order to manage a complex treatment plan, an individual requires a range of health literacy skills to self-manage their medications [74]. Obtaining and filling prescriptions, understanding medication instruction, organising often complex medication regimens and sustaining medication use whilst monitoring for adverse events require a broad range of health literacy abilities [74]. Patients need to be able to find and understand information regarding the risks and benefits of medications, access the necessary health services, communicate effectively with healthcare providers to participate in medication-related decision-making and have the knowledge and support to adhere to and monitor medication regimens over time.

Previous research suggests low health literacy is associated with poorer self-management and medication across a range of conditions [75, 76], though evidence for an association between health literacy and pharmacological management of osteoporosis is currently limited [77]. There is some evidence to suggest low functional health literacy is associated with poorer anti-fracture medication adherence [78, 79]. However, findings from studies utilising multidimensional health literacy assessments to investigate the influence of a broader range of health literacy abilities on anti-fracture medication adherence have demonstrated mixed results [20, 80]. This suggests that different aspects of health literacy may play different roles in

anti-fracture medication uptake and adherence. Interventions to improve anti-fracture medication uptake and adherence among populations with low health literacy should consider the specific health literacy needs of these populations. In addition, mass media has been shown to play a key role in refocusing the conceptualisation of osteoporosis to influence treatment adherence [65].

6.6.2 Healthcare Providers

Regardless of reasons for non-adherence, it is imperative that patients be supported rather than blamed [54]. It is here that practitioners can instigate change in patient attitudes, beliefs and behaviours that are integral to management of osteoporosis. However, physician attitudes, beliefs and knowledge are equally important to the multifaceted issue of postfracture care gap, and practitioner-patient interactions in terms of health communications play a key role in treatment adherence [65]. Adherence is a dynamic process, and as the number of comorbidities increases, so too does the complexity of treatment regimes and the potential for medication-related harms [81]. As identified above, an individual requires a range of abilities to manage a medication plan [74]; however, there is evidence to suggest healthcare providers can support self-management in patients with low health literacy.

Interventions that tailor medication-related information for older adults with osteoporosis have demonstrated relative improvement in medication adherence [82] and have received positive responses from patients and healthcare providers [83]. However, beyond tailoring information, interventions that specifically target health literacy to improve pharmacological management of osteoporosis have been lacking. Health literacy may also be an important factor to consider when prescribing an anti-fracture medication. As previously identified, older adults are more likely to be managing multiple comorbidities and therefore more complex medication regimens. The higher number of comorbidities has demonstrated an inverse relationship with anti-fracture medication use in older adults [84]. Reducing the complexity of medication regimens may be an appropriate mechanism for improving adherence among patients with low health literacy. For instance, in a study of 432 Korean women with a previous low-trauma fracture, low functional health literacy was associated with poorer adherence to weekly oral bisphosphonates but not bisphosphonates delivered intravenously every 3 months [79]. Efforts in the clinical setting to reduce complexity of medication regimens may increase the capacity of individuals to self-manage their osteoporosis with greater effectiveness.

6.7 Health Policy

As discussed earlier, the provision of reimbursement or subsidisation of healthcare services supports greater utilisation by those in greatest need, including those of lower SES. An increasing focus is now being directed towards SES as important to public health research, in order to inform future health policy and disease

intervention or prevention [85]. In other areas, there is evidence that socially disadvantaged groups have benefited from reduced health inequalities due to targeted policies addressing tobacco control [86] and cardiovascular health [87]. Unless the mechanisms underlying and overarching the relationship between SES and poorer health are better understood, we are limited in our ability to intervene effectively to decrease the disproportionate burden of disease in disadvantaged groups. There have been few specific attempts at reducing social inequality in bone health. This requires major promotion of DXA utilisation and osteoporosis therapy to patients and practitioners within Australia's health system and, potentially, revisiting current health policies to examine their focus and implementation.

Where disparities do not exist in subsidisation for DXA scans, there should, in theory, be no difference in referral practices. However, international data examining social inequality, health policy and bone health suggest otherwise. For example, in 1997, Manitoba Health in Canada mandated the creation of a Bone Density Program Committee to develop, implement and oversee a strategic plan for bone densitometry within their province. This plan, which had led to transformational change in testing within the Manitoba healthcare system, includes tracking the utilisation of bone densitometry across different social groups and assessing the impact of this on patient management and outcomes such as fracture [88]. Other examples of the need to promote diagnostic imaging within different SES groups include angiography in Canada [89], as well as general radiology, vascular, computed tomography, MRI and general and obstetric ultrasound [11], CT and MRI in Sweden [90], and mammography in Finland [91] and Guam [92]. One of the major current challenges to health research and policy is to gain a better understanding of the level of equity in the uptake of DXA testing and adherence to osteoporosis medications and treatment plans.

It has been argued that health literacy is a policy choice [93–95], a political choice [93, 94, 96] and indeed a challenge to both [93, 94, 96]. Health policy cannot be considered a niche topic nor applicable only at the individual level; rather it requires an approach that is whole-of-government, whole-of-society and intersectoral for good governance [93, 96]. National action plans to improve health literacy have been developed in various countries [97–100], with the common theme of identifying and removing health literacy-related barriers to healthcare, raising awareness, providing new tools, testing interventions and designing responsive organisations. It is imperative, however, that interventions are codesigned with patients [65], as this will align content of the intervention to the health beliefs that influence non-adherence [65]. Political health literacy will facilitate a health literate, inclusive and sustainable society, '…where no one is left behind' [95, p. 6].

6.8 Conclusion

Disparities between social and ethnic groups exist in screening, diagnosis and treatment of osteoporosis. Various patient- and practitioner-specific factors influence low uptake of testing and poor adherence, many of which relate to health literacy, the quality of patient-practitioner communications and salience of osteoporosis.

To influence the availability of equitable healthcare options and to increase the uptake of services and adherence to treatment plans, health policy must strategically act on health literacy: this requires an approach that is whole-of-government, whole-of-society and intersectoral for good governance.

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Part III

Epigenetic Perspective

Epigenetics: At the Crossroads Between Genetic and Environmental Determinants of Disease

7

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7.1 Epigenetics

The concept of epigenetics was originally defined in 1942 by Conrad Waddington as the study of the causal mechanisms intervening between the genotype and the phenotype [1]. The term refers to all the molecular mechanisms by which DNA, RNA, and proteins are chemically modified, enabling the transformation of one genome into hundreds of different transcriptomes, without the primary sequence being changed, a change which is specific to each cell type and development stage [2, 3].

Even though epigenetic marks might remain stable and be transmitted to subsequent generations, they can also be modified in response to endogenous and exogenous environmental stimuli [3, 4]. The functionality of these marks in the

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establishment of tissue identity during embryonic development, the repair of genomic damage, aging, and cancer have been widely described [5–7]. Epigenetic mechanisms include the covalent chemical modification of DNA (methylation) and chromatin (covalent histone modifications and chromatin compartmentalization) as well as noncoding RNAs, and they are ultimately related to the regulation of gene expression.

7.1.1 DNA Methylation

DNA methylation is a post-replication covalent modification that does not interfere sterically with base pairings, although it can have major consequences in the regulation of gene expression. In mammals, it consists of the addition of a methyl group to the aromatic ring of a single DNA base, mainly cytosines that precede guanines where it generates 5-methylcytosine (5mC). These dinucleotide sites are usually referred to as CpGs [8].

The distribution of methylated DNA throughout the genome shows enrichment at gene bodies and noncoding regions such as centromeric heterochromatin and transposons. While the role of DNA methylation in the promoter regions is well established (hypermethylation being associated with gene repression), the role it plays in the intragenic regions remains poorly understood. Recent works indicate that methylation in intragenic regions could also be involved in the regulation of multiple processes, including the elongation of transcription, the expression of both coding and noncoding intragenic regions, alternative processing, and enhancer activation [7, 9].

DNA methylation is catalyzed by the family of enzymes known as DNA methyltransferases (DNMTs), which transfer a methyl group from S-adenosyl-L-methionine (SAM). Five isoforms have been identified in mammals: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L. The maintenance methyltransferase, DNMT1, specifically recognizes hemimethylated DNA, copies the methylation pattern, and conserves it after each replication. DNMT3a and 3b are de novo methyltransferases, capable of establishing new methylation patterns in strands of DNA that were previously not methylated, and they are unable to discern between hemimethylated and unmethylated strands. They are highly expressed in embryonic stem cells, but this decreases as cells differentiate. DNMT3L, although not catalytically active, plays an important role in the establishment of genomic imprinting during gametogenesis. DNMT2 does not exhibit DNA methyltransferase activity, although there is evidence that it can participate in the covalent addition of methyl groups to transfer RNAs [10, 11].

7.1.2 5-Hydroxymethylation

5-hydroxymethylcytosine (5hmC) was first described in the 1950s, in the study of the nucleic acids of bacteria and viruses [12], and subsequently, in the 1970s, in

vertebrates (rat, mouse, and frog) [13]. But it was not until 2009 that it started to feature more significantly in scientific publications when its presence was described by Kriaucionis and Heintz in Purkinje and granular cells of the mouse cerebellum and by Tahiliani et al. in human and mouse embryonic cells [14, 15].

DNA demethylation is a process that, in recent years, has been proven to be an important regulator of transcription. There are two possible ways for this to happen: (1) passively due to the gradual loss of maintenance DNMT activity, a process which increases with additional replications (and over time), or (2) the active demethylation of DNA, which is catalyzed by the protein family ten-eleven translocation (TET1, TET2, TET3)—enabling 5mC to be transformed into 5-hydroxymethylcytosine (5hmC)—in a process which is independent of replication [16, 17]. According to Métiever and collaborators, DNMT3a and b, in addition to their de novo activity, also participate in the processes of active demethylation of DNA [18].

The family of TET enzymes not only acts on 5mC but can also oxidize 5hmC, transforming it successively into 5-formylcytosine (5fC) and 5-carboxycytosine (5caC), the latter subsequently being converted to cytosine by thiamine-DNA-glycosylase (TDG) [19]. 5mC and 5hmC act as substrates in the deamination reaction carried out by the AID/APOBEC deaminase family, which transforms them into thymine or 5-hydroxymethyluracil (5hmU), respectively [20]. It has been postulated that both products of this reaction could eventually be converted into cytokines by TDG or by the base excision repair (BER) mechanism [20].

Since the recent "rediscovery" of 5hmC, numerous works have been published on the possible role of this epigenetic mark and its regulation. The fact that 5hmC is found in very low amounts in some tissues might lead to the assumption that it is a mere intermediate oxidation product in the demethylation of 5hmC [16].

Globisch and collaborators have carried out the quantification of this base in different tissues using the liquid chromatography-mass spectrometry (LC-MS) method and have detected the presence of 5hmC in all the tissues analyzed. The central nervous system presented the highest levels (from 0.3 to 0.7% depending on the tissue analyzed), with medium levels being found in the kidney, nasal epithelium, bladder, heart, skeletal muscle, and lung (ranging from 0.15 to 0.17%), and the lowest levels in liver, spleen, testes, and pituitary gland tissues (~0.06%) [21]. It has also been discovered that 5hmC is abundant in ESCs, where it is thought that it could regulate the maintenance of their pluripotency and their capacity for self-renewal [22].

Several studies have hypothesized that 5hmC could play an active role in regulating gene expression and contribute to tissue-specific epigenetic regulation [16, 23, 24]. 5hmC is known to be located at promoters with a medium-low level of CpG islands and to be absent in transcription start sites (TSS) [25, 26]. It is also found in regions with a high density of CpG islands in gene bodies, preferentially in exons, where it correlates with active gene expression [27, 28]. In genes with high expression levels and high levels of 5hmC, there is an enrichment of activating histone tags, such as H3K4me1/me3, but an inverse relationship between 5hmC and the repressor tag H3K27me3, which suggests that 5hmC is associated with an active transcriptional state [29, 30]. As with 5mC, there are 5hmC-binding proteins, such

as MeCP2, which seem to favor gene transcription by recognizing 5hmC through the recruitment of chromatin state-modifying proteins [31].

Moreover, this research field contributes to explaining the intricacies of the epigenetic landscape and demonstrates the plasticity which makes genes responsive to changing environmental conditions.

7.1.3 Histone Posttranslational Modifications

The basic structural element of DNA compaction, the nucleosome, is composed of a sequence of 146 base pairs of nucleotides that fold around an octamer of proteins comprising two copies of each of the histones H2A, H2B, H3, and H4, along with the H1 histone, which links the nucleosome with the extranucleosomal DNA to establish the chromatin structure.

Unlike for histones H3 and H4, the posttranslational modifications of histones H2A and H2B have not been widely studied, probably because H2A and H2B have a weaker association due to their continued interchange within the nucleosomal DNA, suggesting that they have less stable histone modifications [32, 33]. Studies conducted in mutated N-terminal domains of histones H2A and H2B evidence an increase in active genes, indicating that they are repressing transcription [34, 35]. This suggests that the histone domains H2A and H2B play a far more important role in the regulation of transcription than has been previously ascribed to them.

Some of the amino-terminal tails of histone proteins extend beyond the DNA–protein octamer, making their amino acid residues accessible to modification [36]. A number of variations in histone residues have been detected, and at least eight different types of change have been described, among them acetylation, methylation, phosphorylation, and ubiquitination [37].

Histone modifications do not occur in isolation but rather in a combinatorial manner. They are evolutionarily conserved proteins responsible for the packaging, organization, and regulation of DNA within the nucleus of all eukaryotes [38].

The interaction between modified histones and DNA can regulate many biological events, including gene expression, DNA repair, chromatin compaction and genomic stability, as well as important genetic processes such as the inactivation of the X chromosome [2, 39, 40].

In addition, one of the main functions of modifications in the histones that make up chromatin is to establish different “environments”: i.e., chromatin with a low state of condensation (euchromatin), which is more “accessible” to transcription factors, or chromatin that presents a high degree of compaction (heterochromatin), which, in contrast to euchromatin, prevents the transcription of genes. Depending on the type of posttranslational modification, the effect on the conformation of chromatin and, therefore, on the regulation of gene expression is different. In general, the addition of acetyl groups to lysines corresponds to a more open conformation of chromatin and, therefore, to an increase in transcription. This is because acetylation cancels the positive charge of lysine. Lysine methylation, on the other hand, does

not alter charge, so any direct effect on chromatin folding would have to occur through non-electrostatic mechanisms [41].

Histone tail modifications are established or erased by the catalytic action of enzymatic systems associated with chromatin. In general, the enzymes that carry out these modifications are part of multiprotein complexes involved in the regulation of transcription or other genomic processes. As such, they directly affect chromatin configuration through the interaction of DNA and histones, or else they constitute signals that are recognized by other complexes [42].

Acetylation is the most widely studied histone modification, and it is generally associated with the active transcription of the gene and is performed by histone acetyltransferase enzymes (HATs) which acetylate specific lysine residues in histone substrate (acetylation of lysine 14 or 19 in histone H3 and lysine 16 of histone H4) and are reversed by the action of histone deacetylases (HDACs) [43]. DNA-bound activators recruit HATs to acetylate nucleosomal histones, while repressors recruit HDACs to deacetylate histones. Other coactivators and corepressors have been shown to possess HAT or HDAC activity or to associate with such enzymes [44, 45].

One of the most widespread modifications of histones in eukaryotic organisms is the acetylation of lysine 16 of histone H4 (H4K16ac). Acetylase hMOF belongs to the MYST family and is responsible for specifically acetylating H4K16, while SIRT1 carries out the opposite reaction [46, 47]. The depletion of hMOF can affect the repair of breaks in double-stranded DNA and abrogate both homologous and nonhomologous recombination [46]. H4K16ac weakens the inter-nucleosome interaction; thus it has a mayor function in chromatin structure [48]. In addition, a loss of acetylation in H4K16ac and a reduction in the trimethylation in H4K20 have been described in relation to the hypomethylation of repetitive sequences and, indeed, were the first alterations described in cancer [49].

Methylation, which is a histone covalent modification, is more complex. It can occur in either lysines or arginines, and the effect on transcriptional expression can be either positive (e.g., in the case of H3K4, H3K36, and H3K79) or negative (e.g., in the case of H3K9, H3K27, and H4K20) depending on the position of the residue within the histone [39]. Two general classes of remodelling enzymes have been described: histone methyltransferases (HMTs) act to add methyl groups to lysine and/or arginine residues in histones, while, another group of enzymes, histone demethylases (HDMs), remove the methylation [50]. An additional level of complexity resides in the possible existence of multiple methylated states on each residue. Lysines can be mono-, di-, or trimethylated, whereas arginines can only be mono- or dimethylated [41].

7.1.4 Chromatin Compartmentalization

The genome is spatially compartmentalized within the nucleus and this is known to play an important role in the transcriptional control of genes [51]. Folding allows interaction between promoters and cis-regulatory elements [52, 53], and, likewise,

the position of genes in the three-dimensional space of the nucleus correlates with expression patterns and function [54].

DNA is wrapped around a histone octamer to form nucleosomes, which are then assembled into 10 nm chromatin fibers. These fibers are, furthermore, folded in such a way that they are organized into evolutionarily conserved higher-order structures and, ultimately, into so-called topologically associating domains (TADs) [53, 55]. In fact, TADs are stable units of the genome and appear to be grouped into A and B compartments [53, 56]. Through genome-wide chromosome conformation capture, the spatially segregated compartments have been identified as active (A) and inactive (B) chromatin [56]. Furthermore, A compartments correlate with early replication and are enriched for transcription binding factors and histone modifications (H3K27ac, H3K4me1/H3K4me3, H3K9me1, and H3K36me3) associated with active gene transcription [55, 57, 58]. B compartments, on the other hand, correlate with late replication and the heterochromatin mark H3K9me3 [55, 57]. Nothjunge and coworkers studied DNA methylation during the differentiation and maturation of cardiac myocytes and showed that A compartments are marked by increased 5-hydroxymethylation and low 5mC regions, while B compartments are partially methylated. Moreover, during cell differentiation A and B, compartments are set first, after which, DNA methylation signatures are established [59].

Topologically associating domains are organized into subcompartments, and a B subset is located close to the nuclear envelope [55]. Contact between DNA and the nuclear lamina has been used to define lamina-associated domains (LADs), which contain transcriptionally inactive genes [60]. Despite TADs and LADs being related, they are, however, independent domains [57].

Several structural proteins, such as CCCTC-binding factor (CTCF), cohesin, and the Mediator complex, have been implicated in the organization and setting of borders between TADs compartments [52, 58, 61, 62]. Boundaries are conserved across cell types and contain CTCF binding sites, which act as insulators and block enhancer-promoter interactions across adjacent TADs [53, 57, 58]. Boundaries are critical to avoid pathogenic enhancer-promoter interactions and are known to be necessary to prevent the formation of limb abnormalities in a mouse model, as was described by Lupiáñez [63].

Perturbation of TAD boundaries through chromosomal rearrangements could lead to changes in the regulatory architecture that results in alterations of gene expression and the appearance of diseases [63]. This field of research represents a new dimension in understanding, and future studies will be able to reveal how genome folding regulates gene expression.

7.1.5 Micro-RNAs

miRNAs are endogenous, small (~22 nucleotides), single-stranded, noncoding RNAs that pair with the 3′ untranslated regions (3′UTR) of their specific target messenger RNA (mRNA). In March 2018, there were 1917 hairpin precursors, and 2654 mature sequences annotated in the human miRBase database [64]. miRNAs

are transcribed from regions that are contained within the introns of coding and noncoding proteins, and a small proportion of miRNAs reside within exons [65]. Normally, to regulate gene expression, miRNAs inhibit translation and/or destabilize the target mRNA [66, 67].

The biosynthesis of miRNAs begins in the cell nucleus and ends in the cytoplasm, where they exert their function. They are encoded by genomic DNA and are most commonly transcribed by RNA polymerase II, initially as long RNA precursors called primary miRNAs (pri-miRNA) which usually have 5′ caps and poly-A tails. pri-miRNA requires the RNase III enzyme Drosha, along with its partner DGCR8 (DiGeorge syndrome chromosomal region 8), to be trimmed in the nucleus into precursor miRNAs (pre-miRNAs), which are characterized by a stem loop, or hairpin, structure of approximately 70 nucleotides [68]. The pre-miRNAs are exported to the cytoplasm by the nuclear export factor Exportin-5 and its cofactor RAN-GTP where they are processed to give the mature miRNAs by another RNase III (Dicer) into mature miRNAs of 21–26 nucleotides in length [69, 70]. Mature miRNA can interact with Argonaute to form RNA-induced silencing complex (RISC), after which it guides the RISC to its target mRNAs, particularly to 3′UTR, and negatively regulates its target mRNA in order to inhibit translation and/or decrease mRNA stability as a result of accelerated uncapping and deadenylation [71, 72]. In order for a miRNA to have functional consequences, 2–8 nucleotides at the 5′ ends must have exactly the same base pairing as the target [73].

Alterations in both histone deacetylation and methylation as a result of chromatin-modifying drugs alter miRNA expression, suggesting that miRNAs are susceptible to epigenetic reprogramming [68]. Several studies have identified alterations in methylation patterns in human cancers that result in changes in miRNA expression [74, 75]. Tao and coworkers suggested there were associations between histone modifications corresponding to gene activation (H3K4me3 and H3K27ac) and promoter hypomethylation, with higher expression levels of pre-miRNA found in genomic regions [76]. Moreover, the double knockdown of DNMT1 and DNMT3b genes in colon cancer cell lines led to a reduction of 5mC levels, which contributed to a threefold upregulation of 18 miRNA genes [77].

In addition to being subjected to epigenetic regulation through chromatin modifications of their corresponding genes, miRNAs may also play a more decisive role in chromatin structure control by directly targeting the posttranscriptional regulation of key factors involved in the epigenetic control of chromatin remodelers [78]. Two miRNAs have been reported to target HDAC4: miR-140, during bone development, and miR-1, during myoblast differentiation [79, 80]. miRNAs may also be involved in establishing DNA methylation as DNMT1, DNMT3a, and DNMT3b have all been predicted to be potential targets of miRNAs [81, 82].

The fact that miRNAs are involved in complex regulatory networks of gene expression suggests that alterations in their expression levels, their cellular location, and their action may have far-reaching effects on cellular physiology and even invoke sustained alterations of cellular function.

7.2 Long-Term Health Effects of Environmental Impacts on the Epigenome

One of the differentiating characteristics of epigenetic marks is their dynamic nature. Even though they constitute stable molecular signals that can be inherited through cell division, there exists a wide array of epigenetic modifiers which can actively add or remove epigenetic marks [83]. These enzymes, while being targets for drug therapy in various settings, such as cancer, are also subject to environmental influence, thus making epigenetic mechanisms a crucial system of communication between the genome and external stimuli [84].

In what follows we will succinctly address some of the most significant findings on the influence of environmental stimuli such as metabolic diseases, alcohol and tobacco consumption, exposure to UV light or heavy metals, and stress (Fig. 7.1) on epigenetic marks.

7.2.1 Metabolic Diseases

Metabolic syndrome (MetS) is a cluster of metabolic disorders, characterized by at least three of the following conditions: dysglycemia, raised blood pressure, elevated

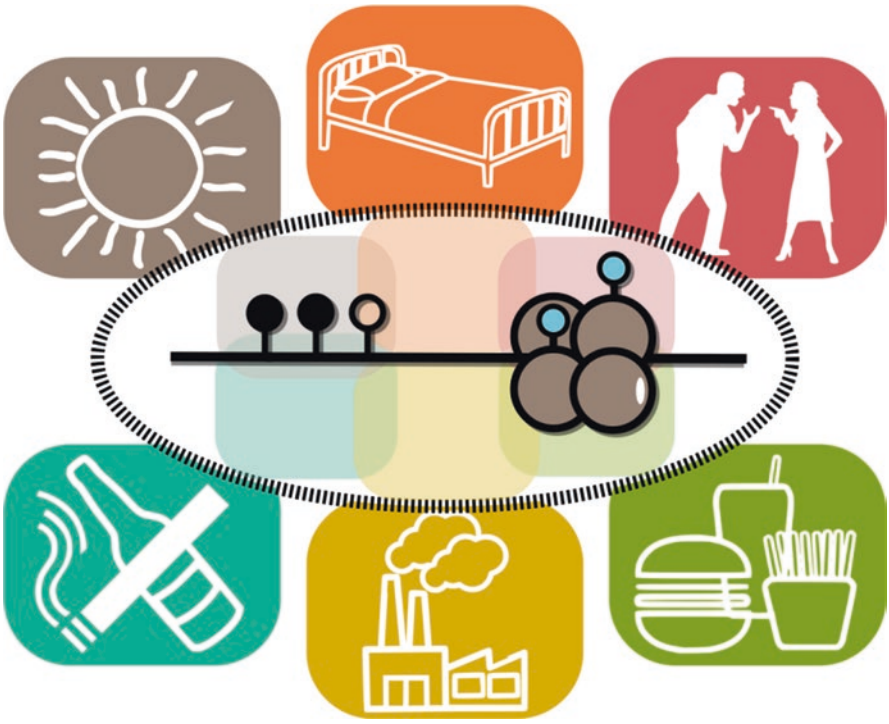

Fig. 7.1 Different aspects of our lifestyle that can influence the epigenome at the level of DNA methylation and histone modifications

triglyceride levels, low high-density lipoprotein cholesterol levels, and obesity (particularly central adiposity) [85, 86]. All these conditions significantly increase an individual's risk of cardiovascular disease and diabetes mellitus type II (DM2) [85]. The latter is a complex and multifactorial disease, which progressively leads to resistance to the action of insulin, glucose intolerance, and, ultimately, the failure of the beta cells of the pancreas, and is accompanied by systemic inflammation, along with other metabolic problems [87–89]. The prevalence of DM2 has increased in recent years and has in fact reached the status of a pandemic and becomes a major health crisis of the twenty-first century [90]. These raised levels of DM2 are concurrent with increasing rates of obesity, and this seems to reflect common environmental and genetic factors underlying both conditions [87, 91]. In fact, obesity or being overweight is widely considered to be the principle driver of DM2 [90]. Although it was traditionally viewed as a disease of adulthood, over the last two decades, the scientific literature has shown a global and dramatic increase in the incidence DM2 in children and adolescents, alongside the concomitant pandemic of childhood obesity [92].

The prevalence of MetS in early ages, however, is difficult to estimate with confidence because of the different criteria used in various studies. The WHO states that the number of overweight or obese children aged 0–5 years increased from 32 million globally in 1990 to 41 million in 2016. The Human Early Life Exposome (HELIX) study aims to measure and describe multiple environmental exposures during pregnancy and early childhood in a prospective cohort across six European countries and associate these exposures with molecular omics signatures and offspring lifespan. Moreover, they recruited from within the full cohort, a subcohort in which one extra follow-up examination was carried out, in order to assess child health outcomes. In this selected subgroup, 24.4% of the pregnant women were overweight, and 14.7% were obese. The offspring of this subcohort were also evaluated when aged between 6 and 11 years and it was observed that 18.8% of the children were overweight and 9.9% were already obese. The percentage of overweight and obese children in the cohort who were from Spain was the highest of any country at 42.3% [93]. Furthermore, in the cross-sectional data obtained from the Third National Health and Nutrition Examination Survey (1988–1994) in the UK, nearly 90% of obese children and adolescents had at least one symptom of MetS [94]. In the SEARCH study, 95% of youth diagnosed with DM2 92% had at least two additional MetS-associated cardiovascular risk factors [95], whereas MetS prevalence in children between 10 and 17 years was 75.8% in the TODAY study [96, 97].

The best evidence relating to the impact of adverse environmental conditions on human development and long-term health outcomes, however, from follow-up studies of the offspring of women pregnant during the great famines, such as the Dutch Hunger Winter (1944–1945) or the Jewish holocaust (1940–1945) [98, 99]. Famine exposure at different stages of gestation has been found to be associated with increased risk of obesity, dyslipidemia, cardiovascular disease, insulin resistance, and other MetS-like symptoms [98, 99]. Similarly, the China Health and Retirement Longitudinal Study (CHARLS) explored the associations between exposure to the Chinese famine (1959–1961) at fetal, infant, and preschool stages with adult health outcomes [100, 101]. It was found that early-life exposure to famine could increase the risk of hypertension in adulthood, and a postnatal obesogenic environment could

further increase the risk [100]. Severe undernourishment during the Chinese famine in fetal (37.5%), infant (43.5%), and preschool (37.9%) cohorts was also found to be linked with increased MetS in adulthood compared to a no-starvation cohort (34%) [101]. Much of the research to date has focused on epigenetic modifications at imprinted regions or metastable regions, both of which seem particularly sensitive to alteration and are areas where changes are maintained throughout adult life [102]. These studies will be discussed in other chapters.

7.2.2 Impact of Maternal Nutrition on the Offspring Epigenome

The risk of developing complex diseases (such as these mentioned above) and disorders during lifetime is an established adaptive response to the intrauterine environment [103]. The placenta is involved in the exchange of nutrients and waste products between the mother and the developing fetus, thus maintaining the uterine microenvironment and ensuring correct organogenesis [104]. There have been many studies on the offspring of gestating animals which have demonstrated that particular developmental stages appear to be more sensitive to epigenetic aggression [105].

A classic example of this is that of the agouti mouse model. These mice carry a locus called agouti viable yellow (Avy) which contains an intracisternal A-particle (IAP) that regulates the expression of a pigment that strikingly alters coat color, among other phenotypical traits. This model has been used to demonstrate that the incorporation of dietary compounds such as aforecited molecules in the diet of a gestating mother leads to epigenetic changes at the IAP loci of the offspring, accompanied by the corresponding changes in phenotype, thus providing evidence of a molecular mechanism through which diet can alter the epigenome, with notable consequences [106]. The variable DNA methylation levels found at the IAP region influence the expression of the agouti gene such that mice are born with coats of varying shades from brown (completely methylated) to bright yellow (completely unmethylated) [107]. Yellow and mottled mice are obese and prone to diabetes and cancer, in contrast to fully agouti mice, which are lean and nondiabetic [108].

Biologically active compounds incorporated through the diet can ultimately affect the molecular pathways of epigenetic modifiers, producing changes which are incorporated into the cell's epigenome. For example, feeding animals with diets enriched or restricted in methyl donors affects the epigenome and consequently influences gene expression. In fact, the pathways relating to DNA methylation, which are examples of one-carbon metabolism, have been much studied. Both DNMTs and HMTs rely on SAM as a universal carbon donor in their catalysis, and molecules such as folate (vitamin B9), cobalamin (vitamin B12), or choline, all of which participate in SAM metabolic pathways, have been investigated due to their capacity to alter these molecular routes [109].

Children taking vitamin B12 and/or folic acid supplementation showed relatively small changes when the methylation data on genomic DNA from peripheral blood cells were analyzed but showed an enrichment in pathways involved in the development of DM2 and related comorbidities [110]. In another study, Sinclair and

colleagues, using restriction landmark genomic scanning (RLGS), analyzed the methylation status of 1400 CpG sites in the offspring of mature female sheep whose periconceptual diet was restricted in terms of specific B vitamins and methionine [111]. They found that the offspring had numerous phenotypic alterations, such as increased body mass, altered immune responses to antigenic challenge, insulin-resistance, and elevated blood pressure. In addition, 4% of the CpG sites analyzed showed altered methylation. These results support the notion that specific maternal diets can lead to widespread epigenetic alterations in DNA methylation in offspring, as well as modify adult health-related phenotypes [111].

Jin et al. stated that increased birthweight and growth rate of the offspring were associated with methyl donor supplementation during pregnancy. Working with newborn piglets, they found that none of the DNMTs analyzed (DNMT1, 3a and 3b) showed changes in their expression, but there was evidence of increased methylation of the hepatic insulin-like growth factor (IGF-1) promoter in the liver. Despite this finding and the fact that increased methylation in promoters is usually correlated with gene repression, higher mRNA expression and protein levels of IGF-1 were found in this case [112].

Although the mechanism of action has not been clearly established, it is known that low levels of circulating IGF-1 can lead to MetS, increased cardiometabolic disease, and DM2 [113]. IGF-1 is involved in mammalian development and glucose homeostasis, and hepatic depletion is known to lead to hyperinsulinemia, hypercholesterolemia, and higher levels of the hormone leptin [114]. Moreover, leptin protein is implicated in regulating energy expenditure and inhibits food intake and is therefore a regulator of body weight [115]. Furthermore, reduction in CpG methylation of the leptin promoter gene in peripheral blood leukocytes is associated with higher body mass index, dyslipidemia, and insulin resistance (characteristic symptoms of MetS) in obese adolescents [116].

Rats exposed to high-fat diet (HFD) in the womb are predisposed to developing obesity and also display symptoms of MetS, both at birth and throughout their life. Obesity in HFD dams was found to be due to higher levels of leptin in plasma [117] and postweaning overnutrition of HFD offspring caused increased glucose intolerance and insulin resistance compared with the low-fat diet dams of reduced-fat maternal diet. In the same study, Ramamoorthy et al. reported that an obesogenic environment in the uterus programs the hypermethylation of pro-opiomelanocortin promoter in the offspring. Pro-opiomelanocortin is part of the central melanocortin system that regulates feeding behavior and it has been found that lack of it leads to early-onset obesity, both in mice and humans [118].

Another study in mice models also found an increment in both leptin resistance and cholesterol and triglyceride levels following long-term feeding on a high sucrose and HFD. In addition, the mice exhibited increased weight and adiposity, as well as displaying a DM2 phenotype. Higher expression of Dnmt1 in adipose tissue was also found, which the authors consider might play an important role in changes in methylation over time on the Glut4 and Leptin gene promoters [119].

Similar results were found in mice exposed to a HFD in utero, where offspring exhibited a MetS-like disorder, with reduced glucose tolerance and insulin

sensitivity and significantly higher total triglyceride and leptin hormone levels. When histone modifications were assessed in the promoter region of the leptin gene in the HFD mice offspring, H4K20 was significantly higher than in controls [120].

A fivefold increase in leptin levels was found in Japanese macaques subjected to a HFD for 4 years [121]. Most of the monkeys (60%) were found to be sensitive to this diet and developed obesity and insulin resistance. These chronically overnutritioned animals were then bred, and their offspring showed an almost twofold increase in body fat percentage and increased fetal hepatic triglyceride levels compared to the control group [121].

The work of Aagaard-Tillery et al. in Japanese macaques revealed that the chronic consumption of a maternal HFD resulted in significant hyperacetylation of H3K14 in fetal hepatic tissues and a trend toward increased acetylation of H3K9 and H3K18 as well. They observed a significant reduction in HDAC1 protein and in vitro HDAC functional activity [122]. The authors thus demonstrated that in utero exposure to specific environmental factors can induce epigenetic changes, which in turn determine specific phenotypic/physiological outcomes in the offspring. Furthermore, the authors carried out a microarray analysis that demonstrated that the expression of the GPT2, Rdh12, Npas2, Hsp, and DNAJ2 genes involved in metabolism and associated responses were appreciably increased [122].

It is also known that epigenetic programming during development occurs not only in utero but also throughout life and across multiple generations. Continuous HFD across multiple generations of female mice has demonstrated that this results in increased adiposity as well as DNA hypomethylation in inflammation-related genes [123].

However, it is not only fetal overnutrition due to an obesogenic maternal environment which influences metabolic health, the effects of undernutrition have also been demonstrated by data from famines resulting from wars and political decisions in the twentieth century.

In animal studies, feed intake restriction during early and late gestation has been shown to affect the development of goat fetuses and lead to hypomethylation in the heart [124]. A higher relative abundance of mRNA in the TET1 gene during late gestation was reported in this same study although levels of DNMT1, DNMT3a, and DNMT3b were not significantly affected and nor was the mRNA expression of imprinting genes, such as IGF2, IGF2R, or DLK1 in fetal organs [124].

Feeding protein-restricted diets (PRD) to pregnant animals induced offspring phenotypes which had characteristics of MetS [125]. The researchers found that the offspring of PRD rat mothers also showed upregulation of the hepatic genes encoding the glucocorticoid receptor (Gr) and hepatic peroxisome proliferator-activated receptor α (PPARα). Hypomethylation of the GR and PPARα promoters has been observed, and the researchers involved considered that this was possibly related to a decrease in the activity level of DNMT1, which is associated with increased gene transcription [126, 127]. Additionally, analysis of the GR promoter has revealed high levels of acetylated histones (H3K9 and H4K9) and methylated H3K4 and low levels of dimethylated H3K9, although high doses of folic acid supplementation reversed these changes [128].

7.2.3 Other Dietary Molecules Which Also Affect the Epigenome

Many phytochemicals have also been studied because of their potential inhibitory activity on HDACs, which has raised interest in their potential antitumoral properties. Substances such as indoles and isothiocyanates, derived from glucosinolates present in vegetables and allyl derivatives from onions and garlic, have been studied in depth [129]. Results have demonstrated some examples of in vivo epigenetic changes linked to decreases in tumor development in mice with sulforaphane-supplemented diets [130]. The most notable compound to date is resveratrol, a polyphenol found in grapes and soy which has shown lifespan-extending effects through SIRT1 activation in yeast and mouse, effects which have yet to be shown in humans [131].

Finally, aside from compounds which are naturally present in food, the diet can also be a route of exposure to xenobiotics (i.e., synthetic or foreign compounds that appear in a given environment), as is the case of bisphenol A or phthalates, which come from the plastic industry and can be found in much of the packaging used for consumer goods. These molecules pose important health risks due to their endocrine-disruptor activities and, moreover, the fact that they can induce epigenetic changes at the level of both DNA and histone modifications, thus providing mechanistic links to their pernicious effects on health [132].

7.3 Other Environmental Factors with a Potential Effect on the Epigenome

7.3.1 Alcohol and Tobacco

Alcohol consumption has been related to alterations in DNA methylation and histone modifications, thus compromising the epigenetic landscape, especially through alterations in one-carbon and energy metabolism pathways. However, the broad spectrum of changes found and the synergism of alcohol with other unhealthy factors, such as smoking, can make it difficult to define clear patterns [133, 134].

On the other hand, smoking is acknowledged as being able to disrupt epigenetic patterns, although the epigenetic landscape of smokers varies greatly due to the diversity of compounds that may be included in tobacco. Global and locus-specific DNA hypomethylation and an aberrant histone code are some of the consequences of exposure to tobacco, which like alcohol, can penetrate the placenta and lead to deleterious effects on the fetus, compromising its development [135–137].

7.3.2 Ultraviolet Light

Ultraviolet light (UV) is a type of radiation that is part of sunlight, and exposure to UV varies depending on factors such as air pollution, ozone layer thickness, and weather conditions. Exposure can induce adverse effects, such as erythema or skin tightness, leading to photoaging and, if the exposure is prolonged or accumulative,

more serious conditions such as skin cancer [138]. The relationships between UV exposure and epigenetic marks are varied. For example, DNA methylation is known to be able to influence the induction of genetic mutations caused by this type of radiation [139] and the UV-induced upregulation of photoaging-related metalloproteinases has been associated with histone methylation changes at their loci [140]. With regard to DNA methylation, there seems to be a tendency to hypomethylation in human non-tumoral skin exposed to sunlight, an effect which is more pronounced in aged subjects, indicating that it may correlate with accumulated exposure [141]. However, mice skin tumors induced by UV radiation present an increase in DNMT activity with subsequent DNA hypermethylation, which is associated with the recruitment of methyl-binding proteins [142].

In addition to classic skin cancer-inducing mechanisms such as mutations in proto-oncogenes and tumor suppressor genes, it is now known that epigenetic mechanisms can play a role in the origin and development of these tumors. This is because UV exposure can compromise the integrity and accessibility of the genome, which can in turn influence gene expression as well as aberrant differentiation

7.3.3 Exposure to Heavy Metals

Exposure to heavy metals such as arsenic, cadmium, lead, or nickel which are found in nature is also produced in industrial activities. These compounds are considered toxic for health because their accumulation in tissues and organs can induce deleterious effects such as cancer or cardiovascular and neurological diseases [143]. Heavy metals can compete with ion enzymatic-binding sites and interfere with ion metabolism, thereby compromising the activity of proteins, which could include epigenetic enzymes such as DNA hydroxymethylases or HDMs which utilize Fe2+ as a cofactor [144, 145]. The effects of heavy metal exposure have mainly been studied in the peripheral blood of exposed industrial workers and populations from Asiatic regions where arsenic and nickel are found in the environment in larger quantities. Global hypomethylation and local hypo- and hypermethylation linked to exposure to these elements have been found in specific genes, some of them important to cell homeostasis, suggesting that cellular metal accumulation could drive the development of tumors [146]. Like methylation, exposure to heavy metals induces perturbations in histone modifications, most probably through the dysregulation of the enzymes responsible for the deposition of the various modifications and the high levels of reactive oxidative species that result from the accumulation of heavy metals in the organism [147].

7.3.4 Psychosocial Stress and Sleep-Wake Rhythms

A stressful lifestyle can lead to diseases of a physical or psychological nature. In situations of stress, glucocorticoids are produced through the hypothalamic/pituitary/adrenal axis and bind to the cytoplasmic GR, a transcription factor which regulates the response to environmental cues. Epigenetic mechanisms related to DNA

methylation and histone modification are implicated in this pathway [148]. Behavior has also been shown to influence epigenetic marks, both in animal models [149] and humans [150], and lifetime stress is known to alter the epigenetic clock [151]. Alterations in circadian rhythms are also known to be detrimental to health [152]. Our molecular clocks are subject to strict regulation, in which epigenetic mechanisms play a part. Very interestingly, the expression of epigenetic modifiers has been associated with circadian patterns, and some clock components are in fact modifiers themselves, such as CLOCK (Circadian Locomotor Output Cycles Kaput), which has histone acetyltransferase activity [153]. Night-shift work has been linked to DNA methylation changes, with mixed results due at least in part to the fact that the effects appear to be small [154, 155]. While night-shift workers constitute an intriguing object of study, it has to be noted that this, and almost all, of the research carried out on human subjects focuses on DNA methylation levels in blood, even though epigenetic marks have tissue-specific distributions, and the consequences of stressors can be experienced in other tissues besides blood.

7.4 Conclusions

Epigenetic mechanisms mediate the interactions between the environment and the genome and can help explain how lifestyle influences health. Thus, epigenetics constitutes a plausible route for intervention toward the improvement of human well-being. Despite the many associations, there is still a long way to go to reach a full understanding of how these mechanisms participate in the cellular response to external cues and what the most strategic and efficient measures are that can be taken to improve health, from an epigenetic perspective. This issue is complicated by the lack of consensus in the research methodologies used and the epigenetic marks studied, to say nothing of their potential inter-dependence and synergism, and, finally, because lifestyle is a combination of various healthy and unhealthy factors.

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The Influence of Maternal and Social Factors During Intrauterine Life

8

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8.1 Introduction

The life course approach, developed in the 1960s for analyzing people's lives within structural, social, and cultural contexts, when applied to biology, implies that developmental trajectories established in early life influence an individual's later responses, such as adult lifestyle. Although our knowledge in this field is still limited and in an early stage, several studies have been carried out, both epidemiological and experimental, showing how the factors to which children are exposed during pregnancy have an effect on the development of chronic illness later in life [1]. In this chapter, we provide an overview of how maternal and social factors during intrauterine life influence health in adult life.

8.2 The Developmental Origins of Health and Disease

The developmental origins of health and disease (DOHaD) concept is based on the premise that the conditions of the prenatal environment influence the risk of suffering a noncommunicable chronic illness. The concept has important consequences both from the biomedical and societal points of view [2].

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Studies published by Barker are considered the epidemiological basis for DOHaD. Barker analyzed the burden of disease in England and Wales and found a direct geographical association between those areas with a higher infant mortality at the beginning of the twentieth century and the prevalence of ischemic cardiopathy during the latter half of that century [3]. Further analysis showed an association between neonatal deaths in 1920, low birth weight, and some stressing intrauterine factors, as well as an increased prevalence of cardiac disease in areas with a lower economic income. This led him to hypothesize that perinatal nutrition could manifest itself pathologically over the adult life after the appearance of a triggering factor [4]. Finally, after reviewing the characteristics of newborn children in the birth registry, Barker found that men who showed a lower birth weight had higher mortality rates for ischemic cardiopathy. Nevertheless, the mortality rates remarkably decreased when there was a notable increment in weight during the first year of life, hinting at the relationship between an insufficient fetal and infant development and an increased risk of ischemic cardiac illness, as well as the possibility of catch-up during infancy (Fig. 8.1).

The hypothesis underlying the DOHaD concept is that throughout the perinatal period, when organogenesis and tissue differentiation occur, alterations in the genetic expression and in the cellular proliferation and differentiation can result in disorders that present themselves later in the adult individual. The life course

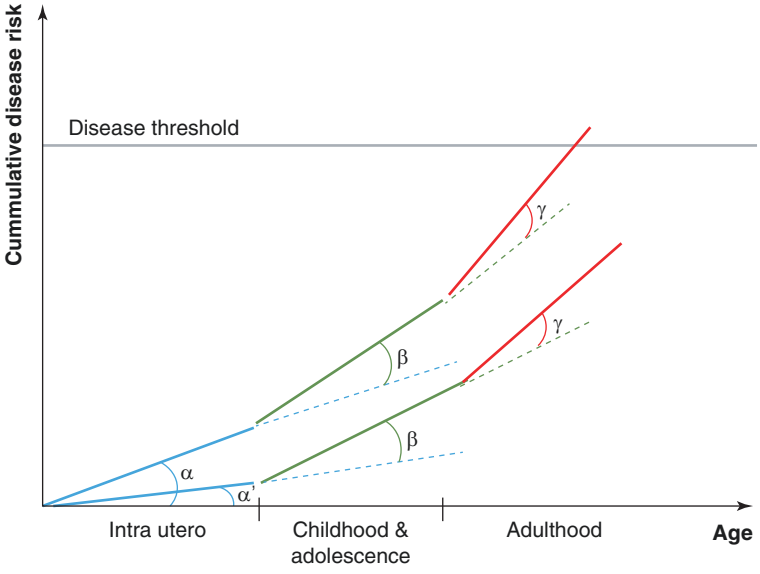

Fig. 8.1 The DOHaD hypothesis—noncommunicable diseases in adults, such as obesity or osteoporosis, are the consequence of the accumulation of risk factors. Those factors act through the life span, beginning at intrauterine life. Deleterious influences determine steeper increase in disease risk. They may impact intrauterine life (determine the alpha angle), during the growth postnatal period (beta angle) or during adulthood (gamma angle). It is shown that better environmental conditions during intrauterine life result in slower accumulation of pathogenetic changes (alpha prime angle) and therefore help in preventing disease in later life, even if postnatal conditions do not change

approach has led to the development of numerous studies that have settled and given form to the concept of "developmental programming" [5, 6]. Also, there is increasing evidence for a role of epigenetic mechanisms in translating the environmental influences into the phenotype.

The plasticity of an organism during perinatal development allows it to adopt a phenotype that adapts to its environment [this plasticity is somewhat lost over time as the individual ages]; this is the reason why the same genotype can culminate in a range of different physiological or morphological states as a response to the specific environmental conditions. This implies that, when under stress, the fetus devotes a large part of its resources to the most critical organs at the expense of others whose vulnerability increases. These short-term, survivability-oriented adaptations often end up being detrimental to the organism and may result in the development of illnesses later in life [6–8].

The pathological processes that a mother undergoes during pregnancy, as well as other stressing events which she may endure, influence developmental programming. As we will show later on this chapter, on that basis, epigenetics and evolutionary biology are providing new knowledge to help comprehend the mechanisms behind DOHaD [9].

8.3 Epigenetics and Intrauterine Environment

The risk of suffering a disease can be transmitted from generation to generation. The mechanisms involved are multiple and include societal and cultural habits (determining lifestyle, nutrition, legal and illicit drug consumption, education, etc.), psychological factors and exposure to stressful situations, and biological factors. Among the latter, heritable factors are certainly important. In fact, gene mutations and polymorphisms are important contributors to determine the risk of monogenic and polygenic disorders, respectively. In polygenic complex disorders, the interaction between genes and the environment seem to be key to determine disease risk, and epigenetic mechanisms are critical to mediate the interaction between environmental and genetic factors.

Epigenetic mechanisms are processes which allow the reversible modulation of the genetic expression, without DNA sequence alteration, immediately adjusting the cellular processes to the changing environmental conditions. Epigenetic modifications, which include cytosine methylation, posttranslational modifications of histone tails and the histone core, and the positioning of nucleosomes (octamer of histone subunits), can be transmitted through cellular division (mitosis) and, in some cases, along generations (meiosis) [10, 11].

Moreover, the regulation of the genetic expression through these modifications can be dynamic, or it can be stable when changes in the chromatin lead to a hypermethylated DNA state, resulting in the formation of transcriptionally silent heterochromatin [12]. In mammals, genetic reprogramming occurs both in primordial germinal cells and in zygotes (immediately following fertilization and extending to the morula stage in preimplantation development), consisting in the elimination and remodeling of epigenetic marks, specifically the elimination of DNA methylation

and the loss of histone alterations [11, 13]. This allows the pre-implant zygote to be in a gene expression state compatible with totipotence.

During embrionary development (when the blastocyst is being formed), epigenetic alterations, specially differential methylation, allow undifferentiated cells to specialize and cause the appearance of the different cellular lines by the expression of specific genes [12, 14]. Additionally, these epigenetic marks produced during pregnancy influence placentary development and have an effect on the genomic imprinting and the suffering of some illnesses in the adult life [15].

Studies of follow-up of individuals exposed to historical famines while in utero provided strong evidence for the role of prenatal environment, and specifically nutrition, on later life. The Dutch famine was the result of a transport embargo on food supplies imposed by the German occupying forces in October 1944. Nutrition in the Netherlands had generally been adequate until then, but official rations, which eventually consisted of little more than bread and potatoes, fell below 900 kcal/day by November 1944 and were as low as 500 kcal/day by April 1945. The famine ceased with liberation in May 1945. Effects of the famine have been documented on the course and outcome of pregnancy as well as on fertility, and follow-up studies have documented persistent consequences among the offspring [16, 17].

The first study which posed the hypothesis that prenatal environmental conditions could result in epigenetic changes in humans, and which would in turn manifest themselves during the adult life, was carried out by Lumey et al. [16, 17]. This group discovered that individuals who were prenatally exposed to the Dutch famine of 1944–1945 showed, six decades later, lower DNA methylation of the insulin-like growth factor II (IGF2) imprinted gene, in comparison to their siblings of the same gender who were not exposed to famine. IGF2 is a key factor in human growth and development. In a later study of this cohort, using a genome-scale analysis, authors showed that nutrition at the periconceptional period persistently influenced DNA methylation levels and appeared to have long-lasting phenotypic consequences. In fact, after studying DNA methylation in peripheral blood cells, they showed that exposure to famine in utero influenced the methylation of a number of genomic regions. Differentially methylated loci were preferentially located at regulatory regions and mapped to genes enriched for differential expression during early development and pathways related to growth and metabolism. For example, those genes were involved in forebrain formation and pancreatic beta cell functioning (SMAD7), growth and insulin signaling (INSR), fatty acid oxidation (CPTIA), and cholesterol metabolism (KLF13). Individuals exposed early in gestation to famine had higher birth weights than controls, and unfavorable later-life metabolic outcomes, such as higher BMI, altered glucose response and higher blood total and LDL cholesterol. DNA methylation at INSR was positively correlated with birth weight. DNA methylation at the CPT1A was positively correlated with LDL cholesterol levels [18]. Those results are consistent with the hypothesis that prenatal malnutrition promotes an adverse metabolic phenotype in later life.

The associations mentioned above were specific to periconceptional exposition, which supports the hypothesis that early development in mammals is a crucial period for setting and keeping epigenetic marks [19, 20]. The periconceptional

period has been defined in various ways, but it is frequently considered to include the completion of meiotic maturation of oocytes, differentiation of spermatozoa, fertilization, and resumption of cell cycles in the zygote [21].

Another source of useful information is the Overkalix cohort in northern Sweden. Food availability during the nineteenth century depended largely on quality and size of harvests. After a poor harvest, the worst period was during early spring the following year. During much of the nineteenth century relief from southern Sweden was difficult at this time of the year as there was no rail service available and the frozen Baltic Sea prevented supplies to reach the north via ship. Conversely, after a good harvest, neighboring parishes did not need to purchase the surplus food, and preserving it to next year was difficult. It was probably consumed creating large variations in food energy for the individual from year to year [22]. A transgenerational effect has been suggested in this cohort. Thus, when a paternal grandmother experienced drastic changes, from good to poor and from poor to good, of food availability as a child, her granddaughters had an increased risk of cardiovascular disease during adulthood [22].

However, some controversy still exists about the effects of intra utero starvation on adult disorders because of the lack of influence found in other famine episodes. For instance, study of the survivors of the Leningrad siege did not find an association between intrauterine starvation and glucose intolerance, dyslipidemia, hypertension, or cardiovascular disease in adult life [23]. Similarly, studies of the Finish 1866–1868 famine did not find long-lasting effects. Survival from birth to age 17 years was significantly lower in cohorts born before and during the famine than in the cohorts born after the famine. At subsequent ages, including old age, mortality was practically identical in the famine-born cohorts and in the five cohorts born before and after the crisis [24].

Besides early development stages in utero, the growth periods during infancy and childhood may also be particularly prone to environmental influences. In fact, among the Leningrad siege cohort, women who were 6–8 years old and men who were 9–15 years old at the peak of starvation had higher systolic blood pressure compared to unexposed subjects, and higher mortality from ischemic heart disease and cerebrovascular disease was noted in men exposed at age 6–8 and 9–15 [25].

8.4 The Role of the Placenta: Adult Health and Disease

During intrauterine development of the fetus, the placenta plays a fundamental role: it allows the exchange of gas and nutrients, and the elimination of waste products, protects the fetus from maternal immune system attacks, and secretes hormones associated with pregnancy and growth factors. The mother is constantly transferring information about the environment to her embryo or fetus through the placenta [15, 26].

Moreover, the placenta, which is primarily composed of tissue derived from the zygote, provides environment for fetal gene products to interact with the maternal circulation. For instance, most of the imprinted genes tested in a study by Charalambous et al. show imprinted expression in the placenta and other

extraembryonic tissues, as one could expect due to their role in the allocation of resources to the fetus. Also, many of these genes were found to be exclusively imprinted in the placenta, suggesting a link between the origin of the placenta and imprinting in mammals [27].

Recently, an epidemiological study has shown that the placental phenotype can predict offspring postnatal disease. This was partly due to the fact that fetal growth, which in turn is dependent on the placenta, is a predictor of the occurrence of disease in the adult life [28]. The main nongenetic variable that determines the size of the term fetus is "maternal constraint," which refers to a set of processes by which maternal and uteroplacental factors act to limit the growth of the fetus, by restricting the availability of nutrients and the metabolic-hormonal response to growth. Low maternal height, maternal age (either being too young or too old), being a first or multiple pregnancies, and an inadequate diet are some of the known causes of an increment in maternal constraint, but, barring those, correct placental function is essential to fetal development [26, 29].

There is increasing scientific evidence that some of the biological mechanisms that regulate growth and placental development are behind the programming of chronic diseases; some examples follow. Several cardiovascular diseases in adults have been associated with the size and shape of the placenta. Barker et al. conducted a study working with the Helsinki Birth Cohort in which they, after examining mortality by cause in 1217 men, concluded that a shorter length of the placental surface was associated with higher mortality; moreover, mortality increased as the difference between length and width decreased, that is, the rounder the surface was [30]. However, in order to assess the effectiveness of the placental function, it is useful to compare the weight of the placenta with the weight of the fetus. This has led to the introduction of the concept of the "efficiency" of the placenta, defined as the grams of fetus produced per gram of placenta [28, 31].

Placental efficiency may change over time due to the surrounding nutritional environment, the variations in the maternal microbiome, and probably other biological factors still unknown that could affect the well-being of the mother. Therefore, placental efficiency is considered a marker of the environmental conditions to which the pregnant woman is exposed and is a predictor of chronic diseases in the offspring. Martyn et al. found in Sheffield, United Kingdom, that the highest risks of coronary heart disease in men appeared when, during their pregnancy, the placenta had had less than 15% of the weight of the fetus or more than 22%. Both higher and lower efficiencies were associated with an increased risk of cardiac death [32].

On the other hand, some authors have described how inflammatory conditions in the placenta can alter its shape and have hypothesized that this could compromise the immune function of the fetus and cause proinflammatory states that last until postnatal life, making the offspring more vulnerable to developing chronic diseases during their adult life [33]. Likewise, placental insufficiency, which causes an inadequate flow of oxygen and substrate to the fetus, limits fetal growth, increases oxidative stress and the production of cytokines, and causes alterations in the endocrine system and in epigenetic signaling. It is also noteworthy that the decrease in the supply of nutrients to the fetus (arising from placental insufficiency) is associated

with a redistribution of cardiac output and the programming generated in this context has been shown to cause hypertension and metabolic diseases in the adult life [28, 34, 35].

Finally, we would like to point out that some recent studies describe the relationship between alterations in epigenetic regulation of the placenta and the development of diseases during pregnancy and childhood. Thus, for example, aberrant methylation patterns in placental gene promoters have been linked to gestational trophoblastic disease and preeclampsia, while alterations in genetic imprinting have been associated with intrauterine growth restriction [36].

8.5 Effects of Maternal Nutrition and Life Habits on the Offspring

8.5.1 Nutrition

The risk of suffering a morbid process during fetal development and in adulthood is affected by maternal nutritional status, both at the time of conception and during pregnancy. Maternal diet is the only source of nutrients for the fetus [37]. In situations of low maternal nutrition, the fetus suffers from intrauterine growth restriction, develops a greater insulin response to food, and, as some authors have described, has lower muscular, nephron, and bone development. In these cases, after birth, if the child kept a proper diet, they would have a greater propensity to gain weight and to suffer type 2 diabetes and metabolic syndrome. Also, the low number of nephrons and the hampered cardiac development would result in an increased risk of hypertension and heart failure [38].

Maternal diet seems to play a fundamental role in the neurological development during the intrauterine period. In humans, the brain begins to form 2 weeks after conception, and it is during these early stages when neuronal proliferation, differentiation, and migration occur. Macronutrients allow the construction of the foundations for the brain system, and micronutrients, including vitamins and minerals, are involved in myelination, synaptogenesis, and the production and transmission of neurotransmitters [37, 39].

Low intrauterine growth has been associated with anatomical alterations at the neurological level (such as lower volume of the hippocampus in children), with a lower-than-normal intellectual capacity, poorer spatial memory, worse school performance, and a lower IQ [39]. The analysis of the effects of the deficit of specific micronutrients has led to inconclusive results [37], yet, it is remarkable that a paper published in 2018 by Eyles et al. showed that vitamin D deficiency during pregnancy is related to an increased risk of suffering schizophrenia in adult life [40].

For its part, maternal obesity causes not only complications during pregnancy (such as gestational diabetes, hypertension, and preeclampsia) but also alterations in fetal development. It is worth remembering that obesity often occurs in people with high caloric density diets, but often with a deficit of some fundamental nutrients. Thus, from an epidemiological perspective, the results that have been found

partially agree with those described in pregnant women with poor diets. Children from obese mothers are at increased risk of fetal death, alteration in the growth pattern, congenital anomalies, hypertension and long-term metabolic diseases. Likewise, maternal obesity is also associated with pathological processes derived from a poor neurological development in children; specifically, it has been linked to a greater risk of cognitive deficit, autism spectrum disorders, developmental delay, a higher incidence of attention deficit hyperactivity disorder (ADHD), and anxiety [41].

Epigenetically, DNA methylation patterns of the offspring have been found to be affected by maternal nutrition. Some authors indicate that the characteristics of the diet can affect the DNA methylation status of the genes associated with the metabolic response, with the regulation of the appetite and with genetic imprinting. The children of underweight and overweight mothers show altered DNA methylation patterns, which affects the levels of adiposity in their adult life. It should be noted that epigenetic stimuli appear to occur independently in each pregnancy; a study that analyzed the methylation status of children whose mothers had undergone bariatric gastrointestinal bypass surgery [which had led to an improvement in their weight and cardiovascular profile] described a change in the methylation status of more than 5500 genes, particularly those related to cardiometabolic pathways, in babies born after the surgery compared to those born before the procedure [14].

There are also works focused on the effect of specific micronutrients. For instance, an analysis of the effects of folate supplementation on the maternal diet of mice found a relationship with Avy metastable epiallele expression, which would lead to obese phenotypes in offspring. On the other hand, in animal tests, a deficit of this nutrient has been linked to genomic DNA hypomethylation in the small intestine. Another example is that magnesium deficit in pregnant rats alters the methylation patterns of their offspring in genes related to glucocorticoid metabolism [42, 43].

Regarding macronutrients, a study on pregnant sheep showed that when fed with high-fiber diet there were increases in DNA methylation at CpG islands of IGF2 and H19 imprinted genes in the adipose and muscle tissues, in comparison with those sheep with a diet rich in starch [42]. On the other hand, the exposure to a diet of high density of lipids and energy in mice causes a global hypermethylation of the genes associated with the metabolism of fatty acids in the liver of offspring [44].

Overall, those results suggest that (a) maternal nutrition plays an important role not only on fetal development but also in determining the risk of chronic disorders in adulthood and (b) epigenetic mechanisms, and specifically DNA methylation, are likely involved in embedding the effect of environmental influences on the genome.

8.5.2 Smoking

Approximately 2% of the population smokes during pregnancy. The highest prevalence of smoking during pregnancy is recorded in European countries (where the average is 8.1%) and the lowest prevalence in African countries (with an average of 0.8%) [45]. Maternal smoking during pregnancy and after birth is the main

modifiable risk factor for morbid processes in the infant. Tobacco consumption in pregnant women, and to a lesser extent her exposure to tobacco smoke, has been related to fetal death; to a higher incidence of ectopic pregnancy; to the suffering of placental abruption, spontaneous abortion, and premature birth; and to a decreased growth of the fetus. Effects that manifest themselves later during childhood have also been described, such as a greater risk of sudden death; suffering from overweight, diabetes, cancer, and pathological upper respiratory tract processes; and of being diagnosed with attention deficit or behavioral disorders [46–48].

Some studies show that smoking cessation interventions during pregnancy reduce the prevalence of low birth weight, premature birth, and infant morbidity and mortality [49]. The biological mechanisms involved in these processes are largely unknown. Some experimental studies show that prenatal nicotine exposure alters the neurological development in animals; it has been suggested that the anorexigenic, hypoxic, vascular, and placental effects of nicotine may have direct teratogenic influences on the fetus and thus result in an incorrect physiological and psychological development [47].

From an epigenetic perspective, most studies have focused on the respiratory system. In mice, a study conducted by Lee et al. showed that environmental exposure to tobacco smoke during pregnancy caused a significant increase in IFN-γ methylation and a significant decrease in IL-13 methylation in the offspring, which in turn could result in an elevated risk of lung inflammation and increased airway hyperreactivity [50]. In the same vein, Cole et al. found alterations in the global DNA methylation and specifically in the methylation of the promoter of IFN-γ and Thy-1 (potentially manifesting in the adult stage as pulmonary fibrosis) in mice with perinatal exposure to tobacco smoke [51].

8.5.3 Alcohol

It is estimated that one in ten pregnant women consume alcohol and one in five who do so drinks enough to cause damage to the fetus. Alcohol use disorders have a high heritability (estimated in several studies around 50%) which would indicate the genetic transmission of risk from parents to children regardless of the environment.

In prenatal alcohol exposure, both the pattern and the duration of consumption are determining factors in the effects on the fetus. Maternal consumption can cause fetal alcohol syndrome which, in the milder cases, would result in neurodevelopmental disorders, cognitive or behavioral anomalies [behavioral and attention deficit disorders being the most common], and, in severe cases, facial anomalies, growth retardation, and central nervous system dysfunction. In addition, prenatal exposure to alcohol can cause psychological disorders in adult life, including addictive disorders [52, 53].

On the other hand, paternal consumption of alcohol before fertilization has been linked to anatomical (decrease in intracranial volumen) and cognitive (lower intellectual performance) alterations in the offspring. However, most works emphasize the difficulty of controlling the social and environmental factors derived from being

raised by a father with an alcohol use disorder and whether the cause of these alterations lies in these factors or in changes acquired in male gametes that are transmitted to offspring.

Results from animal studies support that alcohol induce epigenetic alterations that could be transmitted to the offspring. Different research groups found that paternal ethanol exposition in mammals induces (in the absence of exposure to ethanol by the mother) anomalies in the offspring such as low birth weight, thickening of the layers of the cerebral cortex, low levels of testosterone, learning difficulties, or an increase in anxiety and impulsivity. In addition, Kim et al. found changes in the brain expression of DNMT1 and MeCP2 in the offspring of alcoholic parents, suggesting potentially widespread epigenetic abnormalities in these animals [54]. In the same line, in an experiment performed on descendants of bulls exposed to alcohol, changes in hypothalamic gene expression were observed during adolescence. Parental binge alcohol abuse alters hypothalamic gene expression in the F1 generation in the absence of direct fetal alcohol exposure [55, 56].

8.6 Prenatal Influences on the Epigenome and the Skeleton

Summarizing previous sections, from the moment of conception to adulthood, the environment shapes the phenotypic output. It is thought that there are certain "high-sensitive" windows, especially during development that have major influence on the epigenome [21]. The developmental origins of health and disease concept suggests that poor developmental experience can increase the risk of noncommunicable diseases in later life, including cardiovascular, metabolic, neurological, and skeletal disorders [57]. A variety of mechanisms, including DNA methylation and other long-lasting epigenetic marks, may mediate the influence of the environment on the developing organism [18, 58].

As previously mentioned, malnutrition and starvation endured during famine may affect not only children and adults but also fetuses in utero. Given ethical and practical constraints, there are no interventional studies demonstrating a direct relationship between maternal malnutrition and offspring skeletal status. Nevertheless, several studies showed suggestive associations between intra utero nutrition and bone health [59]. For instance, neonatal bone mass has been associated with maternal food intake at 18 weeks of gestation [60]. Cooper et al. found that low birth and infant weight and diminished growth rates correlate with both a decreased bone mineral content (BMC) and an increased risk of hip fractures in later life [61, 62].

A few studies have explored the role of developmental factors in skeletal disorders. For example, in the Southampton Women's Survey, a higher velocity of 19- to 34-week fetal femur growth was strongly associated with greater childhood skeletal size and BMC [63]. A systematic review of the literature that included nine studies that assessed the relationship between birth weight and bone mass, BMC showed that higher birth weight was associated with greater adult BMC, especially at the lumbar spine [64]. In keeping with this concept, another systematic review found that a positive association between birth weight and bone mass was clear among

children, unclear among adolescents, and weak among adults. The effect was stronger on BMC than on bone mineral density (BMD) regardless of age [65]. Of course, BMC is strongly dependent on bone size. BMD is calculated by DXA machines as the ratio of BMC divided by the projected bone area. Thus, BMD is also partially influenced by skeletal size but to a much lesser degree than BMC. Therefore, those results suggest that intrauterine growth is more closely related to bone size than to bone density and that the effect tends to be mitigated by postnatal influences. In general, bone mass increases during the growth period, reaches a maximum ("peak bone mass") by the third decade of life, and keeps at a steady level for several years. Later, bone mass decreases with advancing age, and the loss is particularly rapid in women in the decade following menopause. It seems that early-life exposures are important for determining peak bone mass, which may be a reflection of the combined influence of intrauterine and early postnatal environmental exposures (Fig. 8.2).

In general, it is expected that genetic and environmental influences during gestation have a stronger impact on the phenotype during early years of life, while their consequences may become progressively obscured by postnatal factors with advancing age. Thus, it has been difficult to demonstrate an association between intrauterine factors and fractures occurring later in life. In this line, investigators of the Helsinki Birth Cohort Study found some signs of nonlinear association of adult hip fractures with childhood growth indices, but not with body weight at

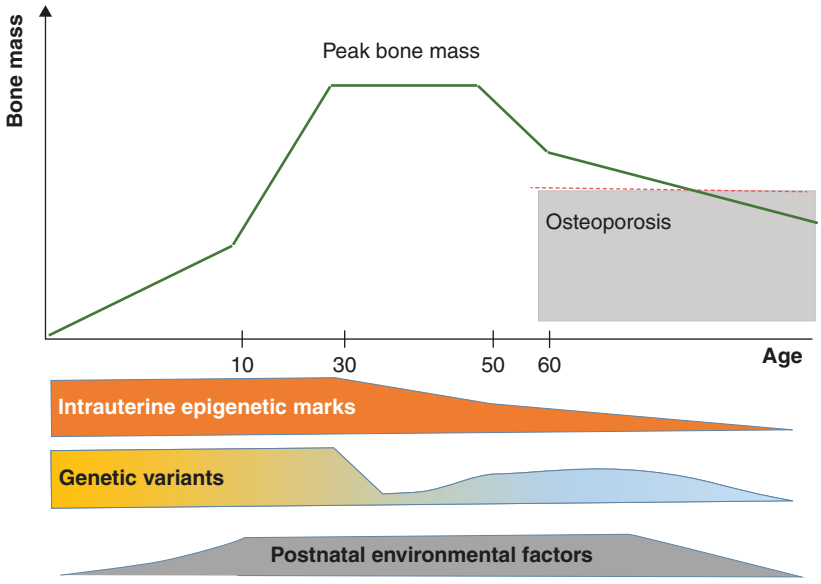

Fig. 8.2 Model of the influences of genetic and environmental factors on bone mass—the relative importance of intrauterine-determined epigenetic marks, genetic variants, and other environmental factors at different age periods is depicted. Genetic factors determining peak bone mass may be different from those determining the loss of bone mass after menopause and with aging

birth [66]. Similarly, in a Swedish study, birth weight was associated with adult BMC, but did not translate into an association with the risk of fractures over 50 years of age [67].

Vitamin D is a particularly important nutrient for bone health. The endogenous synthesis in the skin, induced by sun's ultraviolet radiation, is the main source of vitamin D. Vitamin D is later hydroxylated in the liver to 25-hydroxyvitamin D (25(OH)D) and then in the kidney to form 1,25-dihydroxyvitamin D. This is the most potent vitamin D metabolite, but 25(OH)D is much more abundant in serum, and thus it is the best index of the vitamin D nutritional status. Vitamin D availability and 25(OH)D serum levels fluctuate and are highest in late summer and early autumn and lowest in winter and early spring.

Several studies have suggested an association between maternal 25(OH)D levels and bone mass of the offspring [61]. In the Western Australian Pregnancy Cohort (Raine) Study, maternal serum 25(OH) was positively associated with total body BMC and BMD in offspring at 20 years of age [68]. Additionally, children born in winter may have lower BMC than those born in summer, which is consistent with an anabolic effect of vitamin D availability. Thus, maternal nutrition and specifically the maternal vitamin D status may be an important factor for an adequate intrauterine growth rate and skeletal mass acquisition [69], but studies showed conflicting results [70]. Rather surprisingly, in a recent study of individuals of the Rotterdam cohort, severe maternal 25(OH)D deficiency during midpregnancy was associated with higher offspring BMC and bone area at 6 years of age, while no associations were seen between maternal vitamin D status and offspring BMD [71]. Also, interventional studies have not consistently demonstrated a beneficial effect of vitamin D supplementation to gestating women and fetal or infant growth, even in Asian populations with high frequency of vitamin D deficiency [72, 73].

There is some preliminary evidence that the influence of the early-life environment on bone may be mediated by epigenetic factors. In a study with rodents, Xue et al. found that maternal vitamin D status influences DNA methylation state in the germline, which is transmitted to the unexposed second generation [74]. Also, studies in a British mother-offspring cohort found an association of the methylation levels of several genes (such as eNOS, RXRA and CDKN2A) in cord blood and bone mass at childhood [75–77]. Nitric oxide (NO) is produced by the NO synthases (NOS) family. NOS are expressed in many tissues and modulate the activity of many cells, including those of bone [78]. Harvey et al. studied the methylation level of two CpGs in the promoter of the endothelial-type NOS and found an association between the methylation of one of the two CpGs and the child's whole-body bone area, BMC and BMD at age 9 years [76]. This research group later reported that the methylation of a region within the promoter of the long noncoding RNA ANRIL, encoded by the cyclin-dependent kinase inhibitor 2A (CDKN2) locus, was associated with offspring adiposity and bone mass and size, as assessed by bone area, BMC and BMD at 4 and 6 years of age [77]. ANRIL (official name CDKN2B-AS1) interacts with polycomb-repressive complex-1 (PRC1) and polycomb-repressive complex-2 (PRC2), leading to epigenetic silencing of other

genes. This region is a significant genetic susceptibility locus for cardiovascular disease and has also been linked to a number of other pathologies, including several cancers, intracranial aneurysm, type-2 diabetes, periodontitis, Alzheimer's disease, and frailty.

Harvey et al. also used the Southampton birth cohort to explore the association between vitamin D levels, the methylation of the Retinoid-X receptor-alpha gene (RXRA) in cord blood and bone mass [75]. RXRA forms heterodimers with the vitamin D receptor and thus is an essential cofactor for the action of 1,25(OH)2D on target genes. Harvey's results suggested that perinatal epigenetic marking at the RXRA promoter region in the umbilical cord was inversely associated with offspring size-corrected BMC in childhood. However, the attempt for replication was not completely successful. They found that in 230 children aged 4 years, a higher percent methylation at four of six RXRA CpG sites was correlated with lower offspring BMC. In a second independent cohort ($n = 64$), similar negative associations at two of these CpG sites, but positive associations at the two remaining sites, were observed; however, none of the relationships in this replication cohort achieved statistical significance. The maternal free 25(OH)D was negatively associated with methylation at one of these RXRA CpG sites. Interestingly, vitamin D supplementation during gestation appears to modulate methylation, inducing small but statistically significant changes in the methylation level of the RXR gene [79]. These are exciting results that need to be confirmed in other population cohorts.

Socio-economic status (SES) and other social factors influence bone mass. Indeed, social deprivation during early life (both pre- and postnatal) has a negative impact on the skeleton. The mechanisms involved are likely multiple and include nutritional deficiencies, psychological stress responses, and persistent low-degree inflammation [80–82]. Those responses may be mediated, at least in part, by epigenetic mechanisms, including the methylation of genes encoding the glucocorticoid receptor and several cytokines. Those changes result in exaggerated or persistent secretion of glucocorticoids and pro-inflammatory cytokines that promote bone resorption, while at the same time they have an inhibitory effect on bone anabolism [83] (see next chapter).

The influence of SES in postnatal life and skeletal health is widely recognized and will be described in detail in the next chapter. There are fewer data about the effect of SES during gestation on bone. However, SES is associated with environmental features, such as nutrition or smoking habits, that in turn are associated with skeletal mass acquisition and can mediate the influence of SES while in utero on bone development. Also, low SES is frequently associated with stressful situations, which may have an impact on the epigenome.

In a number of human studies, prenatal exposure to maternal stressful conditions, including acute and chronic stressors, anxiety, or depression (which are all known to be accompanied by elevated cortisol levels), was associated with an increased risk of multiple neurobiological and behavioral problems in adult life, such as autism, anxiety, and schizophrenia. Likewise, maternal psychosocial stress was associated with metabolic abnormalities in the offspring, such as obesity and dysregulated glycemic control [84, 85].

Maternal stress and depression during gestation have been postulated to influence the methylation of several genes, including the glucocorticoid receptor, in the offspring [86–89]. As a form of extreme social deprivation with marked psychological stress and undernutrition, studies of Holocaust survivors have shown that Holocaust exposure had an effect on the methylation of the FK506-binding protein 5 gene (FKBP5). This gene encodes a member of the immunophilin protein family, which plays a role in immunoregulation and the response to glucocorticoids. The methylation changes were present both in the Holocaust-exposed parents and in their offspring [90].

In this line, Dias performed some tantalizing experiments by using olfactory fear conditioning to address when and how the olfactory experience of a parent might influence their offspring. They subjected F0 mice to odor fear conditioning before conception and found that subsequently conceived F1 and F2 generations had an increased behavioral sensitivity to the F0-conditioned odor, but not to other odors. This was associated with changes in the methylation of odor receptors that appeared to be transmitted through gametes to new generations [91].

These results show how the experiences of a parent, even before conceiving offspring, may influence both structure and function in the nervous system of subsequent generations. Such a phenomenon may contribute to the etiology and potential intergenerational transmission of risk for neuropsychiatric disorders, as well as other processes linked to stress responses. Additionally, from an evolutionary perspective, they could represent a mean by which experiences about avoiding dangerous situations could be transmitted to offspring independently of education.

8.7 Intergenerational Transmission of Epigenetic Marks

Epigenetic marks can certainly be transmitted through mitosis during cell divisions. However, there is less evidence of a real heritable transmission of epigenetic marks through several generations. As mentioned above, human studies showed that exposure to famine, endocrine disruptors, or trauma can affect descendants. However, it is to note that fetal tissues, including the gonads, are exposed to the maternal environmental conditions while in utero. Therefore, the environmentally induced changes in the epigenome ("epimutations") can directly affect F0, F1, and F2 without the need of invoking a true hereditary transmission (Fig. 8.3). Hence, transmission into F3 is needed to confirm inheritance when the exposed ancestor is the mother or into F2 when the ancestor is the father [92, 93]. Studies in humans are limited by the low number of successive generations. Animal models have been instrumental to circumvent that limitation. Thus, transgenerational transmission of behavioral and metabolic phenotypes occurred up to the fourth generation in a mouse model of paternal postnatal trauma [94].

DNA methylation signatures have been the main focus in studies of intergenerational transmission of epigenetic marks. However, recent experimental studies point to sperm-derived microRNAs as another epigenetic mark potentially transmitted through generations [95]. Although both intergenerational (from F0 to F1) and

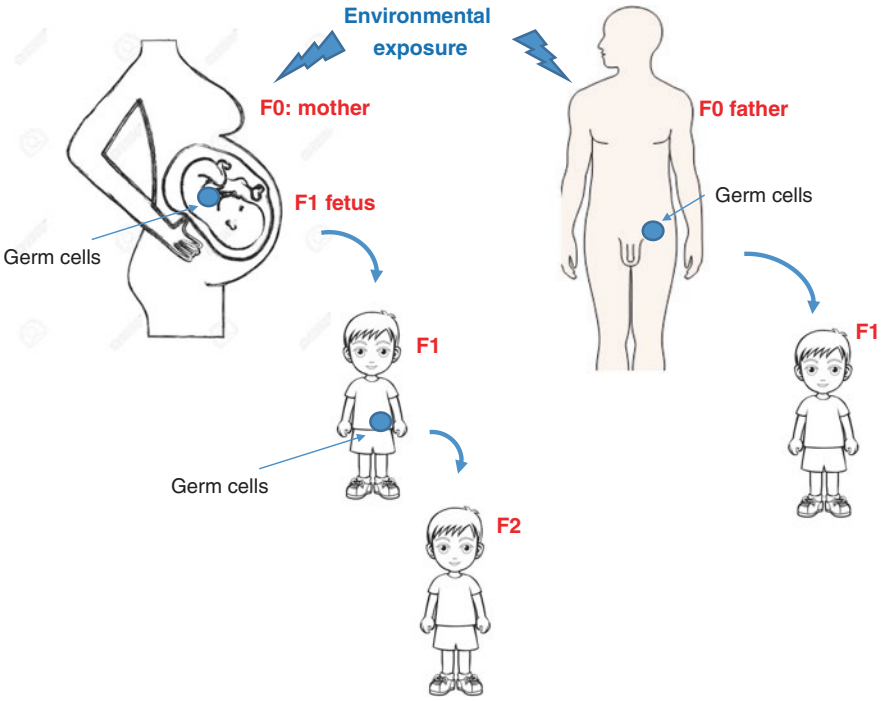

Fig. 8.3 Transgenerational and intergenerational transmission of genomic features—Left: environmental exposures to pregnant mothers (F0) may also directly affect the fetus (F1) and his/her gonads and germ cells. Environmental-induced epigenetic marks can then be transmitted into F2. F2 germ cells are the first not directly exposed to environmental influences, so that marks transmitted into F3 represent tru-inherited transmission. Right: environmental exposures can directly affect men (F0) and their germ cells, which will give place to F1 individuals. The germ cells of F1 can still have those environmentally induced epigenetic marks, but they are no longer exposed. So that marks transmitted into F2 can be considered as truly inherited

transgenerational (from F0 to F3 or F4) transmission of environmental adversity effects have been established in animal models, studies in humans have not yet demonstrated that the effects of social deprivation, trauma, and other exposures are heritable through epimutations.

8.8 Conclusion: A Lifetime Approach to Chronic Disorders

Adult chronic disorders represent an increasing proportion of the global burden of disease. Thus, disorders such as hypertension, diabetes, osteoporosis, or obesity are a very prevalent cause of morbidity and result in a major proportion of deaths. These disorders are the result of complex interactions between genetic and environmental factors. There is increasing epidemiological and experimental evidence showing that the influence of environmental exposures takes place not only after birth but

also during intrauterine life. Although randomized interventional studies are difficult to perform in this setting, a variety of studies suggest that factors such as maternal nutrition and stress result in adaptative changes in the fetus, affect fetal growth and development, and influence the risk of disease in later life. Evidence is stronger for metabolic disorders, such as obesity and related cardiovascular disorders. There are still scarce data about skeletal disorders, but several studies suggest that intrauterine development is associated with postnatal bone size and bone mineral content and therefore likely affects the peak bone mass attained at the end of the postnatal growth period. Whether it translates into a higher risk of osteoporosis and fractures is unclear. However, already available data call for attention to the circumstances of early life, including both prenatal period and childhood, in order to minimize the risk of disease in later life as adults.

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9 Postnatal Social Factors: The Epigenome and the Skeleton

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9.1 Overview of Epigenetic Marks and Their Impact on Gene Expression

The term epigenetics was coined by Waddington to describe the class of internal and external interactions between the environment and the genes leading to the development of phenotype [1]. The current concept of epigenetic mechanisms includes factors, other than DNA sequence, that cause stable changes in gene expression and are maintained during cell divisions [2]. The main epigenetic mechanisms are the methylation of cytosines in DNA, posttranslational changes of histone tails, and noncoding RNAs (ncRNAs), including microRNAs (miRNAs, about 22 nucleotides long) and long noncoding RNAs (lncRNAs, containing more than 200 nucleotides).

9.1.1 DNA Methylation

Most cytosines in DNA are methylated, particularly when they are part of CpG dinucleotides. In somatic cells, more than 80% CpGs are methylated, especially in repetitive sequences in intergenic regions and introns. The promoters of many genes contain CpG-rich regions ("CpG islands"), which may be methylated or not, depending on the transcriptional status of the gene. The methylation of cytosines is a dynamic process. DNA methyltransferases (DNMTs) are a family of enzymes responsible for the methylation of DNA. DNMT1 recognizes

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hemimethylated CpG sites on newly replicated DNA, and thus it is responsible for maintaining the methylation pattern through cell divisions. On the other hand, DNMT3A/3B are the novo methylases. They convert unmethylated CpGs into methylated CpGs in double-strand DNA. This process is particularly important during embryogenesis and cell differentiation. DNA methylation marks can be removed. It follows several steps that involve the ten eleven translocation (TET) family and other enzymes.

In general, the methylation of CpGs in gene promoters is associated with repression of gene expression, whereas unmethylated state of the promoters is a characteristic of actively transcribed genes. However, the methylation of gene bodies and other regulatory regions, such as enhancer regions, has a less predictable effect, because it could either potentiate or decrease gene expression.

9.1.2 Histone Marks

The main components of chromatin are DNA and histone proteins. Nucleosomes are the basic units of the chromatin, formed by histone octamers bound to a DNA segment. DNA-bound histones play major roles in the regulation of gene transcription. Posttranslational modifications of specific amino acids in histone tails, such as methylation, phosphorylation, acetylation, and ubiquitylation, modulate the chromatin conformation and interact with the transcription machinery to activate or repress gene expression. This constitutes the so-called histone code. In general, lysine acetylation and trimethylation of lysines 4, 36, and 79 in histone 3 are associated with active genes. On the contrary, marks associated with repressed genes include trimethylation of lysines 9 and 27 [3].

DNA methylation and posttranslational modifications of histone tails usually act in concert to regulate gene transcription. For example, the methylation of lysine 27 of histone 3 (usually represented as H3K27) tends to be associated with methylated promoters and repressed genes, whereas acetylation of histone tails is usually present in regions with actively transcribed chromatin. A variety of proteins (including acetylases, deacetylases, methylases, etc.) are responsible for maintaining the histone code. For example, the polycomb repressive complex 2 (PRC2) catalyzes the methylation of H3K27. PRC2 includes several structural components and the catalytic subunit enhancer of zeste homolog 2 (Ezh2) [4].

9.1.3 Noncoding RNAs

Messenger RNA (mRNA) contains the information needed to combine amino acids on the ribosomes into protein chains. A variety of noncoding RNAs (ncRNAs) play various roles in the regulation of cell activities. Thus, ncRNAs are also frequently

included among the mechanisms of epigenetic control [5]. They are classified as small RNAs (<200 nucleotides) and long RNAs (>200 nucleotides). Among small RNAs, microRNAs (miRNAs, 18–25 nucleotides) have been most extensively studied. Most miRNAs derive from primary miRNA transcripts (pri-miRNAs) transcribed by RNA polymerase II. Pri-miRNAs are cleaved within the nucleus, by a complex including DROSHA and other proteins, into pre-miRNAs (70 nucleotides). Once transported into the cytoplasm, pre-miRNAs are converted into mature miRNA by Dicer and a helicase. miRNAs are then incorporated into RNA silencing complexes that bind to specific sequences in the 3′-untranslated regions of target mRNAs. This results in mRNA degradation and/or stopping protein translation [6]. There are a few thousand miRNAs, each one contributing to the regulation of several genes. Overall, miRNAs may influence the activity of at least 60% of the protein-coding genes.

Long noncoding RNAs (lncRNAs) are transcribed from specific DNA regions. These regions may be located between protein-coding genes or may partially overlap with protein-coding regions, being transcribed in a sense or antisense way. lncRNAs modulate gene activity by several mechanisms, including both transcriptional and posttranscriptional events. For instance, lncRNAs often serve as scaffolds for transcription factors and other molecules involved in initiation of transcription, including repressive chromatin modifiers such as PRC1 and PRC2, or activating chromatin modifiers. Some lncRNAs are mainly located in the cytosol, where they can target mRNAs and downregulate protein translation. Interestingly, they may also act as "sponges" for miRNAs, thus preventing the inhibitory effect of miRNAs on protein translation.

9.2 Epigenome Molecular Network

There are complex interactions between epigenetic mechanisms that result in the fine regulation of gene activity and the maintenance of the phenotypic features of daughter cells after cell divisions. For example, MeCP2, a protein recognizing methylated CpGs, promotes the activity of histone deacetylases (HDACs). Thus, both gene-repressing marks (methylation of DNA and non-acetylated histones) tend to combine in order to block transcription in inactive genes. The methylation of promoters regulates the transcriptional activity not only of protein-coding genes but also of miRNAs and other noncoding RNAs. In turn, miRNAs contribute to modulate the synthesis of DNMTs and histone-modifying enzymes. lncRNAs also influence the activity of genes encoding chromatin-modifying enzymes and miRNAs [7]. Although the sequence of molecular steps is still unclear, there is evidence for the notion that DNA, RNA, and histone proteins, along with their modifications, act in a concerted fashion to bring about chromatin states that are important for dictating genomic functions [5] (Fig. 9.1).

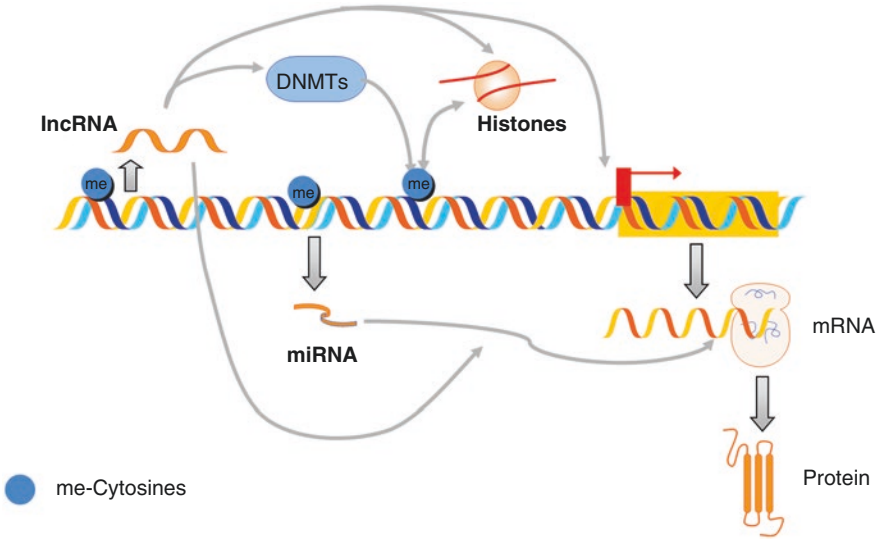

Fig. 9.1 Schematic representation of the interactions between epigenetic mechanisms

9.3 Epigenetic Mechanisms and the Differentiation of Skeletal Cells

The growth and maintenance of bone tissue depends on the activity of several cells, mainly osteoclasts and osteoblasts. Osteoclasts derive from hematopoietic precursors. They are multinucleated highly specialized cells that resorb the bone. On the other hand, osteoblasts are the cells responsible for synthesizing new bone matrix. Osteoblasts derive from mesenchymal cells. Pluripotent mesenchymal stem cells (MSCs) can differentiate into other cell types, besides osteoblasts, such as adipocytes, chondrocytes, and myocytes. During the period of skeletal development, both in uterus and after birth, the activity of chondrocytes and osteoblasts is critical for bone growth. Osteoclasts are also needed to reshape the bones. Once bone acquisition is finished, the skeleton does not remain static. On the contrary, the bone is being continuously removed by a process called bone remodelling. Thus, small volumes of bone tissue are degraded by groups of activated osteoclasts. This phase of bone resorption is followed by a phase of bone formation, characterized by the presence of active osteoblasts that form new bone that fills the cavity previously eroded by osteoclasts. Thus, the adequate balance between the activity of osteoclasts and osteoblasts is critical for the maintenance of the skeletal mass.

9.3.1 DNA Methylation, Histone Code, and Bone Cells

The differentiation of MSCs toward the osteoblastic lineage is induced by the master transcription factors RUNX2 and osterix and is stimulated by ligands of the Wnt and BMP pathways [8]. As it happens in other tissues, epigenetic factors play

critical roles in determining the fate of MSCs, which depends on the activation and repression of specific sets of genes. Specifically, the genes that are characteristic of the osteoblast-osteocyte lineage (such as alkaline phosphatase, sclerostin, RANKL, osteoprotegerin (OPG), etc.) tend to undergo demethylation and derepression during the differentiation of MSCs [9–12].

The differentiation of osteoclast precursors is also associated with marked changes in DNA methylation. PU.1 may play an important role, by recruiting DNMT3B to hypermethylated promoters and TET2, which converts 5-methylcytosine to 5-hydroxymethylcytosine, to genes that become demethylated [13]. Another DNA methyltransferase, DNMT3A, is essential to methylate and repress anti-osteoclastogenic genes, thus allowing osteoclast differentiation to continue [14].

The RANK-RANKL-OPG system plays a critical role in osteoclast differentiation. Osteoclast precursors express the receptor RANK on the cell membrane. Cells of the osteoblastic lineage express RANKL, which binds to RANK expressed by osteoclast precursors and leads to the formation of mature osteoclasts. On the contrary, OPG, also expressed by osteoblasts and other cells, is a decoy receptor for RANKL, preventing its binding to RANK. Therefore, OPG exerts an inhibitory effect on osteoclastogenesis. Thus, the methylation of RANKL and OPG promoters in cells of the osteoblastic lineage indirectly contributes to the regulation of osteoclastogenesis by influencing the expression of RANKL and OPG [11].

A variety of histone-modifying enzymes are involved in the regulation of MSC differentiation. In fact, histone methyltransferases, such as Suv420h2, which methylates lysine 20 in histone 4 (H4K20), are required for the differentiation of MSCs into osteoblasts [15]. On the other hand, other methylases, such as EZH2, tend to have a negative effect on osteoblastogenesis. EZH2 methylates lysines 27 in histone 3. The inhibition of EZH2 stimulates osteogenic differentiation and inhibits adipogenic differentiation in vitro [16], while it has complex effects in vivo, with diverging consequences on the commitment and proliferation of osteoblast precursors [17]. Histone deacetylase (HDAC) family is also involved in MSC differentiation. These enzymes remove acetylation marks in histone tails. Since histone acetylation is generally associated with active chromatin, HDACs tend to inhibit gene transcription. Inversely, HDAC inhibition tends to promote osteogenic differentiation, at least in vitro [18], which likely involves the epigenetic regulation of RUNX2 [19].

Acetylated lysines in histone tails are recognized by the bromodomain and extra-terminal domain (BET) protein family, which binds to acetylated lysines and then acts as a scaffold for molecular complexes leading to gene transcription. The BET family participates in osteoclastogenesis. In fact, the inhibition of BET blocks the expression of NFAT1c (involved in the signaling cascade after the binding of RANKL to RANK) and consequently suppresses osteoclast differentiation and activity in vitro [20].

9.3.2 Noncoding RNAs and Bone Cells

Small and long noncoding RNAs are frequently included as epigenetic factors because they can be transmitted into daughter cells during cell division. They may also act as messengers between neighbor cells passing through gap junctions [21].

miRNAs can be secreted into extracellular vesicles and influence the activity of neighbor or distant tissues. Within the skeletal field, this mechanism participates in signal communication between muscles and bones [22, 23].

Many miRNAs modulate the differentiation and activity of osteoblasts in vitro [24–26]. A few of them have demonstrated skeletal effects in vivo [27]. A few human studies have explored the association of miRNA expression in bone or circulating miRNA levels with osteoporosis and fractures [28–30], but additional data from larger studies are needed to elucidate the actual pathophysiological relevance of those findings.

As with the osteoblastic lineage, several miRNAs influence osteoclast differentiation in vitro. Some of them have been validated in vivo. For instance, miR-503, which targets RANKL, and miR-34a inhibit bone resorption in animal models, whereas miR-148a tends to stimulate resorption [24, 26, 31]. Although less extensively studied than miRNAs, lncRNAs also contribute to the regulation of bone cell differentiation [32, 33].

9.4 The Genetic and Postnatal Environmental Determinants of the Epigenome

Overall, the epigenome shows some random variation both between cells and between individuals. Although not involving DNA sequence, the epigenome is also influenced by genetic variation (see below) and by internal and external environmental influences. Thus, unlike the genome, the epigenome varies across cell types and is not constant but changes with time. This temporal variation also has a stochastic (random) component and may depend on environmental factors. In addition, DNA methylation and other epigenetic marks experiment age-dependent modifications that likely play an important role in normal aging and in aging-associated disorders, such as osteoporosis and osteoarthritis [2, 33, 34]. Specifically, complex interactions between environmental stressors, metabolic pathways, and epigenetic marks may be involved in the regulation of stem cell function and aging [35].

Studies combining genotyping and analysis of DNA methylation have identified DNA polymorphisms that are associated with the methylation level of neighbor regions. DNA methylation at specific loci can be influenced by sequence variations, such that individual genotypes at a given locus may result in different patterns of DNA methylation due to allele-specific methylation. These sites are called methylation quantitative trait loci (meQTLs) and can influence the methylation pattern across an extended genomic region. Most meQTLs mapped to intronic regions, although a limited number appeared to occur in synonymous or nonsynonymous coding SNPs [36]. Overall, genetic variation is estimated to explain about 20–25% of the differences in DNA methylation [37, 38] (Fig. 9.2).

A variety of environmental factors also influence the epigenome and specifically DNA methylation. This happens both in utero and during postnatal life. Some of the postnatal influences are shortly reviewed below while keeping in mind that the epigenome is shaped by the interaction of external factors with genetic characteristics.

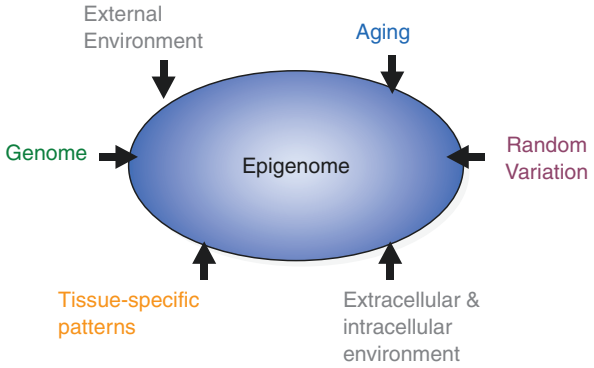

Fig. 9.2 Factors determining the epigenome

9.4.1 Pollution

Although the effects on the respiratory system are the best known consequences of air pollution, contaminants have negative consequences on many organ systems. The mechanisms involved are likely multiple but incompletely understood. Nevertheless, they likely include epigenetic modifications. Most published studies are observational and have explored the influence on DNA methylation. As shown in a recent review of this topic [39], the effects of pollution on the methylome were generally small. Nevertheless, particulate matter levels were positively associated in several studies with global or LINE-1 hypomethylation, a hallmark of several diseases. Air pollution species may also accelerate the epigenetic changes associated with aging [40].

In this line, some recent tantalizing studies suggest that even short-term exposure to air contaminants may impact the epigenome. Li et al. studied the effect of 9-day exposure to high levels of particulate matter (PM2.5) and found that it modified the methylation of several genes involved in oxidative stress, cell survival, inflammation, and glucose and lipid metabolism [41]. Similarly, an acute exposure to ambient ozone can decrease the methylation of the genes encoding angiotensin-converting enzyme and endothelin-1 and increase the circulating levels of this factor, which may be partly responsible for the effects of ozone on blood pressure [42].

9.4.2 Nutrition

As already explained in previous chapters, nutrition impacts the epigenome, both during conception and after birth. Nutrients influence gene expression by a variety of mechanisms, including epigenetic ones.

Some nutrients may have specific effects on epigenetic marks. Folate metabolism is linked to phenotypic changes through DNA methylation, as folate, a water-soluble B vitamin, is a cofactor in the pathways leading to DNA methylation [43]. Thus, long-term supplementation with folic acid and vitamin B12 in elderly subjects resulted in effects on DNA methylation of several genes, including some genes implicated in developmental processes [44].

Vitamin B12 and folate deficiency increases homocysteine levels, which in turn are associated with high cardiovascular risk. Some studies suggest that the link of hyperhomocysteinemia with vascular diseases might be due to the fact that homocysteine competes with the methyl group donor S-adenosylmethionine for binding to DNMT, thus leading to DNA hypomethylation [45].

Moderate calorie restriction appears to extend lifespan through a variety of mechanisms. Among them, increased activity of sirtuins, a histone deacetylase family, plays a role. Resveratrol, a compound present in grapes, has been proposed to have a longevity-promoting effect by activating sirtuins [46]. Sirtuin activity also has a beneficial effect on the skeleton, because it tends to enhance osteoblast differentiation and activity, while osteoclast function is inhibited [47]. However, marked undernutrition has deleterious effects on health, as demonstrated in multiple studies, including those exploring the consequences of historical famines [48].

9.4.3 Smoking

The negative impact of smoking on health outcomes is well known and includes increased risk of cardiovascular disorders, bronchopulmonary diseases, and several cancers. Thus, tobacco use disorders are the largest preventable cause of morbidity and mortality in developed countries. Several studies have explored the effects of smoking on DNA methylation. Those studies have revealed differentially methylated CpG regions in smokers in comparison with non-smokers. One of the genes most consistently pointed out in those studies is aryl hydrocarbon receptor repressor (AHHR). In fact, AHHR methylation may serve as a biomarker for smoking status [49, 50]. Tobacco exposure also influences the methylation of other genes, not only in the airways but in other tissues, such as the adipose one, which may contribute to the metabolic effects of smoking [51].

9.4.4 Stress

Early life exposure to stressful life events is an important risk factor for developmental programming of adverse health outcomes, including metabolic disorders and behavioral and psychiatric conditions such as anxiety and schizophrenia later in life. Increasing evidence from experimental, clinical, and epidemiological studies highlight the importance of epigenetic regulation in mediating these long-term effects [52]. Psychological stress has been consistently shown to have an impact on the epigenome and specifically on the methylation of several genes, both pre-and postnatally. Of course, genes related to the nervous system and psychological reactions have been most studied.

A plethora of experimental studies have confirmed that stressful situations modify the methylation of genes in the central nervous systems [53]. In a widely commented study, Weaver et al. reported that increased pup licking and grooming by rat mothers altered the offspring methylation at the glucocorticoid (GC) receptor gene

promoter in the hippocampus. These differences emerged over the first week of life, were reversed with cross-fostering, persisted into adulthood, and were associated with differences in the hypothalamic-pituitary-adrenal responses to stress, suggesting a causal relation among epigenomic state, GC receptor expression, and the maternal effect on stress responses in the offspring [54]. More recently, in a study with rhesus macaques, Snyder-Mackler et al. found that social status alters the dynamics of GC-mediated gene regulation by a variety of mechanisms, including changes in chromatin accessibility to the transcriptional machinery in response to GC [55].

Studies in humans are much more difficult. Nevertheless, several observational studies are in line with experimental data. McGowan et al. examined epigenetic differences in the neuron-specific GC receptor (NR3C1) promoter in postmortem hippocampus from suicide victims with a history of childhood abuse, suicide victims with no childhood abuse and controls. They found decreased levels of glucocorticoid receptor mRNA, as well as increased cytosine methylation of the NR3C1 promoter. These findings translate previous results from rat to humans and suggest that parental care impacts the epigenetic regulation of hippocampal glucocorticoid receptor expression [56]. Genome-wide analysis has shown that abuse is associated with differential methylation of other genes, including some genes involved in neuronal plasticity [57].

Other studies show that psychosocial stress in adults is a risk factor for various disorders, such as hypertension or cancer, and may have an epigenetic link [58]. Many studies have shown an association of stressful situations with DNA methylation patterns. For example, Holocaust survivors show different methylation levels of FKBP5, a gene involved in the response to glucocorticoids [59]. In men with a mean age of 73 years, Kim et al. found that psychological distress was associated with the methylation of several genes related to stress/inflammatory responses (ICAM-1, TLR2, iNOS, glucocorticoid receptor, γ-interferon, or IL-6) [60]. Posttraumatic stress disorder is associated with low-grade inflammation, which might be related to epigenetic marks. In fact, changes in the methylation of genes involved in immune system pathways has been described in war veterans with posttraumatic disorder [61].

9.4.5 Socioeconomic Status

Socioeconomic groups differ from a variety of perspectives. Several studies have shown that they also differ in some epigenetic marks. The socioeconomic status (SES) may influence the epigenome both during early life (pre- and postnatal) and also during adulthood. A common, but unproven, concept is that early life experiences have a stronger influence on the epigenome than those occurring later in life. In fact, studies in twins have shown that epigenomic differences increase with time, which could suggest a stronger influence of genetic determinants in early life and of ongoing environmental influences during adulthood [38, 62, 63]. However, stochastic variation may be another factor explaining larger differences in individuals of advanced age.

Nevertheless, it is usually difficult to establish which are the specific factors leading to epigenetic differences across socioeconomic groups. In fact, individuals belonging to different socioeconomic strata have different nutrition, exercise patterns, life habits, working duties, exposure to pollution and other contaminants, access to medical care, exposure to stressful situations, etc.

Individuals of low SES across the life course have increased cortisol production and inflammatory activity. In a study of 857 healthy Italian individuals, Stringhini et al. found that several indicators of socioeconomic status were associated with the methylation of genes involved in inflammation. NFATC1, in particular, was consistently less methylated in individuals with low socioeconomic level [64]. This gene is not only involved in inflammation pathways but also in RANKL-mediated osteoclastogenesis.

Investigators in Scotland found a lower DNA methylation in the most socioeconomically deprived individuals, as well as in manual workers, in comparison with non-manual workers. 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 [65].

Needham et al. also found differences in DNA methylation in association with SES, but different genes were identified [66]. In that study, low childhood SES was associated with DNA methylation in three stress-related genes (AVP, FKBP5, OXTR) and two inflammation-related genes (CCL1, CD1D). Low adult SES was associated with methylation of one stress-related gene (AVP) and five inflammation-related genes (CD1D, F8, KLRG1, NLRP12, TLR3). In general, low SES was associated with increased DNA methylation.

Lower SES during adolescence is associated with an increase in methylation of the proximal promoter of the serotonin transporter gene (SLC6A4), which predicts greater increases in threat-related amygdala reactivity and susceptibility to depression [67]. It is to note that serotonergic systems have been postulated as regulators of bone metabolism [68, 69].

9.5 The Epigenome as a Link Between Social Factors and the Skeleton

In previous sections we have shown that (a) epigenetic mechanisms play an important role in the differentiation of bone cells and are likely important in the pathogenesis of skeletal disorders and (b) SES and other factors associated with social strata influence the epigenomic marks, specifically in some genes involved in inflammation and stress responses. So, the question rises whether epigenome changes induced by social factors may influence skeletal status and the susceptibility to disorders such as osteoporosis. There is no clear response to this question yet, but several lines of evidence indeed suggest that may be the case.

9.5.1 Social Factors Are Associated with Bone Mass and the Risk of Osteoporosis

In previous chapters we have discussed extensively the association of social factors with skeletal disorders, such as osteoporosis. Indeed, many studies have shown an increased prevalence of osteoporosis in deprived population groups. The causes are likely multiple, including poor nutrition, inadequate self-care, etc. [70–74].

9.5.2 Skeletal Disorders Are Associated with Specific Epigenetic Signatures

A few studies have explored the association of DNA methylation in blood cells with bone mass. Although one study found some differentially methylated regions in patients with low bone mass [75], most studies reported negative results [76]. This was not unexpected, because the epigenome is cell/tissue-specific. Thus, blood cell methylation does not necessarily reflect the methylation status of bone cells, which is probably more relevant to bone mass. In line with this concept, an accelerated epigenetic aging has been reported in disease-relevant samples of patients with osteoarthritis and with osteoporosis, whereas no differences were found in circulating blood cells [77–79].

In fact, in an epigenome-wide study of DNA methylation in bone tissue of patients with osteoporotic fractures and a comparison group with osteoarthritis, we found several differentially methylated regions. The genes involved were overrepresented in several pathways, including those related to skeletal development [80]. Similarly, differentially methylated regions were found when MSCs grown from patients with hip osteoporotic fractures were compared with MSCs grown from patients with hip osteoarthritis. The genomic analyses revealed that most differentially methylated loci were situated in genomic regions with enhancer activity, distant from gene bodies and promoters. These regions were associated with differentially expressed genes enriched in pathways related to MSC growth and osteoblast differentiation [81]. In another study using bone biopsies of women with low (osteoporotic) or normal BMD, 63 differentially methylated CpGs were found [82].

9.5.3 Pathways Epigenetically Modulated by Social Factors Influence Skeletal Status

As explained above, stressful life experiences and other social-related factors induce changes in the methylation and expression of genes related to inflammation and stress response. Since those genes are also involved in the regulation of bone metabolism, DNA methylation may be a link between social factors and skeletal status.

Inflammatory reactions include the systemic and/or local release of cytokines that have effects on various cell types, including those of the bone. In fact, chronic inflammatory conditions are associated with osteoporosis and increased risk of fractures [83]. Multiple mediators seem involved. For example, cytokines such as IL-1, IL-6, IL-17, and TNF have an inhibitory influence on cells of the osteoblastic lineage, whereas they tend to stimulate osteoclastic bone resorption by direct or indirect, osteoblast-mediated mechanisms [84]. While the association of inflammatory disorders with osteoporosis is well demonstrated, whether low-grade inflammation, such as that associated with social deprivation, induces bone loss or not is still unclear. Thus, studies exploring the association of C-reactive protein and other inflammatory markers with bone mineral density and fractures have shown somewhat controversial results [85, 86].

The stress response includes the activation of the hypothalamus-hypophysis-adrenal axis, with release of glucocorticoids and reduced secretion of growth hormone and sex hormones. Glucocorticoids have a negative influence on bone mass, whereas growth hormone and sex hormones tend to promote bone formation and decrease resorption, with an overall positive influence on bone balance. Therefore, these hormonal changes during stress response have a negative influence on bone homeostasis.

Another component of the stress response is the activation of the sympathetic system with catecholamine release from the adrenal glands and nerve terminals, as well as enhanced release of neuropeptide Y. These factors tend to enhance bone resorption and may impair bone formation [87–89]. The stress response also results in enhanced release of cytokines, such as IL-6, that impact bone negatively.

Stress related to social deprivation and other factors is associated with exaggerated or persistent cortisol responses to stressful situations. Cortisol and other glucocorticoids exert potent effects on bone cells leading to a loss of bone mass and increased fracture risk [90]. The mechanisms are multiple and include direct effects of cells in the bone microenvironment. For instance, glucocorticoids tend to increase osteoclastic bone resorption by increasing the RANKL/OPG ratio and, at the same time, they potentiate apoptosis and impair the survival of cells of the osteoblastic lineage, such as mature osteoblasts and osteocytes. This leads to an uncoupled bone remodelling, with high resorption and low formation, that leads to loss of bone mass. In addition, glucocorticoids decrease the production of sex steroids (which have an anabolic effect on the bone), enhance PTH secretion (which stimulates bone resorption), and impair intestinal calcium absorption, thus further decreasing bone mass and bone strength. Also, glucocorticoids have a negative effect on muscle mass and function, which has a negative impact on the muscle-bone interaction, and increase the risk of falls and subsequent fractures [90].

Thus, the acute stress response, which is initiated by the activation of brain systems, involves a number of neural and humoral changes that tend to mobilize energy stores and increase vigilance to have better chances to “fight or flight.” However, the stress response also has negative influences on tissues, such as the

skeleton, which are not urgently needed. Hence, if stress is persistent in time, those tissues may become compromised. An abnormal stress response may be caused by an inadequate activation or deactivation of the hypothalamic-pituitary-adrenal axis, resulting in inappropriate initiation or termination of the response. This in turn may increase the risk of behavioral problems and mental illness [70, 91]. Although less convincingly demonstrated, chronic stress may also increase the risk of skeletal disorders such as osteoporosis (Fig. 9.3). In fact, persons with high perceived stress have an increased risk of osteoporotic fractures, including hip fractures, even after adjusting for confounding factors, such as comorbidities and medications [92].

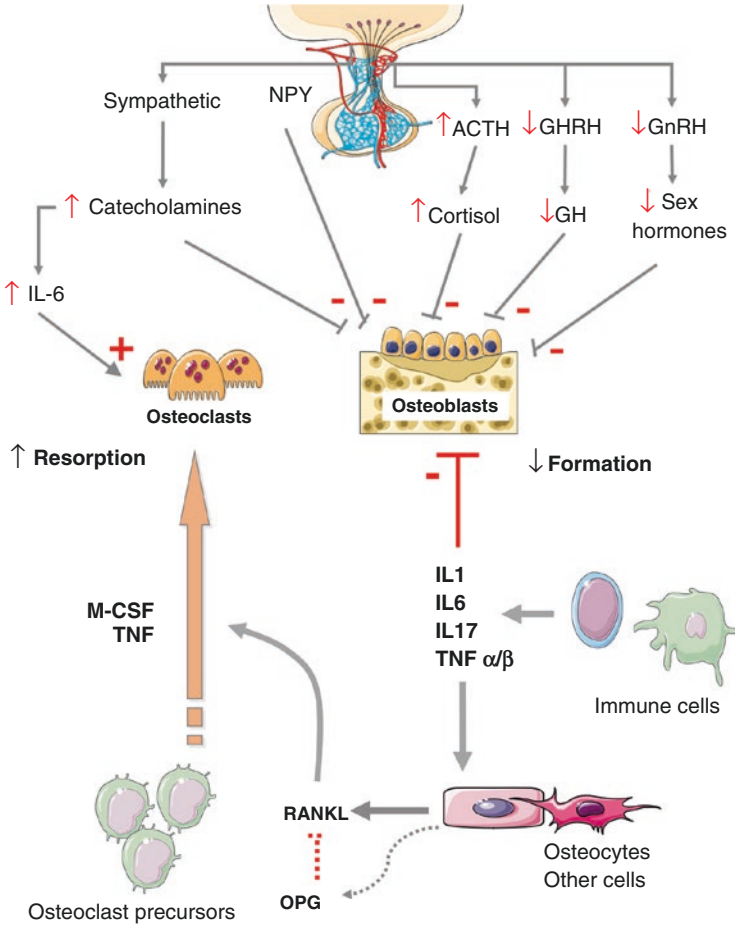

Fig. 9.3 The stress and inflammatory responses and bone cells (from [70] with permission by Springer Verlag)

9.6 Confounding and Reverse Causation in Epigenetic Studies

As discussed above, epigenetic marks modulate gene activity and therefore have an important impact on many cell and organ functions, as well as in determining the health or disease status of the individual. Hence, there is considerable interest in performing association studies that elucidate the links between epigenetic marks and disease and other phenotypic features. In principle, these studies could be considered similar in design to genetic association studies. However, epigenetic association studies are more complex to perform, not only due to the somewhat more complex techniques needed to study epigenetic signatures, but also because those signatures usually are tissue-specific. Moreover, epigenetic association studies may be more difficult to interpret, because of questions about the direction of causal associations.

This is not an issue in genetic studies. The genome (in germ line cells) is determined prior to disease. Therefore, if we find an association between a genetic marker and a phenotypic trait, it is always clear that the genetic feature is causing the phenotype, and not the opposite (of course, this may not always be true when studying diseased tissues). For instance, polymorphisms in the FTO gene have been associated with obesity, which suggests that genetic variants in FTO modulate body mass.

However, that is not the case in epigenetic studies. When an association between an epigenetic mark and a disease is found, it may be hard to establish if the mark is causing the disease, or the opposite is true. This is frequently called "reverse causation." In order to solve this issue, we would need to perform longitudinal studies in which epigenetic marks are analyzed prior to disease development.

A similar problem arises when studying the association of epigenetic marks with environmental factors. For instance, as described in previous sections, smoking has been associated with differences in the methylation of several genes. However, it could be questioned if smoking is influencing DNA methylation, or there is an association in the opposite direction, so that certain methylation marks influence the behavior of the individual and specifically the propensity to smoke.

In the same line, we have previously mentioned that the SES is associated with differentially methylated regions in DNA and assumed that the social environment is driving the changes in methylation. However, the opposite influence cannot be completely excluded. For instance, in a fish experimental model, in which social rank dictates reproductive access, changes in DNA methylation induced pharmacologically were associated with ascents or descents in the social rank [93]. Also, Hamilton et al. recently reported that posttranslational modification of histones in the region of the Fosb gene influences the behavioral responses to social stress. Specifically, Fosb-targeted histone acetylation and methylation in the neurons of the nucleus accumbens induce opposite changes in the resilience to social stress of mice [94]. In this line, the expression of the imprinted gene Cdkn1c modulates social behavior and some aspects of the social structure in mice reared in groups [95].

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 suffering 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 [67, 96]. As an example, the epigenetic regulation of several genes related to the neuroendocrine, serotonergic, and oxytocinergic pathways (such as the glucocorticoid receptor (NR3C1), oxytocin receptor (OXTR), solute carrier family 6 member 4 (SLC6A4), and monoamine oxidase A (MAOA)) has been postulated to modulate the propensity to proactive and reactive aggressive behavior [97].

Several authors proposed to take advantage of the Mendelian randomization concept to help delineating the direction of those associations. This concept is based upon the principle that if a genetic variant (for instance, a FTO gene polymorphism) alters the level of an environmentally modifiable exposure (e.g., obesity), which in turn modifies disease risk (e.g., hypertension), then this genetic variant should also be related to disease risk to the degree predicted by the joint effects of the genetic variant on the modifiable exposure and of the modifiable exposure on the outcome. Common genetic polymorphisms that have a well-characterized biological function (or are proxies for such variants) can therefore be utilized to estimate the causal effect of a suspected environmentally modifiable exposure on disease risk. Of course, the variants should not have an association with the disease outcome except through their link with the modifiable risk process of interest [98, 99]. In this sense, the Mendelian randomization design is considered to be analogous to a randomized clinical trial, where instead of random allocation of participants to interventions (treatments or preventive measures), they are randomized by nature according to the gene variants that regulate susceptibility to a specific exposure they carry [100]. This design is being extensively used in genome-wide genetic association studies, including some studies with skeletal phenotypes as outcomes [100–102], and has also been proposed to avoid reverse causation issues in epigenome-wide studies (Fig. 9.4).

DNA methylation can be considered as an intermediate phenotype, determined in part by genetic architecture. Indeed, DNA methylation patterns can correlate closely with local genetic variants. Mendelian randomization approaches then allow using these genetic variants to assess the direction of causal relationships between the environment or the disease and the epigenome.

Relton et al. proposed a two-step epigenetic Mendelian randomization approach. Genetic variants are used as instrumental variables in a two-step framework to establish whether DNA methylation is on the causal pathway between exposure and disease [98]. It first requires a genetic proxy of the modifiable exposure. This SNP shows association with the exposure. Secondly, a genetic proxy of methylation is used to evaluate the relationship between this methylation mediator and the disease outcome or trait. This is a SNP with allele-specific differences in methylation. Thus, Mendelian randomization helps to distinguish between truly causal relationships and epiphenomena (non-causal associations) which nevertheless may be informative biomarkers.

As a nice example, Jhun et al. used the concept of Mendelian randomization to confirm the causal association of smoking with certain methylation marks that, in turn, modulate the levels of some inflammatory markers, such as interleukin-18 (IL-18) [103]. Specifically, they found that current smoking status was associated with

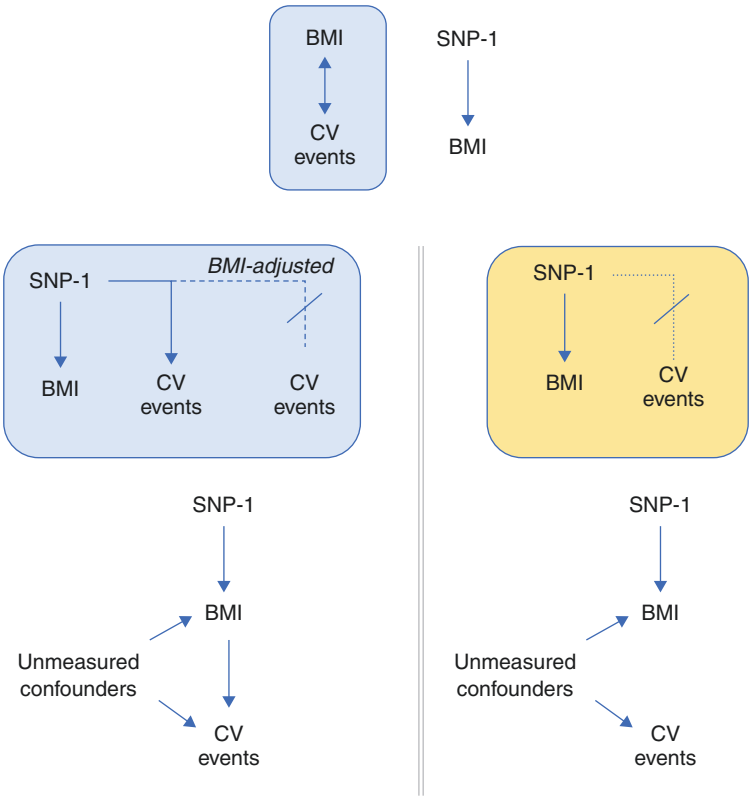

Fig. 9.4 Mendelian randomization in genetic association studies. A hypothetical study to confirm the association between body mass index (BMI) and cardiovascular (CV) risk, taking advantage of genetic markers associated with BMI. If a single-nucleotide polymorphism (SNP) is associated with BMI and CV event risk, but the latter association is lost when adjusted for BMI, then the relationship between BMI and CV can be confirmed (left lower diagram). On the other hand, if the SNP is associated with BMI but not with CV risk, then a causal association between BMI and CV events can be excluded (right lower diagram). This does not exclude the influence on BMI and cardiovascular risk by other non-studied factors

the DNA methylation levels of cg03636183 in the coagulation factor II (thrombin) receptor-like 3 gene (F2RL3) and of cg19859270 in the G protein-coupled receptor 15 gene (GPR15). The DNA methylation levels of cg03636183 in F2RL3 were associated with the levels of the pro-inflammatory cytokine IL-18. Overall, those results suggest that smoking increases IL-18 through decreasing DNA methylation of F2RL3 (Fig. 9.5). Additionally, they represent a potential mechanism to explain the negative association between smoking and bone mass [104].

Inversely, a study using Mendelian randomization provided no support for a causal relationship between the methylation of the DRD4 gene [that encodes the subtype 4 dopamine receptor] and physical aggressive behavior, despite previous studies showing an association between this gene and physical aggression [105].

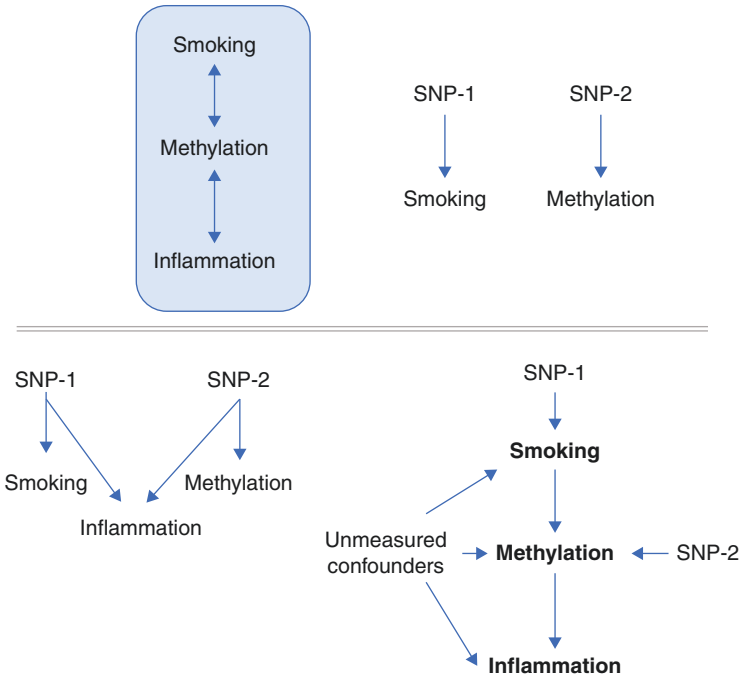

Fig. 9.5 The Mendelian randomization concept in epigenetic studies. A hypothetical study to confirm the causal association between smoking, DNA methylation, and inflammatory response, by using one SNP associated with smoking (SNP-1) and other SNP (SNP-2) associated with DNA methylation at a region presumably also affected by smoking. If SNP-1 is associated with smoking and with inflammation markers and SNP-2 is associated with the methylation level and inflammatory markers, a causal pathway between smoking, methylation, and inflammation is strongly suggested. In real practice, a set of several SNPs are used, not just two SNPs. Of course, other non-studied factors, either genetic or acquired, may also influence risk factors and outcomes

9.7 Conclusions: The Epigenetic Responsibility

Environmental influences, including social-related factors, determine, along with genetic factors and random events, the risk of many diseases, such as cancer, neurological disorders, and skeletal disorders. Epigenetic factors regulate gene activity and are influenced by environmental factors. A whole variety of environmental factors can impact the epigenome and, consequently, shape the phenotype and determine disease risk. Early life stages of the organism, especially intra uterus, may be more susceptible to the influence of environmental factors. However, those factors also impact the individual after birth, through a variety of mechanisms, including the epigenetic ones.

Environmental factors influencing the epigenome vary from chemicals to psychological conditions. In particular, several lines of evidence suggest that factors related to the socioeconomic status impact the epigenome. The factors leading that influence are unclear but may include nutrition, life habits, working conditions, and other ambient exposures, as well as psychological factors (Fig. 9.6).

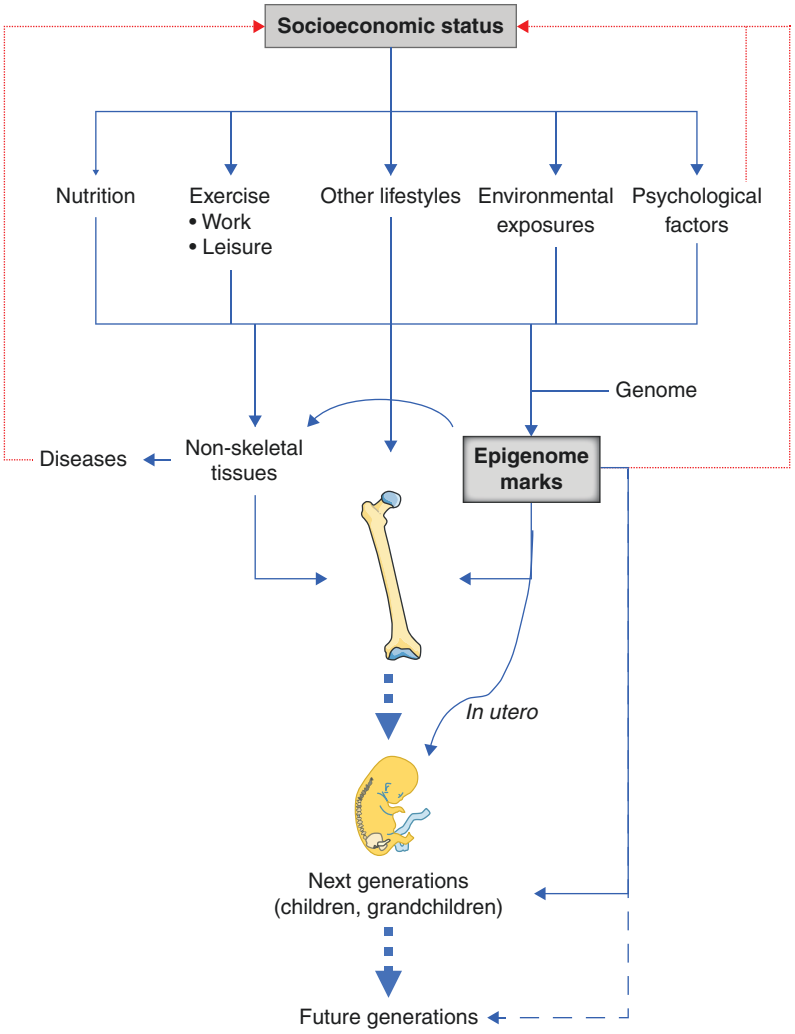

Fig. 9.6 Epigenetic links of the socioeconomic status-related factors and health outcomes of present and future generations (from reference [70] with permission by Springer Verlag)

Social deprivation is associated with low-grade inflammation and activation of the stress response. Changes in gene promoter methylation and other epigenetic marks may be involved in this phenomenon. Those changes likely have a negative impact in bone homeostasis and may be involved in the increased risk of osteoporosis observed in population groups of lower social status.

Epigenetic changes induced by physical or psychological stressful conditions, including those occurring in utero, may persist in later life. Although the transgenerational transmission of epigenetic marks in mammals is unclear, the capability of

environmental influences to determine the epigenome of the exposed individual and his/her offspring calls for responsibility and compels us for procuring to individuals the healthiest physical and psychological environment.

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The Social Context of Bone Health: Conclusions and Future Directions

10

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10.1 Introduction

Just as observed in contemporary society, social status in the Middle Ages was a key determinant of nutrition, health, disease, and general lifestyle [1, 2]. Indeed, there are stark similarities between these two distinct time periods. Ease of access to a nutrient-rich diet and a privileged quality of life can be observed in individuals of upper socio-economic status (SES), whether they be a medieval royal or, for example, a chief executive officer (CEO) of a major corporation in current times. In contrast, individuals with less access to wealth, and thus fewer options for achieving the highest possible quality of life, are more likely to experience poor health, regardless of when they lived. Data ascertained from medieval human skeletal samples deriving from a range of European archaeological sites indicate that, just as observed in contemporary society, a social gradient in bone quality is observed.

However, just as observed in contemporary society, higher SES does not always align with healthier bone. For instance, greater wealth may increase capacity to

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overindulge in food and alcohol consumption by medieval royals and CEOs alike. Similarly, there may be less physical activity for the wealthy medieval upper classes as there is for time-poor, desk-based CEOs. Yet, contemporary data show that poor bone quality does not necessarily result in subsequent fracture, particularly in upper SES groups. This discrepancy across the SES spectrum is worthy of speculation, and, here, medieval data contribute significantly to our understanding, particularly given the clear distinction between upper and lower SES in the Middle Ages times, whereas contemporary society is represented by a much broader spectrum of SES.

10.2 Chronic Stress and Bone

Similar to modern epidemiological data (Part 2 in this volume), the effect of SES on medieval human health can take different directions. Childhood physiological stress episodes related to SES and weaning reconstructed from medieval deciduous dental histology indicated that infants of upper SES (aged 2–8 months) experienced greater stress compared to children of lower SES. However, when considering relationships between childhood developmental disturbances recorded in teeth and bone microstructure density in adulthood, SES manifested in contrasting bone quantity of low and high SES groups from an archaeological site in eleventh- to sixteenth-century Canterbury, UK. Only high SES individuals appeared to develop higher adult bone density despite their experiences of developmental disturbance in the early ontogenetic years. These ancient data suggest that, despite chronic stress during earlier life, those of upper SES still achieved better-quality bone in later life. We may speculate that those of upper SES may have had better coping strategies to deal with chronic stress compared to those of lower SES, thereby reducing the biological impact of chronic stress exposure.

Environmental factors influence disease expression and the epigenome: these factors vary from chemicals to psychological conditions. Several lines of evidence suggest that many SES-related factors impact the epigenome; whilst the biological mechanisms underpinning these associations are unclear, pathways may include nutrition, life habits, working conditions, and other ambient exposures, as well as psychological factors such as chronic stress [3, 4]. Contemporary data [5] suggest that social deprivation is associated with low-grade inflammation and activation of the stress response: changes in gene promoter methylation and other epigenetic marks may be involved in this phenomenon. Those changes likely have a negative impact in bone homeostasis and may be involved in the increased risk of osteoporosis observed in population groups of lower SES. As medieval data here demonstrate, chronic stress during childhood can indeed lead to elevated physiological stress in both lower and higher SES children. However, the impact on epigenetic processes via upregulation of the stress response may not be as great for the higher SES as for lower SES children, considering that the former group attain lower adult bone quantity.

Epigenetic changes induced by physical or psychological stressful conditions, including those occurring in utero, may persist in later life [3, 4]. Although the transgenerational transmission of epigenetic marks in mammals is unclear, the capability of environmental influences to determine the epigenome of the exposed

individual and his/her offspring calls for responsibility and compels us for procuring to individuals the healthiest physical and psychological environment. Clinical practice can contribute much to achieving this "ideal" state of a healthy contemporary environment. For instance, there is much potential to reduce the disproportionate risk of poor bone health experienced by socially disadvantaged persons. Given that the predisposing sex and ethnicity cannot be modified, clinical attention could be focused towards identifying those most at risk of poor bone health and affording extra time to ensure that chronic stress is accounted for, or at least considered, when designing treatment or management plans. In addition, effective health communications between practitioner and patient will ameliorate the negative effect of low health literacy whilst also increasing trust and disclosure regarding chronic stress.

Poor bone health results in an increased fracture risk, itself disproportionately increasing earlier mortality. Disparities in fracture incidence, prevalence, rates, and risk factors exist between social groups. As we have argued, these differences cannot be fully explained by measures of the bone alone. Rather, lower SES may increase exposure to cumulative stressors, influence responses to stressors, and result in a heightened inflammatory state and epigenetic changes, thereby increasing osteoporotic fracture risk. Understanding the mechanisms that underpin the social gradient of fracture may identify various entry points for interventions to reduce the social and ethnic disparities observed in the incidence of osteoporotic fracture.

Community-based health promotion programmes are numerous and encompass an array of lifestyle modification options that will enhance bone health. However, it is imperative that efforts to reduce overall health inequities are prioritised in national health-related and multisectoral policies and strategies. Whilst much expenditure is dedicated to the prevention of non-communicable diseases, large proportions of that investment are commonly targeted towards individual behavioural factors such as physical inactivity or poor nutritional intake. Without a focus on the wider context of health inequities such as the high cost of living and education and the low availability of employment, often referred to as the "causes of the causes", taking a primary focus on behaviours will likely have little impact on reducing health inequities. In addition, disparities in screening and treatment of osteoporosis exist between social groups. Various patient- and practitioner-specific factors influence low uptake of testing and poor adherence, many of which relate to health literacy, the quality of patient-practitioner communications, and salience of osteoporosis. To influence the availability of equitable healthcare options and to increase the uptake of services and adherence to treatment plans, health policy must strategically act on health literacy: this requires an approach that is whole of government, whole of society, and intersectoral for good governance.

10.3 Future Research

Given the inseparable relationship between life course and bone health, themes and outcomes for current and future research, prevention and treatment of increased bone fragility in ageing populations today can be suggested. For example, we have shown the increased risk of fracture that is associated with prevalent diabetes.

Given this, ways of reducing diabetes disparities and improving health outcomes within the social determinants of health framework can also be proposed. There is a need for community-based intervention studies focusing on bone and diabetes. Such research is particularly needed given the high rates of diabetes and subsequent disease sequelae. Cultural tailoring of diabetes prevention educational materials and cultural tailoring of education in group settings may afford the means to increase patients' knowledge of the disease for earlier diagnosis and earlier intervention to prevent diabetes complications. Encouragement of spousal support within the construct of acknowledging cultural norms may provide a means for improving diabetes outcomes and health. The influence of social determinants of health on diabetes outcomes needs to be tested in intervention studies to provide a foundation for effective interventions to impact the current epidemic of diabetes in the United States and around the globe. Prospective interventional studies evaluating the influence of social determinants will be key to lay a foundation for effective interventions and improvement of diabetes and health outcomes.

The educational value of links between lifestyle and peak bone mass accrual in the first few life decades should also be communicated more clearly to the younger generations, so that better-quality bone is built as per individual and community-based, socio-economic background. This can be achieved through incorporation of basic findings, as reported in studies presented in our volume, into school curricula and clinical information sheets. Interdisciplinary efforts by academic and industry researchers, and community organisations and practising clinicians, should also be encouraged and facilitated so that research outcomes are relayed in practice within diverse socio-economic background framework. Ongoing research recording and monitoring bone strength, quantity, and quality in diverse social groups should continue to elucidate lifestyle determinants of skeletal health that can, hopefully, lead to addressing socio-economic inequality and inequity in the future.

In order to have a better understanding of the role of epigenetic mechanisms, and avoiding reverse causation errors, we need large prospective cohorts with epigenetic markers in individuals across different social strata, recruited prior to disease development and with regular follow-ups over time.

10.4 Conclusion

This volume presented three lines of evidence, spanning almost 1000 years, for the effect of socio-economic background on skeletal health. It is well established that social determinants underlie inequality in health and inequity in access to healthcare, education, and resources whether it be in the Middle Ages or today. Therefore, there is an inseparable relationship between bone health and life course which should not be neglected in clinical efforts to manage and prevent bone fragility. Future research will benefit from interdisciplinarity, as we show here that biological anthropology makes contributions to contemporary attempts of osteoporosis and fracture prevention. Collectively, this volume demonstrated that there needs to be a holistic understanding of future risk identification and targeted pharmacological

intervention, which can be achieved by synthesising historical, epidemiological, and epigenetic accounts of social aspects of bone health. Using the social and economic structure of medieval societies as a model for unravelling lifestyle-determined disparities in skeletal health, we further add to the growing body of evidence that modern epidemiological and epigenetic research into bone fragility is imperative to addressing ongoing human social inequality.

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