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UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

MRC Lifecourse Epidemiology Unit

Volume 1 of 1

Understanding the early life and adult determinants of bone microarchitecture using participants of the Hertfordshire Cohort Study

by

Mark Hiley Edwards

Thesis submitted for the degree of Doctor of Philosophy

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UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE

Human Development and Health

Thesis for the degree of Doctor of Philosophy

UNDERSTANDING THE EARLY LIFE AND ADULT DETERMINANTS OF BONE MICROARCHITECTURE USING PARTICIPANTS OF THE HERTFORDSHIRE COHORT STUDY

Mark Hiley Edwards

Osteoporotic fractures are associated with considerable morbidity, mortality and socioeconomic cost. Their primary cause is bone fragility which can be assessed using high-resolution peripheral quantitative computed tomography (HRpQCT), a novel imaging technique that provides detailed *in vivo* data on bone geometry, density and microarchitecture. The aims of this thesis are to use HRpQCT to understand the early life and adult determinants of bone microarchitecture and explore the relationships between bone microarchitecture and fracture status.

376 participants from the Hertfordshire Cohort Study underwent HRpQCT of the non-dominant distal radius and tibia and dual energy x-ray absorptiometry (DXA) to estimate body composition, femoral neck areal bone mineral density (aBMD), and vertebral fracture assessment. Birth weight and weight at 1 year were available from historical records. Information on demographics, lifestyle, and comorbidities was obtained from study questionnaires.

In men and women, mean(SD) age 76.0(2.6) and 76.4(2.6) years respectively, there was a positive association between birth weight and bone area (total and trabecular) in both the radius and tibia ($p<0.05$). Cortical area, cortical thickness and trabecular number were positively associated with lean mass index (LMI) and fat mass index (FMI) in both men and women. However, when both components of body composition were mutually adjusted, LMI was associated solely with cortical geometry and FMI with trabecular microarchitecture. In both sexes, cortical thickness and density were lower in fracture cases, and in men, total and trabecular area were greater. After adjustment for femoral neck aBMD, relationships with cortical and trabecular microarchitecture were partially attenuated but associations in men between fracture status and both total and trabecular area were maintained.

This study confirms the importance of early life to adult bone health in keeping with the developmental origins hypothesis, and highlights novel associations between body composition and bone microarchitecture. It demonstrates that patterns of bone microarchitecture by fracture status differ in men and women, and that some HRpQCT variables may contribute to fracture prediction.

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Declaration of Authorship

I, Mark Hiley Edwards

declare that the thesis entitled

Understanding the early life and adult determinants of bone microarchitecture using participants of the Hertfordshire Cohort Study

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission.

Signed: ……..

Date:……

Project Outputs

Related Publications

MH Edwards, CL Gregson, HP Patel, KA Jameson, NC Harvey, AA Sayer, EM Dennison, C Cooper. Muscle size, strength, and physical performance and their associations with bone structure in the Hertfordshire Cohort Study. *J Bone Miner Res* 2013; 28(11): 2295-2304.

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Awards

National Osteoporosis Society Young Investigator Award – Osteoporosis and Bone Conference 2012.

International Osteoporosis Foundation (IOF) and Servier Young Investigator Award – World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases 2011.

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Definitions and Abbreviations

aBMD:	Areal bone mineral density
ALSPAC:	Avon Longitudinal Study of Parents and Children
AUC:	Area under the curve
BMAD:	Bone mineral apparent density
BMC:	Bone mineral content
BMI:	Body mass index
COPD:	Chronic obstructive pulmonary disease
CSA:	Cross-sectional area
CSMI:	Cross-sectional moment of inertia
CT:	Computed tomography
CV:	Coefficient of variation
DALY:	Disability adjusted life year
DXA:	Dual energy X-ray absorptiometry
EVOS:	European Study of Vertebral Osteoporosis
EWGSOP:	European Working Group on Sarcopenia in Older People
FEA:	Finite element analysis
FMI:	Fat mass index
GOOD:	Gothenburg Osteoporosis and Obesity Determinants
GP:	General practitioner
HAL:	Hip axis length
HCS:	Hertfordshire Cohort Study
HRpQCT:	High resolution peripheral quantitative computed tomography
HRT:	Hormone replacement therapy
HSE:	Health Survey for England
HU:	Hounsfield Unit

IGF: Insulin-like growth factor

IQR: Interquartile range

IRMER: Ionising Radiation Medical Exposure Regulations

ISCD: International Society of Clinical Densitometry

IT: Intertrochanteric

LMI: Lean mass index

MDCT: Multi-detector computed tomography

MRI: Magnetic resonance imaging

NSHD: National Survey for Health and Development

OR: Odds ratio

PCA: Principal component analysis

pQCT: Peripheral quantitative computed tomography

QA: Quality assurance

QC: Quality control

RR: Relative risk

ROI: Region of Interest

SAT: Subcutaneous adipose tissue

SD: Standard deviation

SSI: Stress strain index

SWS: Southampton Women's Survey

TIA: Transient ischaemic attack

US: United States

VAT: Visceral adipose tissue

vBMD: Volumetric bone mineral density

VFA: Vertebral Fracture Assessment

WHO: World Health Organisation

1. Introduction

Fragility fractures are associated with significant morbidity, mortality, and socioeconomic cost(1) . The likelihood of fracture is determined by the strength of a bone and the forces applied to it. Currently, the former is assessed using dual energy x-ray absorptiometry (DXA). However, this technique does not fully assess fracture risk as half of all postmenopausal fractures occur in women with areal bone mineral density (aBMD) values above the World Health Organisation (WHO) threshold for osteoporosis(2, 3).

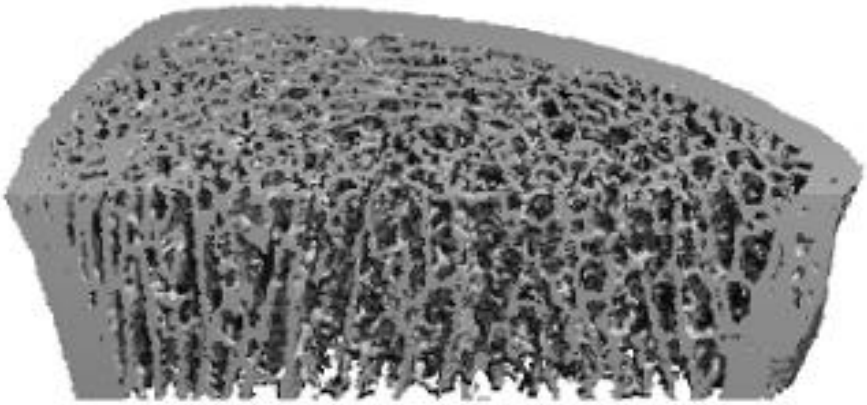

Figure 1.1: Tibial microarchitecture as assessed by HRpQCT. Reproduced with permission from Scanco Medical AG, Bruttisellen, Switzerland.

Over recent years, cross-sectional imaging modalities have been developed to explore both the macrostructure and microstructure of bone. It is hoped that these techniques will provide a more complete assessment of bone structure and strength with potential roles in the understanding of the pathogenesis of metabolic bone disease, monitoring of treatment effects and refining fracture risk prediction. Such techniques include high-resolution peripheral quantitative computed tomography (HRpQCT), a non-invasive imaging modality that permits assessment of the distal radius and tibia (Figure 1.1)(4).

There are several factors that can influence bone structure and strength including simple demographics like age and sex. Bone strength tends to be lower in women, particularly after the menopause, and to decline in older age. Lifestyle factors, such as smoking and alcohol consumption, have also been

shown to affect bone health(5, 6). There is growing awareness of the link between muscle and bone structure(7) and potential common mechanisms in the aetiology of sarcopenia and osteoporosis leading to the possibility of a shared therapeutic intervention. The prevalence of obesity is also rising rapidly with a considerable impact on health. This has similarly fuelled interest in the influence of adiposity on bone tissues (8, 9).

The importance of early life environment in the pathogenesis of adult disease has been well established(10). Specifically, extrinsic factors acting before birth and during infancy can significantly influence later health. These effects rely on developmental plasticity, the ability of one genotype to give rise to a range of different physiological or morphological states in response to different prevailing environmental conditions during development(11). There is good evidence for a positive relationship between birth weight and both bone size and bone mineral content (BMC)(12), however, the relationships between early life and bone microstructure have not previously been assessed in humans. If it is shown that early environment is related to bone microarchitecture in later life, this may lead to the possibility of intervening on a population level with a considerable public health benefit.

This thesis begins by highlighting the burden of osteoporosis and fragility fractures to affirm the importance of research in this field. To provide an overview of the relevant basic science in the area, our current understanding of bone physiology and imaging techniques is then described. This is followed by chapters exploring relationships between bone health and each of the following: demographic and lifestyle factors; body composition; and early life environment. These topics each provide a chapter in the results section. Clearly, the main clinical importance of bone microstructure is through a potential link with fractures. Although aBMD, which is measured by DXA, does predict fracture risk(13), the associations with bone microarchitecture from HRpQCT are far less well defined. The last chapter of the introduction explores our current knowledge in this area, highlights the questions that remain unanswered, and aims to address these in the final section of the results. In summary, this thesis will assess the early life and adult determinants of bone microarchitecture and explore the relationships between bone microarchitecture and fracture.

2. Bone Health and Osteoporosis

2.1 Introduction

Osteoporosis is a skeletal disease characterised by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture(14). The term osteoporosis literally means "porous bone". It was first introduced in France and Germany and implied a histological diagnosis. It now refers to a condition in which bone is normally mineralised but reduced in quantity. A significant leap forward was made with the discovery of non-invasive techniques to assess bone density *in vivo*. Single photon absorptiometry was introduced in the 1960s and was superseded by dual photon absorptiometry and then DXA.

In 1994, a working group of the WHO used bone density measurements by DXA to provide a practical definition of osteoporosis as an aBMD measurement of less than 2.5 standard deviations (SD) below the young normal mean(15). Earlier definitions had incorporated fracture and so, to provide comparability, the subset of women with osteoporosis who had also suffered one or more fragility fractures were deemed to have severe (established) osteoporosis. Osteopenia was defined as an aBMD level between 1 and 2.5 SD below the young normal mean. Lower levels of aBMD have been shown to increase the risk of fracture; specifically, for every 1 SD drop in aBMD there is an approximate doubling of fracture risk(13).

2.2 Epidemiology of Osteoporosis and Fracture

2.2.1 Lifetime risk

Osteoporosis is a very common disease. It has been calculated that it affects around 10 million people over 50 years of age in the US(16) and 200 million women worldwide(17). In 2000, there were approximately 9 million new fragility fractures, of which 1.6 million were hip fractures, 1.4 million were clinical vertebral fractures and 1.7 million affected the distal forearm (18). Other types of osteoporotic fractures include humeral, rib, tibial, pelvic, and non-hip femoral fractures. It is currently estimated that, on average, an

osteoporotic fracture occurs every 3 seconds(18) and, for a given individual, the lifetime risk of one or more of these fractures coming to clinical attention is around 40%(19).

In the UK, approximately half of women and 1 in 5 men over 50 years will, at some point in their life, develop an osteoporotic fragility fracture(20). The lifetime risk of hip fracture is 14% in women and 3% in men. Rates of radiographic vertebral fracture are around twice those for hip. However, many vertebral fractures remain asymptomatic with only one in three coming to clinical attention(21, 22). Therefore, rates of clinically-diagnosed vertebral fractures are lower at 11% and 2% for women and men respectively(23). This is similar to the lifetime risk of distal forearm fractures which affect 1 in 8 women and 1 in 50 men(24).

2.2.2 Age and Sex

There is a bimodal relationship between age and fracture incidence; peaks occur in childhood and in the elderly(25, 26). In children fractures more commonly occur in males than females and incidence peaks around puberty. They usually follow episodes of substantial trauma and tend to affect long bones. Nearly 75% of hip, spine and distal forearm fractures occur in people 65 years old or over(27) and these are more common in women (Figure 2.1), with rates in this age group reaching around twice those in men(28). Overall, 61% of osteoporotic fractures occur in women, with a female to male ratio of 1.6:1 (18).

In most Western populations, hip fracture incidence rises exponentially with age leading to around 90% occurring in those 50 years and older(29). In this group, the female to male incidence ratio is about 2:1(30). This higher incidence, along with the fact that they live longer, means that women account for three quarters of the hip fracture burden(29, 31).

The incidence of vertebral fractures increases with age in both men and women. Overall they are more common in women(32) although most studies show the prevalence to be similar in both sexes between the ages of 50 and 60 years(33, 34). They usually occur due to compressive loading of the spine with

lifting or changing position, or may be found incidentally. Commonly affected vertebral levels are those regions that are most compromised biomechanically(21). These include the midthoracic region, around T8, where the dorsal kyphosis is most pronounced, and T12/L1, the thoracolumbar junction, where the relatively rigid thoracic spine meets the freely moving lumbar segment.

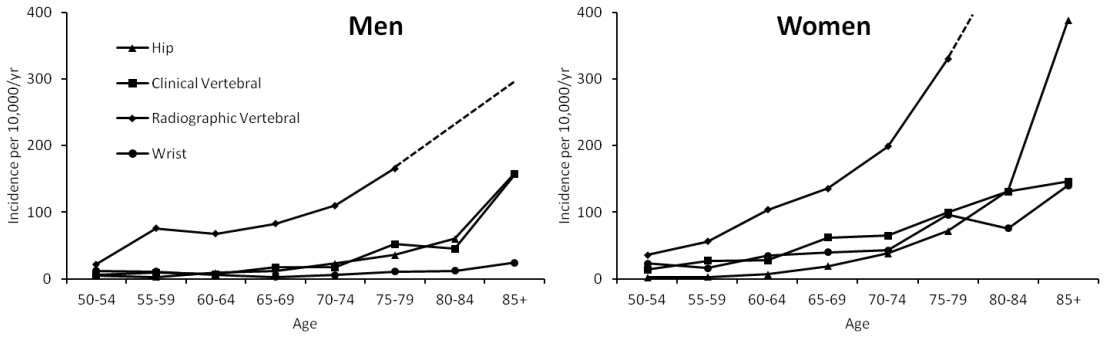

Figure 2.1: Hip, clinical vertebral, radiographic vertebral and wrist fracture incidence by age and sex. Adapted with permission from Edwards et al, Epidemiology and Genetics of Postmenopausal Osteoporosis(35).

Fractures of the distal forearm almost invariably occur following a fall onto an outstretched hand. The incidence in men is low and remains relatively stable between the ages of 20 and 80 years. In women, there is a rapid rise in incidence through the perimenopausal period but a plateau thereafter. Overall the age-adjusted rate of distal forearm fractures is four times higher in women than men. This is a more marked gender disparity than is seen with either hip or vertebral fractures(20).

The incidence of proximal humeral, pelvic, femoral shaft and proximal tibial fractures increases with age and is higher in women than men. The majority of proximal humeral fractures occur due to moderate trauma, such as a fall from standing height or less. Conversely, a significant fraction of proximal tibial fractures and the majority of femoral shaft fractures occur due to severe trauma. This proportion is greater in younger populations.

2.2.3 Geography

There is marked geographical variation in the incidence and prevalence of fragility fractures (Figure 2.2). In 2000, Europe and the Americas accounted for over 50% of all fractures (18). The majority of the remaining fractures occurred in Asian populations, most notably China. There is a lack of data detailing fracture epidemiology in Sub-Saharan Africa. Those studies available suggest that rates are low although this may partly be the result of inaccurate case finding.

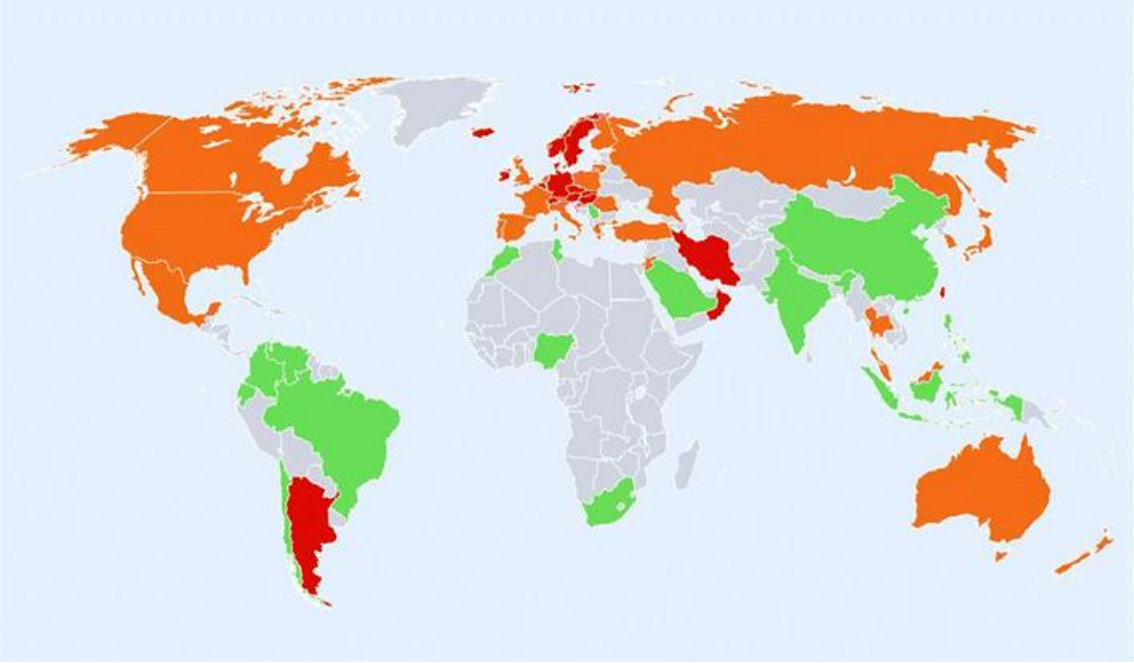

Figure 2.2: Hip fracture rates for men and women combined in different countries of the world categorised by risk. Where estimates are available, countries are colour coded red (annual incidence >250/100,000), orange (150–250/100,000) or green (<150/100,000). Reproduced with permission from Kanis *et al.* A systematic review of hip fracture incidence and probability of fracture worldwide (36).

Although overall rates are broadly similar in Europe, North America, Latin America and Oceania, there are considerable regional differences seen within continents. In Europe, the highest hip fracture incidence is found in Scandinavia where rates are almost seven times greater than in parts of southern Europe(37). In general, when rates of hip fracture are high, this is mirrored by rates of other types of fragility fractures (38, 39). For example,

the European Study of Vertebral Osteoporosis (EVOS) showed a marked variation in the prevalence of vertebral deformities between countries and, akin to hip fractures, the greatest prevalence was found in Scandinavia(33).

Significant variation is also found within countries suggesting that environmental factors are important in fracture pathogenesis. In North America, ecological correlates include socio-economic status, water fluoride content and urbanization(40). Latitude may play a role through sunlight intensity and effects on vitamin D levels although this relationship is inconsistent. Racial variations also exist, with lower fracture rates observed in Black populations than either Caucasians or Asians(41).

2.2.4 Secular Trends

The number of fractures occurring worldwide changes year on year. Between 1990 and 2000, the number of hip fractures rose by nearly 25%(18). At present around 1.6 million hip fractures occur annually but estimates for rates in 2050 range from 4.5 and 6.3 million per year(31, 42). The proportional increase is expected to be greater in men than women(42). The main driving force behind the rise in hip fracture rates is changing population demographics. Life expectancy is increasing around the world and with it the number of elderly men and women. It is predicted that the number of people aged 65 years and older will increase from 323 million to 1555 million by the year 2050.

Estimates of future fracture rates also depend on changes in the age-specific fracture incidence in men and women. With few exceptions, in Western populations including the UK, America and Oceania, age-specific fracture incidences rose until around 1980 and since then have either reached a plateau or decreased. There are a number of possible contributors to this. Initially, levels of inactivity, vitamin D insufficiency and survival of the frailest elderly may all have contributed to rising age-adjusted fracture rates. Towards the end of the twentieth century the favourable changes seen may be in part related to a birth cohort effect, the introduction of bisphosphonates, and better nutrition(43, 44). However, these factors alone are not sufficient to provide a full explanation.

Data available from Asia show a different pattern with age-specific rates continuing to rise in several areas. This, along with a particularly marked demographic shift in this part of the world, is likely to result in a significant change in the distribution of fracture burden. As previously stated, in 1990, half of all hip fractures took place in Europe and North America, however, it is predicted that over 60% will occur in Asian and Latin American populations by 2050(31).

2.2.5 Fracture Morbidity and Mortality

All fractures can result in morbidity. In the US, following a fragility fracture 7% of patients have some degree of permanent disability and 8% require long-term institutional care(45). In Europe, in terms of disability adjusted life years (DALY) lost, fragility fractures lead to greater disability than both rheumatoid arthritis and common neoplastic disorders, with the exception of lung cancer.

Hip fractures represent one of the most severe consequences of osteoporosis. They can be associated with chronic pain, reduced mobility, disability, and increased dependence. 50% of individuals that were ambulatory without assistance prior to the fracture are unable to walk unaided thereafter(45). Nursing home care immediately post discharge is required by 14% of those aged 50-55 years; this rises to over 50% in those above the age of 90 years(45).

Hip fractures invariably require hospital admission and most require surgical intervention. Initially patients are at risk of acute complications such as bronchopneumonia, urinary tract infections and pressure sores. In one US study, 3% of women and 8% of men over 50 years of age did not survive to leave hospital(46). By the end of the first 6 months the excess mortality rates exceed 10% with the additional risk peaking after 1 year at 21% in women and 36% in men(46). However, elevated mortality rates were shown to persist for up to 10 years(47). Similar figures have also been found in the UK(20).

Older individuals and those with poor premorbid health are at greater risk of death following a hip fracture(48). Given the demographic changes described above, and the increasing numbers of comorbidities in hip fracture patients, it

might be expected that mortality rates would be rising. However, comparative analyses from the US Medicare database have shown that they are actually decreasing(49). This may be due to improved perioperative care and rehabilitation.

Vertebral fractures are also attended by excess mortality. In an American study, survival rates at 5 years following a vertebral fracture were only around 80% of those expected for an individual of similar age and sex without a fracture (50). Although the prevalence of fragility fractures is higher in women, it is usually men that have greater rates of fracture-associated mortality(50).

The majority of vertebral fractures do not come to clinical attention but those that do can cause significant problems. These include immobility, height loss, back pain, kyphosis, and compromise of respiratory function. These can adversely influence quality of life and lead to depression, social isolation, loss of self-esteem and distorted body image(51-54). Evidence suggests an inverse dose-response relationship between quality of life and the number of vertebral fractures(55).

In contrast to hip and vertebral fractures, forearm fractures have not been shown to be associated with increased mortality rates(20). By the nature of their position anatomically, they may impact on activities of daily living, such as preparing meals, and approximately half of patients continue to report impaired function 6 months after fracture occurrence(45).

2.2.6 Fracture Burden

Osteoporotic fractures are responsible for a considerable personal, social and economic burden. In 2010 within the EU27, the ecomonic burden of incident and prior fragility fractures was an estimated €37 billion(1). Of this, 66% was due to incident fractures and only 5% pharmacological prevention. Most of the remainder was accounted for by long-term fracture care. As the number of fragility fractures is predicted to increase over the coming decades its burden is also set to rise. This will have substantial implications on an individual and population level.

Hip fractures account for the largest proportion of this due to their inherent need for hospitalization and the substantial number of individuals that subsequently require institutional care. In 1990, it was estimated that 1.66 million hip fractures occurred worldwide; approximately 1.19 million of these were in women and 463,000 in men(56). Due to the associated mortality risk, it is thought that these resulted in around 740,000 deaths(57). A conservative estimate of the annual direct and indirect costs of hip fracture worldwide was $131.5 billion in 1997. In the UK in 2010, the cost of pharmacological fracture prevention, incident fractures, and long-term disability combined was €5.4 billion(1).

2.2.7 Summary

The magnitude of the problem caused by osteoporosis both to the individual, in terms of morbidity and mortality, and economically, by necessitating massive health and social care expenditure, is clearly demonstrated. This highlights the importance of research into bone health to develop our understanding in this area which may allow improvements in risk stratification and illustrate novel targets for therapy both in early and later life.

2.3 Bone physiology

2.3.1 Introduction

In order to understand the biological plausibility of associations identified with bone microarchitecture, it is imperative to have a thorough knowledge of bone physiology. This includes its normal roles and structure at the molecular and cellular levels, and how these components are organised to form a functioning tissue. Furthermore, bones are not static and are continuously remodelling; they begin developing during fetal life and growth persists until skeletal maturation in early adulthood(58, 59).

2.3.2 Bone function, composition and structure

Bones are rigid structures within the body formed mainly of mineralised connective tissue. They also contain bone marrow, endosteum, periosteum, nerves, and blood vessels. They have several roles that include mechanical, synthetic and metabolic functions(60, 61). They provide a mechanical support structure for the body that aids locomotion and protects internal organs. The marrow within the medullary cavity of long bones is the site of haemopoiesis. The most notable metabolic function of bone is mineral storage, particularly calcium and phosphorus; 99% of body calcium is found in the skeleton(61). Bone also contains growth factors and fat, and has a role in acid-base balance.

Bone is made up of an organic matrix, inorganic mineral, and a cellular component (Figure 2.3). The matrix consists of a system of type I collagen (95%) and non-collagenous proteins (5%). Collagen's molecular structure allows its constituent α-chains to arrange themselves in a triple helix with glycine at the centre. It also forms multiple cross-links within and between chains(62). Once the collagen triple helix has been produced within a cell, it is exported into the extracellular environment where it aggregates with similar molecules to form collagen fibres. It is this collagen matrix that contributes largely to bone's elasticity and the triple helix within type I collagen also adds to bone's tensile strength. The non-collagenous proteins within bone include proteoglycan, osteonectin, sialoprotein, osteocalcin and bone morphogenic proteins(63).

The inorganic mineralised component of bone is formed from calcium phosphate laid down in an organised manner as calcium hydroxyapatite crystals ($[Ca_3(PO_4)_2]Ca(OH)_2$). This substance is relatively hard but lightweight. It has a good compressive strength but a poor tensile and shear stress strength. Bone is not homogeneous, it is made up of cortical bone and trabecular bone(63).

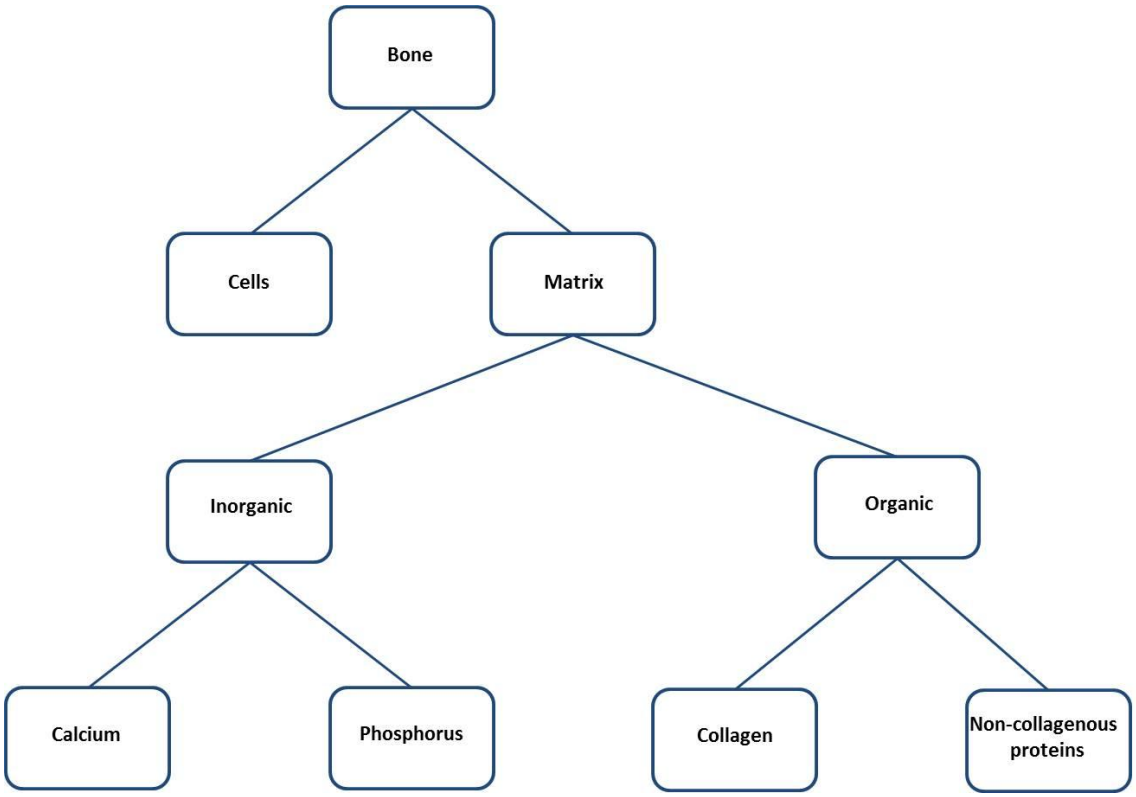

Figure 2.3: The composition of bone

Cortical bone is the solid, tightly packed, ordered, hard layer found around the outside of long bones (Figure 2.4) and accounts for around 80% of the total bone mass in an adult skeleton(64). It contains Haversian systems (osteons) within which collagen fibres adopt an ordered orientation, which leads to the production of concentrically arranged lamellae in cylindrical units around a central Haversian canal. These central canals contain blood vessels, lymphatics, nerves and connective tissue, as do the Volkmann canals which branch off them. Volkmann canals then continue until they reach the periosteum, the endosteum, or another Haversian canal. When intracortical remodelling acts on these canals they can enlarge and increase the porosity of the cortex(65). Osteons also house a fluid-filled lacunar-canalicular system. The lacunae sit between the lamellae of the Haversian systems and are joined to each other by the canaliculi. Each lacuna contains an osteocyte which, using dendritic cytoplasmic projections that extend through the canaliculi, is able to communicate with other osteocytes and bone lining cells(64).

Trabecular bone is a more irregular and porous network of rods and plates which form a three-dimensional mesh and accounts for the remaining 20% of

total bone mass. Despite its relative scarcity, the morphology of trabecular bone results in its surface area being about ten times greater than that of cortical bone. This greater surface area contributes to a higher rate of turnover in trabecular than cortical bone(66). In trabecular bone, as in cortical bone, the collagen fibres are orientated in an ordered manner. In certain disease states, the collagen fibres can have a random arrangement, with non-uniformly sized or distributed osteocytes. This type of bone is known as woven bone(66). Both cortical and trabecular bone are able to react to changes in the strains applied to them in order to adapt to prevailing load patterns. For example, trabeculae can alter their arrangement throughout life and orientate themselves along lines of stress(67).

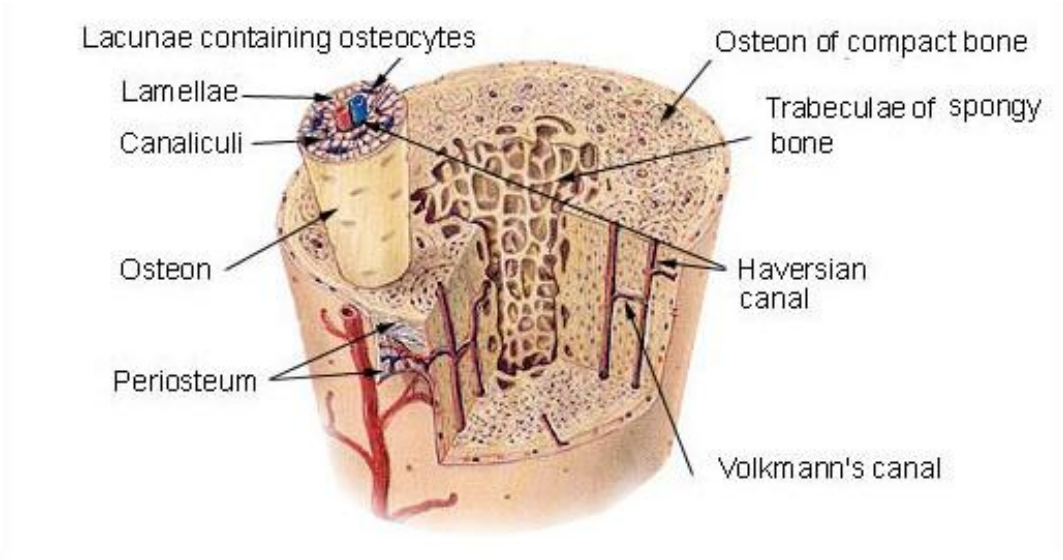

Figure 2.4: Structure of cortical and trabecular bone. Adapted from Wikipedia http://en.wikipedia.org/wiki/File:Illu_compact_spongy_bone.jpg

Bone is a metabolically active tissue being turned over continuously in a process of resorption and formation. It contains several specialised cells with differing functions including: osteoblasts, osteocytes and osteoclasts (Figure 2.5). The role of osteoblasts is in bone formation, producing the organic matrix of bone (including collagen and non-collagenous proteins) and ground substance. The specific function of osteocytes remains uncertain but they are thought to have roles in sensing bone strain and providing signals to other cells when this occurs. Osteoclasts are the cells that are involved in bone resorption. They are giant, multinucleate cells found at the bone surface.

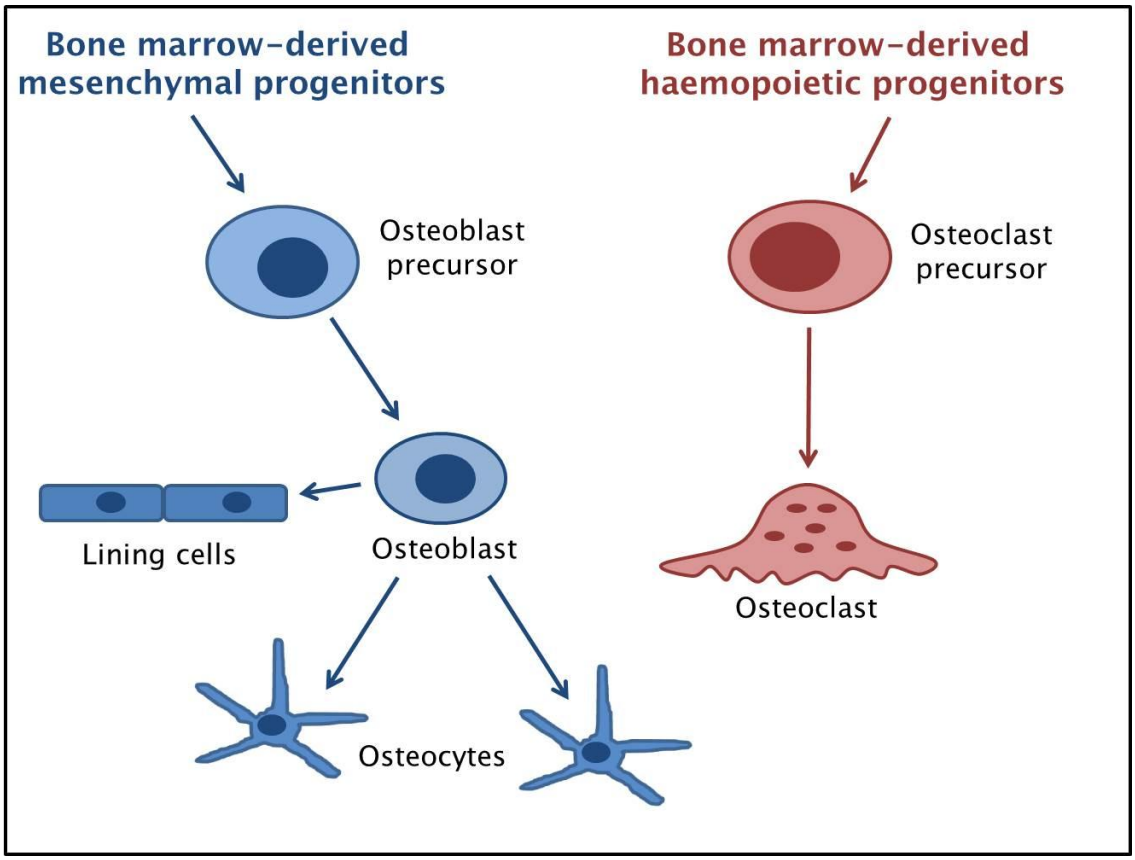

Figure 2.5: Diagrammatic representation of bone cells and their development

In general, bones can be divided into different types by the nature of their structure. Short, flat and irregular bones are composed of trabecular bone surrounded by a thin layer of cortical bone. Long bones are made up of a shaft, known as the diaphysis, which is expanded at each end to form an epiphysis (Figure 2.6). Whilst the bone is growing a cartilaginous, epiphyseal growth plate is present within the metaphysis; a very vascular area. Once bone growth is complete, the plate ossifies and forms the epiphyseal line.

The diaphysis contains a central medullary cavity surrounded by a thick tube of cortical bone. Only a small amount of trabecular bone lines the inner surface of the cortical bone at this point. In healthy adults, the majority of the medullary cavity contains inactive (yellow) marrow, mainly adipose tissue. Active (red) marrow is largely confined to the proximal epiphysis of large long bones. The composition of bone marrow changes through life towards an overall higher proportion of yellow marrow(66).

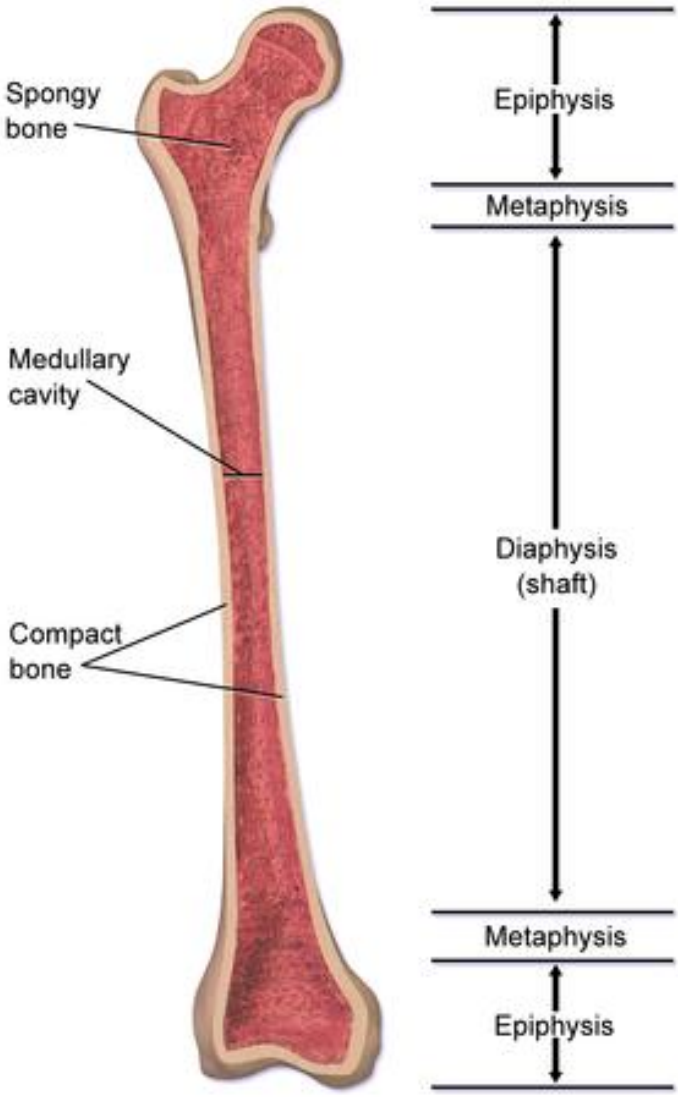

Figure 2.6: Different parts of a long bone. Adapted from Wikipedia http://commons.wikimedia.org/wiki/File:Structure_of_a_Long_Bone.png)

The epiphyses mainly contain trabecular bone and have only a thin outer shell of cortical bone. The ends of the bone are covered with hyaline cartilage at the site of articulation. Elsewhere, the outer surface of the bone is lined by the periosteum, except at the attachments of ligaments and tendons. The periosteum is a membrane consisting of dense, irregular connective tissue. It is made up of an outer "fibrous layer" and an inner "cambium (osteogenic) layer". The fibrous layer is chiefly made up of fibroblasts and collagen fibres. It also contains nerve fibres and blood vessels; these provide pain sensation in response to potentially noxious stimuli and a blood supply to the cells within the bone(66).

The cambium layer contains osteoprogenitor cells that can develop into osteoblasts. The periosteum is attached to underlying bone by strong collagenous fibres called Sharpey's fibres. These extend into the outer circumferential and interstitial lamellae. Branches of blood vessels pass into the bone along Volkmann canals to the vessels in the Haversian canals. The endosteal layer covers the inner surface of the cortex (endosteal component). It also lines the trabeculae (trabecular component) and the Volkmann and Haversian canals (intracortical component). It is a thin connective tissue layer containing osteoblasts, osteoclasts and their progenitors(68).

2.3.3 Determinants of bone strength

The strength of a bone is determined by two main factors: its material composition and its structure (spatial distribution). In terms of material properties, there is a fine balance that must be maintained in bone if it is to serve its required purposes appropriately. Bone must be stiff enough to resist excessive strain (change in shape with loading) in order to permit it to bear loads. Conversely, it must not be so stiff that it is unable to deform at all as it would then become brittle, and brittle substances release energy imposed upon them by structural failure, in this case fracturing.

Bone is generally around 60% mineralised although there is some variation. As this level increases the bone becomes stiffer and thus less flexible. Homeostatic mechanisms within bone aim to ensure adequate strength is maintained. Osteocytes are able to detect changes in bone shape (strain) due to forces applied to that bone. They are also able to detect the direction of the strain(69). As excessive strains can lead to fracture, if these are detected by the osteocytes they respond by increasing bone stiffness, through alterations in bone geometry and/or mineralisation, in order to compensate. Conversely, if bone is not loaded, for example due to immobilisation after a fracture, bone mineral can be lost.

Bone must also be elastic in order to absorb energy, for example during locomotion. Within a specific range of strains a linear relationship is found with stress, as the bone material obeys Hooke's law, and the tissue is able to deform reversibly. Beyond this point the tissue undergoes plastic deformation

which results in a permanent change in shape. This tends to be associated with the development of microfractures. Microdamage caused by the accumulation of multiple microfractures can significantly affect the material properties of bone. It can reduce bone strength by over 80% without changing the bone's appearance, mass or volumetric density(70). Microdamage is the main cause of pseudofractures in osteomalacia and is a potential contributing factor to atypical femoral diaphyseal fractures in patients on long term bisphosphonate therapy.

The structural organisation of the organic matrix (collagenous and non-collagenous proteins) influences the material properties of bone. Collagen cross-linking and composition significantly influence bone strength (71, 72). The microstructure of the bone also has a role to play. Cortical bone with a high rate of turnover is made up of many overlapping osteons that limit the propagation of cracks by obstructing their path(73). Thus, cracks tend to occur more in older, densely mineralised interstitial bone between the osteons(74). Other facets of bone material quality usually vary little between bones within the same individual(75). Major defects can be seen in diseases such as Paget's disease, osteopetrosis and osteogenesis imperfecta.

Bone size, macrostructure, and geometry have a considerable impact on its overall mechanical properties, including its strength. When a long bone undergoes uniaxial compression, it is mainly the total cross-sectional area of bone tissue that is important in resisting the force. In vertebral bodies, which are largely trabecular bone, the number and connectivity of these affects strength independent of bone mass. In bending and torsion, the stiffness and strength depend more on the spatial distribution of the material in cross-sectional area with respect to the bending or torsional axis.

In a tubular structure, such as a long bone diaphysis, the further away from the neutral axis the material is located, the stronger the structure. Thus, doubling the diameter of a tubular structure but maintaining a constant mass, will lead to an eight times increase in bending strength. An assessment of cross-sectional moment of inertia (CSMI) takes into account, not only the spatial distribution of a tubular structure but also its area, in determining its resistance to bending or torsion. In keeping with the understanding of the

determination of CSMI, in long bones the most significant structure providing resistance against fracture is the cortical bone(76).

We currently understand a great deal about how bone strength is derived *ex vivo*. The next step is to be able to measure the important determinants of this in live human subjects in order to assess their bone health. Fortunately, several of these variables can now be measured non-invasively by a variety of methods. These range from simple assessments of bone density to more complex and detailed three dimensional imaging techniques. A description of these will be given in the next chapter.

3. Measurement of bone health

3.1 Early bone health assessment

Bone health can be measured in several ways. Some of the earliest attempts to quantify it involved the use of plain radiographs. Assessments were made of cortical morphometry, most commonly the second metacarpal of the non-dominant hand (metacarpal index)(77). Single- and dual energy photon absorptiometry were subsequently used with radionuclide sources(78). These took over 15 minutes to complete (per site) and as the radionuclide decayed they had a finite lifespan, needing to be replaced at regular intervals. Consequently, around 25 years ago, the radionuclide source was superseded by an X-ray source and DXA scanners were born. These not only had faster scanning times but also greater spatial resolution. Early DXA systems used pencil-beam geometry and a single detector. These slowly scanned across the site of interest. Most current scanners use a fan-beam source and several detectors which reduces scan times yet further.

3.2 Dual energy x-ray absorptiometry

3.2.1 Bone mineral density assessment

In DXA scanning, two beams of x-rays of different energies are directed from a radiation source towards a radiation detector. The participant is placed on a table with the site of interest between the source and detector. The scanner is able to calculate how much of each beam is absorbed by bone mineral and soft tissues. From this the software then calculates the BMC within the beam paths(79, 80). As BMC obtained from DXA is purely an estimate of the bone material in the path of the x-ray beam, it is not a true measure of bone mass. The value produced is, however, a surrogate marker for this. Calculation of BMC using DXA also assumes a constant ratio of fat mass to lean mass in the overlying soft tissue. This can however vary with affects on the results produced. Furthermore, the density that is produced using DXA is not a true volumetric BMD (vBMD), of mass per unit volume measured in g/cm^3, as there is a missing depth value in the calculation. As a result, it is termed areal BMD in order to reflect this and is measured in g/cm^2. To try to correct for the

problem, various methods have been employed, such as calculating an approximate volume for the projected region measured by DXA. This allows for calculation of bone mineral apparent density (BMAD) – the ratio of the BMC to a cuboidal or cylindrical estimation of the volume of bone(81-83). BMAD is used primarily for research purposes and in children and is not yet employed in adult clinical settings.

Overall within a population, higher aBMD and BMC are associated with higher bone strength. On an individual level, however, the predictive power of these measures for bone strength is relatively poor. This is partly related to the inability to measure cortical and trabecular bone separately, and to take into consideration the bone's material quality or structural geometry(84, 85). This is an important issue as it is known that the shape of a long bone also makes a difference to its strength, particularly in torsion and bending. This may be of particular importance when studying bone growth and development; assessing for therapeutic benefits of medications or other interventions; and when attempting to predict the risk of fracture. It is less of a problem in the assessment of vertebral bodies as the main forces they endure are compressive and compressive strength is reasonably well correlated with BMC(86).

Despite the limitations of DXA, it is still recognised as the reference method to measure aBMD with acceptable accuracy errors and good precision and reproducibility(87). Furthermore, it continues to be the measurement used in the WHO definition of osteoporosis(15) and therefore in clinical practice is one of the factors on which treatment decisions may be made. Although current DXA scanners allow measurement of almost any skeletal site, clinical assessments in adults are usually limited to the lumbar spine, proximal femur and forearm(88). Recommendations from the International Society of Clinical Densitometry (ISCD) are that aBMD should be measured at the posteroanterior lumbar spine and the proximal femur (neck or total hip)(89). The lateral spine and Ward's triangle should not be used for diagnosis as they overestimate the prevalence of osteoporosis. In very obese patients, those with primary hyperparathyroidism, or those in whom the hip and spine are difficult to measure or interpret, it can be useful to measure aBMD in the non-dominant radius at a point 33% of the way from distal to proximal.

3.2.2 Vertebral Fracture Assessment

In addition to the assessment of bone health in terms of aBMD and BMC, DXA scanners can also be used to evaluate for vertebral fractures. This is known as vertebral fracture assessment (VFA). A digital X-ray image is taken of the lateral spine that includes the L4 vertebra and extends cranially to T4. Clearly, the alternative to a DXA assessment of vertebral fractures is the use of conventional plain radiography.

The main benefit of plain radiography is that a greater number of vertebrae are visualised sufficiently to permit their assessment. This is a greater problem with VFA, particularly in the upper thoracic spine which tends to be the main site of disagreement between the DXA and radiographic assessments. In those vertebrae deemed to be evaluable by both techniques, good agreement (96.3%, k=0.79) in classifying vertebrae as normal or deformed was found(90). Furthermore, in a similar study, although some vertebral fractures were missed by VFA, all patients with prevalent vertebral fractures were identified. This suggests that in the identification of individuals with one or more fractures, visual assessment of DXA scans had 100% sensitivity(91).

The main advantage of using DXA VFA is that it uses a lower radiation dose. It also reduces parallax errors which, in a conventional radiograph, can give a normal vertebra the appearance of a compression or biconcave fracture(92, 93). When VFA is completed in conjunction with bone density assessment it can be very useful and significantly improve fracture risk stratification(94). However, due to the lower resolution of conventional DXA techniques, they may miss other spinal pathology. As a result, GE Healthcare recently developed the Lunar intelligent DXA, a fan-beam densitometer that uses slightly higher amounts of radiation and has enhanced detector capabilities, with the latter providing improved spatial resolution(95). A recent study has shown that most vertebrae are now evaluable using the iDXA scanner, even to discriminate mild vertebral fractures(96). Furthermore, whilst VFA is capable of assessing the spine in one image, conventional plain radiography requires two images; one of the lumbar and one of the thoracic spine. This can make accurate identification of vertebral levels more difficult.

Images from VFA can be assessed in two main ways, either by vertebral morphometry (97-99) or a semi-quantitative technique(100). In the vertebral morphometric assessment, the scanner software is used in a semiautomated way to mark points on the superior and inferior vertebral endplates allowing determination of the dimensions of the vertebral body in sagittal section. These values are then compared to the expected normal ranges to assess for the presence of deformities consistent with fracture. However, it is sometimes difficult to discriminate true fractures from normal variants of vertebral shape(101). Specifically, there is variation in vertebral size and shape at different levels of the spine and between individuals(102, 103). Furthermore, there are many possible causes of vertebral deformities and thus there is a wide differential. They can be caused by osteoporosis, degenerative disease, Scheuermann's disease, trauma, congenital abnormalities, Paget's disease, tumours, and infections. Currently the only way to confirm whether a vertebral fracture is present is by visual inspection and interpretation by an experienced reviewer(104, 105). Quantitative methods alone may therefore be insufficient to accurately assess whether a fracture is present and thus methods have been developed to include visual evaluation by an experienced reviewer to provide a "semi-quantitative" assessment (100).

Although it is recognized that visual interpretation of radiographs is subjective, an expert assessment can better distinguish between true fractures and vertebral anomalies than quantitative morphometry. For example, only an experienced observer can distinguish a fractured endplate from a deformity associated with Schmorl's nodes or a wedge-shaped deformity resulting from remodelling in degenerative disc disease(106, 107). In order to reduce the subjective nature of an assessment by a reviewer, semiquantitative techniques have been developed that provide more specific guidance. The most widely used was devised by Genant and colleagues(100).

The Genant method is based on evaluation by radiologists or experienced clinicians to correctly identify and then classify vertebral fractures. Vertebrae T4-L4 are graded from 0 to 3 based on visual inspection and not direct measurement. Normal vertebrae are grade 0; mild but "definite" fractures (20-25% reduction in anterior, middle, and/or posterior height, and 10-20% reduction in area) are grade 1; moderate fractures (25-40% reduction in any

height and 20-40% reduction in area) are grade 2; and severe fractures (40% or greater reduction in any height and area) are grade 3 (Figure 3.1). A grade of 0.5 is applied to any vertebra in which the deformity is borderline and may or may not represent a fracture. This method shows good sensitivity in identifying moderate or severe fractures (91.9%) and has an excellent negative predictive value (98%) to discriminate those without fracture from those with possible or definite fractures(90). It also provides excellent agreement when solely assessing for the presence or absence of fracture, however, differentiation by severity grade often shows relatively poor reproducibility(108). Specific problems may arise with subtle abnormalities, particularly wedge-like deformities in the mid-thoracic region, and bowed endplates in the lumbar spine. In these cases, differentiation between a borderline deformity and definite mild fracture can be extremely difficult. The reviewer must be able to distinguish normal variations from true fractures and this is clearly reliant on the expertise and experience.

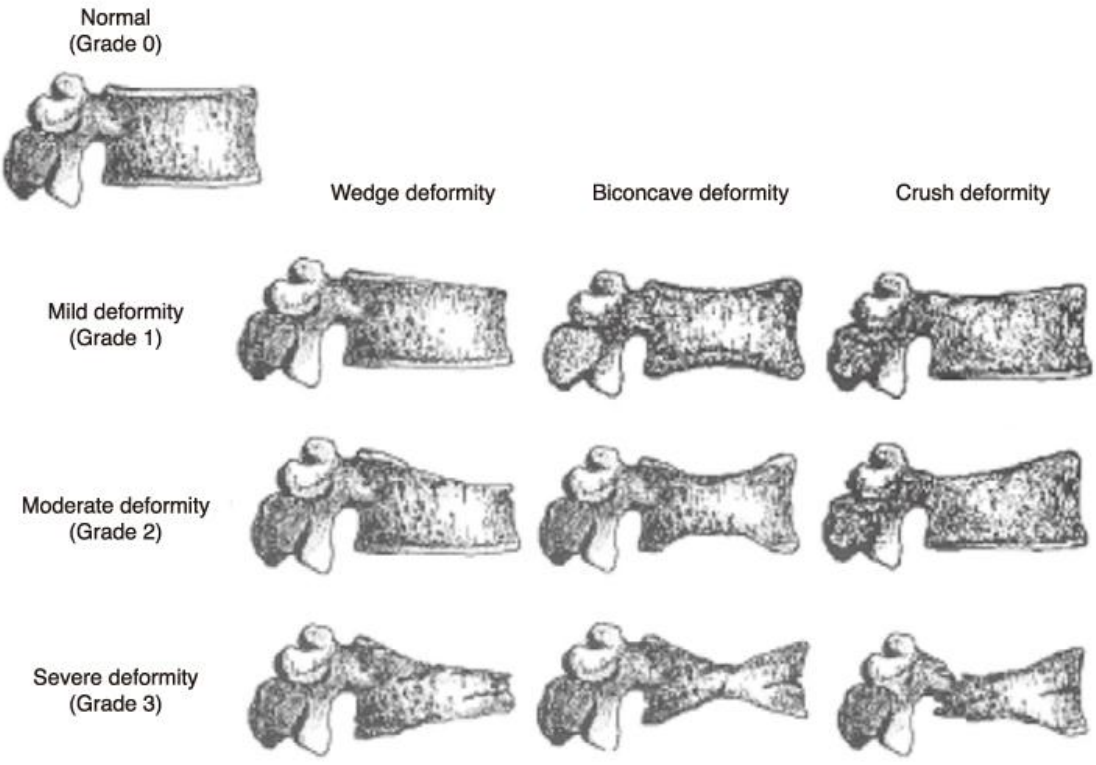

Figure 3.1: Semiquantitative method to assess for vertebral fractures. Reproduced from Genant *et al.* Vertebral fracture assessment using a semiquantitative technique(100).

Clearly there are benefits and limitations to both morphometric and semiquantitative assessments as described above. Given the additional potential to discriminate the various causes of vertebral deformity, it may be argued that the Genant semiquantitative method may be preferable for use in a group such as the Hertfordshire Cohort Study (HCS). Interestingly, most comparative studies have found a high concordance between different quantitative morphometric approaches and visual semiquantitative evaluation techniques for prevalent moderate or severe vertebral fractures using VFA(90, 109).

3.3 Peripheral quantitative computed tomography

3.3.1 Introduction to computed tomography scanning

Computed tomography (CT) was first introduced in the 1970s. Head scanning was initiated in 1973(110) and soon after whole body CT scanners were brought in(111). More recently, scans have been carried out at peripheral sites, such as the radius and tibia, using a peripheral CT scanner. These are much smaller than conventional scanners and are also less expensive with a smaller radiation dose (<0.5μSv). Peripheral quantitative computed tomography (pQCT) is used in studies investigating bone growth and development; assessing the effects of pathology and treatments; and exploring the relationships between assessments of bone strength and the risk of fracture.

3.3.2 Image acquisition and analysis

CT is a three-dimensional imaging technique. In order to produce an image, there is first the acquisition of a series of radiographic projections from a range of angles around the object being assessed. As X-rays pass through the body, they are absorbed and attenuated at different levels. As the beams are transmitted and detected at many points around the structure, this creates a matrix of X-ray beams of different strengths. The cross-sectional image of the field of view is then reconstructed from these (Figure 3.2).

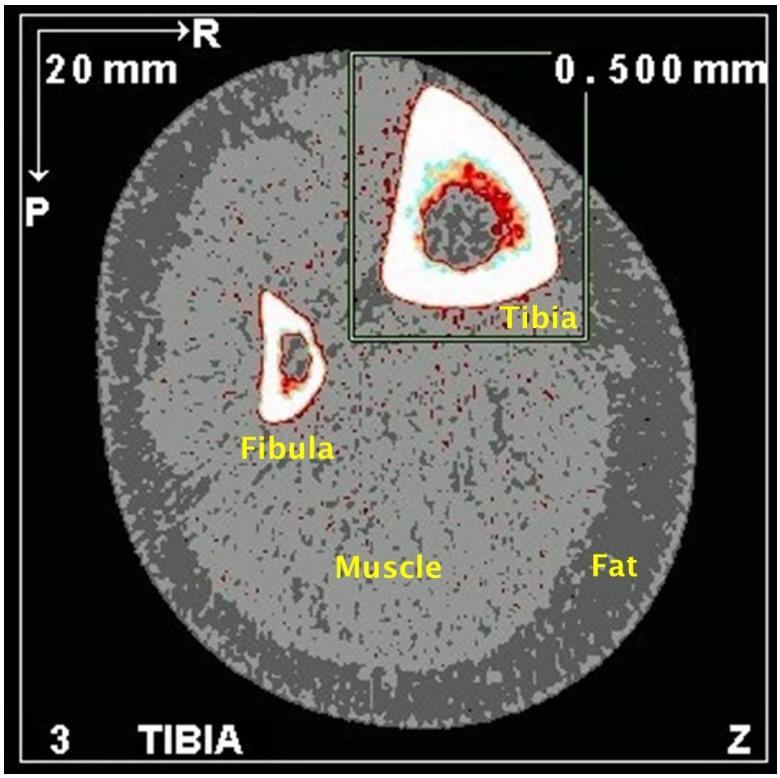

Figure 3.2: pQCT scan of tibial diaphysis

As in simple radiography, the reconstructed voxel intensity values are representative of the specific X-ray attenuation; this is related to the electron density of the material being scanned. High atomic number materials absorb more x-rays, and appear white on the image. As bone (calcium hydroxyapatite) has a particularly high electron density, it is highly contrasted from the surrounding soft tissues. To transform the radiodensity in Hounsfield Units (HU) into bone mineral equivalents (mg/cm^3) a calculation is performed by the scanner using a predetermined ratio and is calibrated by the regular scanning of a bone mineral phantom.

3.3.3 Outcome variables from pQCT scanning

The images produced are cross-sectional slices through the limb. Usually these are taken at several different points along the length of the bone. At distal sites (the metaphysis) the structure is largely made up of trabecular bone within a thin cortical shell. Within the shaft of the bone (the diaphysis) there is mainly cortical bone with a marrow cavity centrally and varying amounts of trabecular bone between them. The marrow can be either red marrow or

yellow marrow and the proportions of these can affect the results of pQCT scans(112). Furthermore, the structural variation in bone structure by location must also be taken into consideration when the methods of analysis for the various images are chosen. In general, trabecular parameters are assessed in the metaphysis and cortical parameters in the diaphysis.

At the distal (4%) slice of the pQCT scan, assessment is made of total cross-sectional area (CSA), total vBMD, and trabecular vBMD. At more proximal sites (radius 66%; tibia 14%, 38% and 66%) assessment is made of total CSA, cortical CSA, cortical thickness, periosteal circumference, endosteal circumference, and polar stress-strain index (SSI). An explanation of how SSI is calculated is shown in appendix 1. At the 66% level an assessment is also made of muscle CSA.

3.3.4 Comparison of DXA and pQCT

pQCT has several advantages over the use of DXA. Firstly, as a cross-sectional slice is imaged through the bone, an assessment of vBMD can be made. This is more accurate than aBMD obtained from DXA as it is not influenced by bone size. Secondly, the technique allows assessment of cortical bone and trabecular bone separately. It also provides structural information, such as the diameter and thickness of the cortex, allowing a more complete assessment of bone strength to be made. Importantly, aBMD only explains about 70% of the variance in bone strength, whereas the remaining variance is due to the influences of other factors, such as bone geometry(113). Similar deficits are found when aBMD is used to assess the therapeutic benefit of drugs in trials; it explains less than half of the effect(114).

DXA also has some benefits over pQCT. Firstly, the sites that are studied by DXA – the proximal femur and spine – are of particular clinical significance. Both are the sites of fractures shown to reduce survival rates(29, 50). The distal radius, one of the sites of pQCT scanning, is also an important site of osteoporotic fractures. Secondly, in longitudinal studies repositioning can be more problematic with pQCT than with DXA.

3.4 High-resolution peripheral quantitative computed tomography

3.4.1 Introduction to high-resolution scanning

In order to fully assess bone strength, we must explore not only a bone's macrostructure, as can be determined by pQCT, but also its microarchitectural makeup. A good example of this is the large variability in the biomechanical properties of trabecular bone that cannot be explained purely by measurement of bone mass(115, 116). The trabecular region assessed is made up of both trabecular bone and bone marrow; consequently it is not homogeneous and hence further information on structure is required.

Although trabecular bone morphometry can be assessed by iliac crest biopsies(117, 118) these are invasive and lead to localised bone damage which limits repeat measurements. Thus there is a need for high resolution imaging techniques that allow for the non-invasive assessment of bone microstructure. One example of this is magnetic resonance imaging (MRI). MRI is technically challenging due to sensitivity to motion and artefacts are not uncommon. It requires complex image processing, significant operator expertise and there are also issues with scanner availability. Although, MRI does permit the imaging of central sites, such as the proximal femur, and does not expose the subject to ionizing radiation, it usually has suboptimal spatial resolution(119).

For many years basic researchers have used desktop micro-CT to investigate bone specimens and small animal models of skeletal disease(120). *In vivo* CT images of whole human bones can be obtained using multi-detector computed tomography (MDCT), which is available in the majority of radiology departments as it does not require a dedicated scanner. There are, however, limitations to this technique in particular spatial resolution with a minimum slice thickness in clinical studies of around 0.6mm. Furthermore, although these scanners permit imaging of clinically relevant central sites, such as vertebrae and proximal femora, this is associated with a substantial radiation dose and requires specialised software and phantoms for analysis.

Figure 3.3: Scanco Xtreme HRpQCT Scanner

In the last few years, a high-resolution, limited field of view CT device has become commercially available, specifically for the assessment of bone microstructure in the peripheral skeleton, known as a HRpQCT(4, 121, 122). HRpQCT uses a dedicated imaging system to assess trabecular and cortical structure at distal peripheral sites. There is currently only one manufacturer of such a system: XtremeCT, Scanco Medical AG, Bruttisellen, Switzerland (Figure 3.3). It has greater spatial resolution and signal-to-noise ratio than both MDCT and MRI techniques, and has been shown to correlate well with findings from transiliac bone biopsies(123).

3.4.2 Image acquisition and analysis

The XtremeCT scanner has a microfocus x-ray source with a 70μm focal spot size (the size of the x-ray beam source). The larger this is the bigger the penumbra and thus the greater the loss of image definition. The source utilises an x-ray tube. This is a vacuum tube (a device controlling electric current through a vacuum in a sealed container) that produces x-rays. The range of photonic energies emitted by the system could theoretically be

adjusted by changing the applied voltage however in the Xtreme scanner the there is a fixed voltage of 60kVp and a fixed current of 900μA.

There are filters of 0.3mm copper and 1mm aluminium positioned at the aperture of the source to filter out the soft x-rays which would not penetrate the tissues but would otherwise still add to the radiation dose to the subject. This process also reduces beam hardening and the resultant image artefacts. Beam hardening occurs because lower energy photons are preferentially absorbed and thus as the x-rays pass through an object the beam becomes of higher average energy. Its properties thus change, for example it attenuates less quickly, and this makes image analysis more difficult. By removing low energy photons near the source, this reduces the propensity for hardening to occur within the scanned object.

When the x-rays leave the source they form a cone beam. A motorised gantry allows movement along the z-axis of the scanner and axial rotation. The source is thus able to move in a helical path around the object. A two dimensional array of detectors measure the transmitted radiation. The detectors then interface with a computer workstation and data storage facilities. This method of image acquisition is in contrast to the pQCT scanner that images one slice at a time as the source rotates around the object with a one dimensional array of detectors. The benefits of the helical scan are lower radiation doses and faster scanning for a given resolution.

The standard scan protocol and analysis produces 82μm isotropic voxels. A voxel is a volume element that represents a value on a regular grid in three dimensional space. In the radius, the default scan location is found to partially overlap with the most common site for fracture in the distal radius(124). Unfortunately, hardware constraints for this device prevent imaging of diaphyseal sites in the radius or tibia although cortical bone can still be assessed at distal sites due to the higher resolution of the technique.

3.4.3 Outcome variables from HRpQCT scanning

When assessments are made of the distal radius and tibia and analysed using the standard, manufacturer-recommended method, several variables are

created including cortical and trabecular area, cortical thickness, and periosteal circumference (Figure 3.4).

Density is calculated in the bone as a whole; the cortical and trabecular regions; and the inner 60% and outer 40% of the trabecular region separately. The ratio of outer trabecular density to inner trabecular density is then calculated. The proportion of trabecular area that constitutes bone tissue is also determined (bone volume/tissue volume; BV/TV). Trabecular microarchitecture is assessed in terms of trabecular thickness, number, and separation although only trabecular number is measured directly. An estimate is also made of the distribution (SD) of trabecular separation.

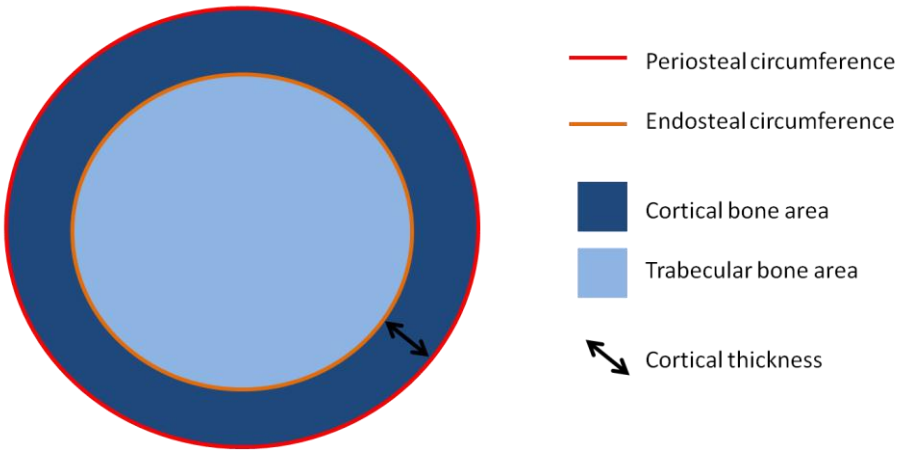

Figure 3.4: Schematic representation of bone geometry in cross-section

More recently, a technique was developed by Burghardt *et al* to more accurately delineate the cortical region(125). Using this technique, an improved assessment of cortical parameters can be made. Cortical volume can be determined as total cortical volume including pores and total cortical bone volume excluding pores. Similarly, cortical density is measured as cortical bone mineral density including pores and cortical tissue mineral density excluding pores. Other geometric measurements include periosteal circumference, endosteal circumference, total area, cortical area, cortical thickness and trabecular area. Cortical porosity is assessed as pore volume, mean pore diameter, and as a proportion of total cortical volume. There is also an estimation of the variation in cortical pore diameter. Precisely how

variables are derived will be discussed in detail in the methods section. Further software has been produced that allows assessment of bone mechanical properties using finite element analysis (FEA). However, this is not available on all machines as standard.

3.4.4 Comparison of pQCT and HRpQCT

The radiation dose for both pQCT and HRpQCT is a very small fraction of that required for a whole body CT and the radiation exposure does not involve the most radiosensitive organs. The HRpQCT scan is limited to more distal areas of the radius and tibia than a pQCT scan. It is therefore not possible to image the diaphyseal region or muscles. The data provided regarding the distal radius and tibia is, however, far more detailed and the technique permits acquisition of a stack of 3D slices which provides a larger block of tissue to analyse than with standard single slice pQCT. HRpQCT can therefore produce information on trabecular morphometry, distal cortical area and cortical porosity. Due to the higher resolution and thus smaller voxels there are fewer problems with partial volume effect. The partial volume effect occurs because the scanner is unable to differentiate between a small amount of high density tissue and a large amount of low density tissue within a given voxel. Instead it averages the densities of whatever tissues are in the voxel and, as a result, information is lost. This is therefore a greater problem with larger voxels.

3.5 Conclusion

Each of the imaging techniques described has its own individual advantages and disadvantages in the assessment of bone health. All measurements including those of size, geometry, density, and microarchitecture can contribute to bone strength. Using pQCT does allow for the calculation of stress strain index based on certain assumptions and gives an indication of bone strength. Similar overall strength variables are also available with HRpQCT but only when FEA software is accessible.

There are a total of 29 HRpQCT variables created at both the radius and tibia with some measured by both methods of analysis. Although, detailed phenotypic information is one of the main strengths of this high-resolution

technique, it is important to focus on those outcomes with the most clinical significance and ensure that the correct image analysis method is used for each. The choice of variables to use in analyses will be discussed in the methods section. However, given the large number of outcomes available care must be taken when interpreting associations as chance findings may occur.

Having developed an appreciation of the burden of osteoporosis, the normal physiology of bone, and how bone health can be measured, the next step is to understand what is known about the determinants of bone health. Simple demographic and lifestyle factors will be explored first.

4. Demographic and lifestyle determinants of bone health

4.1 Introduction

Although the process of bone formation is similar in all individuals the resulting skeleton can vary considerably depending on multiple factors. Peak bone mass is achieved in early adulthood. Thereafter there is an initial period of stability followed by a gradual decline with alterations in bone density and structure. The acquisition of peak bone mass and the course of bone loss in later life will be described in this chapter along with some of the factors that influence them.

4.2 The path to peak bone mass

The amount of cortical and trabecular bone, and thus total BMC, increases from conception until the epiphyseal growth plates close and skeletal mineralisation and growth are complete. Peak bone mass is defined as the amount of bony tissue present at the end of skeletal maturation(58) and is important in determining the risk of osteoporotic fracture occurrence in the future. It has been estimated that a 10% increase in peak mass would reduce the risk of fracture in postmenopausal women by around 50%(126). In contrast to childhood, in which bone turnover is very high and the rate of formation exceeds resorption, in young adult life formation and resorption rates are similar and so for the period around peak bone mass there is maintenance of a relatively stable skeletal makeup. This is followed by a further period of stability before a subsequent decline in the amount of bone tissue. Thus, the point from which this decline starts is an important determinant of bone health at older ages.

It has been suggested by twin and family studies that 50% to 85% of the variance in aBMD is determined by heritable factors(127-129); these include both genetic and shared environmental exposures. Specific estimates vary depending on the skeletal site, with lumbar spine aBMD demonstrating a greater heritable component than distal forearm aBMD (127;129;130).

Although there was initial optimism that genetic factors might be able to explain a large proportion of the peak bone mass variance, to date only a very small amount has been accounted for (1-3%).

There is a tendency for bone parameters to track during the growth phase. Thus, those that were in the lowest centiles before puberty are still likely to remain in a similar ranking at maturation(127). However, peak bone mass can also be modified by non-genetic factors such as physical activity, diet (e.g. calcium, vitamin D and protein intake), lifestyle (e.g. alcohol consumption and smoking), endocrine factors (e.g. sex steroids), and specific risk factors (e.g. certain medications).

4.3 The importance of sex in the determination of peak bone mass

At birth, bone mass appears to be similar in males and females with no evidence to suggest a significant gender difference(128). After adjustment for age, nutrition, physical activity, and size, bone mass in the axial and appendicular spine remains equivalent in boys and girls up to the onset of puberty (129-131). Interestingly, Wang *et al* noted that in prepubertal children, the amount of periosteal apposition was the same in those with bigger and smaller tibial CSAs(132). However, those with larger cross-sectional areas had greater endosteal resorption which led to a thinner cortex. Those with smaller CSAs tended to form relatively denser bones with greater cortical thickness which would offset the reduced bending strength due to their lesser size(132).

Between infancy and puberty, the rate of appendicular growth is approximately double that of axial growth. Thus, the increase in height at this point is driven mainly by lengthening of the legs(133, 134). During this period, leg length remains the same in both sexes. However, as males enter puberty two years later than females, the gender disparity in adult leg length is greater than trunk length(135). During the pubertal growth spurt bone mineral accrual is accelerated. This increase is more marked in the femoral neck and lumbar spine than in the long bone diaphyses(136). For the first two years of puberty,

axial and appendicular growth is similar, whereas in late puberty axial growth predominates.

Most sex differences in bone width, mass and strength develop during puberty. In boys, diaphyseal bone width increases due to periosteal apposition(137) with a corresponding increase in endosteal resorption although the latter occurs to a lesser extent. Consequently there is an overall increase in cortical thickness. The endosteal resorption does, however, help to maintain the lightness of the bones whilst moving the cortex away from the neutral axis, which in turn increases the bone's bending strength.

In girls, periosteal apposition occurs to a lesser extent and medullary size remains stable or even reduces due to endosteal bone formation. Thus, bone width becomes smaller than in boys although cortical thickness and vBMD remain comparable (137-139). The greater increase in bone width in boys is driven mainly by their greater height. Consequently, the resulting bones tend to be appropriate for adult size, whether male or female.

In boys, puberty corresponds with an increase in serum testosterone levels although many other endocrine changes are also seen. These are likely to contribute to the pattern of diaphyseal bone growth. In post-pubertal girls, oestrogen levels are high and it may be this that results in the reduced periosteal and increased endosteal apposition compared with boys(140, 141). A study of male rats has confirmed that oestrogen reduces the osteogenic response at the periosteal surface to exercise but increases endocortical bone formation(142). These differing hormonal patterns may partly explain the observed sexual dimorphisms in bone structure.

Metaphyseal growth differs from that in the diaphysis as bone in this region is mainly trabecular in nature. During growth the metaphyseal cortex is resorbed at the periosteal surface and formed at the endosteal surface by trabecular coalescence(143). Consequently, the disparity between metaphyseal and diaphyseal CSA reduces. Similarly to diaphyseal bone, vBMD, in this case trabecular, remains unchanged from early childhood to adulthood in both boys and girls(144).

Vertebral bodies are largely made up of trabecular bone. Throughout growth vBMD is similar in boys and girls(145, 146). However, the CSA of the vertebrae is greater in boys than girls and this disparity increases up to skeletal maturity. Thus, vertebral bodies are stronger in adult men but this is purely a function of their greater size and BMC, and not due to variation in vBMD(147). Peak bone mass is therefore also higher in men than women due to greater bone size whereas total vBMD is relatively similar in both sexes(58). Race is an important factor determining peak bone density; overall black people have a higher vBMD than white people(148). They tend to have similar numbers of trabeculae but overall these are thicker(149).

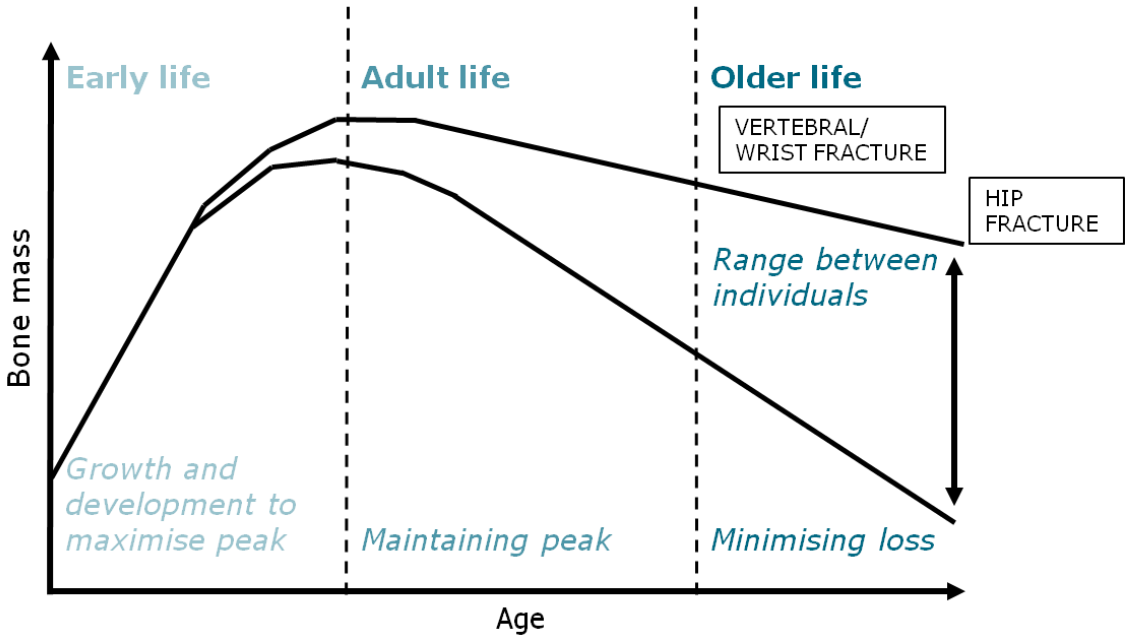

Figure 4.1: Graph describing bone mass across the lifecourse. Adapted from Cooper *et al*. Epidemiology of Osteoporosis(56).

The growth phase produces about 90% of the peak bone mass attained during adult life (Figure 4.1). Following the growth spurt, linear growth slows dramatically whereas bone mineralisation continues(150). During this consolidative phase it is thought that bone density can rise for 5-15 years(151, 152). However, the magnitude of this effect on vBMD is likely to be small. One study showed that female subjects had no further significant gains in the 2 years after puberty at either the lumbar spine or femoral neck(136) and another found no significant difference in men or women between vBMD in the lumbar spine of 16 year old and 30 year old subjects(153). It is therefore

thought that peak bone mass in Caucasian females with adequate intakes of calcium, protein, and energy can be almost completely achieved by late adolescence (136, 154). In males, total body BMC is shown to plateaux at the age of 20 years, approximately 1-2 years after peak bone area is achieved(59).

4.4 Age-related changes in bone geometry and density

Once the consolidation phase has occurred and peak bone mass has been achieved there is a period during which bone mass remains stable. At the age of around 35 years, there then begins a gradual decline(155). The balance between formation and resorption shifts to favour the latter; this results in a net bone loss. In women, this phenomenon is increased in the years immediately following the menopause. The oestrogen deficiency that ensues at this point is thought to be the main cause.

It has been estimated that postmenopausal women lose bone at a rate of 0.32% to 0.95% per year in the total hip region with similar rates at other sites in the proximal femur(156-158). In the elderly, rates of loss progressively increase with age reaching around 1.64% per year in the hips of those above 85 years. It has been estimated that, between the time of peak bone mass and death, aBMD has dropped in the femoral neck of women by 58% and in men by 39%(159). Rates of decline in the lumbar spine are more difficult to assess due to artefactual changes related to spondylosis. As a higher rate of turnover is found in trabecular than cortical bone this is one reason why bone loss occurs earlier and relatively rapidly in the spine.

In later life, although the loss of bone density is a significant problem, it varies little between individuals of the same sex, with one SD in the rate of loss equating to just 1% of the mean(160). The variation in bone morphology, including mass and size is large. The difference between the 5th and 95th percentiles for bone size is around 50%. Structural changes with ageing include thinning of the cortex, increasing intracortical porosity, and reducing trabecular thickness and connectivity(160). It is believed by some that periosteal apposition acts as an adaptive process to compensate for the loss of bone strength that occurs due to endosteal resorption(161).

In premenopausal women, there is significant endosteal resorption and periosteal apposition within long bones. The latter occurs at a lower rate than the former and thus there is a net loss in cortical thickness(162). However, as the bone circumference increases and cortical thickness reduces the amount of bone mineral remains fairly constant. Consequently, the bending strength actually increases as the bone tissue becomes distributed further from the neutral axis.

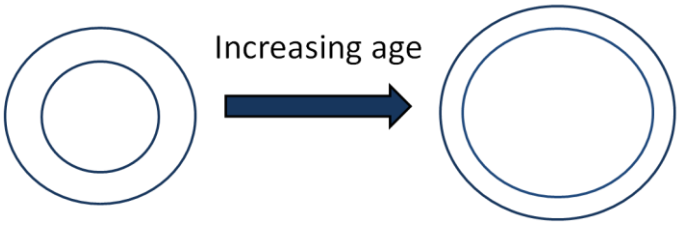

Figure 4.2: Changes in cortical geometry with age in later life

In the perimenopausal period, as expected, the rate of endosteal bone resorption increases. However, the rate of periosteal apposition decreases and thus there is further cortical thinning(163). At this point, bending strength remains stable, again due to redistribution of the bone mineral away from the neutral axis (Figure 4.2). If periosteal apposition was purely a compensatory mechanism in response to endosteal resorption, it would be expected to increase rather than decrease at this point. Thus, this is not the full explanation. Studies on primates have shown that gonadectomised females have higher numbers of osteoclasts at the periosteal surface compared with intact controls(164). It is therefore possible that oestrogen deficiency attenuates periosteal apposition after the menopause. This is in contrast to its role during bone growth.

As ageing continues, the pattern of changes is maintained with further increases in the rate of endosteal resorption and decreases in periosteal apposition. Consequently, the cortex continues to thin and at this point bending strength also reduces. This, in addition to a concurrent reduction in bone density, leads to increased bone fragility and greater fracture risk.

Similar changes with age occur in men although the effect of progressive oestrogen deficiency is far less marked(165). Interestingly, the continuous,

slight expansion of bone periosteal circumference is more prominent in men than women and affects both the axial and peripheral skeleton(166, 167).

Both the path to peak bone mass and changes in bone health with age are influenced by the bone's structure and density. This highlights the complexity of assessment needed to accurately determine bone strength and thus fracture risk. However, gross structure and density are by no means the only determinants of this. Bone microarchitecture also plays an important role.

4.5 Age, sex and bone microarchitecture

4.5.1 Age, sex and bone geometry

Although it is well established that changes occur in bone with age and that there are clear differences between men and women, previous studies have focused mainly on bone density and to a lesser extent on overall bone structure. With the introduction of HRpQCT we are now able to investigate in greater detail the influence of these factors at the distal radius and distal tibia, including assessments of bone geometry, vBMD in the distal cortex and trabecular regions, and bone microarchitecture.

Macdonald *et al* studied the distal radius and tibia of 644 Canadian adults (442 women and 202 men) aged 20 to 99 years(168). They found that young men had greater bone size and cortical thickness when compared with young women. This was also the finding of a study of adolescent boys and girls (15-20 years) investigated at the distal tibia(169). Khosla *et al* used the prototype HRpQCT scanner to investigate the distal radius of 278 men and 324 women between the ages of 21 and 97 years. These were an age-stratified, random sample of adults living in Rochester, US(4). In young men and women (20-29 years) although both total area and trabecular area were larger in men, contrary to the two studies above, cortical thickness did not differ significantly(4). This is in keeping with the findings of another smaller study by Dalzell *et al*(170) of 58 men and 74 women aged 20-79 years. When they compared parameters by gender across adult life, total CSA was significantly lower in women in both the radius and tibia at almost all time points but

measures of cortical thickness were indistinguishable until the age of 35 years in the tibia and 70 years in the radius(170). After these points, they became greater in men than women. Similarly, Burghardt *et al* compared bone structure across the majority of the adult lifecourse (20-78 years). Overall they found that cortical area and cortical thickness were higher in men than women at the distal radius and tibia(171).

In a cross-sectional study of 108 premenopausal (19-45 years) and 148 postmenopausal (52-88 years) women, Boutroy *et al* examined the effects of ageing at the distal radius and tibia(121). At both anatomical sites, in those with greater age total area was higher and cortical thickness was lower. Interestingly, when analyses were limited to premenopausal women, there were no significant correlations identified. With advancing age, Khosla *et al* also found that the total area and trabecular area were higher and cortical thickness was lower in both men and women(4). The magnitude of the associations of total area and trabecular area with age did not differ by gender. In contrast, the relationship between lower cortical thickness and greater age was more pronounced in women(4) which may explain why gender differences in cortical thickness are more consistently found at older ages.

Table 4.1: Summary of gender differences in bone geometry and density

Bone parameter	Sex with greater level in young adulthood	Direction of change with age	Sex with greater rate of change with age
Total area	Men	Higher	Similar
Cortical area	Similar/Men	Lower	Women
Cortical thickness	Similar/Men	Lower	Women
Trabecular area	Men	Higher	Similar
Cortical vBMD	Similar/Women	Lower	Women
Trabecular vBMD	Men	Lower	Women

Although the primary aim of the study by Vico and colleagues was to use HRpQCT to compare women with wrist fractures (n=50) or hip fractures (n=62) to controls (n=54), they also assessed the associations of age to radius and tibial bone parameters in the group as a whole (43 to 93 years)(172). They too

found that cortical thickness and cortical area were lower in older individuals at both the distal tibia and radius. Burghardt *et al* demonstrated these findings in women at both the distal radius and tibia, but in men the association was only shown for cortical area in the tibia(171).

In summary, total and trabecular area are higher in men than women (Table 4.1). Cortical area and thickness tend to be higher in men but less so at younger ages. Older individuals have greater total and trabecular area. The inverse relationship between cortical thickness and age is more pronounced in women than men, particularly after the menopause.

4.5.2 Age, sex and bone density

Cross-sectional studies have shown that in the distal radius of young adults (20-29 years), trabecular density was higher in men(4, 168, 173). By contrast, Khosla *et al* also showed at this age that cortical density was higher in women(4). Burghardt *et al* looked at cortical density in men and women between the ages of 20 and 78 years (mean(SD) 47(16) years)(171). They found that overall it was similar in both sexes in the radius but significantly higher in women in the tibia. It was significantly lower in older women, when compared to younger, but this association was much weaker in men and only reached statistical significance in the tibia(171). This is in keeping with the findings of Khosla *et al* who showed that in individuals aged between 21 and 97 years the difference in cortical density by age was significantly greater in women(4).

Interestingly, Dalzell *et al* further described that cortical density was higher in women than men around the fourth and fifth decades and this difference was significant in the radius (Figure 4.3). Thereafter, cortical density appeared to drop more rapidly in women and became higher in older men, although the study was cross-sectional and so there are other possible explanations for these associations(170).

Several cohorts have confirmed that cortical bone density is generally found to be lower in older people(4, 121, 170, 172, 173). However, part of this association may be caused by a coexistent lower cortical thickness. Cortical

thickness is only around 5 to 10 times greater than voxel width in HRpQCT. Thus, as it is negatively associated with age, the partial volume effect has a greater influence in older individuals which can result in lower cortical density readings.

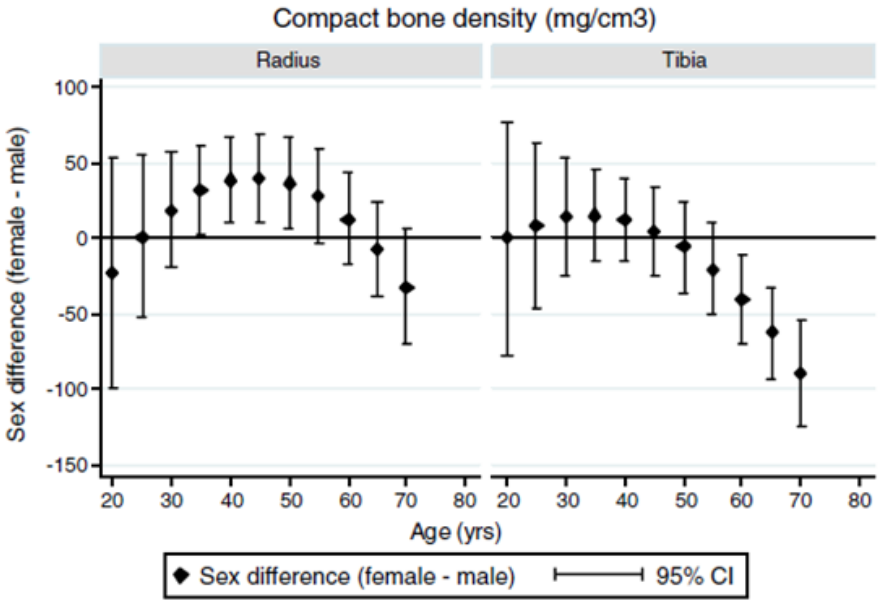

Figure 4.3: Sex differences (female–male) in 3D pQCT parameters evaluated at 5-year intervals. Reproduced with permission from Dalzell *et al.* Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT(170).

Trabecular bone density has also been shown to be lower in older individuals(4, 121, 170, 172, 173). This was also true when the trabecular region was subdivided into the inner 60% and outer 40%(173). As trabecular density is a function of both trabecular thickness and number, further information regarding microarchitecture is required to fully understand the specific nature of this association. This will be discussed in the next section.

In summary, trabecular density is higher in men than women and appears to be negatively associated with age. In younger individuals, cortical density does not differ by sex or is higher in women. It subsequently appears to reduce more rapidly in women than men and so, in some cases, is higher in older men than women.

4.5.3 Age, sex and trabecular architecture

In children aged 5-18 years, Wang *et al* found that trabecular thickness was higher in boys than girls(174). However, Burrows *et al* showed that in the distal tibia of adolescents (15-20 years) it was trabecular number, and not trabecular thickness, that was higher in boys(169). In young adults (age 20-29 years), MacDonald *et al* found that trabecular thickness and trabecular number at the distal radius were higher in men than women(168) whereas other studies in young adults only showed the former association(4, 173). Studies that have included individuals all the way from young adulthood through to later life have shown that trabecular number and thickness tended to be greater in men than women overall(170, 173).

When microarchitecture was compared in adults of different ages, it was found in both the radius and tibia that trabecular number and thickness were lower in elderly people than young adults and that trabecular separation was correspondingly higher(173). In keeping with these findings, Boutroy *et al* also showed, in both the radius and tibia of women, that with increasing age the number and thickness of trabeculae was lower(121). Vico and colleagues studied the distal radius and tibia but in postmenopausal women only. They found that only trabecular number showed a negative relationship with age(172).

Khosla noted that the inverse association between trabecular thickness and age was more pronounced in men than women but trabecular number was lower in older women only ($p<0.001$)(4). Furthermore, Dalzell *et al* confirmed that trabecular thickness appeared to be lower in older age in women but did not find any associations at all between age and trabecular structure in men(170).

In summary, at younger ages trabecular thickness and number are similar or higher in men than women. Both of these parameters have been shown to be lower in older age. Interestingly, there is some evidence that trabecular thickness may be more strongly associated with age in men than women.

4.5.4 Age, sex and cortical porosity

Burghardt and colleagues studied cortical porosity in 151 subjects aged 20 to 78 years(171). Overall they found that it was significantly higher in men than women and in the tibia than in the radius. This gender difference was most marked at younger ages and attenuated after the age of 50 years. Cortical porosity was also significantly higher in older adults compared to younger men and women, in both the radius and tibia(171).

MacDonald *et al* confirmed that cortical porosity was positively associated with age but to a lesser extent in men than women(168). They too found that cortical porosity was greater in men than women, although no significant difference was found in the eighth decade(168). It appears that although cortical porosity is greater in men overall, its relationship with age is less pronounced in men and this may explain the attenuation of sex differences in more elderly individuals.

4.6 Lifestyle determinants of bone health

4.6.1 Calcium intake

There are several lifestyle factors that can influence skeletal health. For example, a great deal of research has focussed on determining the relationship between calcium intake and bone health. Bone is the major calcium store within the body and a key organ in calcium homeostasis. Specifically, both bone forming and resorbing cells use calcium signals as regulators of differentiation and activity(175).

Calcium can be obtained from the diet and also through supplementation, and the latter is often recommended for patients with osteoporosis along with vitamin D. However, DXA studies examining the effects of calcium supplementation have been inconsistent(176-178). Although some have shown positive effects of calcium on aBMD(179, 180), a meta-analysis of calcium supplementation in healthy children showed differing effects depending on pubertal stage and site examined(178). It has been suggested that calcium supplementation may be more beneficial in those that have lower

baseline levels although this has not been confirmed(181). Furthermore, it is uncertain whether any gains in bone mass remain once the supplementation ceases with evidence, particularly in children, pointing to a lack of sustained effect(182).

Observational studies examining the relationships between calcium intake and bone health assessed by pQCT have also been inconsistent(183, 184). In one study of 129 community-based women, aged 70-87 years, calcium intake was not found to be an independent predictor of radial cortical or trabecular vBMD(183). In contrast, in a larger study of 1172 men, aged 69 to 97 years, positive associations were identified between total daily calcium intake and both cortical and trabecular vBMD in the radius and tibia ($p<0.05$)(184). In a randomised controlled trial of daily calcium and vitamin D supplementation for 6 months in children around puberty, supplementation was associated with increased trabecular area, density and SSI in the distal radius and tibia, and increased cortical area in the tibial midshaft(185). There is therefore still uncertainty in this area.

4.6.2 Physical activity

Physical activity and exercise appear to mainly affect cortical rather than trabecular bone, and can influence both mass and geometry. Most interventional and observational studies show increases in cortical density and area, both distally and within the midshaft(186-190). This can result in a rise in overall bone strength(191). Effects on trabecular bone have been far less consistent (186-188, 190, 192). In both regions, the size of the effects tends to be modest and site-specific. Interestingly, recent evidence has suggested that the increase in bone density in men related to exercise can be maintained for up to three decades following the cessation of high-level physical activity(193).

Several types of exercise have been shown to influence bone health. These include high-impact, rapid and forceful loading (e.g. running and jumping); changing, diverse, or novel loading angles (e.g. ball sports); weight-bearing with high forces (e.g. dancing and weight lifting); and direct impacts on bones (e.g. ball striking in tennis)(194). The greatest benefits tend to occur with

high-impact loading exercise(186, 187). Further research is required to more fully understand the amounts and types of activity that are required to optimise bone health, and to confirm whether it prevents fractures.

4.6.3 Smoking

In general, aBMD measured by DXA tends to be lower in smokers than non-smokers and aBMD accrual has been shown to be impaired in those that take up smoking in early adulthood before peak bone mass is achieved(195, 196). Studies utilising pQCT of the radius and tibia have demonstrated that the main deficits lie in trabecular vBMD and cortical thickness(184, 197-199). Associations between smoking and lower cortical vBMD are more inconsistent and appear to be site and sex-specific(184, 198, 200) or completely absent(195-197, 199). Only one study has looked at trabecular microarchitecture using HRpQCT and showed a tendency towards lower trabecular thickness, but not number, with smoking(196). Further studies are required to confirm the microarchitectural changes that underlie the relationship between smoking and trabecular density, and to further explore whether a true association with cortical density exists.

4.6.4 Alcohol consumption

Studies that assess the effect of alcohol consumption on bone health tend to show that the outcome is highly dependent on the amount of alcohol consumed. For example, fracture rates have been shown to be lower in those that drink more than 0.5-1 unit of alcohol per day but greater in those that drink more than 2 units of alcohol per day, when compared to those that abstain completely(201). Furthermore, pQCT studies that have explored relationships with alcohol consumption have not shown consistent effects on either vBMD or BMC(184, 199). Whereas in DXA studies, moderate alcohol consumption was positively associated with aBMD(202, 203). Attempts have been made to see whether factors such as smoking, diet and physical activity could explain the beneficial effects of moderate alcohol as described through confounding(204). Interestingly, however, the positive effects were maintained independent of lifestyle factors.

One study chose to look at men that, on average, drank more than one drink per week and compared them to those that drank less than this, rather than just those that abstained completely(198). Although they showed a trend towards lower trabecular vBMD in both the radius and tibia of those that drank more, these associations did not reach statistical significance. It appears that no studies to date have examined relationships between bone geometry and alcohol consumption. Further work is therefore required in this area.

4.6.5 Socioeconomic status

Although social inequalities in health are well recognised(205), little is known about their effects on osteoporosis. A recent systematic review examined the associations between aBMD and socioecomonic status, assessed using income, education, and occupation(206). It found that although there was some evidence to support an association between educational attainment and aBMD in women, no such evidence was available for income or occupation in either sex. In a similar vein, Barbour and colleagues showed greater educational attainment (above high school) was related to greater tibial trabecular and cortical vBMD(184). Despite these associations, the evidence that socioeconomic status influences fracture rates is also limited(207). Clarification of these relationships is clearly required, particularly with respect to definitions of socioeconomic status that are not directly related to education.

4.7 Diseases and bone health

Several diseases have been shown to be secondary causes of osteoporosis and are therefore included in fracture prediction models, such as FRAX(208, 209). These include type I (insulin dependent) diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition or malabsorption, and chronic liver disease. Several studies have also shown deleterious effects of chronic kidney disease, both in adults and children, in part related to secondary hyperparathyroidism (210-213). Clearly, these diseases will be affecting bone density directly, whereas in other cases it may occur due to confounding

through factors such as medication use. Type I and type II diabetes are both risk factors for fracture; however, the mechanism through which this occurs is likely to be different. Although type I diabetes may directly affect bone density, the influence of type II diabetes is likely to be more complex, as described below.

In adolescents from the Avon Longitudinal Study of Parents and Children (ALSPAC) study, fasting insulin levels were inversely associated with cortical vBMD, as assessed by pQCT(214). Whereas, in a study of 1171 men aged over 65 years, cortical density did not differ by diabetic status(215). Prior to adjustment for weight, radial and tibial trabecular density were higher in those with type II diabetes. However, after adjustment this relationship was fully attenuated but instead tibial total and cortical area were significantly lower in those with type II diabetes. Furthermore, in a HRpQCT study of 28 postmenopausal women, those with type II diabetes did not differ significantly from those without the condition in terms of bone microarchitecture in either the tibia or radius, although there was a tendency towards lower cortical area in the tibia ($p=0.06$)(216). Interestingly, another study showed that cortical pore volume was greater in individuals with type II diabetes than controls(217).

Relationships with bone health have also been assessed for other common conditions including hypertension, ischaemic heart disease, cerebrovascular disease, and chronic obstructive pulmonary disease (COPD). Associations between hypertension and bone health have been very inconsistent (184, 198, 218). One study showed no difference compared with normotensive individuals(198) whereas another showed higher radial trabecular and cortical density(184). When systolic and diastolic hypertension were investigated individually, the former showed no associations and the latter was related to lower radial trabecular BMC(218). Ischaemic heart disease has not been shown to be related to trabecular or cortical vBMD in either the radius or tibia(184, 198).

Cerebrovascular disease has rarely been studied in the absence of stroke causing paresis and disuse. However, one large study of over 1000 men did not show any significant difference overall between those individuals that had suffered a stroke and those that had not(184). In patients with a persistent

limb paresis, there is evidence to support declines in cortical thickness and trabecular density with a resultant reduction in bone strength(219-222). Interestingly, men with COPD have been shown to have significantly lower radial trabecular and cortical density, and tibial cortical density(184). Furthermore, when individuals with untreated COPD were stratified by the presence or absence of hypercapnia, it was found that those with higher carbon dioxide levels had significantly lower trabecular and total vBMD(223).

Premature menopause affects around 1% of women. It is usually idiopathic but can also be secondary to genetic, autoimmune or iatrogenic causes. Several studies have explored the relationships between time since menopause and bone health with others focussing on the period of maximal bone loss(224-227). Cross-sectional studies of postmenopausal women have shown that both trabecular and cortical vBMD are lower in women with a greater time since the menopause, although associations are invariably stronger for the former(226, 227). Clearly, those women who are further from the menopause will, on average, be older and this may contribute to the associations found.

Immediately following the menopause, there is a decrease in oestrogen levels and a rise in gonadotrophins. It has been shown that at this point bone loss is at its highest rate and it appears to be trabecular density, rather than cortical density, that declines most dramatically(224). Furthermore, a longitudinal analysis using micro-MRI has shown that during this period, there is a considerable decline in trabecular microarchitecture which can be abated by the administration of estradiol(225).

Specifically, DXA studies have shown that HRT has a positive effect on aBMD(228, 229) and it has also been shown to reduce fracture rates(230, 231). The increase in aBMD is, however, relatively small when compared to the corresponding decrease in fracture risk. It was therefore hypothesised that favourable structural changes are also occurring with HRT administration and this has been investigated using pQCT. In a study of 24 monozygotic twin pairs, distal radial and tibial bone density were higher in those on HRT for a mean of 8.3 years(232). Similarly, midshaft tibial cortical area and density were also higher with predominant effects on the endosteal surface. These changes led to greater estimates of bone strength in the treated group. Muller

and colleagues similarly showed changes at the endosteal surface within 6 months of treatment(233).

Another group of commonly used treatments that improve bone health are bisphosphonates. These have been shown to improve aBMD and reduce fracture risk(114, 234). More recently studies using pQCT and HRpQCT have investigated in greater detail the nature of these beneficial effects. Alendronate has been shown to prevent the decline in radial trabecular and cortical density, and cortical thickness over a 12 month period, with similar changes seen in the tibia(235). Analogous effects were demonstrated by Burghardt and colleagues with relationships stronger in the tibia(236). These findings are also consistent with pQCT studies showing alendronate to prevent the decline in trabecular density over a 1 year period(237, 238).

4.8 Adult anthropometry and bone health

Anthropometric factors have been shown to influence bone health. It might seem intuitive that larger individuals would have bigger bones, however, larger can mean taller, heavier, or greater adiposity. Furthermore, the specific relationships between anthropometry and other facets of bone health, such as bone density and microarchitecture, may be less predictable. To date, several studies have investigated the associations between body mass index (BMI) and bone density and geometry as assessed by cross-sectional imaging.

Sukumar and colleagues recruited 211 women aged 25-71 years(239) and found that higher BMI was associated with greater distal tibial trabecular vBMD and midshaft cortical area and SSI as assessed by pQCT. Interestingly, a negative association was shown between BMI and midshaft cortical vBMD(239). Similar results were found in both the radius and tibia in a much younger cohort of 427 children aged 7-10 years(240). The principal difference was that no associations were identified between BMI and cortical vBMD at either site. Additionally, total area was determined distally and in the midshaft and found to be positively associated with BMI.

To look more closely at bone microstructure, Hoy and colleagues carried out an analogous study using radial and tibial HRpQCT as the outcome in both men and women after stratification by BMI(241). A normal BMI is defined as between 18.5 and 25kg/m^2 and those with a BMI between 25-30 and 30-35kg/m^2 are defined as overweight and obese respectively. Therefore, Hoy and colleagues grouped 103 men and 85 women (mean age 17 and 18 years respectively) into those with a BMI over 25kg/m^2 and those below that threshold.

Overweight men had higher trabecular vBMD and number than healthy weight men in both the distal radius and tibia. There was no evidence of a difference in total area or trabecular thickness. Cortical parameters were not assessed. A different pattern was found in women. Overweight women had higher total area in the upper and lower limbs, and greater trabecular number in the radius(241). It appears therefore that relationships may be sex specific.

Few studies have assessed adult height as a determinant of bone microarchitecture, as assessed by HRpQCT. In premenopausal and postmenopausal women, in the radius and tibia taller women had larger total area; cortical and trabecular density were higher; and trabecular number was greater(121).

Weight has been studied in a similar way to height and BMI. Boutroy and colleagues showed that in the radius, weight was associated with total area and there was a weak negative association with trabecular thickness. In the tibia, weight had several effects. Again, with greater weight total area was higher but additionally cortical thickness and cortical density were lower. Trabecular number was greater and trabecular thickness was lower. There was no significant association between weight and trabecular density. Vico *et al* found that in both the radius and tibia of postmenopausal women those with greater weight had a larger total bone area, higher trabecular density and higher trabecular number ($p<0.01$)(172). There was no relationship between weight and cortical thickness or density identified.

One recent longitudinal study assessed growth velocity (height and weight) in relation to bone outcomes (DXA and pQCT) and showed that pre- and post-

pubertal height and weight velocities were both associated with greater bone size and strength. In adulthood, weight gain velocity was positively associated with total and trabecular vBMD(242).

In summary, studies utilising both pQCT and HRpQCT techniques have tended to show BMI associated with greater distal total area and trabecular density in both the radius and tibia. This was found in conjunction with greater trabecular number in men. The midshaft can only be assessed by pQCT scanners. In these studies, cortical area and bone strength were higher with greater BMI whereas cortical density shows a negative relationship or none at all. Greater height has been associated with larger total area, cortical density, and trabecular density and number. Relationships shown between weight and bone microarchitecture appear to be driven partly by associations with height and in part by BMI. Weight gain in adulthood, appears to be associated with higher trabecular, but not cortical, bone density.

4.9 Summary

Peak bone mass differs in young men and women mainly due to differences in bone size rather than overall bone tissue density. When assessed in more detail, in younger adults, trabecular density tends to be higher in men but cortical density is similar or higher in women. The higher trabecular density in men is accompanied by higher trabecular number or thickness depending on the cohort studied. Total and trabecular area are invariably higher in men than women throughout adult life; differences in cortical area and thickness are more pronounced in older age.

Older individuals have been shown to have greater bone CSA with lower cortical thickness. The latter is particularly marked in women. Both cortical and trabecular density reduce with age, again more rapidly in women than men. In general, trabecular architecture shows more pronounced deterioration in women although there is some evidence that trabecular thickness may reduce more rapidly in men. Over adult life as a whole, cortical porosity tends to be higher in men. However, it appears to also increase less rapidly with age in men and this may explain the attenuation of gender differences in more elderly individuals.

Several lifestyle factors have shown associations with bone health although these are often inconsistent. The most reliable relationships appear to be shown for physical activity although these vary depending on the type of exercise undertaken. Further research is required to delineate the effects of calcium intake, smoking, alcohol consumption and social class.

In general, taller individuals have bones with greater total area, cortical and trabecular density, and trabecular number. Weight in postmenopausal women has been consistently associated with total area and trabecular number but there are several points of inconsistency. The most consistent relationships for BMI tend to be positive associations with cortical area and trabecular density. In some studies positive relationships with total area and negative associations with cortical density are also demonstrated.

Although BMI is a widely used measure and does appear to be associated with bone structure, it does not differentiate between muscle and fat mass. As these may have differing influences on bone microarchitecture, their effects will be explored separately in the next chapter.

4.10 Areas of Uncertainty

Although relationships have been investigated between bone microarchitecture and both age and gender, these studies have looked at general trends across most of adult life. We know, however, that changes in bone health with age are not linear. A more detailed assessment of these associations in an older population is therefore likely to provide additional useful information.

A few studies have examined the relationships between adult anthropometry and bone microarchitecture. However, there are many areas of inconsistency between studies. This is particularly true for relationships with weight. Further research is therefore required to determine how the variation in study results can be explained.

4.11 Questions to be addressed

1. What are the relationships between age, sex, and bone microarchitecture in a cohort of older men and women?

2. What are the relationships between lifestyle factors and bone microarchitecture in older men and women?

3. What are the relationships between adult anthropometry (height, weight and BMI) and bone microarchitecture in older adults?

5. Body composition and bone

5.1 Clinical relevance of fat and muscle in body composition

There is a growing appreciation of the relationship between bone metabolism and body composition. Bone itself constitutes a relatively small proportion of the connective tissue within the body. The main component is soft tissue which is made up chiefly of skeletal muscle and adipose tissue (fat). These are of particular clinical importance at present due to the changes that have occurred in the body composition of our population over the last several decades.

Firstly, levels of obesity are rising and have been doing so at a rapid rate(8, 9). As a result the WHO has declared it as one of the top 10 risk conditions in the world and one of the top 5 in industrialised countries. Obesity increases the likelihood of various diseases including ischaemic heart disease, type 2 diabetes, obstructive sleep apnoea, certain types of cancer, and osteoarthritis(243).

Secondly, evidence also suggests that anorexia nervosa, an important cause of low BMI in the developed world, is increasing in prevalence in the UK(244). Low BMI is an established risk factor for osteoporosis and osteoporotic fractures including hip, vertebral, and wrist(245, 246). This effect tends to be more marked at lower BMIs(245). Although greater BMI may have a positive effect on fracture risk in thinner individuals, there is evidence that fracture rates might be higher in those with the highest BMIs particularly at regions such as the ankle, pelvis and ribs(246).

Lastly is the issue of sarcopenia. This condition is defined as the age-related loss of muscle mass and function(247). It is associated with not only disability and morbidity but also increased mortality rates(248-252). Given the demographic shifts towards greater life expectancy and subsequently greater numbers of older individuals, sarcopenia constitutes a major health and social care burden both now and in the future.

Potential relationships between sarcopenia and osteoporosis are two-fold. Firstly, there is evidence accruing for a direct relationship between both muscle size and strength, and bone macrostructure(7). Secondly, sarcopenia can increase the risk of falls which may result in injury or fracture (253). These are therefore both mechanisms by which sarcopenia could increase fracture risk. The interrelationship between muscle and bone is a topic of much interest and a potential area in which we might successfully intervene clinically(254).

5.2 Assessment of body composition with DXA

It is now possible to estimate the amounts of lean and fat tissues within an individual using DXA making it easier to investigate these components. The differential attenuation of the two X-ray energies is used to estimate soft tissue composition as well as BMC. As only two energies are used, only two tissue compartments can be assessed in any one area. Therefore, soft tissue measurements (i.e. fat and lean body mass) can only be made in regions where no bone is present. Potential errors resulting from this issue have been investigated through cadaver studies and comparisons with three-dimensional imaging techniques(255). The estimations made are also based in part on assumptions; for example, that the hydration of fat-free mass remains constant at 73%. In reality, hydration can naturally vary between 67% and 85%, and can differ further in disease states. Furthermore, it is not possible to measure the amount of intramuscular fat. When interpreting DXA scans, it is therefore important to recognise the potential artefacts and disease processes that can influence the results. This will allow optimal interpretation.

DXA total body composition assessment provides data on total fat mass, lean mass, and BMC, and percent fat and lean mass. It can also provide values for individual parts of the body such as the android/gynoid region, arms, legs and trunk (Figure 5.1). The android region is particularly important as it can measure central, or abdominal, obesity which is important in health outcomes such as coronary artery disease. The understanding of the relative amounts of fat, lean mass and bone in different regions also allows for investigation of interrelationships between these tissues at a local level.

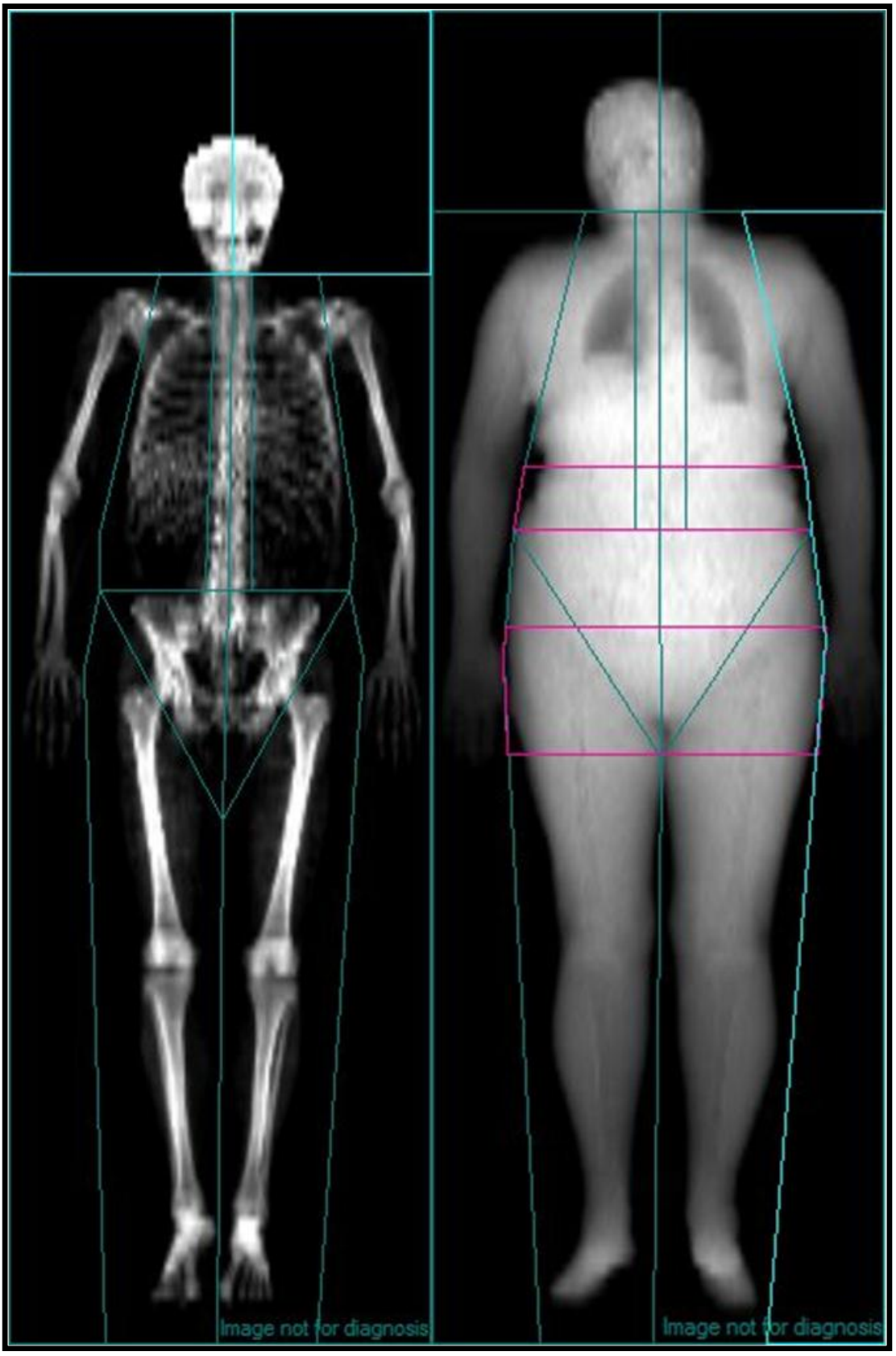

Figure 5.1: DXA body composition assessments illustrating segmentation

5.3 Lean mass, fat mass and bone mass over the lifecourse

Both lean mass and bone mass increase throughout childhood and into adulthood (Figure 5.2). In a study of over 3000 men and women aged 20 to 96 years, assessed by DXA, bone mass was found to peak between the ages of 20-29 years in the femur and 30-39 years in the spine and total body in women and between 20-29 years at all three sites in men(256). Lean mass peaked in the 5th decade and percentage fat in the 8th decade in both men and women(256).

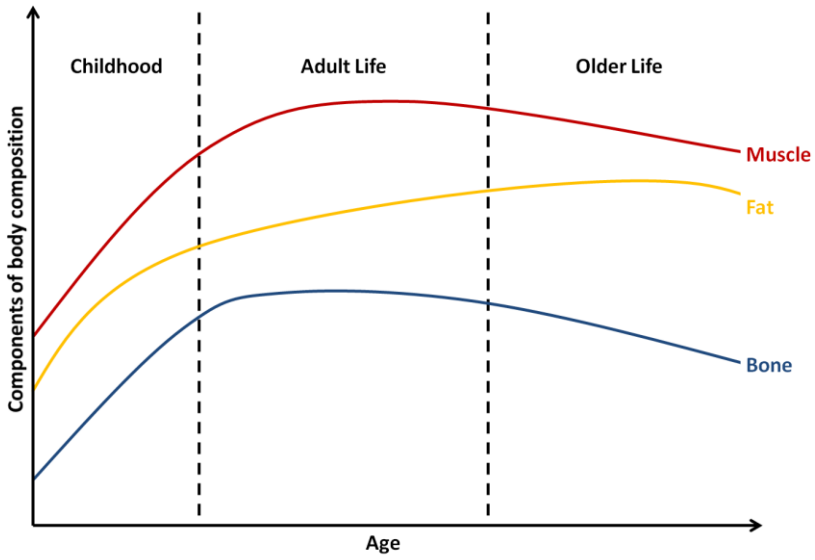

Figure 5.2: A lifecourse model for body composition

This is commensurate with another study of postmenopausal women aged 50-77 years which showed that fat mass remained stable across this age range but lean mass decreased significantly(257). Specifically, studies have estimated that muscle mass declines between the ages of 40 and 80 years by between 30 and 50%(258-260). There are clearly considerable differences in the changes in muscle, bone and fat throughout the lifecourse. How these different components relate to one another will now be discussed.

5.4 Muscle and fat: associations with DXA bone densitometry

5.4.1 Lean mass and fat mass (univariate)

In a study of children aged 8-18 years, bone quality, as assessed by ultrasonography using amplitude-dependent speed of sound (Ad-SoS) in the hand phalanges, was positively correlated with fat-free mass in both boys and girls(261). This was determined by subtracting fat mass, calculated from skin fold thickness at the triceps and subscapular regions(262), from weight. As fat-free mass includes bone mass this would strengthen any association with bone quality. Associations with fat mass were weaker and did not reach statistical significance in boys(261). In a study in which body composition was assessed using bioelectrical impedance, similar findings were identified(263). Bone quality was more strongly associated with fat-free mass than with fat mass and again the latter relationship did not reach statistical significance in boys(263).

It has been suggested that developmental changes in bone strength occur secondary to the increasing loads imposed by larger muscle forces and thus increases in muscle strength should precede the augmentation of bone strength(264). When this theory was investigated in boys and girls during puberty, it was indeed found that the peak in total body lean mass accrual did precede the peak in BMC accumulation by several months in both sexes(264). In a similar fashion, Ashby and colleagues assessed changes in BMC and lean mass between the ages of 5 and 18 years(265). They confirmed that in both sexes, as puberty proceeds, more BMC was accrued in relation to lean mass at multiple skeletal sites. Interestingly, females accrued more BMC in relation to lean mass, compared to males, at most skeletal sites. Similarly, Crabtree and Hogler both found that girls had significantly greater BMC per unit lean mass(266, 267). However, boys had greater lean mass to height ratio than girls(267).

In several adult studies, lean mass has been found to be associated with aBMD in the hip, spine, and total body independent of age group or sex(256, 268-274). The various components of muscle health were investigated further by

Kim *et al* in men and women over 65 years. They found that femoral neck aBMD was positively correlated with DXA-derived muscle mass, but also thigh extensor muscle strength, measured using an isokinetic device(275).

Several investigators have explored the relationships between fat mass and aBMD in adults. In one such study of twins and their related family members from the Healthy Twin Study, mean(SD) age 43.2(12.6) years, strong positive associations between fat mass and aBMD at several sites were identified(268). When postmenopausal Turkish women were examined, a similar positive association between fat mass and aBMD (lumbar spine and proximal femur) was also found(269). However, in a study of postmenopausal Finnish women, no relationship was found between fat mass and femoral neck aBMD(270) and in a Brazilian study of postmenopausal women, there was evidence of a relationships between fat mass and femoral neck aBMD but not lumbar spine aBMD(276).

Interestingly, in a Chinese cohort of younger and older adults, fat mass was significantly correlated with aBMD in postmenopausal women and older men only; and percentage fat negatively correlated with aBMD in younger people(256). It is of note here that higher percentage fat mass could be the result of two main factors; greater fat mass or lower muscle mass. Given the findings described above, we might suggest that the former would lead to greater aBMD whereas the latter might lead to lower aBMD. Consequently, we could hypothesise that those with higher percentage fat in the study above are those with predominantly lower muscle mass.

High bone mass represents an extreme phenotype of bone health and is defined as present in individuals with 1) both L1 Z-score of ≥ +3.2 and total hip Z-score of ≥ +1.2; or 2) both total hip Z-score ≥ +3.2 and L1 Z-score of ≥ +1.2. In a case-control study by Gregson *et al*, it was found that women with high bone mass had greater fat and lean mass than their controls(277). These differences remained after adjustment for age, gender, height, alcohol consumption, smoking status, malignancy, steroid use, physical activity, hypothyroidism, and menopausal status and oestrogen replacement use in women. These findings further corroborate the relationships between bone, muscle and fat.

In summary, lean mass in both boys and girls appears to correlate well with bone health. Conversely, associations between fat mass and bone appear weaker, particularly in boys. In adults, it can be clearly seen that lean mass is invariably correlated with aBMD but relationships between fat mass and bone are less consistent and may differ depending on the age of the cohort studied.

5.4.2 Lean mass and fat mass (mutually adjusted)

When attempting to determine whether lean mass or fat mass has the predominant influence on bone health, some investigators have used both variables within the same analyses. For example, in stepwise regression models, which included both fat mass and lean mass, lean mass maintained the strongest relationships with aBMD (hip, spine, and total body) in both men and women(256). Similarly, in a study of 104 women across the lifecourse (37 young (23.3+/-2.6 years), 28 middle-age (49.2+/-5.4 years), and 39 older (68.3+/-6.4 years)), relationships were found between lean mass and both lumbar spine and femoral neck aBMD after adjustment for fat mass, physical activity, and hormonal status. However, no such associations were maintained between fat mass and aBMD at either site after adjustment for lean mass, physical activity, and hormonal status(278).

In an older Spanish cohort (65-92 years), relationships between lean mass and aBMD were shown in both men and women, and these were maintained after adjustment for covariates including fat mass(271). In contrast, fat mass was only associated with aBMD in women and completely attenuated by adjustment for covariates including lean mass(271).

Other investigators have identified sex-specific relationships. In a study of older Austrian men and women (60–92 years), multiple regression models that included age, height, fat mass, and total skeletal muscle mass showed that aBMD was independently associated with fat mass in women but muscle mass in men(279). When 1448 postmenopausal Thai women were studied, and both lean mass and fat mass were included in the same model with age and height, both showed positive associations with lumbar spine, femoral neck, and total femur aBMD but not whole body aBMD(272).

One of the possible explanations for the association between fat mass and bone health may be the increased force applied to weight bearing bones. Consequently, several investigators have tried to elicit the effect of fat mass above and beyond that due to body weight alone. In order to do this, the associations between fat mass, or percentage fat, and aBMD were adjusted for body weight. In all cases, negative relationships were then found(280-282). As body weight and fat mass are highly correlated, their inclusion in the same model significantly affects its validity and thus these results must be interpreted with caution(283, 284).

A different approach was adopted by investigators in the fourth Korean National Health and Nutrition Examination Survey of 2008-2009 who studied 3945 adult men(274). Exploring the relationship between fat mass and aBMD (whole body, femoral neck, and lumbar spine) they identified consistent positive associations that were maintained after adjustment for age, height, smoking status, alcohol consumption, physical activity, income, education, calcium intake, and serum vitamin D concentration(274). Then, in order to take account of the weight-bearing effects of fat, they stratified by weight using 10kg bands and, similarly to those investigators that adjusted for body weight, found a negative association between fat mass and aBMD(274). They suggested several mechanisms by which fat mass might have detrimental effects on bone but there is another possible explanation. In individuals of a given weight, if fat mass is higher than lean mass must be lower, if the latter has a stronger effect on bone that the former, this could also explain the study findings.

In summary, in the majority of cases, when lean mass and fat mass are mutually-adjusted, relationships are maintained between lean mass and aBMD. In some studies of older women, relationships may be maintained with fat mass instead of or, in addition to, lean mass. In analyses where fat mass is adjusted for body weight, a negative association is usually demonstrated with aBMD. As discussed, these results must be interpreted with caution(283, 284). When alternate methodologies are used, similar findings are elicited. This may suggest a negative metabolic effect on bone or that lean mass has the more dominant positive effect.

5.4.3 Lean mass and fat mass (longitudinal analyses)

So far, all associations described have been from cross-sectional analyses and thus it is difficult to determine the direction of causality. In order explore this issue further a few investigators have looked at relationships between body composition and bone health in longitudinal studies. One group assessed the importance of body composition to the rate of change of aBMD over the next year in post-menopausal women(285). They found that greater BMI and appendicular muscle mass were both associated with a less rapid decline in aBMD, and thus showed a possible protective effect. No such correlations were found with fat mass or percentage fat(285).

A similar study in older men (70-97 years) in Australia looked at change in hip aBMD over a 2.2 year follow up and related this to change in body composition over the same period. In a mutually adjusted model, loss of fat mass and lean mass both correlated with loss of hip aBMD; however, associations were stronger for changes in fat mass(273). In summary, few longitudinal studies exist but from those that have been completed, both lean and fat mass may be related to changes in bone health.

5.5 Muscle and fat: associations with CT bone structure

5.5.1 Muscle cross-sectional area

Relationships have been shown in several studies using pQCT between muscle size and both bone size and strength. Specifically, in children(286), young adults(287) and older adults(7) positive associations were identified between muscle size and total bone area in the tibia, both distally and in the midshaft, with similar trends seen in the radius(7, 288). In the analyses that utilised the HCS, these relationships were shown to remain robust after rigorous adjustment for potential confounding factors including age, anthropometry, social class, smoking status, alcohol consumption, calcium intake, physical activity, diabetes mellitus, and in women, time since menopause and use of hormone replacement therapy (HRT)(7). Furthermore, diaphyseal bone strength has also been found to increase in relation to muscle size at a range of ages and in both sexes(286, 288-290).

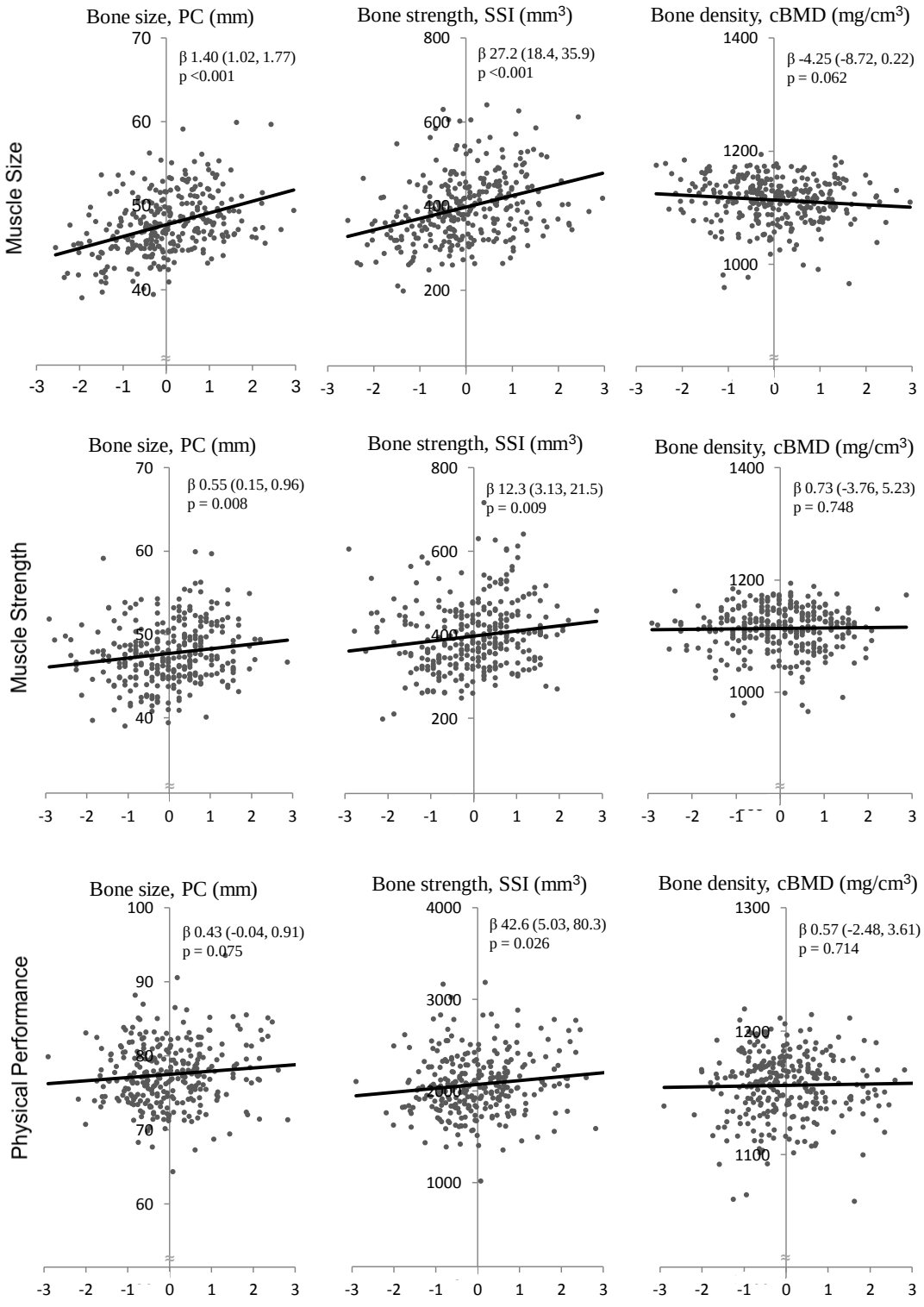

Figure 5.3: Scatter plots showing relationships between standardized forearm muscle size, muscle strength and physical performance, and bone size, bone strength, and volumetric bone density in men. Reproduced with permission of Edwards *et al.* Muscle size, strength, and physical performance and their associations with bone structure in the Hertfordshire Cohort Study(7).

When periosteal and endosteal circumferences were investigated individually, it was found that, in both the upper and lower limbs, greater muscle size was invariably associated with a higher periosteal circumference, a marker of larger bone CSA (Figure 5.3). Conversely, endosteal circumference tended to be lower after adjustment for periosteal circumference. Thus, it is not purely the greater total bone size that is driving the positive relationship between muscle size and cortical area(7). Relationships between muscle size and vBMD are less consistent. From the analyses of the HCS, no association was found between cortical vBMD and muscle size(7). This was consistent with the results of a similar assessment in pre- and early pubertal children in which no relationship was identified in either sex(286). In contrast, a positive univariate association was shown between trabecular vBMD and muscle size in the lower limbs of females in these two studies(7, 286). In both cases the relationships were completely attenuated by adjustment for a combination of anthropometric, demographic, and lifestyle covariates(7, 286).

In summary, larger muscle size is associated with greater bone CSA and strength with possible differential modulation at endosteal and periosteal surfaces. However, there is limited evidence of an association with vBMD once relevant covariates are taken into account. This is in keeping with animal studies that showed that bone preferentially adapts its strength to mechanical loading by increasing bone size rather than vBMD(291) although effects of exercise on trabecular vBMD, number and thickness have also been demonstrated(292).

5.5.2 Lean mass and fat mass (univariate)

In the Gothenburg Osteoporosis and Obesity Determinants (GOOD) study, a population of 1068 young men (mean age 18.9 years) was investigated. Bone structure was assessed by pQCT and lean and fat mass by DXA(293). In univariate analyses, both lean mass and fat mass were found to be associated with cortical area and thickness in the radius and tibia and trabecular vBMD in the tibia. Interestingly negative associations were identified between cortical vBMD and both lean mass and fat mass(293).

Pollock and colleagues found similar associations between lean mass and bone geometry in late adolescent girls (mean age 18.2 years)(294). However, in contrast, they identified positive associations between lean mass and tibial cortical density. Furthermore, in contrast to men in the GOOD study, there were positive associations between fat mass and both total area and SSI rather than cortical geometry(294). Farr and colleagues also assessed the lower limb bone structure of 396 girls, aged 8-13 years(295). Again, positive associations were identified between fat mass and tibial total area and SSI. In addition, there were positive associations with cortical and trabecular density. No assessment was made of cortical area or thickness(295).

Ng and colleagues investigated men and women separately ranging in age from 20-97 years and with distal radial microstructure as one of the outcomes of interest(296). They also stratified by age in men and menopausal status in women. Total fat mass was again derived from DXA body composition assessment. In post-menopausal women, total fat mass was positively associated with distal radial total area, cortical area and thickness, and trabecular density, number, and thickness. There was no evidence of an association with cortical density. In young men (20-49years), total fat mass was positively associated with total area, trabecular area, and trabecular number. Interestingly, it was negatively associated with cortical vBMD and trabecular thickness(296).

In summary, in univariate analyses, both lean mass and fat mass appear to be most consistently associated with total area, cortical area and thickness, and trabecular density. Relationships between soft tissue mass and total area may occur due to confounding by the individual's height. Associations between cortical density and both lean mass and fat mass are very inconsistent with both positive and negative associations shown. In the one study that examined trabecular architecture, fat mass was consistently related to trabecular number. Relationships with trabecular thickness were variable.

5.5.3 Lean mass and fat mass (mutually adjusted)

In an attempt to separate out the effects of lean mass and fat mass, Taes and colleagues included both variables in a mixed effects model, in addition to age

and height, with bone density and geometry as the outcomes(297). They found that lean mass maintained relationships with midshaft cortical area, cortical thickness, periosteal circumference and endosteal circumference. Radial trabecular area and density were also positively associated with lean mass. However, there was a negative relationship between lean mass and midshaft cortical density in both the radius and tibia(297). In contrast, fat mass tended to be negatively associated with cortical area, thickness, and bone size. There was no evidence of an association between fat mass and density measurements(297).

Farr and colleagues included both muscle CSA and fat mass in the same model, and found that relationships between muscle and bone were maintained whereas relationships with fat mass were completely attenuated(295). In a similar vein, Lorentzon and colleagues performed mutual adjustment in their study of fat mass and lean mass as predictors of bone structure(293). They found that significant associations remained between lean mass and radial periosteal circumference, endosteal circumference, cortical area and thickness, and trabecular density. However, all associations between fat mass and radial microstructure were entirely attenuated. In the tibia, relationships were maintained for both lean mass and fat mass with periosteal circumference, endosteal circumference, cortical area, and trabecular density although stronger for lean mass. A negative association was identified between fat mass and cortical bone density.

In summary, after adjustment for fat mass, strong consistent associations were maintained between lean mass and bone size, cortical area, cortical thickness, and trabecular density. Relationships between lean mass and cortical density again varied by study. After adjustment for lean mass, relationships between fat mass and bone size, cortical area and thickness, and trabecular density were either significantly attenuated, completely lost or, in one case, became inverse associations. No studies were available assessing relationships with trabecular microarchitecture in this way.

5.6 Summary

DXA studies have shown us that, in both children and adults, lean mass correlates more strongly than fat mass with bone health. When cross-sectional imaging is employed, we have found that larger muscle CSA is associated with greater bone size and strength but less consistently with bone density. In univariate analyses, both lean mass and fat mass are consistently associated with total area, cortical area and thickness, and trabecular density. It is possible that relationships between total area and both lean mass and fat mass may occur partly through confounding by height. Relationships between cortical density and both lean mass and fat mass are very inconsistent. In one study examining trabecular architecture, fat mass was found to be related to trabecular number whereas associations with trabecular thickness varied by the age and sex of the study group.

Bivariate models have been used to try to separate out the effects of lean mass and fat mass as the two are correlated. After adjustment for fat mass, strong consistent associations have been shown to be maintained between lean mass and bone size, cortical area, cortical thickness, and trabecular density. Conversely, when fat mass was adjusted for lean mass, relationships between fat mass and bone size, cortical area and thickness, and trabecular density were either significantly attenuated or, in some cases, negative associations were seen. This is consistent with the results of DXA studies. No evidence was available assessing potential relationships with trabecular microarchitecture in this way. As fat mass is highly correlated with body weight care must be taken when interpreting the results of studies that include both in the same model(283, 284). In general, studies that have performed this analysis have shown fat mass to be negatively associated with bone health.

5.7 Areas of uncertainty

Although studies exist which describe the associations between lean mass, fat mass and bone health as assessed by DXA or pQCT, there are very few that look at associations between body composition and bone microstructure as assessed by HRpQCT. In particular, no studies are available that address the associations between lean mass and bone microarchitecture or any body

composition measure with cortical porosity specifically. There is consequently no evidence regarding the independent relationships between components of body composition and trabecular architecture. Furthermore, in the majority of studies, total lean mass and fat mass are used without taking into account their correlations with height. Therefore, there is currently a lack of evidence of the importance of body composition independent of height.

5.8 Questions to be addressed

1. What are the associations between lean mass and fat mass, and bone microstructure?

2. Are these relationships maintained after adjustment for lifestyle and demographic factors?

6. Early life influences on bone health

6.1 The lifecourse approach

Recognition is growing that extrinsic factors acting at multiple points during life can significantly influence an individual's health outcomes. Specifically it has been shown that the phenotypic characteristics of an organism, including propensity to disease, can start to be modified as early as conception. It is largely factors that affect development during critical periods in early life that permanently determine the structure and function of the body's tissues, organs and systems. This is known as programming and relies on the principle of developmental plasticity(11).

6.2 Developmental plasticity

Developmental plasticity is defined as the ability of one genotype to give rise to a range of different physiological or morphological states in response to the prevailing environmental conditions during development. One of the earliest and simplest examples of this is Walton and Hammond's study of Shetland and Shire horses(298). Reciprocal crosses were made between small Shetland ponies and large Shire horses using artificial insemination. Although foals from the Shire mare were genetically similar to those from the Shetland mare, maternal regulation of foetal growth was marked and at birth the foals were proportional in weight to their mothers and not their fathers. It is therefore the environment during foetal life that is controlling the offspring size.

Another example of developmental plasticity is found in the meadow vole (*microtus pennsylvanicus*). The thickness of the offspring's coat at birth is determined by the photoperiod experienced by the mother; thus when days are shorter the coat grows thicker and when days are longer the coat becomes thinner. Again it is the environmental conditions that determine this characteristic in the offspring.

The benefit of programming from an evolutionary stand point is that it gives the organism the potential to alter its phenotype over one generation.

Environmental conditions, such as food security, can change over a relatively short period of time which may be inadequate to allow adaptation through natural selection with a resultant genomic shift.

It is during periods of rapid growth that organisms are most predisposed to changes due to programming. In humans, the highest growth rates within the fetal period occur at around 2-months gestation. Thereafter growth gradually slows and continues to do so into childhood. In adulthood plasticity is reduced and functional capacity becomes more fixed. Research into programming is therefore focused on early life.

6.3 Early growth and childhood bone health

Several studies have investigated the effects of growth in early life on bone health in children. For example, the Southampton Women's Survey (SWS) investigated the relative importance of growth rates at various time points in intrauterine and early postnatal life(299). They showed that conditional growth in abdominal circumference *in utero*, in particular in late pregnancy, had positive effects on both bone area and BMC at birth. Interestingly, when the same bone parameters were assessed at 4 years, it was found that growth in the first two years of life had a greater positive effect on BMC than growth between years 2 and 4.

Shortly after birth, height and weight begin to track along centiles. In the absence of disease states, this tends to remain set and controlled by homeostatic mechanisms. Longitudinal studies have shown that this also applies to bone traits in children across the pubertal growth spurt(300). This provides evidence that effects occurring *in utero* and early postnatal life are likely to persist into adulthood.

6.4 Early growth and adult bone health

Some of the earliest epidemiological evidence that adult bone health might be programmed early in life came from a cohort study of 153 women born in Bath, Somerset, between 1968 and 1969(301). The women were traced at the

age of 21 years and assessed by DXA at the femoral neck and lumbar spine. Weight at one year was associated with higher BMC, but not aBMD, at both sites scanned, independent of adult weight and BMI. This association was replicated in an older cohort (60-75 years) of 439 men and women who were born in Hertfordshire(302).

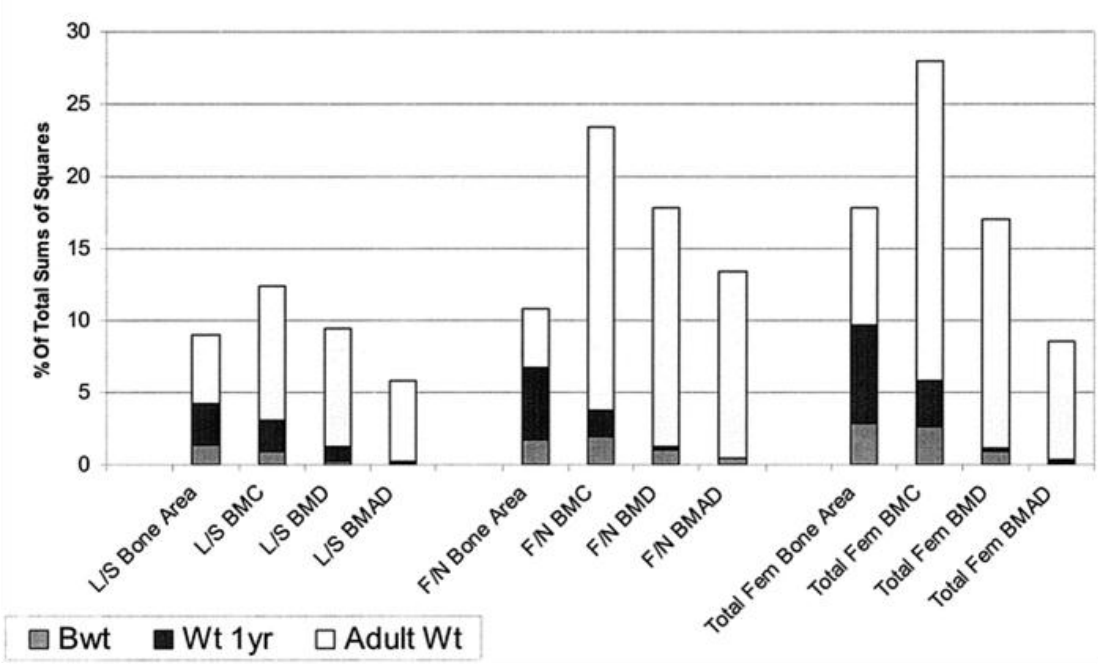

Figure 6.1: Partitioning of effects of weight throughout the life course on bone mass in Hertfordshire men. Reproduced with permission from Dennison *et al.* Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study(303).

More recently, a cohort of 966 patients from East Hertfordshire was used to investigate the effects of prenatal and postnatal life on bone health in older age(303). Those with greater birth weight, had a higher BMC; this relationship was seen in both men (proximal femur: $r = 0.16$, $p = 0.0003$; lumbar spine: $r = 0.10$, $p = 0.03$) and women (proximal femur: $r = 0.16$, $p = 0.0008$; lumbar spine: $r = 0.11$, $p = 0.03$). Similar trends were found with aBMD but were only significant in men at the proximal femur. Associations between weight at 1 year and BMC were stronger (proximal femur: men $r = 0.22$, $p < 0.0001$; women $r = 0.14$, $p = 0.002$). In men, 2.8, 6.8, and 8.2%, of the variance in proximal femur bone area was explained by birth weight, weight at 1 year, and adult weight respectively (Figure 6.1). In women, birth weight explained 6.7%

of the variance with smaller contributions for weight at 1 year and adult weight. These results imply that early life influences are likely to have a larger effect on bone size rather than density. Studies from Scandinavia and the United States have found similar associations(304, 305).

In order to investigate the role of genotype in the association between early growth and bone health, Antoniades and colleagues carried out lumbar spine, hip and radius DXA on 4008 twins, mean age 47.5 years, with discordant birth weights(306). In the purely monozygotic twin cohort, BMC in the spine, total hip, femoral neck, and radius were higher in the twins with higher birth weight, after adjustment for adult height and weight. Higher birth weight was associated with higher aBMD in the spine, total hip and femoral neck but these associations were fully attenuated by adjustment for adult height and weight. These data confirm that the relationship between birth weight and adult bone health is not driven solely through a shared genotype and is instead likely to be related to the intrauterine environment.

A meta-analysis was carried out by Baird *et al* to assess the strength and magnitude of the association between birth weight and adult bone mass(12). A significant association was found between birth weight and BMC in both the lumbar spine and hip in adulthood; for every 1kg increase in birth weight, BMC increased by 1.49g and 1.41g respectively (Figure 6.2). Associations were stronger in women than in men. No significant association was identified between birth weight and aBMD at either the lumbar spine or hip. Three studies considered the association between birth weight and bone mass at the radius and ulna with conflicting results(304-306). As there were so few studies, no meta-analysis was attempted. Three studies also assessed the relationship between weight at 1 year and bone mass in the lumbar spine and hip in adulthood (301-303). In all three, there was a positive association with BMC. As with birth weight, evidence of a similar association with aBMD was not identified.

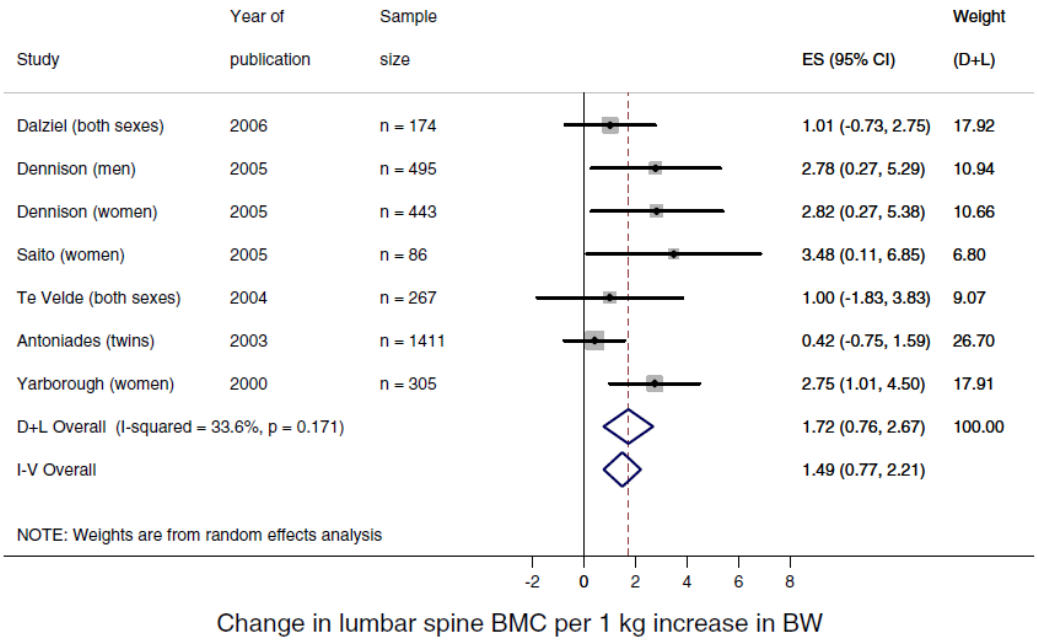

Figure 6.2: Forest plot of studies assessing the association between birthweight (kg) and BMC of the lumbar spine in adulthood. Reproduced with permission from Baird *et al.* Does birthweight predict bone mass in adulthood? A systematic review and meta-analysis(12).

Recently it is has been discovered that the association between early life and bone health is not homogeneous in all individuals as there is a significant interaction with genotype. In particular, one study utilising the HCS has shown that the relationship between lumbar spine aBMD and VDR genotype varies according to birth weight. Among individuals in the lower third of birth weight, spine aBMD was higher (p=0.01) in individuals of genotype "BB" after adjustment for age, sex and weight at baseline. In contrast, spine aBMD was reduced (p=0.04) in individuals of the same genotype who were in the highest third of the birth weight distribution(307). Thus the relationship between genotype and phenotype is not linear and can be significantly affected by other factors.

Oliver and colleagues investigated the effect of birth weight and conditional weight at 1 year on radial and tibial structure determined by pQCT in the HCS(308). The mean age of the population at the time of scanning was 69.2 and 69.5 years in men and women respectively. They found that birth weight was associated with radial and tibial total bone area in both men and women and that this relationship was maintained after adjustment for age, BMI,

smoking, alcohol consumption, physical activity, dietary calcium intake, social class, and in women, HRT use and time since menopause. Radial cortical area and estimates of bone strength also tended to be higher in men with greater birth weight. Interestingly, trabecular density tended to be lower in those with higher birth weight although this only reached statistical significance in the radius in women and the tibia in men after adjustment as described previously. There was a tendency towards a positive association between conditional weight at 1 year and total bone area in the radius and tibia; however in both cases this only reached statistical significance in men. There was also a trend towards higher bone strength estimates in both men and women with greater conditional weight at 1 year. Similar associations between birth weight and radial bone total area and strength in men and women age 60-64 years were also found in the National Survey for Health and Development (NSHD)(242).

Femoral geometry is an independent predictor of hip strength and fracture risk. Javaid and colleagues derived this from DXA scans of the proximal femur, using Hip Structure Analysis software, in a group of 333 men and women (age 60-75 years) in whom records of early life were available(309). They found associations between weight at 1 year and intertrochanteric (IT) CSMI, IT width, IT buckling ratio, femoral neck CSMI and femoral neck width reaching or approaching statistical significance in both men and women. The associations with IT width and IT buckling ratio remained after adjustment for lifestyle factors, height and weight, whereas the association with IT CSMI did not. Similar but weaker unadjusted trends were seen for birth weight. However, with the exception of IT width, these only reached statistical significance in women. Buckling ratio was not associated with birth weight in men or women.

To investigate possible explanations for these associations, hormonal factors have been explored. Results from several cohorts have shown that birth weight and weight in infancy both predicted growth hormone(310) and cortisol(311) levels in late adult life. These hormones are known to be skeletally active and studies have confirmed that they are predictors of the rate of bone loss(312). It is therefore possible that one of the mechanisms by which adverse early life factors influence bone health in adult life is by programming an unfavourable endocrine profile which later leads to an accelerated deterioration in bone health.

6.5 Summary

Developmental plasticity provides an organism with the ability to alter their phenotype depending on prevailing environmental conditions during development. These may be favourable or adverse, with one example of an adverse environment being suboptimal nutrition. Current evidence suggests that there is a link between early life events and bone health in adulthood. This has been shown using DXA, pQCT, and hip structural analysis. With DXA higher birth weight was associated with higher BMC; with pQCT associations were found with radial cortical area and bone strength; and with hip structural analysis early life was associated with femoral geometry. In all of these cases, it appears that bone size has an important role. As bone size has been linked to bone strength, early life may also have an effect on fracture risk.

6.6 Areas of uncertainty

To date, no studies in humans have investigated the effect of early life influences on bone microarchitecture as assessed by HRpQCT. The data collected as part of this doctorate will considerably advance current knowledge as this is one of the main foci of this thesis.

As discussed, a positive relationship between growth in early life and bone size may explain many, if not all, of the associations described above. At present there is little evidence as to whether early growth might influence bone structure independently of bone size.

6.7 Questions to be addressed

1. What are the relationships between growth in early life, as assessed by birth weight and conditional growth in the first year, and adult bone microarchitecture?

2. Specifically, are there associations between growth in early life and adult bone structure above and beyond bone size?

7. Relationships between bone microarchitecture and fracture

7.1 Introduction

The thesis so far has focussed on the understanding of those factors that may determine bone microarchitecture including age, sex, lifestyle, early life and body composition. In relation to metabolic bone diseases, such as osteoporosis, the clinical importance of these associations would be largely dependent on potential relationships between bone microarchitecture and fracture risk. At present, these relationships have not been fully delineated. This chapter will focus on imaging modalities that have shown associations or predictive capacity in this regard.

7.2 Bone densitometry and fracture

The importance of low aBMD in the estimation of fracture risk has been established for many years. In 1996, Marshall and colleagues published a meta-analysis in the *British Medical Journal* describing how assessments of aBMD predicted the occurrence of osteoporotic fractures(13). In general, they found that all measurement sites had similar predictive abilities with relative risks (RR) of around 1.5 for every 1 SD below the age-adjusted mean. The exceptions were stronger relationships between spine aBMD and vertebral fractures (RR 1.9-2.8), and hip aBMD and hip fractures (RR 2.0-3.5).

Although aBMD is an important predictor of fracture, approximately half of all postmenopausal fractures occur in women with aBMD values above the WHO threshold for osteoporosis(2, 3). Consequently, there is considerable interest in novel methods of assessment of bone health that might improve fracture prediction, such as HRpQCT.

7.3 HRpQCT and fracture

Several studies have investigated the differences in bone microarchitecture by fracture status in postmenopausal women. In case-control studies that have compared those with any postmenopausal fracture and those without, results have been fairly consistent. In terms of cortical parameters, radial and tibial cortical thickness were lower in those with a fracture(313-315). Cortical density also tended to be lower in this group but the trend did not always reach statistical significance (121, 313). Cortical porosity has not been found to differ by fracture status(316, 317).

When trabecular parameters were assessed, trabecular density and thickness were invariably lower in those with a prevalent fracture(121, 313-315). Interestingly, trabecular number tended to be lower in the radius but not the tibia(121, 313, 315). Total cross-sectional bone area in the radius and tibia did not differ between women with and without a fracture(121, 313, 314).

Differences have also been assessed in the radius between those individuals with and without a prevalent wrist fracture. Results have been very similar to the findings for any fracture, with those that have suffered an event having lower cortical thickness and density; lower trabecular density, number and thickness; and no difference in total cross-sectional area(172, 315, 317, 318). This was also the outcome when individuals were stratified by vertebral fracture severity(319).

Recently, new image analysis methods have been developed to provide further detail regarding the nature of the trabeculae. Local topological analysis and individual trabecula segmentation methods allow differentiation of rods, plates, and nodes(320, 321). The proportion of trabecular bone in the form of plates was higher in those without fracture and, as one might expect, the proportion that was in the form of rods showed the opposite relationship. Consequently, having a greater ratio of rods to plates is associated with a greater likelihood of prevalent fracture(320, 321).

FEA is a technique that involves computationally modelling individual scans to estimate bone mechanical competence (e.g. stiffness and failure load)(121). This requires an additional software package that is not available as standard

on all HRpQCT scanners. Radial and tibial estimated failure load have been found to be significantly lower in women with a prior fracture(314). Significant differences have also been shown in estimated stiffness to uniaxial compression and sheer stress in both the radius and tibia, with relationships being stronger in the former(313). Similar disparities were noted in those postmenopausal women with and without wrist fractures(317, 318).

As discussed, in routine practice the imaging modality of choice for assessing fracture risk is aBMD by DXA. Thus several investigators have attempted to determine whether HRpQCT differences by fracture status are independent of aBMD, and therefore might potentially add to fracture risk prediction. In the radius, analyses have been adjusted for aBMD at the ultradistal radius. Although most relationships are maintained, the difference in trabecular and cortical thickness by fracture status are no longer significant(313, 315). For analyses in the tibia, aBMD at the total hip has been the chosen covariate. Adjustment for this more distant site has not materially affected the study findings(313, 315). When vertebral fractures were assessed, differences in radial microarchitecture were adjusted for lumbar spine aBMD. This did not result in any significant relationships being lost(319).

HRpQCT produces many outcome variables and, as described, several of these have been found to differ between women with and without fractures. Investigators have assessed how each parameter individually discriminates fracture status using ROC curve analyses. For conventional HRpQCT analysis variables, the areas under the curve (AUC) range from 0.55 to 0.67(313) and for FEA models 0.50 to 0.70(313, 320). In an attempt to improve discriminatory performance, researchers have used machine learning techniques, which learn from a dataset with a given output, and are then able to predict the output in previously unseen datasets. When such models were produced in a study using radial HRpQCT outcomes, including FEA, the AUC was 0.82 for wrist fractures(316).

All of the studies discussed above have been carried out in women. To date, only one study has assessed the differences in bone microarchitecture by fracture status in men(322). Ostertag and colleagues recruited men aged 40 to 70 years with idiopathic male osteoporosis; 31 had suffered a fracture after the

age of 40 year and 37 had not. Those with a fracture were older and had lower bone density at the femoral neck and total hip. In a similar pattern to women, those with fractures had lower cortical thickness and density. In contrast, with the exception of radial trabecular density, trabecular parameters did not differ between those men with and without prevalent fractures. Furthermore, those with fractures also had a higher total area in both the radius and tibia and this remained of borderline significance after adjustment for age, height and weight(322).

7.4 Summary

In summary, several studies have demonstrated significant differences in bone microarchitecture between postmenopausal women with and without a prevalent fracture. Furthermore, in these women the nature of the fracture studied (e.g. wrist, vertebral, or any) did not appear to have a substantial effect on the results. There is far less evidence available in men although from what has been undertaken there is a suggestion that there may be subtle differences in the pattern of findings.

In general, differences in microarchitecture by fracture status are maintained after adjustment for aBMD suggesting that HRpQCT may provide additional information to that obtained from DXA alone. However, when the covariate is assessed at the same site as bone microarchitecture there can be attenuation of differences in cortical thickness and trabecular thickness.

In an attempt to augment the information obtained from HRpQCT, investigators have developed new image analysis techniques, including FEA, local topological analysis, individual trabecula segmentation and have employed machine learning techniques. At present it is uncertain how much additional benefit these methods provide and whether they will significantly improve fracture prediction.

7.5 Areas of uncertainty

In addition to the role of novel image analysis and modelling techniques, it is currently uncertain whether men and women show the same relationships between bone microarchitecture and fracture status. More evidence is therefore required in studies of men, and preferably those including both men and women in order to allow direct comparisons.

7.6 Questions to be addressed

1. How does bone microarchitecture differ by fracture status in men and women?

2. Are differences in bone microarchitecture by fracture status robust to adjustment for aBMD in both men and women?

8. Aims and Objectives

8.1 Objectives

The overall objectives of this thesis are to identify the early life and adult lifestyle and anthropometric determinants of bone microarchitecture and to explore the relationships between bone microstructure and fracture.

8.2 Aims

The specific aims of the study are:

1. Describe the relationships between age, sex, and bone microarchitecture in a cohort of older men and women.

2. Examine the relationships between lifestyle factors and bone microarchitecture in older men and women.

3. Explore the relationships between adult anthropometry (height, weight and BMI) and bone microarchitecture.

4. Investigate the associations between lean mass and fat mass, and bone microstructure.

5. Determine whether relationships between body composition and bone microstructure are maintained after adjustment for lifestyle and demographic factors.

6. Describe the relationships between growth in early life, as assessed by birth weight and conditional growth in the first year, and adult bone microarchitecture.

7. Assess associations between early growth and adult bone structure above and beyond bone size.

8. Determine whether bone microarchitecture differs by fracture status in men and women.

9. Ascertain whether differences in bone microarchitecture by fracture status are robust to adjustment for aBMD in both men and women.

8.3 Study planning and choice of cohort

In order to address the above aims and objectives it was decided to recruit participants from the Hertfordshire Cohort Study (HCS). The main strengths of this cohort that made it suitable to answer these questions are as follows. Firstly, data were available from infancy, such as birth weight and weight at 1 year, and this information permitted the influence of early growth on adult bone health to be examined without the possibility of recall bias. Secondly, the cohort had been extensively phenotyped in terms of musculoskeletal health. Specifically, the dataset includes several covariates which may act as confounders or mediators of the types of associations studied. This allowed for their effects to be explored in the analyses. Thirdly, the HCS has been shown to be fairly representative of the UK population by comparison with the Health Survey for England (HSE) and consequently the results are likely to be generalisable to this population. Lastly, the majority of participants have remained involved in studies run by the MRC Lifecourse Epidemiology Unit over many years and were therefore felt to be a group that would be likely to engage with the current project.

9. Methods

9.1 Background of the Hertfordshire Cohort Study

The HCS was established to investigate whether growth *in utero* and in the first year of life influenced the risk of adult diseases. To achieve this aim in an appropriate timeframe, a cohort of older men and women was identified for whom records were available that detailed their early life. These records were then located through a search of the national archives. They had been written, during the early twentieth century, by Ethel Margaret Burnside (Hertfordshire's first 'chief health visitor and lady inspector of midwives') and her team of midwives and nurses. The group had been formed, in 1911, in response to concern about a physical deterioration in the British people (1). Their aim was to improve the health of children in Hertfordshire. Each time a midwife attended a child's birth she recorded their weight on a card. A health visitor subsequently recorded the child's illnesses and method of infant feeding, and weighed them again at 1 year of age. This information was transcribed into ledgers at the Hertfordshire county office (Figure 9.1). These ledgers describe all births up to 1948.

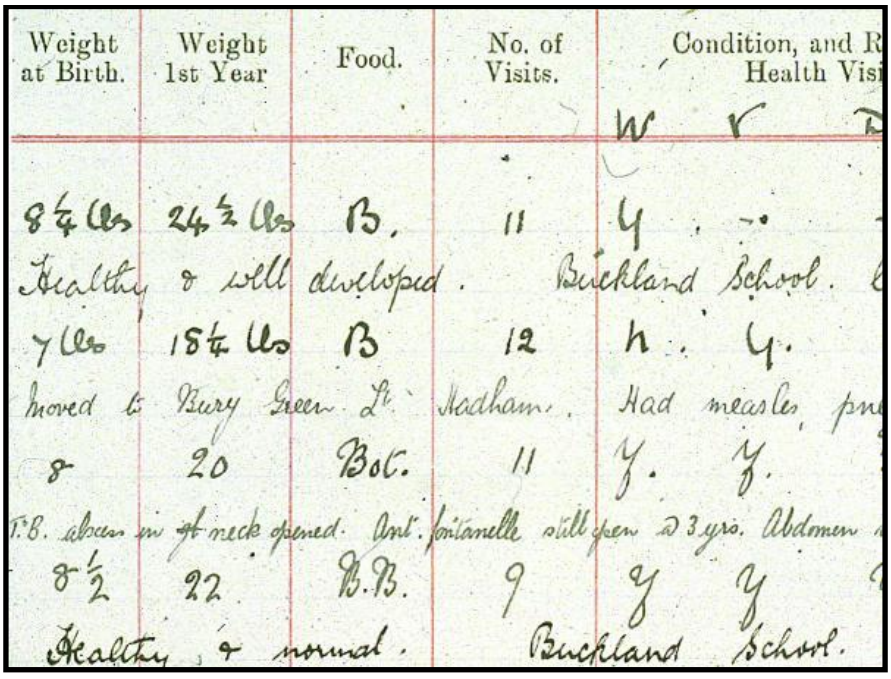

Figure 9.1: Extract from Hertfordshire Ledgers

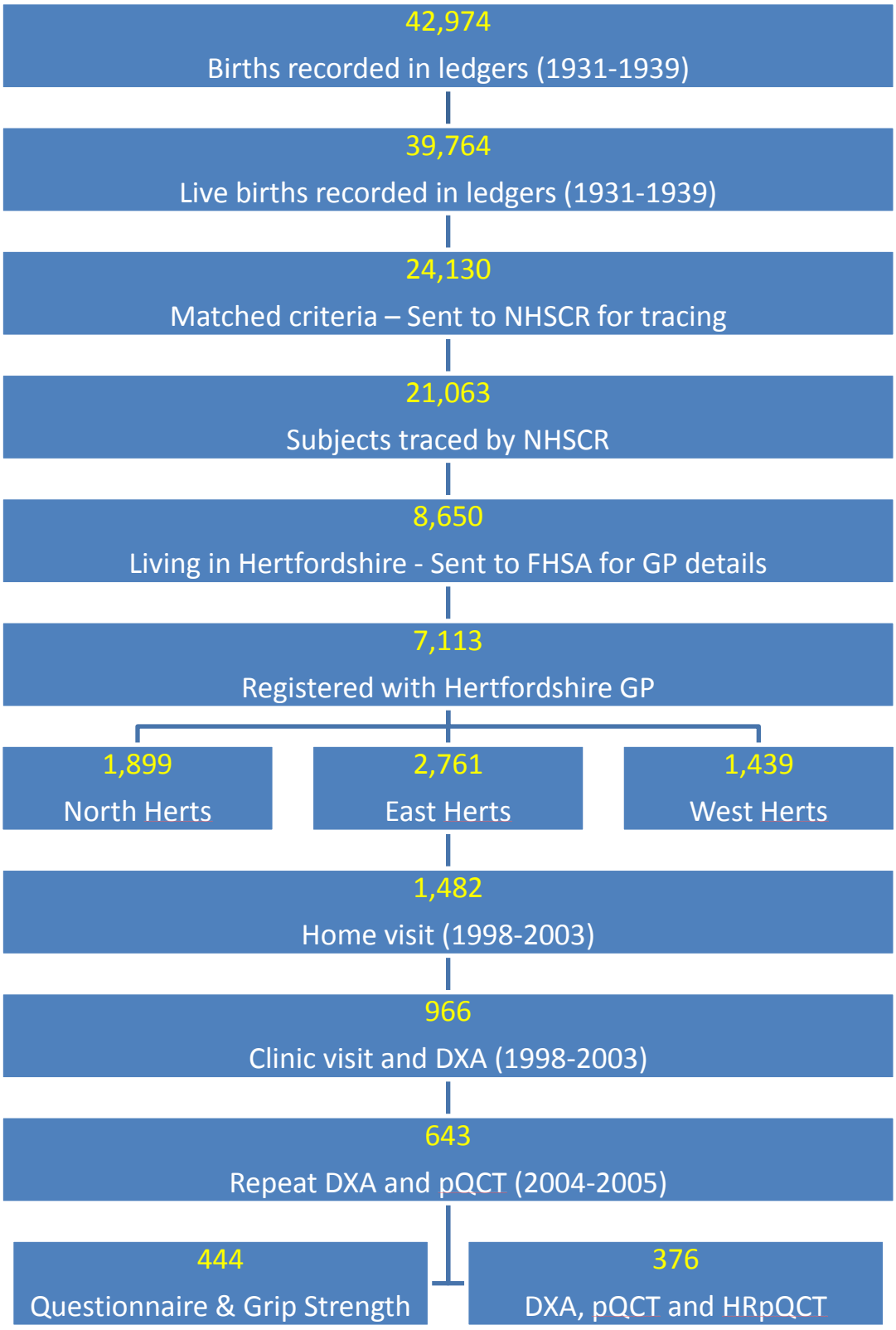

Figure 9.2: Sample selection within the Hertfordshire Cohort Study

9.2 Sample Selection

The selection of participants is illustrated in Figure 9.2. The HCS includes those individuals born between 1931 and 1939; in total there were 42,974 births recorded in this period and 39,764 were live births. Subjects whose birth weight or weight at 1 year was missing were not included. Multiple births and deaths during childhood were also excluded. Naturally, if there was insufficient tracing information available the participant could not be enrolled in the study.

In 1998, details of the remaining 24,130 men and women were sent to the National Health Service Central Registry in Southport for tracing. It was found that 8,650 individuals were still alive and living in Hertfordshire. Of those, the Hertfordshire Family Health Services Authority determined that 7,106 were registered with a Hertfordshire General Practitioner (GP). A letter was sent to each individual's GP and permission was given to contact 6,099 men and women; 3,225 (53%) agreed to enter the study and 2,997 also attended a baseline clinic. The representativeness of this group is assessed in section 10.4.1 and discussed in section 16.1.1.

The initial home interview included participants from throughout Hertfordshire and information was obtained on demographics, lifestyle, family history, reproductive health, quality of life, medication history and comorbidities. Specifically, physical activity, calcium intake, social class, and in women, age at menopause and HRT use were collected.

Physical activity was calculated as a standardised score ranging from 0-100 derived from frequency of gardening, housework, climbing stairs and carrying loads in a typical week. Higher scores indicated greater levels of activity(323). Dietary calcium intake was assessed using a food frequency questionnaire (324). Social class was determined by own current or most recent occupation of the participant in men and single women, and of the husband in ever-married women based on the OPCS Standard Occupational Classification scheme for occupation and social class. Time since menopause was calculated as the number of years since the reported time of menopause. HRT use was assessed as a binary outcome; those that had never used it and those that had (currently or previously).

Subsequently the cohort was divided into East, North and West. The small South Hertfordshire region was excluded because its boundaries changed over time with the potential to cause confusion. Of the 1,482 individuals from East Hertfordshire, who agreed to the above interview, 966 (68%) returned for bone densitometry using a Hologic QDR 1000 instrument (Lunar Prodigy Advanced, GE Medical Systems, UK) and bilateral AP and lateral knee x-rays.

In 2004-5, a subgroup of 643 attended a follow up clinic, where a detailed lifestyle questionnaire was administered, along with measurement of grip strength, repeat bone densitometry and a pQCT scan of the non-dominant radius and tibia using a Stratec 2000XL instrument (Stratec Medizintechnik GmbH, Pforzheim, Germany). The participant was asked which hand they wrote with and the alternate side was considered to be the non-dominant one. The questionnaire included information on smoking status and alcohol consumption. Smoking status was classified as never smoker, ex-smoker or current smoker. Alcohol consumption was assessed in units per week and this was subsequently categorised into groups.

Between 2005 and 2011, 51 people became ineligible for the study as they had either moved out of Hertfordshire or had died. In March 2011, the remaining 592 men and women were approached and 444 consented to a further survey, which included questions on osteoarthritis, social participation, mastery, healthcare utilization, physical function, falls and fractures. Grip strength was repeated along with a short physical performance battery(2).

9.3 Recruitment

By the start of recruitment to the current study, in October 2011, a further 21 individuals had become ineligible. Therefore, 571 men and women of the 592 described above were sent an invitation letter (Appendix 2). This was accompanied by a participant information sheet (Appendix 3) and a study conduct information leaflet (Appendix 4). The potential participants were asked to return a reply slip, in a prepaid envelope, indicating whether or not they wished to be included. The responses were logged and those who had stated a willingness to take part were telephoned. A mutually convenient time was arranged for them to attend the MRC Human Nutrition Research in

Cambridge and a taxi was booked to transport them to and from the unit. A total of 376 individuals agreed to take part in the study. How these individuals compared with those that attended the baseline clinic is assessed in section 10.4.1 and discussed in section 16.1.1.

9.4 Informed Consent

On arrival at the research unit, written informed consent was obtained. The researcher checked that the participants had read the information leaflets and addressed any further questions. The participants were then given the consent form to read, initial and sign (Appendix 5). A copy of the completed consent form was given to them.

9.5 Anthropometric measurements

All study measurements were performed in accordance with standard operating procedures. Height was measured with the subject standing using a wall-mounted SECA stadiometer (SECA Ltd, Hamburg, Germany). The subject removed their shoes and stood with their back to the wall. They were asked to stand as tall and straight as possible with their feet together and their arms held loosely at the side. Their shoulders were relaxed to avoid creating an excessive lordosis. The head was placed in the Frankfurt Plane, such that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye would be horizontal. The headpiece was rested gently on the participant's head and the height was measured off to the nearest 0.1cm.

Weight was measured using calibrated SECA 770 digital floor scales (SECA Ltd, Hamburg, Germany). These were placed on a level surface and then zeroed. Participants removed their shoes and any items of heavy clothing or jewellery before standing on the scales. Weight was measured to the nearest 0.1kg.

9.6 Dual energy x-ray absorptiometry (DXA)

9.6.1 Preparation

A Lunar Prodigy Advance densitometer (GE Medical Systems Lunar) was used. Before commencing the scan, the procedure was explained to the participant and they were updated on their progress at intervals throughout. Pillows were used as necessary to ensure participants were as comfortable as possible. They were asked to remove all metal jewellery, glasses and anything else that could significantly attenuate the x-ray beam. If their clothes contained metal fasteners then a dressing gown was provided for them to wear instead. They were asked to try to remain completely still and not to talk during the procedure.

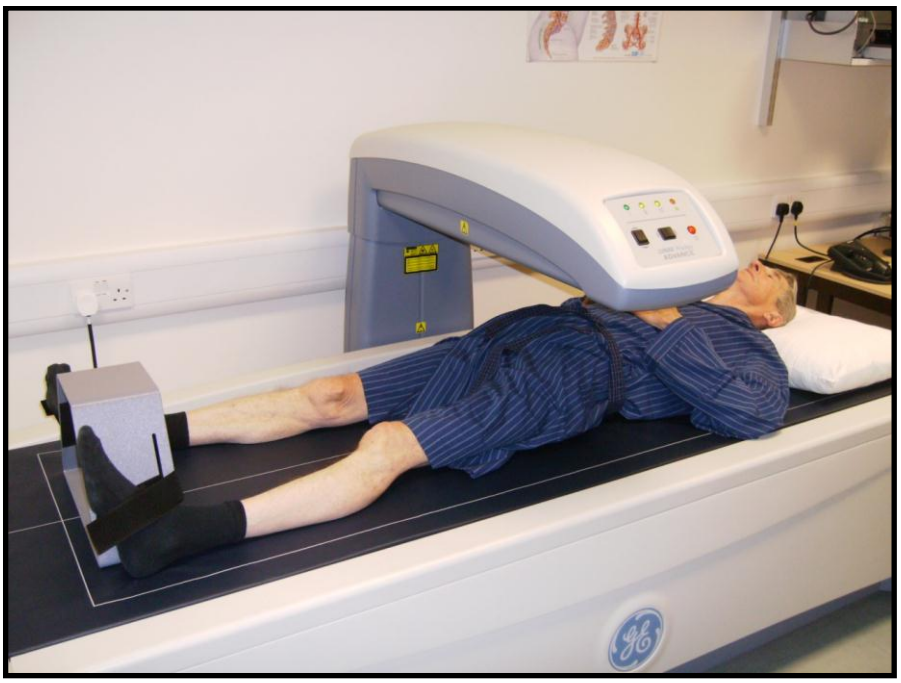

Figure 9.3: Dual Femur DXA Scan

9.6.2 Dual femur scan

The subject lay supine on the scanner table, in the centre of a white rectangle, with their head 3cm from the top. They were asked to internally rotate both hips causing their patellae to turn inwards. The legs were secured in this position by attaching the feet to a foot brace with Velcro straps (Figure 9.3). The participant's arms were placed across their chest to prevent them entering the scan field.

On initiation, the scanning arm directed an illuminated red cross at the left upper thigh of the participant. The position of this cross was then manually adjusted to rest 28-30cm below the subject's waist; the waist being defined as equidistant between the lower border of the ribs and the iliac crest, in the midaxillary line. The cross was then moved in a transverse plane until it was two thirds of the way from the lateral border of the thigh to the midline.

When the cross was appropriately positioned the scan was commenced. If there were not three clear sweeps before reaching the base of the pelvis/lesser trochanter, or at least 2 sweeps above the greater trochanter, then the scan was aborted and repeated. Once the first scan was complete, the arm was repositioned and the contralateral side was assessed using the same process. Imaging both hips took approximately 2 minutes. If the subject had a hip replacement, the involved side was not scanned.

Following the scan, image quality was assessed. Rotating the hips prior to initiation should have lead, in each case, to the lesser trochanter appearing as a small protuberance only. If the degree of rotation was not correct the scanner displayed a message which prompted the operator to repeat the image. The Region of Interest (ROI) was also assessed by the operator. The position of the neck box was reviewed and if necessary, it was moved to lay over the middle of the neck of femur, ideally with an equal amount of space in the box either side of the bone. The box was not permitted to overlie the trochanters or bones of the pelvis. The Hip Axis Length (HAL) line was checked and moved if incorrect.

9.6.3 Whole body scan

The participant remained lying supine on the bed within the white rectangle (Figure 9.4). The hips were rotated until the long axis of each foot was at right angles to the bed. This alignment was maintained by fitting a Velcro strap around both ankles. The participant's arms were by their sides and their hands were positioned with palms downwards. If this caused any part of their upper limbs to be outside of the white rectangle then their hands were held flat against their body. The body wrap was used if part of the subject remained outside the rectangle despite this change in position. The scan was

then initiated and took between 5 and 10 minutes to complete. The ankle straps were then removed.

Once the scan was completed, the operator checked that the scan mode used was appropriate for the average tissue thickness calculated by the scanner software and checked the image for anomalies such as the subject being outside of the scan field and artefacts. The latter included jewellery left on by mistake and showed up as areas of unusually high density. If any such error was seen, and it was possible to correct it, then the scan was repeated. If not (e.g. knee replacement), there was a standardised protocol in place to deal with the issue.

Figure 9.4: Whole body DXA scan

Firstly the problem was defined and then, using the protocol devised at the MRC Human Nutrition Research (Appendix 8), the data were altered to compensate for the issue, where possible, assuming that right and left sides were symmetrical in terms of body composition. The ROIs were then assessed. The scanner software automatically generated dividing lines on the image demarcating body regions (Figure 5.1). If the lines were incorrectly positioned they were manually adjusted. *In vivo* precision (%CV) for this machine has been determined for lean mass (1.87%) and fat mass (1.75%) by measuring 35 individuals twice on the same day with re-positioning.

9.6.4 Vertebral Fracture Assessment

The subject moved into the left lateral position. A backrest was placed behind them, parallel to the long axis of the scanner bed. They were then asked to ensure their shoulders and pelvis were against the backrest and this was checked by the operator. The hips and knees were flexed to 90 degrees with the knees and feet together. A cube-shaped positioning pad was placed behind the knees to maintain flexion and a wedge was placed between or under the knees to minimise spinal rotation and projectional error.

The iliac crest was located by the operator in the midaxillary line. The illuminated red cross was positioned 5cm inferior and 5cm posterior to this point. During the 5-minute scan, the operator ensured that the spine was in the centre of the screen, the pelvis appeared within the first 1-2 sweeps and the image ended above T4. If any of these did not occur the scan was terminated and repeated after appropriate adjustments had been made.

To analyse the images, a visual assessment was performed by two trained assessors using the Genant method(3) as described in section 3.2.2. This is a semi-quantitative technique, used to confirm the presence of a fracture and assess its severity by visual determination of the extent of vertebral height reduction and morphologic change. In particular, it allowed the differentiation of vertebral fractures from other non-fracture deformities. In cases when one reviewer felt there was a borderline fracture, the assessment of the other reviewer was taken. When there were other discrepancies, the assessment of the senior reviewer (MHE) was used. The Genant method has been shown to have good inter-rater agreement(325). This was also the case in the current study, as described in section 14.3.

9.6.5 Quality assurance and quality control

Quality assurance (QA) testing was performed on a daily basis using a block phantom. Quality control (QC) testing was performed on Monday and Friday of each week using the spine phantom.

9.7 High resolution peripheral quantitative computed tomography

9.7.1 Preparation

HRpQCT was performed using a Scanco Xtreme CT scanner (Scanco Medical AG, Bassersdorf, Switzerland). The height of the chair was adjusted to ensure the participant was made as comfortable as possible. The participants were asked not to talk during the scan and to try to remain still to prevent motion artefact. Clothing and jewellery that may have interfered with the scans were removed. The non-dominant limb was used, as determined at the time of pQCT scanning in 2004-2005, unless there was a contraindication (e.g. previous fracture). The participant had been asked which hand they wrote with and the alternate side was considered to be the non-dominant one. Prior to scanning, the instrument was precalibrated with the participant located a safe distance away. Precalibration allowed measurement of both the dark bias signal in the detector (x-ray shutter closed) and the reference intensity of the x-ray source with an empty field of view (x-ray shutter open). All measurements were taken following the manufacturer's protocols.

9.7.2 Tibial HRpQCT

The lower leg was placed within a carbon fibre cast. Appropriately-sized rubber moulds inside the cast held the foot comfortably in position and prevented inferior and lateral movement (Figure 9.5a). With the participant sitting in a chair, the cast was connected with the gantry, and slowly slid into the scanner and locked in place (Figure 9.6). The wheels of the chair were also locked to maintain stability.

A single dorsal-palmar projection image of the distal tibia was then acquired as a scout view and the reference line was set along the plateau found approximately half way along the tibial endplate (Figure 9.7a). The scan was commenced 22.5mm proximal to this point. The need to remain still was then reiterated before the 2.8 minute scan was commenced.

Figure 9.5: Casts for (a) tibial and (b) radial HRpQCT scans

Figure 9.6: Tibial HRpQCT scan

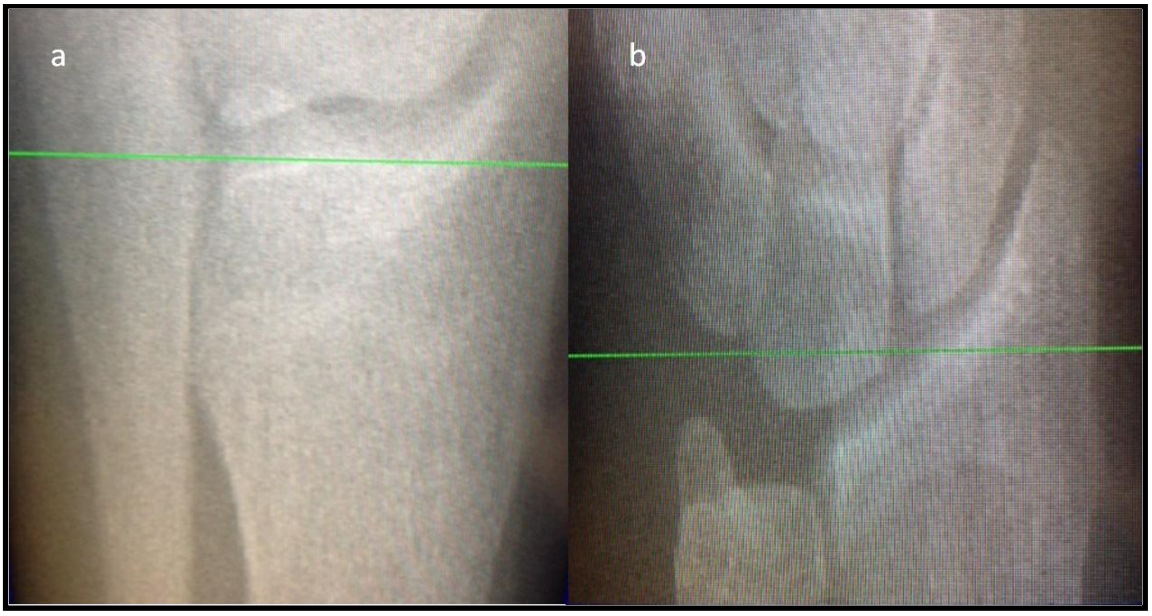

Figure 9.7: Scout views for (a) tibial and (b) radial HRpQCT scan

9.7.3 Radial HRpQCT

The arm to be scanned was positioned with the ulnar border of the forearm resting on the cast. The participant was asked to loosely grip a fixed rod within the cast (Figure 9.5b) ensuring that the first web space was in contact with the rod. Pieces of foam were placed around the forearm and within the cast to restrict movement. A stable position was also maintained by Velcro straps over the wrist and mid-forearm. The cast was connected to the gantry and then slowly slid into the scanner, before being locked in place (Figure 9.8). The wheels on the chair were also locked. The scout view was reviewed and the reference line was set along the plateau located approximately one third to halfway along the radial endplate (Figure 9.7b). Imaging was commenced 9.5mm proximal to this point. The scan was started and lasted for 2.8 minutes. On completion the limb was removed from the gantry and cast.

Figure 9.8: Radial HRpQCT scan

9.7.4 Image acquisition

In both the radius and tibia, the scan region spanned 9.02mm (110 slices) and for this acquisition, 750 projections were acquired over 180 degrees with a 100ms integration time (the time taken to make an observation) at each angular position. The effective radiation dose for each site was 3μSv.

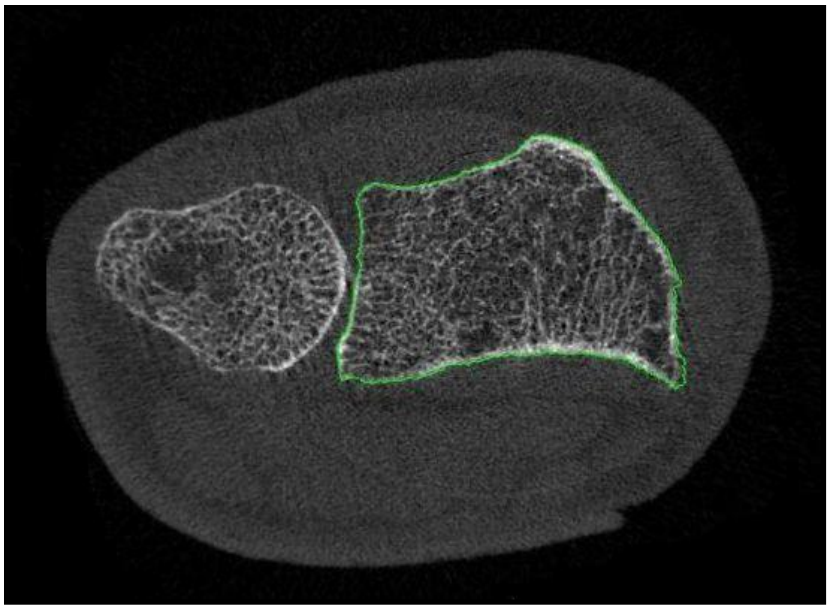

Figure 9.9: High resolution image of distal radius and ulna with line delineating outer margin of cortex

9.7.5 Motion artefact

The scanning time for each site was around 3 minutes. During this time, on some occasions the participant moved slightly causing motion artefact. As the images were of such high resolution, the effect of movement on the results would have been significant, impacting on both accuracy and reproducibility. Therefore, once the images had been acquired, their quality was assessed visually. If there was excessive streaking at the edges of the bones, signifying motion artefact, the scans were repeated. In some cases, however, despite repeating the scan, this problem could not be avoided.

This has been found in other studies using HRpQCT and therefore several manual grading techniques to quantify motion artefact have been developed(326–329). Before image analysis took place all images were reviewed by a single reader and graded using the Pialat method(327) and those images with excessive movement artefact were excluded from analyses. This is particularly important as the effects of movement on outcomes are not systematic and thus are not amenable to correction algorithms(326).

9.7.6 Scanner operators

As the images were acquired by two separate operators, an assessment was made to ensure that the results obtained from both were comparable. When bone microarchitecture was compared by operator, there was no difference in the majority of variables. Although radial trabecular thickness, radial cortical porosity and radial cortical thickness were significantly different in the two groups, the latter difference was completely attenuated after adjustment for participant age, sex, height, weight, calcium intake, physical activity level, alcohol consumption, smoking status, social class, bisphosphonate utilisation and number of comorbidities. Given the number of analyses completed, it would be likely that such differences would occur purely due to chance and they would not remain statistically significant if a strict Bonferroni correction was applied.

9.7.7 Standard image analysis

9.7.7.1 Segmentation

The reconstructed images were initially analysed using a standard protocol provided by the manufacturer. The distal-most slice was accessed and the operator manually drew a line just outside the cortex, ensuring to enclose the whole of the bone (Figure 9.9). This line was subsequently modified by the scanner software using a simple attenuation threshold method so that it accurately delineated the perimeter of the cortex. Thereafter, the software automatically repeated this process for the remainder of the slices which were then visually checked by the operator and amended if required. The mean of this area for all slices was defined as the total area.

The cortical compartment was then separated from the rest of the bone by first using a Gaussian filter to blur the trabecular bone with the marrow while preserving the cortical shell. Gaussian smoothing is commonly used in graphics software to reduce noise and detail. Then a single fixed threshold was used to delineate the cortical shell from the surrounding tissue. The trabecular region was subsequently defined by digital subtraction of the cortical bone from that area contained within the periosteal contour. Once

segmentation had been completed, the regions were subjected to densitometry and morphometric analyses(4, 121).

9.7.7.2 Bone density assessment

In order to produce hydroxyapatite mineral densities, linear attenuation values of the tomographic images were converted using phantom calibration and corrected for beam hardening. The calibration phantom (Scanco Medical AG, Bruttisellen, Switzerland) included 5 cylinders containing a mixture of hydroxyapatite and resin. The mineral concentrations of these cylinders were 0, 100, 200, 400 and 800 mgHA/cm^3. The value of 0mgHA/cm^3 equated to a soft tissue background devoid of mineral.

This information was used to calculate values for cortical vBMD, trabecular vBMD and total vBMD. The trabecular region was then further divided into inner (60%) and outer trabecular (40%) regions by area alone and densities were also provided for these.

9.7.7.3 Assessment of trabecular microarchitecture

Morphometric analysis of trabecular bone involved assessment of trabecular number, separation, thickness and bone volume fraction (the proportion of volume within the trabecular region that was composed of bone). This was carried out as follows.

As HRpQCT has a high isotropic resolution (82µm) it allows direct, assessment of the inter-trabecular distances (Figure 9.10). This uses a 3D ridge extraction method which picks out ridges (i.e. trabeculae) and draws spheres between them. It then calculates the mean sphere diameter and from this calculates trabecular number(330).

The average trabecular thickness is only 100-150µm which equates to less than 2 voxels. As a result, if this measure was assessed directly there would be significant error in the measurement. Thus, instead trabecular thickness was derived from trabecular number and trabecular vBMD using plate-model assumptions. Bone volume fraction was also derived from the trabecular vBMD(331). The derivation assumes a fixed mineralisation of 1200mgHA/cm^3 for bone (bone volume fraction = trabecular vBMD/1200).

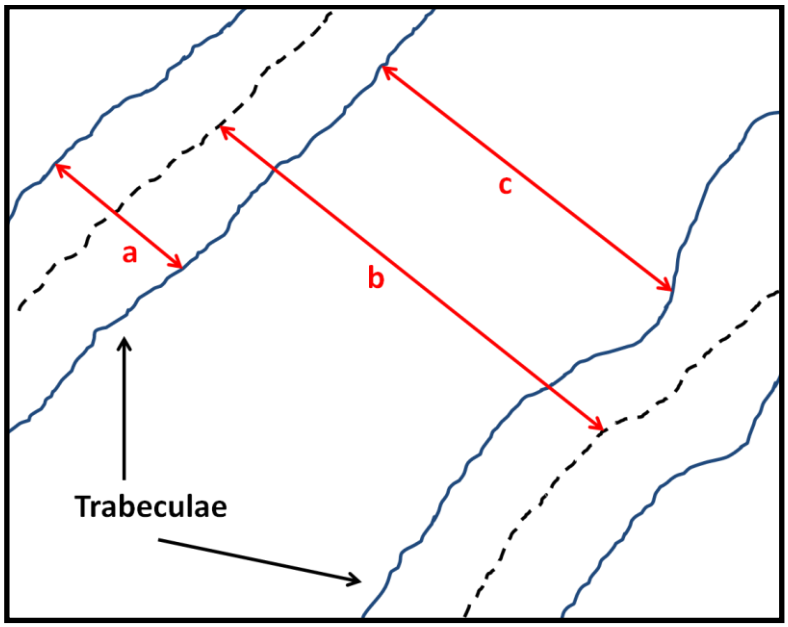

Figure 9.10: Schematic representation of measurements of trabecular microarchitecture derived from HRpQCT

Key: a = trabecular thickness, b = 1/trabecular number, c = trabecular separation.

9.7.8 Additional cortical image analysis

9.7.8.1 Segmentation

The method of data acquisition above concentrated on assessment of trabecular bone morphometry. The assessment of cortical microstructure, including porosity, required a different method of analysis. In order to do this software was utilised which had been designed based on the method of Burghardt and colleagues(171).

The periosteal and endosteal boundaries were described using an automated contouring method and verified by the operator. The region between these two surfaces was defined as the cortical compartment volume of interest. To exclude void voxels on the periosteal and endosteal margins from the estimation of intracortical porosity, only void voxels unconnected to the background in each 2D axial slice were included as part of the cortical compartment.

Once the cortical region had been segmented out, geometric measurements were determined. Cortical volume was defined as the total cortical volume

(including pores). Cortical bone volume was determined as the total volume of mineralised cortical bone within the cortex (excluding pores). Periosteal and endosteal circumference were the distances around the outer and inner perimeter of the cortex respectively. Total area, cortical area, cortical thickness and trabecular area were the mean values for each of these measures over the 110 slices.

9.7.8.2 Bone density assessment

Cortical bone mineral density was measured as cortical vBMD in a similar way to the standard image analysis. Cortical tissue mineral density was then calculated as the density of the bone mineral alone, excluding pores.

9.7.8.3 Determination of cortical porosity

The determination of the presence of a pore required the use of several assumptions. It has also been shown that smaller pores are missed by this method in which total pore volume is measured using the number of void voxels. Cortical porosity was defined as the volume of the intracortical pore space normalised by the sum of the pore and cortical bone volumes. Mean cortical pore diameter and the within individual variation in this measurement were also determined.

9.7.9 Choice of HRpQCT variables to study

As there were a total of 29 variables produced by the above image analysis in both the radius and tibia, it was important to focus on those thought to provide information of the greatest clinical relevance. This would reduce the number of relationships tested and mitigate the inherent problems associated with multiple testing. In the following sections, I will explain my decision making process in choosing which variables to study. Ultimately, it transpired that these were very similar to those used by other authors in this area(4, 121, 125, 170, 172, 326, 332, 333). To aid clarity, the variables considered are divided into those describing bone geometry, density, trabecular microarchitecture, and cortical microstructure.

9.7.9.1 Bone geometry

Taking geometry first, there are two main components – cortical and trabecular. Size parameters can be measured as volumes, areas, or circumferences. Clearly, volume and area would be directly proportional as the length of bone examined is constant. Similarly, both would be closely related to estimates of circumference assuming the bone approximates to a circle. Therefore, only one type of size measurement was chosen as follows.

Total and trabecular size were not available as volumes and therefore to maintain consistency in all regions this outcome was not used. Periosteal circumference was clearly related to total area and endosteal circumference to trabecular area. However, there was no such similar circumferential measure which related to the amount of cortical bone. Consequently, total area, cortical area and trabecular area were chosen as the optimal methods to assess the geometry of the different bone regions.

All three area measurements were determined by the standard and additional cortical image analysis methods. As those factors relating to trabecular microarchitecture were derived solely from the standard image analysis, this technique was also used to define trabecular area. However, delineation of the cortical region was more precise using the second method(125) and therefore this was used for total and cortical area.

Cortical area is an important factor in bone health particularly because it contains the majority of bone mineral, which is in turn directly related to a bone's compressive strength(86). It is, however, influenced by two factors, firstly the cross-sectional area of the bone as a whole and secondly the average thickness of the cortex. To distinguish these two features, an assessment of cortical thickness was therefore also required. The short term precision (%CV) for cortical thickness ranged from 1.1 to 3.9 and was significantly better in the tibia than the radius(328).

9.7.9.2 Bone density

To minimise the number of variables assessed, it was decided to only include one estimate of bone density from cortical bone and one from trabecular bone.

Total density was highly dependent on the proportion of cortical to trabecular bone in addition to their individual densities. This would make interpretation difficult and it was therefore not assessed.

Given the more accurate delineation of the cortical region using the additional cortical image analysis based on the method of Burghardt(171), cortical density measurements were taken from this analysis. Density measurements were available for cortical bone tissue alone (excluding void voxels) and for the cortical region as a whole (including void voxels). In order to provide the most representative assessment of the cortical compartment in situ, the density of the cortical region as a whole was chosen (including void voxels).

As for trabecular area, trabecular density was also derived from the standard image analysis. Inner and outer trabecular densities were not assessed individually to minimise the number of variables. As expected, they were both significantly correlated with trabecular density. The ratio of outer and inner trabecular densities was heavily skewed. It is likely that the most extreme values occurred when the inner trabecular density tended towards zero. This made it difficult to utilise as an outcome variable and it was not felt that it would provide additional useful clinical information.

In individuals above 70 years, short-term precision values (%CV) for cortical and trabecular density have been shown to range from 0.3 to 1.2 with results for cortical density significantly better in the tibia and in men(328).

9.7.9.3 Cortical microarchitecture

Cortical microarchitecture was only assessed using the method of Burghardt and colleagues. The variables created were cortical porosity, mean cortical pore diameter, and variation in pore diameter (expressed as a standard deviation). Although all three provide additional information, by far the most widely used is cortical porosity. This gives an indication of the proportion of the cortical volume which is devoid of bone mineral and is therefore likely to be the most clinically relevant. Short-term precision (%CV) for cortical porosity ranges from 11.2 to 12.6 in the radius and 4.9 to 7.4 in the tibia(328). These

values for radial cortical porosity are higher than for all other HRpQCT variables.

9.7.9.4 Trabecular microarchitecture

Trabecular microarchitectural parameters were derived solely from the standard image analysis. This provided bone volume fraction, trabecular number, trabecular thickness, trabecular separation, and a measure of the spread of trabecular separation. Bone volume fraction is a measurement of the proportion of volume within the trabecular region that is made up of bone mineral. Due to the way it is estimated, this value is directly proportional to trabecular density and therefore would not have provided additional information in analyses. Trabecular number, thickness, and separation have all been linked to mechanical properties of bone above and beyond density and therefore are important outcomes to measure(334). Short term precision (%CV) for trabecular number, thickness, and separation has been shown to range from 4.6 to 6.8(328). The spread of trabecular separation was not included in the assessed variables. The reasons for this are that it was heavily skewed, not intuitively related to bone strength, and strongly correlated to trabecular density.

9.7.10 Summary of HRpQCT variables

Based on the above rationale, the variables chosen for study in the radius and tibia were total area, trabecular area, cortical area, cortical thickness, cortical density, cortical porosity, trabecular density, trabecular number, trabecular thickness, and trabecular separation.

9.8 Choice of covariates

In epidemiological studies that examine associations there is invariably the possibility of confounding. In order to try to minimise this, all analyses were adjusted for those factors which were felt to be potential confounders. In terms of demographic factors, age was felt to be important as it is associated with bone health and also with body composition and fracture status.

Adult anthropometry has similarly been shown to affect bone geometry(239-242). Calcium intake, physical activity, smoking and alcohol consumption are also lifestyle factors with probable effects on bone health (179, 180, 187, 188, 198, 199, 202, 203). It was felt important to take comorbidities into consideration as several diseases, including type I (insulin dependent) diabetes, untreated long-standing hyperthyroidism, hypogonadism, and chronic malnutrition, have been shown to adversely affect bone health. Unfortunately, these had not been formally assessed in the HCS. As a result, a pragmatic decision was taken to use a combination of those diseases on which data were available. These included ischaemic heart disease, hypertension, diabetes mellitus, chronic bronchitis, and stroke or transient ischaemic attack (TIA). A score was created (0-5) to indicate the number of these conditions that each participant had reported. It was therefore not possible to distinguish the influence of one disease over another and if two of them had opposing actions on bone health one may cancel out the effect of the other.

Previously in the HCS, participants had undergone DXA scanning and results had been fed back to GPs which would, in some cases, highlight the need for osteoporosis treatment. Consequently, we would expect that a greater than average proportion of individuals in our cohort would be taking bisphosphonates. As these drugs alter bone metabolism they may affect the results of analyses(114, 234). It was therefore felt necessary to include this as an additional covariate in the models.

In women, HRT is another bone active drug(228, 229). Indications for use would include low bone density but it could also be used for other clinical reasons. As a result, it would potentially lead to a population with augmented bone health. Time since menopause is influenced by a women's age and the age at which she went through the menopause(226, 227). Its potential importance is as a measure of the duration for which she is likely to have been deficient in oestrogen with the resultant deleterious effects on bone. Both HRT utilisation and time since menopause were included as covariates in women. In men, it is known that drugs given for the treatment of prostate cancer can adversely affect bone health. However, in the current study only 3 men had

been diagnosed with this condition, of which just one was taking medication. As a result, this was not used as a covariate in the analysis.

9.9 Data management and statistical analyses

Demographic variables from previous studies in the HCS were merged with the HRpQCT parameters and current anthropometric data. Age was calculated using visit date and date of birth. HRpQCT variable names were changed to be in line with previous conventions for naming variables in this cohort. Each new variable was viewed as a histogram using the study population as a whole and then separately by sex. Normality of the data was then assessed. Any outlying values were scrutinised. The data cleaning process continued during data analysis. The statistical analyses utilised will be explained at the start of each results section.

9.10 Ethical approval and research governance

Ethical approval for the study was obtained from the Hertfordshire Research Ethics Committee on 7th July 2011 (Appendix 6). The study was sponsored by the University of Southampton who provided the required Professional Indemnity and Clinical Trials Insurance (Appendix 7).

9.11 Author's contribution to the project

I was heavily involved in the design of the study. I produced all of the study documentation and obtained ethical approval for the project. During the data collection process, I travelled to the MRC Human Nutrition Research, in Cambridge, to take part in the recruitment of over half of the study participants. I regularly took informed consent and soon after the commencement of the study, attended an Ionising Radiation Medical Exposure Regulations (IRMER) course after which I was permitted to learn scanning techniques and was able to perform these scans unsupervised after an appropriate training period. I learnt how to grade VFAs using the Genant method(100) before grading the scans of all study participants. I was involved in the cleaning of data, data management, and the performing of statistical

analyses with the help and advice of statisticians at the MRC Lifecourse Epidemiology Unit. The thesis was planned and written entirely by me with initial guidance and subsequent comments from each of my supervisors.

10.Results: Description of Study Participants

10.1 Aim

To assess for evidence of selection bias and to describe the demographic, anthropometric, lifestyle characteristics, and radial and tibial bone microarchitecture of participants recruited to the study.

10.2 Summary of methods

Full details of the study methodology can be found in section 9 and the cohort is defined in figure 9.2. Participants that attended the MRC Human Nutrition Research in Cambridge underwent detailed assessment of bone microarchitecture in both the radius and tibia using the Scanco Xtreme HRpQCT scanner. Height and weight were also assessed at the visit.

Additional demographic and lifestyle variables were obtained from previous studies within the HCS described in section 9.2. Smoking status, alcohol consumption and bisphosphonate use were obtained from data collection in 2004-2005. Calcium intake, physical activity, social class, time since menopause, HRT use and number of comorbidities were obtained from baseline HCS data collection (1998-2003). Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

10.3 Analyses

In order to assess for potential selection bias in participant recruitment, differences were firstly assessed between those individuals registered with a Hertfordshire GP that attended the baseline clinic (n=2997) and those that did not (n=4109). The only data available for all of these individuals was that from the ledgers; therefore, comparisons were made of birth weight and weight at 1 year using a Student's t-test as both were normally distributed.

Results

The second analysis was to compare those individuals that took part in the baseline clinic alone (n=2621) to those that also took part in the current study (n=376). Demographic and lifestyle data were available on all subjects from the baseline clinic. These included age, sex, height, weight, BMI, calcium intake, physical activity, smoking status, alcohol consumption, social class and number of comorbidities. In this group, both calcium intake and BMI were not normally distributed. Consequently they are presented as median and interquartile range (IQR) in descriptive tables and differences were assessed by Student's t-tests of the log-transformed data. Differences between categorical variables were assessed using Chi-squared tests.

Thirdly, an assessment was made of whether a selection bias had been introduced at the point of recruitment for the current study. Thus, those that took part (n=376) were compared to those that were approached but did not take part (n=195). In these two groups, all continuous variables were normally distributed and so differences were assessed using Student's t-tests. Differences between categorical variables were assessed using Chi-squared tests.

Characteristics of the study participants were then explored by sex. Continuous variables including height, weight, BMI, calcium intake, physical activity, time since menopause and bone microarchitectural parameters were assessed for normality. They were found to have a normal distribution and have therefore been presented as means and SDs in men and women. Differences between men and women were assessed using Student's t-tests. Sex differences for smoking status, social class and number of comorbidities were assessed using a Chi-squared test; sex differences for alcohol consumption were assessed using Fisher's exact test.

Differences in bone microarchitecture between men and women were assessed using Student's t-tests. Pearson's correlation coefficients were determined between each bone microarchitectural component in the radius and tibia separately.

10.4 Results

10.4.1 Assessment of selection bias

Those that attended the baseline HCS clinic (1998-2003) differed from those that were identified as living in Hertfordshire and registered with a Hertfordshire GP. Although birth weight did not differ between the two groups, weight at 1 year was on average 110g higher in those that took part ($p<0.001$) (Table 10.1).

Table 10.1: Comparison of individuals who were recruited to the Hertfordshire Cohort Study and those who were eligible but did not take part

	HCS Participants (n=2997) Mean (SD)	Non-participants (n=4109) Mean (SD)	p value
Birth weight (kg)	3.43 (0.53)	3.42 (0.55)	0.389
Weight at 1 year (kg)	10.00 (1.06)	9.89 (1.09)	<0.001

Those that took part in the current study also differed from those that only attended the baseline HCS clinic (Table 10.2). The former were significantly heavier at birth, younger, had a lower weight and BMI, and higher levels of physical activity. They were less likely to be a current or ex-smoker and to abstain from alcohol. They also had fewer comorbidities.

Five hundred and seventy one individuals were approached to take part in the study of which 376 consented and were assessed between September 2011 and October 2012. Those individuals that did not participate were lighter at birth, on average around 8 months older and 2.32cm shorter with lower physical activity than those that took part. They were also more obese with a BMI that was 1.2kg/m^2 higher (Table 10.3).

Table 10.2: Comparison of individuals who took part in the current study and those who were recruited to the Hertfordshire Cohort Study but did not take part in the current study

	Participants		Non-participants		
	Total N	**Mean (SD)**	**Total N**	**Mean (SD)**	**p value**
Birth weight (kg)	**376**	**3.49**	**2621**	**3.42**	**0.023**
Weight at 1yr (kg)	**376**	**9.97**	**2621**	**10.0**	**0.635**
Age (years)	**376**	**64.6 (2.7)**	**2621**	**66.3 (2.8)**	**<0.001**
Height (cm)	376	168.5 (8.8)	2612	167.7 (9.1)	0.107
Weight (kg)	**376**	**75.1 (12.6)**	**2613**	**77.5 (14.3)**	**0.002**
BMI (kg/m^2)[1]	**376**	**26.0 (23.9, 28.5)**	**2611**	**27.1 (24.4, 30.0)**	**<0.001**
Daily calcium intake (mg)[1]	376	1166 (974, 1346)	2620	1130 (944, 1348)	0.154
Physical activity score	**376**	**64.0 (13.7)**	**2621**	**29.4 (15.7)**	**<0.001**
	Total N	**n (%)**	**Total N**	**n (%)**	**p value**
Sex	376		2621		
Male		198 (52.7)		1381 (52.7)	
Female		178 (47.3)		1240 (47.3)	0.991
Smoker status	**376**		**2619**		
Never		**198 (52.7)**		**1187 (45.3)**	
Ex		**146 (38.8)**		**1087 (41.5)**	
Current		**32 (8.5)**		**345 (13.2)**	**0.006**
Alcohol intake	**376**		**2620**		
None		**20 (5.3)**		**352 (13.4)**	
≤Recommend[2]		**304 (80.9)**		**1912 (73.0)**	
>Recommend		**52 (13.8)**		**356 (13.6)**	**<0.001**
Social status[3]	368		2580		
I-IIINM		161 (43.8)		1052 (40.8)	
IIIM-V		207 (56.3)		1528 (59.2)	0.278
Comorbidities[4]	**361**		**2497**		
0		**211 (58.5)**		**1148 (46.0)**	
1		**106 (29.4)**		**859 (34.4)**	
2+		**44 (12.2)**		**490 (19.6)**	**<0.001**

[1]Median and IQR displayed, p value relates to t test of log-transformed data; [2]Recommended maximum weekly consumption of alcohol (14 units for women, 21 units for men); [3]I-IIINM (I to III non-manual), IIIM-V (III manual to V). [4]Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

Table 10.3: Comparison of those individuals that participated in the current study and those that were approached but did not take part

	Recruited individuals (n=376)	**Approached but not recruited (n=195)**	
	Mean (SD)	**Mean (SD)**	**p value**
Birth weight (kg)	**3.49**	**3.40**	**0.047**
Weight at 1yr (kg)	**9.97**	**9.97**	**0.971**
Age (years)[1]	**69.1 (2.6)**	**69.8 (2.6)**	**0.002**
Height (cm)	**167.9 (8.9)**	**165.5 (10.2)**	**0.005**
Weight (kg)	76.57 (12.9)	77.85 (16.1)	0.304
BMI (kg/m^2)	**27.15 (4.0)**	**28.35 (5.0)**	**0.002**
Daily calcium (mg)	1183 (342)	1200 (319)	0.571
Physical activity	**64.0 (13.7)**	**61.5 (14.5)**	**0.047**
	n (%)	**n (%)**	**p value**
Sex			
Female	178 (47.34)	109 (55.90)	
Male	198 (52.66)	86 (44.10)	0.052
Smoker status			
Never	197 (52.67)	100 (51.55)	
Ex	154 (41.18)	83 (42.78)	
Current	23 (6.15)	11 (5.67)	0.923
Alcohol intake			
None	44 (11.73)	36 (18.56)	
≤Recommend[2]	295 (78.67)	141 (72.68)	
>Recommend	36 (9.60)	17 (8.76)	0.085
Social status[3]			
I–IIINM	161 (43.75)	76 (40.43)	
IIIM–V	207 (56.25)	112 (59.57)	0.453
Comorbidities[4]			
0	211 (58.5)	94 (50.0)	
1	106 (29.4)	62 (33.0)	
2+	44 (12.2)	32 (17.0)	0.122

[1] At time of scans; [2] Recommended maximum weekly consumption of alcohol (14 units for women, 21 units for men); [3] I–IIINM (I to III non–manual), IIIM–V (III manual to V). [4]Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension. For recruited individuals and approached but not recruited individuals respectively, n=374 and 194 for smoker status; n=375 and 194 for alcohol intake; n=368 and 188 for social status; and n=361 and 188 for comorbidities.

10.4.2 Summary characteristics of study participants

10.4.2.1 Demographics

The mean (SD) age of women and men that took part in the study was 76.5(2.6) and 76.1(2.6) years respectively. There were 198 (52.7%) men and 178 (47.3%) women recruited.

10.4.2.2 Anthropometry

Anthropometric measures at the time of assessment are summarised in table 10.4. Men were taller and heavier than women. The mean BMI for both women and men was within the overweight range (27.7 and 27.3kg/m^2 respectively) and did not differ significantly by sex.

10.4.2.3 Lifestyle and diet

Information on smoking status, alcohol consumption, social class and time since menopause was not available within the dataset on 2, 1, 8 and 3 individuals respectively. Rates of smoking were higher in men than women; 58.6% of men and only 34.7% of women were current or ex-smokers. Alcohol consumption was also greater in men with around 1 in 6 drinking above the recommended amount of alcohol (21units/week for men). This figure was only 1.7% in women. The mean daily dietary calcium intake was 1137mg and 1225mg in women and men respectively, significantly higher in the latter. Physical activity score was also higher in men than women. Women had gone through the menopause a mean of 28.1 years previously. Social class and number of comorbidities did not differ by sex with just over half of participants in class IIIM-V.

10.4.3 Radial and tibial microarchitecture by sex

Table 10.5 shows summary characteristics for the radius and tibia by sex. Significant differences were found between the bone microarchitecture of men and women. Men tended to have larger bones; specifically, total area, cortical area and trabecular area were all greater in men than women (all $p<0.001$). Men also had greater bone density than women in both the cortical and trabecular regions, with the exception of cortical density in the radius.

Sexual dimorphism was also apparent in trabecular microarchitecture. Men had higher radial trabecular number and thickness and higher tibial trabecular number. Trabecular separation was greater in women than men in both regions studied.

Table 10.4: Summary of participant characteristics

	Men		Women		
	Total N	**Mean (SD)**	**Total N**	**Mean (SD)**	**p value**
Age[1]	198	76.1 (2.5)	178	76.5 (2.6)	0.186
Height (cm)	**198**	**173.4 (6.6)**	**178**	**160.0 (6.3)**	**<0.001**
Weight (kg)	**198**	**82.2 (12.0)**	**178**	**70.9 (12.8)**	**<0.001**
BMI (kg/m^2)	198	27.3 (3.8)	178	27.7 (4.8)	0.438
Daily calcium intake (mg)	**198**	**1225 (298)**	**178**	**1137 (381)**	**0.012**
Physical activity score	**198**	**65.7 (13.6)**	**178**	**62.1 (13.7)**	**0.012**
Time since menopause (years)	NA	NA	175	28.1 (6.7)	NA
	Total N	**n (%)**	**Total N**	**n (%)**	**p value**
Smoker status	**198**		**176**		
Never		**82 (41.4)**		**115 (65.3)**	
Ex		**102 (51.5)**		**52 (29.6)**	
Current		**14 (7.1)**		**9 (5.1)**	**<0.001**
Alcohol intake	**198**		**177**		
None		**10 (5.0)**		**34 (19.2)**	
≤Recommend[2]		**155 (78.3)**		**140 (79.1)**	
>Recommend		**33 (16.7)**		**3 (1.7)**	**<0.001**
Social status[3]	190		178		
I-IIINM		84 (44.2)		77 (43.3)	
IIIM-V		106 (55.8)		101 (56.7)	0.854
Comorbidities[4]	188		173		
0		112 (59.6)		99 (57.2)	
1		51 (27.1)		55 (31.8)	
2+		25 (13.3)		19 (11.0)	0.563
HRT (ever use)[5]	NA	NA	178		
Yes				77 (43.3)	
No				101 (56.7)	NA

[1] At time of scans; [2] Recommended maximum weekly consumption of alcohol (14 units for women, 21 units for men); [3] I-IIINM (I to III non-manual), IIIM-V (III manual to V); [4] Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension. [5] Hormone Replacement Therapy as measured at HCS baseline (1998-2003).

Cortical microarchitecture showed site-specific sex differences. In the radius, cortical porosity was higher in men (4.2%) than women (3.6%). Conversely, in the tibia it was higher in women (10.0%) than men (9.2%).

Table 10.5: Description of HRpQCT parameters by sex in the radius and tibia

		Men Mean (SD)	Women Mean (SD)	p value
Total area (mm^2)	R	**425.79 (76.74)**	**284.65 (43.51)**	**<0.001**
	T	**906.84 (144.51)**	**712.60 (102.42)**	**<0.001**
Trabecular area (mm^2)	R	**350.11 (73.73)**	**235.70 (44.85)**	**<0.001**
	T	**764.51 (148.54)**	**618.37 (108.48)**	**<0.001**
Cortical area (mm^2)	R	**71.39 (14.03)**	**47.36 (8.41)**	**<0.001**
	T	**141.75 (27.62)**	**93.08 (16.20)**	**<0.001**
Cortical thickness (µm)	R	**832.27 (193.64)**	**686.35 (163.85)**	**<0.001**
	T	**1213.82 (261.18)**	**919.68 (191.22)**	**<0.001**
Cortical density (mg/cm^3)	R	906.16 (50.39)	904.85 (58.72)	0.818
	T	**868.86 (61.87)**	**811.28 (63.68)**	**<0.001**
Cortical porosity (%)	R	**4.16 (1.44)**	**3.58 (1.40)**	**<0.001**
	T	**9.20 (2.72)**	**10.00 (2.59)**	**0.004**
Trabecular density (mg/cm^3)	R	**179.33 (37.19)**	**142.61 (42.29)**	**<0.001**
	T	**188.59 (34.85)**	**170.52 (39.69)**	**<0.001**
Trabecular number (cm^{-1})	R	**23.46 (2.49)**	**20.68 (4.13)**	**<0.001**
	T	**24.43 (3.16)**	**22.57 (3.54)**	**<0.001**
Trabecular thickness (µm)	R	**63.44 (10.31)**	**56.87 (10.52)**	**<0.001**
	T	64.48 (9.83)	63.07 (11.93)	0.213
Trabecular separation (µm)	R	**368.74 (62.32)**	**453.59 (145.62)**	**<0.001**
	T	**351.83 (55.49)**	**392.65 (85.93)**	**<0.001**

Key: R, radius; T, tibia.

10.4.4 Correlations between bone microarchitectural parameters

Tables 10.6 and 10.7 show the correlations between different bone components, as measured by HRpQCT, in the radius and tibia of men and women. The relationships shown were similar in both sexes and at both sites. Total area describes the total cross-sectional area of the bone enclosed within the periosteal circumference. It was clearly highly correlated with trabecular area (r=0.90 to 0.99). This is illustrated for the tibia in men and women in figure 10.1.

Table 10.6: Correlations between bone parameters in the radius in men (blue) and women (red)

	ToA	TrA	CoA	CoT	CoD	CoP	TrD	TrN	TrT	TrS
ToA	1									
	1									
TrA	**0.90**	1								
	0.98	1								
CoA	0.02	**-0.26**	1							
	-0.03	**-0.21**	1							
CoT	**-0.47**	**-0.64**	**0.83**	1						
	-0.48	**-0.62**	**0.85**	1						
CoD	**-0.40**	**-0.51**	**0.43**	**0.63**	1					
	-0.43	**-0.52**	**0.48**	**0.67**	1					
CoP	**0.14**	0.10	**0.26**	0.10	**-0.55**	1				
	-0.02	-0.05	**0.17**	**0.15**	**-0.44**	1				
TrD	**-0.17**	**-0.25**	**0.40**	**0.41**	**0.16**	**0.39**	1			
	-0.23	**-0.29**	**0.33**	**0.39**	**0.22**	**0.32**	1			
TrN	0.01	-0.02	**0.29**	**0.19**	-0.06	**0.41**	**0.67**	1		
	-0.03	-0.07	**0.29**	**0.23**	0.06	**0.26**	**0.82**	1		
TrT	**-0.22**	**-0.31**	**0.34**	**0.41**	**0.27**	**0.23**	**0.87**	**0.23**	1	
	-0.34	**-0.39**	**0.21**	**0.37**	**0.31**	**0.23**	**0.77**	**0.31**	1	
TrS	0.04	0.09	**-0.33**	**-0.27**	-0.07	**-0.37**	**-0.74**	**-0.94**	**-0.39**	1
	-0.07	0.11	**-0.22**	**-0.20**	-0.07	**-0.23**	**-0.80**	**-0.95**	**-0.37**	1

Key: ToA, Total area; TrA, Trabecular area; CoA, Cortical area; CoT, Cortical thickness; CoD, Cortical density; CoP, Cortical porosity; TrD, Trabecular density; TrN, Trabecular number; TrT, Trabecular thickness; TrS, Trabecular separation; Results in bold if $p<0.05$.

Table 10.7: Correlations between bone parameters in the tibia in men (blue) and women (red)

	ToA	TrA	CoA	CoT	CoD	CoP	TrD	TrN	TrT	TrS
ToA	1									
	1									
TrA	**0.96**	1								
	0.99	1								
CoA	-0.11	**-0.32**	1							
	-0.21	**-0.34**	1							
CoT	**-0.54**	**-0.69**	**0.85**	1						
	-0.57	**-0.68**	**0.87**	1						
CoD	**-0.37**	**-0.44**	**0.39**	**0.55**	1					
	-0.31	**-0.40**	**0.53**	**0.56**	1					
CoP	**0.19**	**0.18**	0.09	-0.09	**-0.75**	1				
	-0.05	-0.04	0.02	0.04	**-0.69**	1				
TrD	**-0.19**	**-0.25**	**0.30**	**0.31**	0.14	**0.15**	1			
	-0.23	**-0.28**	**0.36**	**0.35**	0.23	**0.18**	1			
TrN	**0.16**	0.09	**0.37**	0.13	0.00	**0.28**	**0.60**	1		
	0.11	0.07	**0.34**	0.09	**0.15**	0.11	**0.61**	1		
TrT	**-0.36**	**-0.38**	0.06	**0.26**	**0.16**	0.03	**0.74**	-0.09	1	
	-0.30	**-0.40**	**0.18**	**0.36**	**0.16**	**0.15**	**0.75**	0.06	1	
TrS	-0.07	0.00	**-0.37**	**-0.18**	-0.06	**-0.23**	**-0.74**	**-0.96**	-0.12	1
	-0.04	0.00	**-0.30**	-0.12	**-0.18**	-0.09	**-0.71**	**-0.91**	-0.12	1

Key: ToA, Total area; TrA, Trabecular area; CoA, Cortical area; CoT, Cortical thickness; CoD, Cortical density; CoP, Cortical Porosity; TrD, Trabecular density; TrN, Trabecular number; TrT, Trabecular thickness; TrS, Trabecular separation. Results in bold if $p<0.05$.

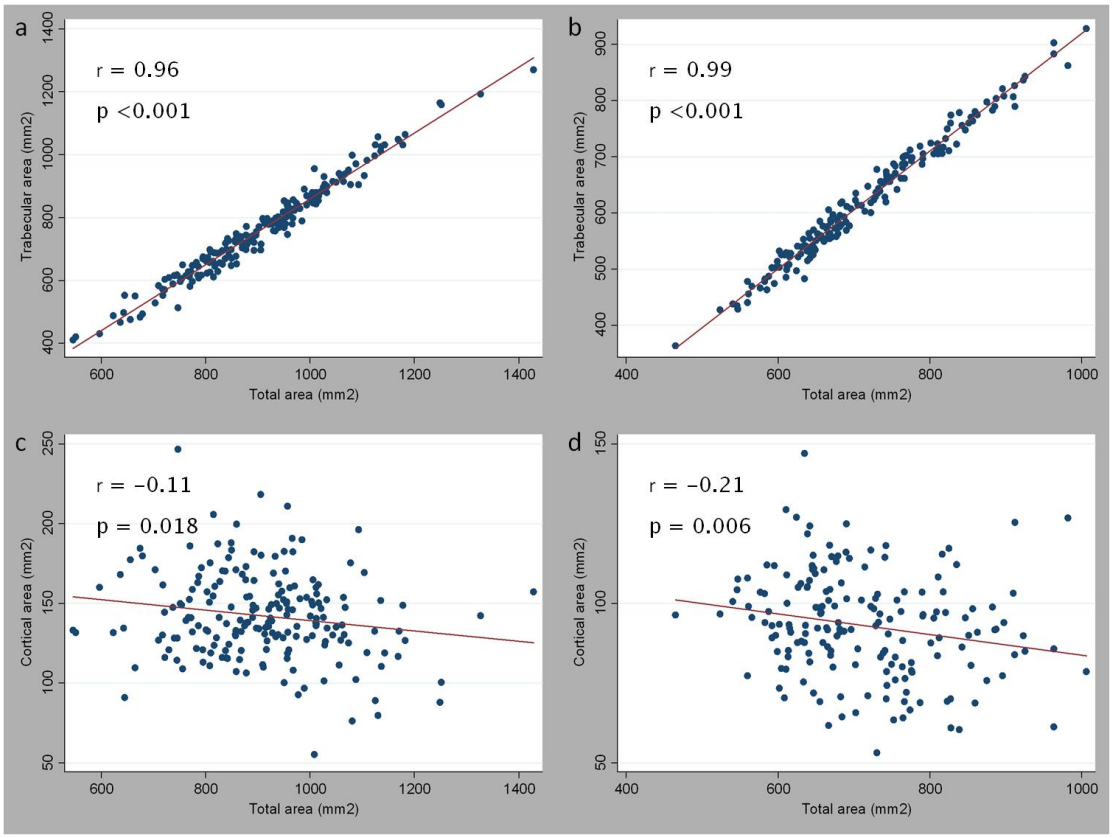

Figure 10.1: Scatter plot of tibial total area and (a) tibial trabecular area in men, (b) tibial trabecular area in women, (c) tibial cortical area in men, and (d) tibial cortical area in women

In contrast, there was a negative correlation between total area and cortical area in the tibia (Figure 10.1), and a much stronger negative correlation between total area and cortical thickness in both the radius and tibia. There was also an inverse correlation between total area and trabecular thickness. Thus, in those individuals with larger bones in CSA, both the cortex and trabeculae were thinner. This is likely to explain why trabecular density was also negatively correlated with bone size.

Cortical thickness was positively associated with both cortical density and trabecular density. As expected, cortical density was strongly negatively correlated with cortical porosity, and trabecular density was positively correlated with trabecular number and thickness. Trabecular separation was strongly negatively correlated with trabecular number (r=-0.94 to -0.96) and less strongly with trabecular thickness (r=-0.12 in tibia; r=-0.37 to -0.39 in radius).

10.5 Results Summary

Those individuals that were recruited to the HCS differed from those that did not take part. There was also significant disparity between participants of the current study and those that only attended the baseline HCS clinic. These differences were consistent with a healthy participant bias. In the group that did participate, men were heavier and taller than women with a similar BMI. They were also more likely to smoke and to be heavier drinkers.

With few exceptions, tibial and radial parameters of size, density, and trabecular microarchitecture were higher in men than women. Interestingly, relationships with cortical porosity appeared to be site specific. There were strong positive correlations between total area and trabecular area in both men and women but inverse correlations between total area and each of cortical thickness, cortical density, trabecular thickness, and trabecular density. Cortical thickness was positively associated with cortical density. Trabecular number and trabecular thickness were both positively correlated with trabecular density.

11.Results: Determinants of bone microarchitecture

11.1 Aim

To explore the relationships between bone microarchitecture at the radius and tibia, and demographic, lifestyle, and anthropometric factors in older men and women.

11.2 Summary of methods

Full details of the study methodology can be found in section 9 and the cohort is defined in figure 9.2. Participants that attended the MRC Human Nutrition Research in Cambridge underwent measurement of height and weight. BMI was calculated as weight divided by height squared. Detailed bone microarchitecture in both the radius and tibia was assessed using the Scanco Xtreme HRpQCT scanner. Age was calculated from date of scan visit and date of birth. Smoking status, alcohol consumption and bisphosphonate use were obtained from data collection in 2004-2005. Calcium intake, physical activity, social class, time since menopause, HRT use and number of comorbidities were obtained from baseline HCS data collection (1998-2003). Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

11.3 Analyses

Radial and tibial microarchitectural parameters were assessed for normality and found to be normally distributed. With the exception of number of units of alcohol per week which was heavily positively skewed, all other continuous variables were assessed for normality and distributions were deemed acceptable for utilisation as independent variables in regression models. Smoker status was categorised as current smoker, ex-smoker, or lifelong non-smoker. Alcohol consumption was categorised into bands. Those that drank

<1 units of alcohol per week were defined as minimal alcohol. Those that drank more alcohol than this were subdivided into those that drank under half of the maximum recommended amount of alcohol (<10units in men, <7 units in women), hereafter defined as low alcohol, and those that drank more than half of the maximum recommended amount of alcohol (>10units in men, <7 units in women), hereafter defined as moderate-high alcohol.

As bone microarchitecture, height and weight differed significantly by sex, men and women were evaluated separately. Regression analyses were completed to explore relationships between bone microarchitecture and age, lifestyle factors (calcium intake, physical activity, smoking status, alcohol consumption, social class), number of comorbidities, bisphosphonate use, anthropometry (height, weight and BMI) and, in women, years since menopause and HRT use. All analyses were then repeated with adjustment for these covariates. When regression analyses were carried out in men and women separately, an additional analysis was completed to assess for interaction by sex.

Although several associations were tested, the HRpQCT variables have clearly been shown not to be independent and therefore a Bonferroni correction was felt to be over conservative. A p value of <0.05 was therefore described as statistically significant although the clinical relevance of each finding was carefully interpreted on a case by case basis and the possibility of a chance finding was always considered.

11.4 Results

11.4.1 Relationships between age and bone microarchitecture

Table 11.1 shows the associations between age and microarchitecture in the radius and tibia. Through the relatively narrow age range of the study (approximately 10 years) the only significant associations in men were higher radial cortical porosity and lower tibial trabecular density with advancing age.

Table 11.1: Cross-sectional associations between bone microarchitecture and age in men and women

		Men		Women	
		β (95% CI)	p	β (95% CI)	p
Total area (mm^2)	R	0.44 (-3.95,4.84)	0.843	2.16 (-0.30,4.62)	0.085
	T	-1.44 (-9.53,6.65)	0.726	4.54 (-1.27,10.34)	0.125
Trabecular area (mm^2)	R	-0.98 (-5.21,3.24)	0.647	**2.75 (0.22,5.27)**	**0.033**
	T	0.81 (-7.50,9.12)	0.848	5.68 (-0.45,11.80)	0.069
Cortical area (mm^2)	R	0.08 (-0.73,0.88)	0.849	**-0.57 (-1.04,-0.10)**	**0.018**
	T	-1.01 (-2.55,0.53)	0.196	-0.90 (-1.81,0.02)	0.054
Cortical thickness (μm)	R	-0.11 (-11.21,10.98)	0.984	**-13.00 (-22.13,-3.87)**	**0.006**
	T	-4.26 (-18.88,10.35)	0.566	**-11.77 (-22.53,-1.01)**	**0.032**
Cortical density (mg/cm^3)	R	-2.41 (-5.28,0.46)	0.099	**-4.81 (-8.08,-1.54)**	**0.004**
	T	-2.04 (-5.49,1.42)	0.246	**-4.84 (-8.40,-1.28)**	**0.008**
Cortical porosity (%)	R	**0.09 (0.01,0.18)**	**0.024**	0.01 (-0.07,0.09)	0.761
	T	0.07 (-0.08,0.22)	0.345	0.08 (-0.07,0.23)	0.281
Trabecular density (mg/cm^3)	R	-1.90 (-4.01,0.22)	0.078	**-3.64 (-5.99,-1.29)**	**0.003**
	T	**-2.06 (-3.99,-0.13)**	**0.036**	**-2.84 (-5.07,-0.62)**	**0.013**
Trabecular number (cm^{-1})	R	-0.05 (-0.19,0.09)	0.502*	**-0.35 (-0.58,-0.12)**	**0.003***
	T	-0.11 (-0.29,0.06)	0.202	-0.14 (-0.34,0.06)	0.158
Trabecular thickness (μm)	R	-0.53 (-1.11,0.06)	0.078	**-0.60 (-1.19,-0.00)**	**0.049**
	T	-0.40 (-0.95,0.15)	0.149	-0.66 (-1.33,0.01)	0.055
Trabecular separation (μm)	R	0.67 (-2.85,4.18)	0.709**	**13.56 (5.51,21.61)**	**0.001****
	T	2.26 (-0.83,5.35)	0.150	3.95 (-0.91,8.81)	0.111

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Most of the relationships in women were common to both the radius and the tibia although the strength of these associations was invariably stronger in the radius. At both sites, there was a negative relationship between cortical and trabecular density and age (all p<0.05). There was also a tendency towards lower trabecular number and trabecular thickness with age although this only reached statistical significance in the radius. This was attended by a correspondingly higher trabecular separation (p=0.001). Total and trabecular area tended to be higher with advanced age in women whereas cortical area was lower; however, these associations failed to reach statistical significance in the tibia. The most consistent relationship was with cortical thickness, present in both the radius (β=-13.00μm/year, p=0.006) and tibia (β=-11.77μm/year, p=0.032). Cortical porosity did not differ by age in women. The relationship between radial trabecular number and age was significantly different in men and women, with women, but not men, showing a negative association.

11.4.2 Relationships between lifestyle and bone microarchitecture

There were no consistent relationships identified when bone microarchitecture was regressed on calcium intake and physical activity (Appendix 9; Tables A1 and A2). Only 14 men and 9 women were current smokers whereas ex-smokers made up 51.5% of men (n=102) and 29.6% of women (n=52). Smoking status was also not found to be associated with bone structure. This was when comparing lifelong non-smokers to current smokers and ex-smokers separately (Appendix 9; Table A3) and when comparing lifelong non-smokers to ever smokers (Appendix 9; Table A4). The proportion of men and women in social class I-IIIn was similar at 44.2% and 43.3% respectively. There were no significant relationships between social class and bone microarchitecture (Appendix 9; Table A5). Consistent sex interactions were not identified with any of the above lifestyle factors in the determination of bone microarchitecture.

A total of 30, 90 and 78 men drank minimal, low and moderate-high amounts of alcohol respectively. These figures were 74, 80 and 23 respectively in women. Men that drank low or moderate-high alcohol had lower radial cortical thickness and density, and lower radial trabecular thickness and density than those that drank minimal alcohol (Table 11.2). Although similar tends were shown in women these did not reach statistical significance. When relationships were explored comparing those that drank low or moderate-high alcohol separately with those that drank minimal alcohol, similar trends were seen (Table 11.3). However, with few exceptions these were only significant for low alcohol in men. There were no consistent interactions by sex for the relationships between alcohol consumption and bone microarchitecture.

11.4.3 Bisphosphonates, comorbidities and bone microarchitecture

As only two of the 198 men were taking bisphosphonates, the regression model was not felt to be stable and so results are not presented for men. Women taking bisphosphonates had lower trabecular density, number and thickness, and greater trabecular separation (Table 11.4). Trabecular density was 42.15 (-63.85,-20.44) and 34.81 (-55.37,-14.25) mg/cm^3 lower in those

Table 11.2: Regression analysis of bone microarchitecture on alcohol consumption in men and women

		Men				Women			
		Unadjusted β (95% CI)[2]	p value	Adjusted[1] β (95% CI)[2]	p value	Unadjusted β (95% CI)[2]	p value	Adjusted[1] β (95% CI)[2]	p value
Total area (mm^2)	R	**30.38 (0.14,60.63)**	**0.049**	21.14 (-8.09,50.37)	0.155	2.53 (-10.76,15.82)	0.708	2.84 (-10.13,15.81)	0.666
	T	9.75 (-46.89,66.39)	0.735	4.94 (-42.55,52.43)	0.838	-3.92 (-34.54,26.71)	0.801	-7.41 (-35.41,20.59)	0.602
Trabecular area (mm^2)	R	**33.53 (4.57,62.50)**	**0.024**	26.23 (-2.18,54.65)	0.070	3.61 (-10.09,17.30)	0.604	3.37 (-10.25,16.98)	0.626
	T	20.63 (-37.50,78.77)	0.485	16.55 (-31.10,64.20)	0.494	-3.14 (-35.50,29.22)	0.848	-7.80 (-37.68,22.09)	0.607
Cortical area (mm^2)	R	-4.68 (-10.23,0.86)	0.097	**-5.73 (-11.32,-0.14)**	**0.044**	-0.46 (-3.03,2.10)	0.722	0.22 (-2.48,2.92)	0.871
	T	-9.04 (-19.79,1.72)	0.099	-10.15 (-20.56,0.26)	0.056*	-0.37 (-5.24,4.51)	0.882	1.16 (-3.73,6.05)	0.640*
Cortical thickness (μm)	R	**-88.11 (-164.19,-12.03)**	**0.023**	**-89.34 (-167.6,-11.04)**	**0.026**	-16.07 (-66.09,33.95)	0.527	-7.01 (-60.85,46.83)	0.797
	T	-90.09 (-191.70,11.53)	0.082	-96.64 (-193.40,0.12)	0.050	-8.31 (-65.58,48.95)	0.775	4.54 (-53.72,62.80)	0.878
Cortical density (mg/cm^3)	R	**-26.92 (-46.62,-7.23)**	**0.008**	**-25.80 (-46.29,-5.31)**	**0.014**	-13.58 (-31.38,4.22)	0.134	-11.52 (-31.16,8.12)	0.248
	T	-6.64 (-30.88,17.60)	0.590	-10.31 (-35.10,14.48)	0.413	-9.33 (-28.60,9.94)	0.340	-7.95 (-28.19,12.29)	0.439
Cortical porosity (%)	R	0.24 (-0.33,0.81)	0.404	0.06 (-0.48,0.60)	0.820	0.24 (-0.18,0.66)	0.268	0.22 (-0.24,0.69)	0.348
	T	-0.59 (-1.65,0.47)	0.277	-0.63 (-1.70,0.44)	0.244	0.23 (-0.55,1.02)	0.555	0.41 (-0.45,1.27)	0.348
Trabecular density (mg/cm^3)	R	**-14.88 (-29.54,-0.22)**	**0.047**	**-17.88 (-33.08,-2.67)**	**0.021**	-8.00 (-20.79,4.79)	0.219	-9.07 (-22.36,4.22)	0.180
	T	-5.60 (-19.24,8.03)	0.419	-5.74 (-19.42,7.94)	0.409	-10.55 (-22.33,1.24)	0.079	-8.52 (-20.81,3.77)	0.173
Trabecular number (cm^{-1})	R	-0.51 (-1.50,0.47)	0.307	-0.67 (-1.69,0.34)	0.191	-0.44 (-1.69,0.81)	0.492	-0.34 (-1.62,0.94)	0.598
	T	-0.10 (-1.34,1.13)	0.870	-0.18 (-1.24,0.88)	0.737	**-1.14 (-2.20,-0.08)**	**0.035**	-0.84 (-1.88,0.20)	0.113
Trabecular thickness (μm)	R	**-4.06 (-8.13,0.01)**	**0.050**	**-4.69 (-8.92,-0.45)**	**0.030**	-1.48 (-4.68,1.73)	0.364	-1.93 (-5.33,1.47)	0.263
	T	-1.76 (-5.60,2.09)	0.368	-1.60 (-5.46,2.26)	0.415	-0.28 (-3.84,3.29)	0.879	-0.14 (-4.00,3.73)	0.944
Trabecular separation (μm)	R	15.95 (-8.36,40.26)	0.197	20.49 (-5.30,46.27)	0.119	17.47 (-26.63,61.57)	0.435	9.18 (-37.16,55.53)	0.696
	T	2.63 (-19.11,24.37)	0.812	3.90 (-15.72,23.52)	0.695	**33.26 (7.64,58.88)**	**0.011**	**26.98 (0.76,53.20)**	**0.044**

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, social class, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use). [2]Regression coefficient are for intake ≥1unit per week (low or moderate–high) compared to those with <1 unit per week (minimal or none). Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 11.3: Regression analysis of bone microarchitecture comparing individuals with low alcohol consumption and moderate-high alcohol consumption to those with minimal alcohol consumption.

		Low alcohol consumption[2] vs minimal/none[1]				Moderate-high alcohol consumption[3] vs minimal/none[1]			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	**32.47 (0.23,64.71)**	**0.048**	-0.29 (-14.38,13.80)	0.968	28.00 (-4.80,60.81)	0.094	11.96 (-8.66,32.59)	0.254
	T	17.48 (-42.90,77.86)	0.569	-10.34 (-42.83,22.14)	0.531	0.93 (-60.51,62.37)	0.976	17.60 (-30.04,65.24)	0.467
Trabecular area (mm^2)	R	**38.68 (7.87,69.49)**	**0.014***	1.08 (-13.46,15.61)	0.884	27.66 (-3.69,59.01)	0.083*	12.08 (-9.19,33.36)	0.264
	T	28.69 (-33.22,90.60)	0.362	-9.22 (-43.57,25.13)	0.597	11.34 (-51.75,74.43)	0.723	17.21 (-33.16,67.58)	0.501
Cortical area (mm^2)	R	-5.76 (-11.66,0.13)	0.055	-0.57 (-3.30,2.16)	0.681	-3.45 (-9.45,2.55)	0.258	-0.11 (-4.11,3.89)	0.956
	T	**-12.44 (-23.83,-1.05)**	**0.032**	-0.66 (-5.85,4.53)	0.803	-5.15 (-16.74,6.44)	0.382	0.60 (-7.01,8.21)	0.876
Cortical thickness (μm)	R	**-98.30 (-179.30,-17.30)**	**0.018**	-16.41 (-69.65,36.83)	0.544	-76.46 (-158.88,5.97)	0.069	-14.94 (-92.86,62.99)	0.706
	T	**-118.31 (-226.1,-10.47)**	**0.032**	-9.71 (-70.68,51.25)	0.754	-57.88 (-167.62,51.85)	0.299	-3.63 (-93.04,85.77)	0.936
Cortical density (mg/cm^3)	R	**-23.15 (-44.09,-2.21)**	**0.030**	-13.87 (-32.82,5.07)	0.150	**-31.24 (-52.55,-9.93)**	**0.004**	-12.60 (-40.33,15.13)	0.371
	T	-8.64 (-34.50,17.22)	0.511	-7.80 (-28.31,12.70)	0.453	-4.36 (-30.68,21.95)	0.744	-14.44 (-44.51,15.62)	0.344
Cortical porosity (%)	R	-0.05 (-0.65,0.55)	0.866	0.16 (-0.28,0.61)	0.469	0.58 (-0.03,1.19)	0.062	0.48 (-0.17,1.14)	0.148
	T	-0.72 (-1.85,0.41)	0.210	0.14 (-0.69,0.98)	0.738	-0.43 (-1.58,0.72)	0.459	0.55 (-0.68,1.77)	0.378
Trabecular density (mg/cm^3)	R	**-17.97 (-33.55,-2.40)**	**0.024**	-12.62 (-26.07,0.83)	0.066	-11.34 (-27.19,4.50)	0.160	7.49 (-12.20,27.17)	0.454
	T	-6.05 (-20.59,8.49)	0.413	**-12.94 (-25.4,-0.44)**	**0.043**	-5.08 (-19.90,9.73)	0.499	-2.54 (-20.87,15.80)	0.785
Trabecular number (cm^{-1})	R	-0.83 (-1.88,0.21)	0.119	-0.73 (-2.05,0.60)	0.280	-0.15 (-1.21,0.91)	0.781	0.54 (-1.40,2.48)	0.582
	T	-0.39 (-1.70,0.93)	0.562	**-1.17 (-2.30,-0.04)**	**0.042**	0.22 (-1.11,1.56)	0.741	-1.03 (-2.69,0.63)	0.221
Trabecular thickness (μm)	R	**-4.44 (-8.77,-0.11)**	**0.044**	-2.70 (-6.07,0.66)	0.115	-3.62 (-8.03,0.78)	0.107	2.62 (-2.30,7.55)	0.295
	T	-1.13 (-5.23,2.96)	0.586	-1.22 (-4.99,2.54)	0.522	-2.48 (-6.65,1.69)	0.242	2.90 (-2.63,8.42)	0.302
Trabecular separation (μm)	R	23.12 (-2.62,48.87)	0.078	29.55 (-17.07,76.17)	0.213	7.75 (-18.45,33.95)	0.560	-22.98 (-91.21,45.26)	0.507
	T	7.11 (-16.00,30.22)	0.545	**35.28 (8.02,62.55)**	**0.012**	-2.54 (-26.09,21.01)	0.832	26.49 (-13.50,66.47)	0.193

Key: R, radius; T, tibia. [1]Minimal/none, <1 unit/week; [2]Low alcohol consumption, ≥1 unit/week and <8 units/week in women or <11 units/week in men; [3]moderate-high alcohol consumption, ≥8 units/week in women or ≥11 units/week in men. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 11.4: Regression analysis of bone microarchitecture by bisphosphonate therapy utilisation in women.

		Women			
		Unadjusted		Adjusted[1]	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	2.98 (-20.28,26.24)	0.801	7.58 (-16.23,31.39)	0.530
	T	8.62 (-46.11,63.35)	0.756	5.22 (-46.91,57.34)	0.843
Trabecular area (mm^2)	R	5.87 (-18.10,29.83)	0.629	8.25 (-16.74,33.24)	0.515
	T	15.22 (-42.71,73.16)	0.605	4.10 (-51.53,59.74)	0.884
Cortical area (mm^2)	R	-2.84 (-7.32,1.64)	0.213	-0.60 (-5.58,4.38)	0.812
	T	-6.38 (-14.98,2.23)	0.145	1.66 (-7.44,10.76)	0.719
Cortical thickness (µm)	R	-52.56 (-139.81,34.69)	0.236	-23.78 (-123.12,75.56)	0.637
	T	-32.39 (-134.49,69.70)	0.532	35.09 (-73.25,143.43)	0.523
Cortical density (mg/cm^3)	R	-16.98 (-48.27,14.32)	0.286	-11.85 (-48.13,24.42)	0.519
	T	-30.02 (-63.76,3.72)	0.081	-12.77 (-50.46,24.93)	0.504
Cortical porosity (%)	R	-0.16 (-0.91,0.58)	0.667	-0.32 (-1.18,0.55)	0.470
	T	0.46 (-0.92,1.84)	0.511	0.85 (-0.75,2.45)	0.294
Trabecular density (mg/cm^3)	R	**-42.15 (-63.85,-20.44)**	**<0.001**	**-29.63 (-54.31,-4.95)**	**0.019**
	T	**-34.81 (-55.37,-14.25)**	**0.001**	-15.59 (-38.58,7.39)	0.182
Trabecular number (cm^{-1})	R	**-4.07 (-6.19,-1.95)**	**<0.001**	**-2.44 (-4.80,-0.08)**	**0.043**
	T	**-2.65 (-4.49,-0.80)**	**0.005**	-0.85 (-2.78,1.09)	0.389
Trabecular thickness (µm)	R	**-6.94 (-12.47,-1.41)**	**0.014**	-6.30 (-12.61,0.00)	0.050
	T	-5.76 (-12.08,0.56)	0.074	-2.93 (-10.11,4.25)	0.421
Trabecular separation (µm)	R	**148.12 (73.52,222.72)**	**<0.001**	**96.07 (10.49,181.64)**	**0.028**
	T	**63.75 (18.82,108.67)**	**0.006**	21.81 (-27.28,70.90)	0.381

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, alcohol consumption, social class, number of comorbidities, time since menopause and HRT use. [2]Regression coefficient for user of bisphosphonate when compared to non-user.

on bisphosphonates in the radius and tibia ($p \leq 0.001$). These relationships were attenuated after adjustment for demographic and lifestyle covariates. There were no consistent relationships shown between number of comorbidities and bone microarchitecture, in either men or women, and no consistent interactions by sex (Appendix 9; Table A6 and A7).

11.4.4 Factors affecting bone microarchitecture in women only

There were negative associations between the number of years since the menopause and trabecular density, trabecular number and trabecular thickness in both the radius and tibia (Table 11.5). As women that are further past the menopause tend to be older, the associations were initially adjusted for age. This led to attenuation although relationships were maintained with trabecular density, thickness and separation. Further adjustment for covariates led to greater attenuation. In all cases, regression coefficients remained greater for the radius than the tibia. There were no consistent relationships between HRT utilisation and bone microarchitecture (Appendix 9; Table A8).

Table 11.5: Associations between bone microarchitecture and time since menopause in women

		Unadjusted		Adjusted for age		Adjusted[1]	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	0.00 (-1.01,1.01)	1.000	-0.41 (-1.50,0.68)	0.454	-0.22 (-1.28,0.83)	0.675
	T	0.99 (-1.34,3.32)	0.401	0.19 (-2.34,2.73)	0.880	0.39 (-1.94,2.71)	0.744
Trabecular area (mm^2)	R	0.06 (-0.97,1.10)	0.903	-0.45 (-1.57,0.67)	0.429	-0.31 (-1.42,0.79)	0.577
	T	1.24 (-1.22,3.71)	0.321	0.28 (-2.41,2.96)	0.839	0.29 (-2.20,2.78)	0.816
Cortical area (mm^2)	R	-0.04 (-0.23,0.15)	0.690	0.06 (-0.15,0.27)	0.567	0.12 (-0.10,0.34)	0.278
	T	-0.26 (-0.63,0.11)	0.166	-0.14 (-0.54,0.26)	0.490	0.07 (-0.34,0.48)	0.736
Cortical thickness (μm)	R	-0.47 (-4.27,3.33)	0.808	1.90 (-2.15,5.96)	0.355	2.22 (-2.18,6.62)	0.320
	T	-1.44 (-5.81,2.94)	0.518	0.59 (-4.15,5.34)	0.805	1.69 (-3.17,6.54)	0.494
Cortical density (mg/cm^3)	R	-0.16 (-1.52,1.20)	0.816	0.73 (-0.71,2.18)	0.318	1.11 (-0.49,2.71)	0.171
	T	-0.63 (-2.07,0.82)	0.395	0.24 (-1.32,1.80)	0.761	0.74 (-0.95,2.42)	0.387
Cortical porosity (%)	R	-0.02 (-0.05,0.02)	0.344	-0.02 (-0.06,0.01)	0.242	-0.03 (-0.06,0.01)	0.192
	T	-0.02 (-0.08,0.04)	0.443	-0.05 (-0.11,0.02)	0.167	-0.05 (-0.12,0.02)	0.191
Trabecular density (mg/cm^3)	R	**-1.77 (-2.71,-0.83)**	**<0.001**	**-1.41 (-2.44,-0.39)**	**0.007**	**-1.15 (-2.25,-0.05)**	**0.040**
	T	**-1.53 (-2.42,-0.65)**	**0.001**	**-1.28 (-2.24,-0.31)**	**0.010**	**-1.08 (-2.11,-0.06)**	**0.038**
Trabecular number (cm^{-1})	R	**-0.19 (-0.28,-0.10)**	**<0.001**	**-0.16 (-0.26,-0.07)**	**0.001**	**-0.13 (-0.24,-0.03)**	**0.014**
	T	**-0.12 (-0.20,-0.04)**	**0.004**	**-0.11 (-0.20,-0.03)**	**0.010**	-0.07 (-0.16,0.01)	0.100
Trabecular thickness (μm)	R	**-0.26 (-0.50,-0.02)**	**0.036**	-0.19 (-0.45,0.07)	0.153	-0.17 (-0.45,0.11)	0.240
	T	-0.25 (-0.52,0.02)	0.072	-0.16 (-0.46,0.13)	0.273	-0.20 (-0.52,0.12)	0.226
Trabecular separation (μm)	R	**6.97 (3.77,10.18)**	**<0.001**	**5.75 (2.28,9.22)**	**0.001**	**4.74 (0.94,8.54)**	**0.015**
	T	**2.70 (0.77,4.63)**	**0.006**	**2.48 (0.36,4.60)**	**0.022**	1.77 (-0.47,4.00)	0.121

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, smoking status, alcohol consumption, social class, bisphosphonate use, comorbidity score (and in women, HRT use). [2]Regression coefficients per year increase in time since menopause.

11.4.5 Relationships between adult anthropometry and bone microarchitecture

Strong positive relationships were shown between height and both total and trabecular area in both men and women (Table 11.6). However, the magnitude of these associations was significantly greater in men than women, with the exception of radial trabecular area. Inverse associations were also found between height and tibial cortical thickness in both sexes. Other relationships with height were limited to tibial parameters in men. Taller men had significantly lower tibial cortical density and trabecular thickness with higher cortical porosity and trabecular number.

In contrast, BMI did not show relationships with total or trabecular area (Table 11.7). It was positively associated with cortical area, cortical thickness, trabecular density, and trabecular number in both men and women. Prior to adjustment, relationships between BMI and cortical area were of significantly greater magnitude in men than women. In men, cortical porosity was positively associated with BMI in both the radius and tibia.

Similarly to height, positive relationships were found between total body weight and both total and trabecular area, although this does not reach statistical significance for radial trabecular area in men (Table 11.8). As with BMI, weight was also positively associated with cortical area, trabecular density and trabecular number. As a result, an inverse association was shown with trabecular separation.

Height and BMI had opposing associations with cortical thickness; consequently, there were no consistent relationships between cortical thickness and weight (Table 11.8). Relationships between weight and cortical porosity showed sexual dimorphism in both the radius and tibia. In men, cortical porosity showed a positive association with weight whereas in women no such relationship was demonstrated.

Table 11.6: Associations between bone microarchitecture and height in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[2]	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	**4.63 (3.13,6.13)**	**<0.001***	**6.02 (4.35,7.69)**	**<0.001***	**2.71 (1.76,3.66)**	**<0.001***	**3.44 (2.31,4.56)**	**<0.001***
	T	**12.62 (10.13,15.11)**	**<0.001****	**13.68 (10.96,16.39)**	**<0.001****	**7.22 (5.04,9.39)**	**<0.001****	**9.10 (6.64,11.57)**	**<0.001****
Trabecular area (mm^2)	R	**4.31 (2.86,5.76)**	**<0.001**	**5.32 (3.69,6.95)**	**<0.001**	**2.63 (1.64,3.61)**	**<0.001**	**3.30 (2.13,4.48)**	**<0.001**
	T	**12.98 (10.42,15.54)**	**<0.001****	**14.21 (11.48,16.93)**	**<0.001****	**7.52 (5.20,9.83)**	**<0.001****	**9.51 (6.90,12.12)**	**<0.001****
Cortical area (mm^2)	R	0.22 (-0.07,0.52)	0.142	0.33 (-0.00,0.66)	0.052	0.08 (-0.12,0.28)	0.442	0.14 (-0.09,0.38)	0.233
	T	-0.02 (-0.60,0.57)	0.951	0.10 (-0.55,0.75)	0.764	-0.25 (-0.63,0.13)	0.196	-0.29 (-0.75,0.16)	0.206
Cortical thickness (μm)	R	-3.38 (-7.48,0.73)	0.106	-3.96 (-8.60,0.68)	0.094	-2.33 (-6.19,1.54)	0.236	-2.63 (-7.25,2.00)	0.263
	T	**-12.18 (-17.5,-6.92)**	**<0.001**	**-11.9 (-17.6,-6.08)**	**<0.001**	**-8.23 (-12.6,-3.87)**	**<0.001**	**-9.92 (-15.1,-4.75)**	**<0.001**
Cortical density (mg/cm^3)	R	-0.71 (-1.78,0.36)	0.194	**-1.44 (-2.62,-0.27)**	**0.017**	-0.32 (-1.71,1.07)	0.649	-0.95 (-2.64,0.74)	0.267
	T	**-1.83 (-3.12,-0.54)**	**0.006**	**-1.73 (-3.15,-0.31)**	**0.017**	-1.12 (-2.62,0.38)	0.143	-1.59 (-3.37,0.18)	0.078
Cortical porosity (%)	R	0.01 (-0.02,0.04)	0.390	**0.04 (0.01,0.07)**	**0.017**	-0.01 (-0.04,0.02)	0.589	0.01 (-0.03,0.05)	0.638
	T	**0.06 (0.00,0.12)**	**0.040**	0.06 (-0.00,0.12)	0.062	-0.01 (-0.08,0.05)	0.658	-0.01 (-0.08,0.07)	0.881
Trabecular density (mg/cm^3)	R	0.25 (-0.54,1.04)	0.537	0.03 (-0.85,0.92)	0.939	-0.29 (-1.29,0.71)	0.566	-0.48 (-1.64,0.69)	0.421
	T	-0.01 (-0.75,0.73)	0.986	-0.31 (-1.11,0.49)	0.444	0.61 (-0.33,1.54)	0.202	0.43 (-0.65,1.52)	0.433
Trabecular number (cm^{-1})	R	0.03 (-0.02,0.08)	0.277	0.04 (-0.02,0.09)	0.242	0.04 (-0.06,0.13)	0.454	0.02 (-0.09,0.13)	0.712
	T	**0.10 (0.03,0.16)**	**0.003**	**0.10 (0.03,0.17)**	**0.004**	0.08 (-0.01,0.16)	0.072	0.07 (-0.03,0.16)	0.175
Trabecular thickness (μm)	R	0.01 (-0.21,0.23)	0.933	-0.08 (-0.33,0.16)	0.493	-0.22 (-0.46,0.03)	0.087	-0.28 (-0.57,0.02)	0.063
	T	**-0.25 (-0.46,-0.05)**	**0.016**	**-0.37 (-0.59,-0.14)**	**0.002**	0.01 (-0.27,0.30)	0.918	-0.02 (-0.36,0.32)	0.896
Trabecular separation (μm)	R	-0.44 (-1.75,0.87)	0.508	-0.47 (-1.97,1.04)	0.541	-0.50 (-3.95,2.95)	0.775	0.66 (-3.37,4.69)	0.747
	T	**-1.43 (-2.59,-0.27)**	**0.016**	**-1.42 (-2.67,-0.17)**	**0.026**	-1.79 (-3.81,0.23)	0.081	-1.56 (-3.96,0.84)	0.201

Key: R, radius; T, tibia. [1] Adjusted for age, smoking status, alcohol consumption, calcium intake, physical activity, social class, number of comorbidities and bisphosphonate use; [2] As adjusted 1 and time since menopause and HRT use. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 11.7: Associations between bone microarchitecture and BMI in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[2]	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	−1.47 (−4.35,1.41)	0.314	−2.48 (−5.87,0.91)	0.151	1.32 (−0.02,2.66)	0.054	1.09 (−0.51,2.69)	0.182
	T	−1.54 (−6.95,3.86)	0.574	−0.47 (−6.64,5.71)	0.881	2.44 (−0.72,5.60)	0.129	1.07 (−2.60,4.74)	0.565
Trabecular area (mm^2)	R	−1.95 (−4.70,0.81)	0.165	−2.68 (−5.92,0.56)	0.105	1.00 (−0.40,2.39)	0.159	0.84 (−0.81,2.49)	0.317
	T	−4.42 (−9.95,1.10)	0.116	−3.91 (−10.16,2.35)	0.219	1.36 (−2.00,4.72)	0.427	−0.11 (−3.98,3.77)	0.957
Cortical area (mm^2)	R	**0.93 (0.42,1.44)**	**<0.001**	**0.96 (0.37,1.55)**	**0.001**	**0.41 (0.16,0.67)**	**0.002**	**0.34 (0.04,0.64)**	**0.027**
	T	**2.79 (1.83,3.74)**	**<0.001****	**2.99 (1.90,4.08)**	**<0.001**	**1.26 (0.80,1.73)**	**<0.001****	**1.35 (0.81,1.90)**	**<0.001**
Cortical thickness (μm)	R	**11.73 (4.65,18.81)**	**0.001***	**13.58 (5.46,21.70)**	**0.001**	2.80 (−2.30,7.90)	0.281*	1.68 (−4.25,7.60)	0.577
	T	**19.95 (10.58,29.32)**	**<0.001***	**20.58 (10.09,31.06)**	**<0.001**	**6.88 (1.04,12.72)**	**0.021***	**9.24 (2.49,15.98)**	**0.008**
Cortical density (mg/cm^3)	R	−0.61 (−2.50,1.28)	0.528	0.22 (−1.93,2.37)	0.839	−0.37 (−2.20,1.47)	0.695	−0.63 (−2.80,1.53)	0.565
	T	0.51 (−1.81,2.82)	0.666	0.87 (−1.74,3.47)	0.513	2.16 (0.21,4.11)	0.030	1.89 (−0.39,4.16)	0.103
Cortical porosity (%)	R	**0.07 (0.02,0.12)**	**0.009**	**0.06 (0.01,0.12)**	**0.030***	0.01 (−0.03,0.05)	0.685	0.00 (−0.05,0.05)	0.976*
	T	**0.14 (0.04,0.24)**	**0.005***	**0.15 (0.03,0.26)**	**0.011**	0.01 (−0.07,0.09)	0.756*	0.05 (−0.05,0.14)	0.335
Trabecular density (mg/cm^3)	R	**1.52 (0.14,2.90)**	**0.032**	**1.65 (0.07,3.22)**	**0.040**	**1.65 (0.35,2.95)**	**0.013**	**1.52 (0.06,2.99)**	**0.042**
	T	**1.86 (0.58,3.14)**	**0.005**	**2.08 (0.66,3.49)**	**0.004**	**1.01 (−0.21,2.23)**	**0.104**	1.04 (−0.34,2.42)	0.138
Trabecular number (cm^{-1})	R	**0.16 (0.07,0.25)**	**0.001**	**0.16 (0.05,0.26)**	**0.003**	**0.21 (0.09,0.34)**	**0.001**	**0.19 (0.05,0.34)**	**0.007**
	T	**0.40 (0.29,0.50)**	**<0.001***	**0.41 (0.30,0.53)**	**<0.001**	**0.24 (0.13,0.34)**	**<0.001***	**0.26 (0.14,0.37)**	**<0.001**
Trabecular thickness (μm)	R	0.12 (−0.27,0.50)	0.556	0.14 (−0.29,0.58)	0.517	0.05 (−0.28,0.38)	0.764	0.03 (−0.35,0.41)	0.886
	T	**−0.34 (−0.71,0.02)**	**0.067**	−0.31 (−0.72,0.10)	0.139	−0.26 (−0.63,0.11)	0.166	−0.34 (−0.77,0.09)	0.116
Trabecular separation (μm)	R	**−3.01 (−5.27,−0.75)**	**0.009**	**−3.23 (−5.88,−0.58)**	**0.017**	**−5.52 (−9.99,−1.05)**	**0.016**	**−5.24 (−10.33,−0.16)**	**0.043**
	T	**−5.78 (−7.69,−3.87)**	**<0.001**	**−6.10 (−8.20,−4.01)**	**<0.001**	**−4.29 (−6.88,−1.70)**	**0.001**	**−5.13 (−8.10,−2.16)**	**0.001**

Key: R, radius; T, tibia. [1] Adjusted for age, smoking status, alcohol consumption, calcium intake, physical activity, social class, number of comorbidities and bisphosphonate use; [2] As adjusted 1 and time since menopause and HRT use. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 11.8: Associations between bone microarchitecture and weight in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[2]	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	**0.92 (0.02,1.81)**	**0.046**	1.02 (-0.01,2.06)	0.053	**1.08 (0.59,1.56)**	**<0.001**	**1.14 (0.55,1.72)**	**<0.001**
	T	**3.20 (1.56,4.83)**	**<0.001**	**3.80 (2.01,5.60)**	**<0.001**	**2.45 (1.32,3.59)**	**<0.001**	**2.32 (0.97,3.67)**	**0.001**
Trabecular area (mm^2)	R	0.68 (-0.19,1.54)	0.125	0.76 (-0.23,1.76)	0.131	**0.95 (0.44,1.45)**	**<0.001**	**1.02 (0.41,1.63)**	**0.001**
	T	**2.44 (0.73,4.15)**	**0.005**	**2.98 (1.12,4.84)**	**0.002**	**2.13 (0.90,3.35)**	**0.001**	**1.97 (0.53,3.41)**	**0.008**
Cortical area (mm^2)	R	**0.35 (0.19,0.50)**	**<0.001**	**0.37 (0.20,0.55)**	**<0.001**	**0.17 (0.07,0.26)**	**0.001**	**0.15 (0.04,0.27)**	**0.008**
	T	**0.83 (0.53,1.13)**	**<0.001***	**0.88 (0.54,1.21)**	**<0.001***	**0.40 (0.23,0.58)**	**<0.001***	**0.44 (0.23,0.65)**	**<0.001***
Cortical thickness (μm)	R	**2.55 (0.29,4.81)**	**0.027**	**2.77 (0.23,5.30)**	**0.033**	0.51 (-1.41,2.42)	0.604	0.07 (-2.19,2.33)	0.953
	T	2.39 (-0.66,5.44)	0.124	2.35 (-0.96,5.67)	0.163	0.72 (-1.51,2.94)	0.526	1.32 (-1.30,3.95)	0.321
Cortical density (mg/cm^3)	R	-0.40 (-0.99,0.19)	0.186	-0.34 (-1.00,0.32)	0.310	-0.20 (-0.89,0.49)	0.568	-0.41 (-1.23,0.41)	0.329
	T	-0.37 (-1.09,0.36)	0.322	-0.23 (-1.03,0.57)	0.569	0.55 (-0.19,1.29)	0.143	0.38 (-0.49,1.25)	0.389
Cortical porosity (%)	R	**0.03 (0.01,0.04)**	**0.003***	**0.03 (0.01,0.04)**	**0.001***	0.00 (-0.02,0.02)	0.917*	0.00 (-0.02,0.02)	0.921*
	T	**0.06 (0.03,0.09)**	**<0.001****	**0.06 (0.02,0.09)**	**0.001****	0.00 (-0.03,0.03)	0.892**	0.02 (-0.02,0.05)	0.386**
Trabecular density (mg/cm^3)	R	**0.53 (0.09,0.96)**	**0.017**	0.48 (-0.00,0.96)	0.052	**0.51 (0.02,1.00)**	**0.040**	0.45 (-0.11,1.01)	0.114
	T	**0.55 (0.15,0.96)**	**0.007**	**0.49 (0.06,0.93)**	**0.026**	**0.50 (0.04,0.96)**	**0.032**	0.49 (-0.03,1.01)	0.067
Trabecular number (cm^{-1})	R	**0.06 (0.03,0.08)**	**<0.001**	**0.05 (0.02,0.09)**	**0.001**	**0.08 (0.04,0.13)**	**0.001**	**0.08 (0.02,0.13)**	**0.005**
	T	**0.15 (0.12,0.18)**	**<0.001**	**0.15 (0.11,0.18)**	**<0.001**	**0.10 (0.06,0.14)**	**<0.001**	**0.11 (0.07,0.15)**	**<0.001**
Trabecular thickness (μm)	R	0.04 (-0.08,0.16)	0.544	0.02 (-0.12,0.15)	0.798	-0.04 (-0.16,0.09)	0.552	-0.06 (-0.20,0.09)	0.450
	T	**-0.18 (-0.29,-0.06)**	**0.002**	**-0.20 (-0.32,-0.07)**	**0.002**	-0.09 (-0.23,0.05)	0.200	-0.13 (-0.29,0.04)	0.129
Trabecular separation (μm)	R	**-1.03 (-1.74,-0.32)**	**0.005**	**-1.04 (-1.85,-0.23)**	**0.012**	**-2.03 (-3.71,-0.35)**	**0.018**	-1.74 (-3.68,0.20)	0.079
	T	**-2.15 (-2.73,-1.58)**	**<0.001**	**-2.14 (-2.76,-1.53)**	**<0.001**	**-1.94 (-2.89,-0.98)**	**<0.001**	**-2.22 (-3.34,-1.10)**	**<0.001**

Key: R, radius; T, tibia. [1] Adjusted for age, smoking status, alcohol consumption, calcium intake, physical activity, social class, number of comorbidities and bisphosphonate use; [2] As adjusted 1 and time since menopause and HRT use. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

11.5 Results Summary

Relationships between age and bone architecture were generally more pronounced in women than men. The strongest relationships were between greater age and lower cortical thickness, cortical density and trabecular density. Alcohol consumption was associated with lower radial cortical thickness and density and radial trabecular thickness and density in men before and after adjustment for covariates. In women, a greater time since menopause and use of bisphosphonates were associated with poorer bone microarchitecture. In both cases, these relationships were attenuated after adjustment.

Greater height was consistently associated with greater total and trabecular area. Cortical area and thickness, and trabecular density and number, tended to be greater in those with a higher BMI. Relationships between bone microarchitecture and total body weight appeared to demonstrate a combination of those relationships identified with height and BMI. Interestingly, weight was positively associated with cortical porosity in men but not women. Adjustment for demographic and lifestyle factors had little effect on the relationships between anthropometry and bone microarchitecture.

12.Results: Relationships between body composition and bone microarchitecture

12.1 Aim

To explore the associations between body composition – lean mass and fat mass – and bone microarchitecture at the radius and tibia.

12.2 Summary of methods

Full details of the study methodology can be found in section 9 and the cohort is defined in figure 9.2. Participants that attended the MRC Human Nutrition Research in Cambridge underwent assessment of body composition by DXA, and detailed bone microarchitecture in both the radius and tibia using the Scanco Xtreme HRpQCT scanner. Height and weight were also assessed at the visit. Additional covariates within the analysis were obtained from previous studies within the HCS described in section 9.2. Smoking status, alcohol consumption and bisphosphonate use were obtained from data collection in 2004-2005. Calcium intake, physical activity, social class, time since menopause, HRT use and number of comorbidities were obtained from baseline HCS data collection (1998-2003). Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

12.3 Analyses

Total lean mass is significantly correlated with height and taller individuals also tend to have bones of greater size. Thus, associations between body composition and, for example, total area may occur through confounding by height. Therefore, to define sarcopenia and muscle size, previous investigators have elected to produce a measure of relative muscle mass. Specifically, a method was devised by Baumgartner and colleagues to divide lean mass by height squared as this function was found to minimise the correlation with height across all sex, ethnic and age groups within the

populations studied, and is directly analogous to the use of BMI for grading relative adiposity(335). This function also minimised the correlation with height in our cohort. Using this method, lean mass divided by height squared is termed lean mass index (LMI) and fat mass divided by height squared is fat mass index (FMI). LMI has already been adopted by the European Working Group on Sarcopenia in Older People (EWGSOP) as a component of sarcopenia(247). Although, unlike lean mass, fat mass is not strongly correlated with height, FMI was used to be comparable to LMI. Another option would have been to run analyses and then adjust relationships for height. This lean mass variable would however have been less amenable to use in definitions of sarcopenia.

Total lean mass, total fat mass, LMI, FMI, and percentage fat mass were all assessed for normality and found to be normally distributed. Body composition in the arms, legs, and whole body were described in men and women; differences between the two sexes were assessed using a Student's t-test. As body composition and bone microarchitecture differed significantly by sex, men and women were evaluated separately. Pearson's correlation coefficients were determined between components of body composition and anthropometric measurements. Body composition variables and bone microarchitecture variables were standardised to allow direct comparisons of effect sizes.

Regression models were fitted to explore relationships between bone microarchitecture (radius and tibia) and LMI and FMI, before and after adjustment for covariates (age, smoking status, alcohol consumption, calcium intake, physical activity, social class, bisphosphonate use, number of comorbidities and, in women, time since menopause and use of HRT). Next, LMI and FMI were included in the same model to determine their independent effects on bone microarchitecture. Given the correlation between LMI and FMI ($r=0.45$ in men, $r=0.53$ in women, $p<0.01$), further analyses were completed using variable residuals to confirm whether, when completely independent components of LMI and FMI were used, similar associations were identified. Firstly, FMI was regressed against LMI and the FMI standardised residuals were calculated. LMI and FMI standardised residuals were then included in the same model before and after adjustment for covariates as

described above. This process was then repeated to produce a model of FMI and LMI standardised residuals before and after adjustment. When regression analyses were carried out in men and women separately, an additional analysis was completed to assess for interaction by sex. Statistical significance was determined as described in section 11.3.

12.4 Results

Three hundred and seventy six participants were eligible for DXA body composition assessment. Of these, 2 declined scanning and 1 was unable to tolerate the procedure. A further 8 had artefacts in both upper limbs and 23 had artefacts in both lower limbs preventing inclusion of that region, thus whole body composition was available in 342 (91%). This group did not differ from the overall cohort (n=376) in terms of demographic and lifestyle factors.

Table 12.1: Summary of body composition by dual energy X-ray absoptiometry in men and women

Variable	Men Mean (SD)	Women Mean (SD)	p value
Arm	n=190	n=175	
Fat Mass (kg)	2.59 (1.01)	3.32 (1.16)	<0.001
Fat Mass Index (kg/m^2)	0.86 (0.33)	1.29 (0.45)	<0.001
Fat Mass (% of tissue)	27.44 (7.02)	43.93 (6.97)	<0.001
Lean Mass (kg)	6.62 (0.84)	4.05 (0.63)	<0.001
Lean Mass Index (kg/m^2)	2.20 (0.24)	1.58 (0.23)	<0.001
Lean Mass (% of tissue)	72.56 (7.01)	56.07 (6.97)	<0.001
Tissue Mass (kg)	9.21 (1.52)	7.37 (1.64)	<0.001
Leg	n=182	n=168	
Fat Mass (kg)	6.82 (2.60)	10.55 (3.68)	<0.001
Fat Mass Index (kg/m^2)	2.26 (0.84)	4.11 (1.40)	<0.001
Fat Mass (% of tissue)	27.18 (6.51)	44.84 (7.31)	<0.001
Lean Mass (kg)	17.66 (2.14)	12.40 (1.63)	<0.001
Lean Mass Index (kg/m^2)	5.85 (0.59)	4.83 (0.55)	<0.001
Lean Mass (% of tissue)	72.82 (6.51)	55.16 (7.31)	<0.001
Tissue Mass (kg)	24.49 (4.06)	22.95 (4.75)	0.001
Whole body	n=175	n=167	
Fat Mass (kg)	25.12 (8.35)	29.63 (9.28)	<0.001
Fat Mass Index (kg/m^2)	8.35 (2.76)	11.55 (3.59)	<0.001
Fat Mass (% of tissue)	31.18 (6.78)	42.48 (6.79)	<0.001
Lean Mass (kg)	53.72 (5.27)	38.62 (4.41)	<0.001
Lean Mass Index (kg/m^2)	17.82 (1.44)	15.06 (1.55)	<0.001
Lean Mass (% of tissue)	68.83 (6.78)	57.51 (6.79)	<0.001
Tissue Mass (kg)	78.84 (11.55)	68.24 (12.24)	<0.001

The body composition of study participants is shown in table 12.1. Lean mass, LMI and tissue mass were all greater in men than women in the whole body,

arms, and legs. Conversely, fat mass, FMI and percentage fat mass were all greater in women than men.

The Pearson's correlation coefficients of body composition and anthropometric measures are shown in table 12.2. Results are similar in men and women. Lean mass is strongly positively correlated with height, weight, and fat mass. Weight is highly correlated with percentage fat mass, lean mass and fat mass, and this might explain the positive correlation between lean mass and percentage fat mass. LMI and FMI are positively correlated in both men and women (r=0.45 and 0.53 respectively).

Table 12.2: Pearson's correlation coefficients of body composition and anthropometry in men (blue) and women (red)

	Height (m)	Weight (kg)	Lean Mass (kg)	Fat Mass (kg)	LMI (kg/m^2)	FMI (kg/m^2)	PFM (%)
Height (m)	1						
	1						
Weight (kg)	**0.32**	1					
	0.29	1					
Lean Mass (kg)	**0.59**	**0.72**	1				
	0.46	**0.77**	1				
Fat Mass (kg)	0.09	**0.91**	**0.41**	1			
	0.16	**0.93**	**0.54**	1			
LMI (kg/m^2)	**-0.20**	**0.57**	**0.67**	**0.41**	1		
	-0.23	**0.61**	**0.75**	**0.47**	1		
FMI (kg/m^2)	**-0.14**	**0.82**	**0.27**	**0.97**	**0.45**	1	
	-0.08	**0.86**	**0.42**	**0.96**	**0.53**	1	
PFM (%)	-0.11	**0.70**	0.06	**0.92**	**0.17**	**0.94**	1
	0.00	**0.74**	**0.19**	**0.91**	**0.21**	**0.93**	1

Key: LMI, Lean mass index; FMI, Fat mass index; PFM, Percentage fat mass. Result in bold if $p<0.05$.

Table 12.3 shows the associations between standardised LMI and FMI, and bone architecture at the radius and tibia in men and women. In terms of cortical parameters, there was a consistent positive relationship shown between LMI and cortical area in both men and women. Very similar relationships were identified for FMI. Associations with cortical thickness were weaker and there was no evidence of an association between radial cortical thickness and either LMI or FMI in women. When trabecular microarchitecture was assessed, positive associations were found between trabecular number and both LMI and FMI in men and women. This was seen in conjunction with a negative association between trabecular separation and both LMI and FMI,

although this only tended to reach statistical significance in the tibia. Interestingly, trabecular density was positively associated with FMI but not LMI in both men and women. Associations were also noted in women between LMI and radial total area and between FMI and tibial cortical density.

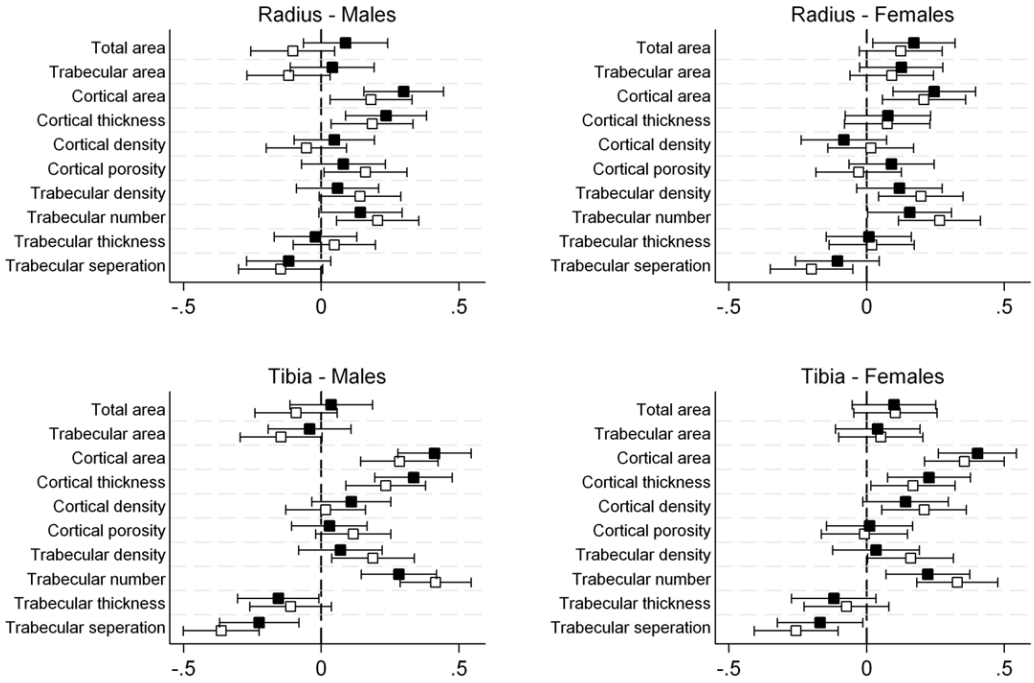

Figure 12.1: Regression coefficients and 95% confidence intervals for relationships between bone architecture and both lean mass index (black) and fat mass index (white)

Figure 12.1 illustrates that the effect sizes relating to cortical parameters tend to be greater with LMI whereas the opposite is true when trabecular number is considered. Furthermore, associations tend to be stronger in the tibia than the radius in both men and women. The relationships between body composition and bone architecture were then adjusted for demographic and lifestyle factors that might confound such associations (Table 12.4). This led to a small modulation of relationships with both cortical and trabecular parameters. Interestingly, after adjustment, FMI was significantly associated with tibial cortical density in women. There were also weak negative associations shown between FMI and radial total area and tibial trabecular area in men ($p=0.037$ and 0.039 respectively). The former relationship showed a significant interaction by sex, with no association found between FMI and radial total area in women.

Table 12.3: Univariate regression analysis of bone microarchitecture on lean mass index and fat mass index (all variables expressed as z-scores)

		Lean mass index				Fat mass index			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total	**R**	0.09 (-0.06,0.24)	0.260	**0.17 (0.02,0.32)**	**0.031**	-0.11 (-0.26,0.04)	0.163*	0.12 (-0.03,0.27)	0.125*
area	**T**	0.04 (-0.11,0.19)	0.632	0.10 (-0.05,0.25)	0.197	-0.09 (-0.24,0.06)	0.229	0.11 (-0.05,0.26)	0.175
Trabecular	**R**	0.04 (-0.11,0.19)	0.595	0.12 (-0.03,0.28)	0.124	-0.12 (-0.27,0.03)	0.115	0.09 (-0.07,0.24)	0.271
area	**T**	-0.04 (-0.19,0.11)	0.577	0.04 (-0.11,0.19)	0.601	-0.15 (-0.29,0.00)	0.054	0.05 (-0.10,0.21)	0.506
Cortical	**R**	**0.30 (0.16,0.45)**	**<0.001**	**0.25 (0.10,0.40)**	**0.001**	**0.18 (0.03,0.33)**	**0.018**	**0.21 (0.06,0.36)**	**0.006**
area	**T**	**0.42 (0.28,0.56)**	**<0.001**	**0.40 (0.26,0.54)**	**<0.001**	**0.29 (0.15,0.43)**	**<0.001**	**0.35 (0.21,0.50)**	**<0.001**
Cortical	**R**	**0.23 (0.09,0.38)**	**0.002**	0.08 (-0.07,0.24)	0.282	**0.18 (0.03,0.33)**	**0.017**	0.08 (-0.07,0.24)	0.295
thickness	**T**	**0.34 (0.20,0.48)**	**<0.001**	**0.22 (0.07,0.37)**	**0.004**	**0.24 (0.09,0.38)**	**0.002**	**0.17 (0.02,0.32)**	**0.03**
Cortical	**R**	0.05 (-0.10,0.20)	0.526	-0.08 (-0.23,0.08)	0.336	-0.05 (-0.20,0.10)	0.488	0.02 (-0.14,0.17)	0.813
density	**T**	0.11 (-0.04,0.26)	0.135	0.14 (-0.01,0.29)	0.075	0.02 (-0.13,0.17)	0.828	**0.21 (0.06,0.36)**	**0.008**
Cortical	**R**	0.08 (-0.07,0.23)	0.323	0.09 (-0.07,0.24)	0.278	**0.15 (0.00,0.30)**	**0.044**	-0.03 (-0.18,0.13)	0.717
porosity	**T**	0.03 (-0.12,0.18)	0.672	0.01 (-0.14,0.17)	0.883	0.13 (-0.02,0.28)	0.096	-0.01 (-0.16,0.14)	0.905
Trabecular	**R**	0.06 (-0.09,0.21)	0.467	0.12 (-0.03,0.28)	0.126	0.14 (-0.01,0.29)	0.069	**0.20 (0.04,0.35)**	**0.012**
density	**T**	0.07 (-0.08,0.22)	0.369	0.03 (-0.12,0.19)	0.660	**0.19 (0.04,0.33)**	**0.014**	**0.16 (0.00,0.31)**	**0.046**
Trabecular	**R**	0.14 (-0.01,0.29)	0.073	**0.16 (0.01,0.31)**	**0.042**	**0.21 (0.06,0.35)**	**0.006**	**0.27 (0.12,0.42)**	**0.001**
number	**T**	**0.30 (0.15,0.44)**	**<0.001**	**0.22 (0.07,0.37)**	**0.005**	**0.44 (0.30,0.57)**	**<0.001**	**0.33 (0.18,0.47)**	**<0.001**
Trabecular	**R**	-0.02 (-0.17,0.13)	0.759	0.01 (-0.15,0.17)	0.896	0.04 (-0.11,0.19)	0.595	0.02 (-0.13,0.17)	0.803
Thickness	**T**	**-0.16 (-0.30,-0.01)**	**0.038**	-0.12 (-0.27,0.04)	0.131	-0.11 (-0.26,0.04)	0.142	-0.08 (-0.23,0.08)	0.335
Trabecular	**R**	-0.11 (-0.26,0.04)	0.143	-0.11 (-0.27,0.04)	0.156	-0.15 (-0.30,0.00)	0.055	**-0.20 (-0.35,-0.05)**	**0.009**
separation	**T**	**-0.23 (-0.37,-0.08)**	**0.002**	**-0.16 (-0.32,-0.01)**	**0.035**	**-0.37 (-0.51,-0.23)**	**<0.001**	**-0.25 (-0.40,-0.10)**	**0.001**

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 12.4: Univariate regression analysis of bone microarchitecture on lean mass index and fat mass index with adjustment for demographic and lifestyle covariates (all variables expressed as z-scores)

		Lean mass index				Fat mass index			
		Men[1]		Women[2]		Men[1]		Women[2]	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area	**R**	0.08 (-0.10,0.25)	0.386	0.17 (-0.01,0.35)	0.068	**-0.19 (-0.37,-0.01)**	**0.037***	0.10 (-0.08,0.28)	0.281*
	T	0.10 (-0.07,0.27)	0.259	0.06 (-0.12,0.24)	0.499	-0.11 (-0.28,0.07)	0.236	0.04 (-0.13,0.21)	0.642
Trabecular area	**R**	0.05 (-0.13,0.22)	0.601	0.13 (-0.06,0.31)	0.172	-0.18 (-0.36,-0.00)	0.050	0.07 (-0.11,0.25)	0.424
	T	0.01 (-0.16,0.17)	0.945	-0.00 (-0.18,0.17)	0.967	**-0.18 (-0.35,-0.01)**	**0.039**	-0.01 (-0.18,0.16)	0.893
Cortical area	**R**	**0.31 (0.15,0.47)**	**<0.001**	**0.23 (0.05,0.40)**	**0.012**	0.16 (-0.02,0.33)	0.076	**0.18 (0.01,0.35)**	**0.040**
	T	**0.43 (0.27,0.58)**	**<0.001**	**0.45 (0.28,0.61)**	**<0.001**	**0.31 (0.15,0.48)**	**<0.001**	**0.37 (0.21,0.54)**	**<0.001**
Cortical thickness	**R**	**0.26 (0.10,0.43)**	**0.002**	0.06 (-0.12,0.24)	0.511	**0.21 (0.04,0.38)**	**0.018**	0.06 (-0.12,0.23)	0.529
	T	**0.33 (0.17,0.48)**	**<0.001**	**0.29 (0.11,0.46)**	**0.001**	**0.27 (0.11,0.44)**	**0.001**	**0.22 (0.05,0.39)**	**0.012**
Cortical density	**R**	0.11 (-0.06,0.27)	0.216	-0.06 (-0.24,0.13)	0.551	0.03 (-0.15,0.20)	0.747	-0.01 (-0.19,0.17)	0.919
	T	0.10 (-0.06,0.27)	0.218	0.14 (-0.04,0.32)	0.123	0.08 (-0.09,0.25)	0.374	**0.20 (0.03,0.37)**	**0.025**
Cortical porosity	**R**	0.06 (-0.09,0.22)	0.421	0.00 (-0.18,0.19)	0.982	0.10 (-0.05,0.26)	0.197	-0.03 (-0.21,0.14)	0.699
	T	0.06 (-0.11,0.22)	0.506	0.03 (-0.15,0.22)	0.742	0.09 (-0.09,0.26)	0.323	0.05 (-0.13,0.23)	0.612
Trabecular density	**R**	0.06 (-0.11,0.23)	0.468	0.12 (-0.06,0.29)	0.181	0.13 (-0.04,0.30)	0.141	**0.18 (0.01,0.35)**	**0.034**
	T	0.06 (-0.10,0.23)	0.448	0.05 (-0.13,0.22)	0.597	**0.19 (0.02,0.35)**	**0.028**	0.16 (-0.01,0.33)	0.061
Trabecular number	**R**	0.14 (-0.03,0.31)	0.110	**0.17 (0.00,0.34)**	**0.045**	**0.18 (0.01,0.36)**	**0.041**	**0.24 (0.08,0.40)**	**0.004**
	T	**0.26 (0.10,0.42)**	**0.001**	**0.26 (0.09,0.43)**	**0.003**	**0.41 (0.26,0.56)**	**<0.001**	**0.34 (0.18,0.50)**	**<0.001**
Trabecular Thickness	**R**	-0.01 (-0.18,0.15)	0.863	-0.01 (-0.19,0.17)	0.925	0.04 (-0.13,0.22)	0.629	0.02 (-0.15,0.19)	0.815
	T	-0.13 (-0.30,0.03)	0.104	-0.15 (-0.32,0.03)	0.109	-0.09 (-0.26,0.08)	0.274	-0.09 (-0.27,0.08)	0.309
Trabecular separation	**R**	-0.14 (-0.31,0.04)	0.122	-0.13 (-0.30,0.04)	0.142	-0.13 (-0.31,0.05)	0.150	**-0.18 (-0.35,-0.02)**	**0.030**
	T	**-0.20 (-0.36,-0.05)**	**0.012**	**-0.23 (-0.40,-0.05)**	**0.011**	**-0.35 (-0.51,-0.19)**	**<0.001**	**-0.28 (-0.45,-0.11)**	**0.001**

Key: R, radius; T, tibia; [1] Adjusted for age, smoking status, alcohol consumption, calcium intake, physical activity, social class, number of comorbidities and bisphosphonate use; [2] As adjusted 1 and time since menopause and HRT use. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Overall patterns of associations identified were similar for LMI and FMI. However, when both components were included in the same model the independent relationships differed considerably. LMI was positively associated with cortical area and thickness in both men and women although this did not reach statistical significance for radial cortical thickness in women. Conversely, FMI was positively associated with trabecular density and number and inversely related to trabecular separation, although associations with radial trabecular density and separation did not reach statistical significance in men (Table 12.5). These findings are very similar to associations between bone microarchitecture and LMI residuals and FMI residuals respectively (Table 12.6).

These associations were additionally adjusted for demographic and lifestyle variables and found to be very similar to those found in the unadjusted analyses (results not shown). Again, relationships were stronger in the tibia than the radius. This may be due to the former being a weight-bearing bone or reflect the greater precision of measurements in the tibia than the radius.

12.5 Results Summary

The main findings of this analysis were the associations of both LMI and FMI with cortical area, cortical thickness, and trabecular number. Effect sizes tended to be greater for cortical parameters with LMI and cortical geometry was associated with LMI but not FMI in the mutually adjusted model. In contrast, effect sizes for trabecular number tended to be greater for associations with FMI than LMI and, in the model including both LMI and FMI, strong relationships were shown between both trabecular density and number and FMI only. In the majority of cases, relationships in the tibia were stronger than those in the radius.

Table 12.5: Regression analysis of bone microarchitecture on lean mass index and fat mass index when mutually adjusted (all variables expressed as z-scores)

		Lean mass index				Fat mass index			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total	**R**	**0.17 (0.00,0.34)**	**0.046**	0.15 (-0.04,0.33)	0.114	**-0.18 (-0.35,-0.02)**	**0.031***	0.04 (-0.14,0.22)	0.634*
area	**T**	0.10 (-0.07,0.26)	0.251	0.06 (-0.12,0.24)	0.501	-0.14 (-0.30,0.03)	0.112	0.07 (-0.11,0.25)	0.428
Trabecular	**R**	0.12 (-0.05,0.29)	0.156	0.10 (-0.08,0.29)	0.260	**-0.18 (-0.34,-0.01)**	**0.041**	0.03 (-0.15,0.21)	0.732
area	**T**	0.03 (-0.14,0.20)	0.730	0.02 (-0.16,0.20)	0.841	-0.16 (-0.33,0.01)	0.062	0.04 (-0.14,0.22)	0.649
Cortical	**R**	**0.28 (0.12,0.44)**	**0.001**	**0.19 (0.02,0.37)**	**0.033**	0.05 (-0.11,0.21)	0.542	0.11 (-0.06,0.29)	0.208
area	**T**	**0.36 (0.21,0.52)**	**<0.001**	**0.29 (0.13,0.46)**	**0.001**	0.13 (-0.03,0.28)	0.104	**0.20 (0.03,0.36)**	**0.018**
Cortical	**R**	**0.19 (0.02,0.35)**	**0.025**	0.06 (-0.13,0.24)	0.538	0.09 (-0.07,0.26)	0.262	0.05 (-0.13,0.23)	0.573
thickness	**T**	**0.29 (0.13,0.45)**	**<0.001**	**0.19 (0.01,0.36)**	**0.038**	0.11 (-0.05,0.26)	0.191	0.07 (-0.11,0.25)	0.440
Cortical	**R**	0.09 (-0.08,0.26)	0.284	-0.12 (-0.30,0.06)	0.201	-0.10 (-0.26,0.07)	0.269	0.08 (-0.10,0.26)	0.381
density	**T**	0.13 (-0.03,0.30)	0.119	0.04 (-0.14,0.22)	0.654	-0.04 (-0.21,0.12)	0.609	**0.19 (0.01,0.36)**	**0.042**
Cortical	**R**	0.01 (-0.16,0.17)	0.937	0.14 (-0.04,0.32)	0.134	0.15 (-0.02,0.32)	0.080	-0.10 (-0.28,0.08)	0.271
porosity	**T**	-0.03 (-0.20,0.14)	0.713	0.02 (-0.16,0.21)	0.804	0.14 (-0.03,0.31)	0.099	-0.02 (-0.20,0.16)	0.817
Trabecular	**R**	-0.01 (-0.18,0.16)	0.909	0.02 (-0.16,0.20)	0.801	0.14 (-0.03,0.31)	0.096	**0.18 (0.00,0.36)**	**0.044**
density	**T**	-0.02 (-0.18,0.15)	0.821	-0.07 (-0.25,0.11)	0.465	**0.19 (0.03,0.36)**	**0.022**	**0.19 (0.01,0.37)**	**0.038**
Trabecular	**R**	0.05 (-0.11,0.22)	0.529	0.03 (-0.15,0.20)	0.779	**0.18 (0.02,0.35)**	**0.032**	**0.25 (0.08,0.43)**	**0.005**
number	**T**	0.12 (-0.03,0.28)	0.104	0.06 (-0.11,0.23)	0.475	**0.38 (0.23,0.53)**	**<0.001**	**0.29 (0.12,0.47)**	**0.001**
Trabecular	**R**	-0.05 (-0.22,0.12)	0.537	-0.00 (-0.18,0.18)	0.999	0.07 (-0.10,0.24)	0.451	0.02 (-0.16,0.20)	0.832
Thickness	**T**	-0.13 (-0.30,0.03)	0.115	-0.11 (-0.29,0.07)	0.240	-0.05 (-0.22,0.12)	0.544	-0.02 (-0.20,0.16)	0.845
Trabecular	**R**	-0.06 (-0.22,0.11)	0.505	-0.00 (-0.19,0.18)	0.956	-0.12 (-0.29,0.05)	0.161	**-0.20 (-0.38,-0.02)**	**0.028**
seperation	**T**	-0.08 (-0.23,0.08)	0.325	-0.04 (-0.22,0.13)	0.637	**-0.33 (-0.49,-0.18)**	**<0.001**	**-0.23 (-0.41,-0.05)**	**0.011**

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 12.6: Regression analysis of bone microarchitecture on lean mass index residual and fat mass index residual when explored in models with fat mass index and lean mass index respectively (all variables expressed as z-scores)

		Lean mass index residuals				Fat mass index residuals			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total	**R**	**0.15 (0.00,0.30)**	**0.047**	0.12 (-0.03,0.28)	0.114	**-0.17 (-0.32,-0.02)**	**0.029***	0.04 (-0.12,0.19)	0.633*
area	**T**	0.09 (-0.06,0.24)	0.255	0.05 (-0.10,0.21)	0.500	-0.12 (-0.27,0.03)	0.109	0.06 (-0.09,0.22)	0.430
Trabecular	**R**	0.11 (-0.04,0.26)	0.161	0.09 (-0.07,0.24)	0.262	**-0.16 (-0.31,-0.01)**	**0.039**	0.03 (-0.13,0.18)	0.730
area	**T**	0.03 (-0.12,0.17)	0.738	0.02 (-0.14,0.17)	0.839	-0.14 (-0.29,0.01)	0.059	0.04 (-0.12,0.19)	0.650
Cortical	**R**	**0.25 (0.11,0.39)**	**0.001**	**0.16 (0.01,0.31)**	**0.033**	0.04 (-0.10,0.19)	0.542	0.10 (-0.05,0.24)	0.208
area	**T**	**0.33 (0.19,0.46)**	**<0.001**	**0.25 (0.11,0.39)**	**0.001**	0.11 (-0.02,0.25)	0.098	**0.17 (0.03,0.31)**	**0.018**
Cortical	**R**	**0.17 (0.02,0.32)**	**0.025**	0.05 (-0.11,0.20)	0.537	0.08 (-0.06,0.23)	0.259	0.04 (-0.11,0.20)	0.575
thickness	**T**	**0.26 (0.12,0.40)**	**<0.001**	**0.16 (0.01,0.31)**	**0.039**	0.10 (-0.05,0.24)	0.182	0.06 (-0.09,0.21)	0.444
Cortical	**R**	0.08 (-0.07,0.23)	0.284	-0.10 (-0.26,0.05)	0.200	-0.08 (-0.24,0.07)	0.270	0.07 (-0.09,0.22)	0.390
density	**T**	0.12 (-0.03,0.27)	0.122	0.03 (-0.12,0.19)	0.657	-0.04 (-0.19,0.11)	0.601	**0.16 (0.00,0.31)**	**0.043**
Cortical	**R**	0.01 (-0.14,0.16)	0.940	0.12 (-0.04,0.27)	0.131	0.13 (-0.02,0.28)	0.080	-0.08 (-0.24,0.07)	0.281
porosity	**T**	-0.03 (-0.17,0.12)	0.741	0.02 (-0.13,0.17)	0.801	0.13 (-0.02,0.28)	0.092	-0.02 (-0.17,0.14)	0.829
Trabecular	**R**	-0.01 (-0.16,0.14)	0.915	0.02 (-0.13,0.17)	0.799	0.13 (-0.02,0.28)	0.095	**0.16 (0.00,0.31)**	**0.044**
density	**T**	-0.02 (-0.16,0.13)	0.827	-0.06 (-0.21,0.10)	0.462	**0.17 (0.03,0.32)**	**0.022**	**0.16 (0.01,0.31)**	**0.038**
Trabecular	**R**	0.05 (-0.10,0.20)	0.528	0.02 (-0.13,0.17)	0.779	**0.16 (0.01,0.31)**	**0.032**	**0.21 (0.07,0.36)**	**0.005**
number	**T**	0.11 (-0.02,0.25)	0.102	0.05 (-0.09,0.20)	0.474	**0.34 (0.20,0.47)**	**<0.001**	**0.25 (0.10,0.40)**	**0.001**
Trabecular	**R**	-0.05 (-0.20,0.10)	0.543	0.00 (-0.16,0.16)	0.997	0.06 (-0.09,0.21)	0.447	0.02 (-0.14,0.17)	0.830
Thickness	**T**	-0.12 (-0.27,0.03)	0.116	-0.09 (-0.25,0.06)	0.238	-0.04 (-0.19,0.10)	0.551	-0.02 (-0.17,0.14)	0.842
Trabecular	**R**	-0.05 (-0.20,0.10)	0.505	-0.00 (-0.16,0.15)	0.957	-0.11 (-0.26,0.04)	0.159	**-0.17 (-0.32,-0.02)**	**0.028**
separation	**T**	-0.07 (-0.21,0.07)	0.324	-0.04 (-0.19,0.11)	0.638	**-0.30 (-0.44,-0.16)**	**<0.001**	**-0.19 (-0.34,-0.05)**	**0.011**

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

13.Results: Relationships between early life and bone microarchitecture

13.1 Aim

Explore the associations between early life (birth weight, conditional growth in the first year, and weight at 1 year) and bone microarchitecture in the radius and tibia.

13.2 Summary of methods

Full details of the study methodology can be found in section 9 and the cohort is defined in figure 9.2. All individuals recruited into the HCS were identified through birth records – these included data on birth weight and weight at 1 year. From this information conditional growth in the first year was calculated as described below. Those participants who attended the MRC Human Nutrition Research in Cambridge underwent a detailed examination of bone microarchitecture in both the radius and tibia using the Scanco Xtreme HRpQCT scanner. Height and weight were also assessed during the visit. Additional covariates within the analysis were obtained from previous studies within the HCS described in section 9.2. Smoking status, alcohol consumption and bisphosphonate use were obtained from data collection in 2004-2005. Calcium intake, physical activity, social class, time since menopause, HRT use and number of comorbidities were obtained from baseline HCS data collection (1998-2003). Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

13.3 Analyses

Conditional growth in the first year was calculated to take account of birth weight when assessing growth in the first year using the method of Cole(336). Smaller babies would be expected to grow more than larger babies due to "catch up" growth and regression to the mean. Conditional growth is

independent of birth weight and thus gives a measure of how the baby grew in comparison to what would be expected.

Birth weight, conditional growth in the first year, and weight at 1 year were assessed for normality and found to be normally distributed. These variables are described in table 13.1; differences between the two sexes were assessed using a Student's t-test. Birth weight and weight at 1 year were then standardised.

As bone microarchitecture differed significantly by sex, men and women were evaluated separately. Regression analyses were completed to explore relationships between bone microarchitecture (radius and tibia) and birth weight, conditional growth in the first year, and weight at 1 year. These associations were then subject to adjustment for adult size, and subsequently for additional covariates including age, calcium intake, physical activity, alcohol consumption, smoking status, social class, and in women, time since menopause and use of HRT. To ascertain whether there was a relationship between early life and bone microarchitecture independent of bone size, the regression analysis were adjusted for total bone area at the site being investigated(337, 338). When regression analyses were carried out in men and women separately, an additional analysis was completed to assess for interaction by sex.

As the results of the initial analyses suggested that associations between early life and bone microarchitecture showed some similar patterns to those for adult body composition (LMI and FMI), the relationships between early life and body composition were then assessed using linear regression in men and women. Thereafter, multiple linear regression was used to assess associations between important bone outcomes, such as tibial cortical thickness, and birth weight, conditional growth in the first year, LMI and FMI. The outcome of this analysis was used to create an equation to determine tibial cortical thickness. Furthermore, in order to assess how much of the outcome each variable explained, partial correlation coefficients were also ascertained. Statistical significance was assessed as described in section 11.3.

13.4 Results

The mean(SD) birth weight was 3.53(0.56)kg and 3.44(0.50)kg in men and women respectively. Although birth weight did not differ by sex, men were heavier at 1 year of age than women (Table 13.1).

Table 13.1: Summary of birth weight and weight at 1 year

	Men Mean (SD)	Women Mean (SD)	p value
Birth weight (kg)	3.53 (0.56)	3.44 (0.50)	0.085
Weight at 1 year (kg)	10.2 (1.09)	9.73 (1.01)	<0.001

In the unadjusted analyses, birth weight was strongly associated with total and trabecular area in both men and women (Table 13.2; Figure 13.1). In general, regression coefficients tended to be greater in the tibia than the radius although confidence intervals overlapped. Adjustment for height and weight, did lead to attenuation of these relationships but significant associations were maintained in all cases with the exception of the radius in women. Additional adjustment for covariates including calcium intake, physical activity, smoking status, alcohol consumption, social class, bisphosphonate use, number of comorbidities, and in women, time since menopause and HRT use, led to attenuation of relationships between birth weight and radial total and trabecular area in men (Table 13.5).

In women, there was a negative association between birth weight and tibial cortical thickness (Table 13.2; Figure 13.1). Assessment of the trabecular region also showed a negative relationship between birth weight and trabecular density and trabecular thickness in women. There was little change in these relationships after adjustment for adult size (height and weight). After this adjustment, interactions by sex were shown for both tibial cortical thickness and radial trabecular density.

As cortical thickness, trabecular density and trabecular thickness were all negatively correlated with total area (Table 10.6, Chapter 10) it was thought possible that these relationships were purely driven by the greater bone size in individuals with higher birth weight. To explore this hypothesis further, all associations were adjusted for total area in the corresponding bone and this

lead to considerable attenuation of the negative relationships described (Table 13.6). Interestingly, after similar adjustment in men, positive associations were evident with cortical area and thickness.

The relationships between conditional growth in the first year and bone microarchitecture were less consistent. Although positive associations were seen with total area and trabecular area in men, the relationship did not reach statistical significance for trabecular area in the radius, and there was no evidence of such associations in women (Table 13.3; Figure 13.1). All relationships between bone geometry and conditional growth in the first year are fully attenuated after adjustment for adult height and weight.

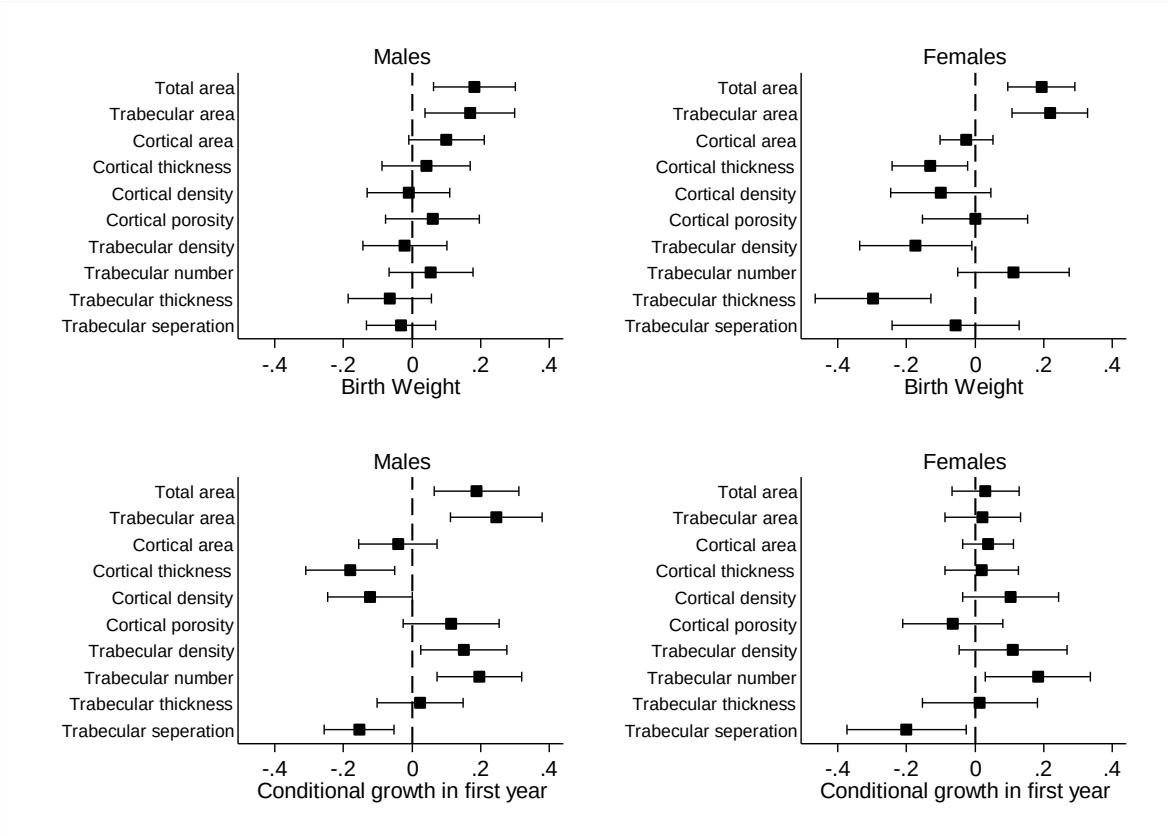

Figure 13.1: Regression coefficients and 95% confidence intervals for relationships between early life environment and tibial microarchitecture

Table 13.2: Regression analysis of bone microarchitecture on birth weight (all variables expressed as z-scores)

		Men				Women			
		Unadjusted		Adjusted for height and weight		Unadjusted		Adjusted for height and weight	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area	**R**	**0.14 (0.03,0.25)**	**0.014**	**0.12 (0.02,0.22)**	**0.023**	**0.08 (0.01,0.15)**	**0.033**	0.04 (-0.02,0.11)	0.203
	T	**0.18 (0.06,0.30)**	**0.003**	**0.14 (0.04,0.24)**	**0.006**	**0.19 (0.09,0.29)**	**<0.001**	**0.14 (0.05,0.23)**	**0.002**
Trabecular area	**R**	**0.13 (0.01,0.25)**	**0.029**	**0.11 (0.00,0.22)**	**0.043**	**0.09 (0.01,0.18)**	**0.034**	0.05 (-0.03,0.13)	0.182
	T	**0.17 (0.04,0.30)**	**0.012**	**0.13 (0.02,0.24)**	**0.020**	**0.22 (0.11,0.33)**	**<0.001**	**0.16 (0.06,0.26)**	**0.002**
Cortical area	**R**	0.10 (-0.01,0.21)	0.082	0.07 (-0.04,0.18)	0.198	-0.02 (-0.10,0.06)	0.595	-0.04 (-0.12,0.04)	0.341
	T	0.10 (-0.01,0.21)	0.076	0.07 (-0.03,0.17)	0.191	-0.03 (-0.10,0.05)	0.510	-0.04 (-0.11,0.03)	0.306
Cortical thickness	**R**	0.06 (-0.07,0.20)	0.373	0.05 (-0.09,0.18)	0.479	-0.08 (-0.22,0.05)	0.234	-0.08 (-0.21,0.06)	0.268
	T	0.04 (-0.09,0.17)	0.525*	0.04 (-0.08,0.16)	0.494*	**-0.13 (-0.24,-0.02)**	**0.019***	**-0.11 (-0.22,-0.01)**	**0.037***
Cortical density	**R**	-0.04 (-0.16,0.09)	0.577	-0.02 (-0.15,0.10)	0.707	-0.00 (-0.18,0.17)	0.983	0.01 (-0.17,0.18)	0.933
	T	-0.01 (-0.13,0.11)	0.867	0.00 (-0.12,0.12)	0.959	-0.10 (-0.25,0.05)	0.179	-0.10 (-0.25,0.05)	0.176
Cortical porosity	**R**	0.10 (-0.04,0.23)	0.153	0.07 (-0.06,0.21)	0.274	-0.10 (-0.25,0.06)	0.224	-0.09 (-0.25,0.06)	0.241
	T	0.06 (-0.08,0.20)	0.390	0.03 (-0.11,0.16)	0.706	-0.00 (-0.15,0.15)	0.999	0.00 (-0.15,0.16)	0.968
Trabecular density	**R**	0.00 (-0.12,0.12)	0.992	-0.02 (-0.13,0.10)	0.779	**-0.20 (-0.36,-0.05)**	**0.009**	**-0.22 (-0.37,-0.07)**	**0.005**
	T	-0.02 (-0.14,0.10)	0.736	-0.04 (-0.16,0.08)	0.514	**-0.17 (-0.34,-0.01)**	**0.037**	**-0.21 (-0.38,-0.05)**	**0.011**
Trabecular number	**R**	0.06 (-0.04,0.15)	0.243	0.03 (-0.06,0.13)	0.455*	-0.16 (-0.34,0.02)	0.077	**-0.21 (-0.38,-0.03)**	**0.024***
	T	0.06 (-0.07,0.18)	0.377	-0.01 (-0.11,0.10)	0.891	0.11 (-0.05,0.27)	0.176	0.05 (-0.10,0.21)	0.494
Trabecular Thickness	**R**	-0.03 (-0.16,0.10)	0.678	-0.03 (-0.16,0.10)	0.625	**-0.17 (-0.32,-0.02)**	**0.031**	-0.15 (-0.31,0.00)	0.054
	T	-0.07 (-0.19,0.06)	0.292	-0.04 (-0.16,0.08)	0.519	**-0.30 (-0.47,-0.13)**	**0.001**	**-0.30 (-0.47,-0.12)**	**0.001**
Trabecular seperation	**R**	-0.04 (-0.11,0.03)	0.312	-0.02 (-0.10,0.05)	0.489*	**0.21 (0.01,0.40)**	**0.040**	**0.24 (0.04,0.44)**	**0.018***
	T	-0.03 (-0.13,0.07)	0.518	0.01 (-0.08,0.10)	0.840	-0.06 (-0.24,0.13)	0.545	-0.00 (-0.18,0.18)	0.998

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 13.3: Regression analysis of bone microarchitecture on conditional growth in the first year (all variables expressed as z-scores)

		Men				Women			
		Unadjusted		Adjusted for height and weight		Unadjusted		Adjusted for height and weight	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total	**R**	**0.15 (0.04,0.27)**	**0.007**	0.03 (-0.08,0.15)	0.579	0.03 (-0.04,0.10)	0.336	-0.01 (-0.07,0.06)	0.873
area	**T**	**0.19 (0.06,0.31)**	**0.003**	-0.02 (-0.13,0.09)	0.707	0.03 (-0.07,0.13)	0.551	-0.03 (-0.12,0.06)	0.471
Trabecular	**R**	0.11 (-0.02,0.23)	0.091	-0.03 (-0.16,0.09)	0.617	0.04 (-0.04,0.12)	0.362	-0.01 (-0.08,0.07)	0.893
area	**T**	**0.25 (0.11,0.38)**	**<0.001**	0.03 (-0.09,0.15)	0.618	0.02 (-0.09,0.13)	0.691	-0.05 (-0.15,0.05)	0.362
Cortical	**R**	0.08 (-0.03,0.20)	0.165	0.03 (-0.09,0.16)	0.584	0.01 (-0.07,0.09)	0.767	0.00 (-0.07,0.08)	0.911
area	**T**	-0.04 (-0.16,0.07)	0.479	-0.08 (-0.19,0.04)	0.186	0.04 (-0.04,0.11)	0.322	0.05 (-0.02,0.12)	0.172
Cortical	**R**	-0.08 (-0.22,0.06)	0.247	-0.07 (-0.21,0.08)	0.391	-0.03 (-0.16,0.10)	0.692	-0.01 (-0.14,0.12)	0.871
thickness	**T**	**-0.18 (-0.31,-0.05)**	**0.007**	-0.10 (-0.23,0.03)	0.139	0.02 (-0.09,0.13)	0.727	0.06 (-0.04,0.16)	0.247
Cortical	**R**	-0.12 (-0.25,0.01)	0.060	-0.10 (-0.24,0.04)	0.161	0.02 (-0.14,0.19)	0.779	0.03 (-0.14,0.20)	0.700
density	**T**	-0.12 (-0.25,0.00)	0.053	-0.06 (-0.20,0.07)	0.341	0.10 (-0.04,0.24)	0.143	0.13 (-0.01,0.27)	0.068
Cortical	**R**	**0.16 (0.03,0.30)**	**0.021**	0.15 (-0.00,0.29)	0.054	-0.02 (-0.17,0.13)	0.813	-0.01 (-0.16,0.14)	0.897
porosity	**T**	0.11 (-0.03,0.25)	0.109	0.05 (-0.10,0.19)	0.538	-0.07 (-0.21,0.08)	0.383	-0.06 (-0.21,0.09)	0.426
Trabecular	**R**	**0.15 (0.03,0.27)**	**0.013**	**0.15 (0.02,0.27)**	**0.025**	-0.01 (-0.16,0.14)	0.894	-0.00 (-0.15,0.15)	0.971
density	**T**	**0.15 (0.03,0.28)**	**0.019**	**0.16 (0.03,0.30)**	**0.019**	0.11 (-0.05,0.27)	0.169	0.09 (-0.07,0.25)	0.255
Trabecular	**R**	**0.10 (0.01,0.20)**	**0.038**	0.08 (-0.02,0.18)	0.130	-0.00 (-0.18,0.17)	0.977	-0.02 (-0.19,0.15)	0.820
number	**T**	**0.20 (0.07,0.32)**	**0.002**	0.10 (-0.01,0.22)	0.081	**0.18 (0.03,0.34)**	**0.019**	**0.16 (0.01,0.31)**	**0.032**
Trabecular	**R**	0.11 (-0.02,0.24)	0.102	0.12 (-0.02,0.27)	0.091	0.01 (-0.13,0.16)	0.843	0.04 (-0.11,0.19)	0.571
Thickness	**T**	0.02 (-0.10,0.15)	0.719	0.11 (-0.02,0.24)	0.099	0.01 (-0.15,0.18)	0.870	0.01 (-0.16,0.18)	0.881
Trabecular	**R**	-0.05 (-0.12,0.02)	0.172	-0.04 (-0.12,0.04)	0.333	-0.03 (-0.22,0.16)	0.736	-0.03 (-0.22,0.17)	0.792
separation	**T**	**-0.15 (-0.26,-0.05)**	**0.003**	-0.09 (-0.19,0.01)	0.068	**-0.20 (-0.37,-0.03)**	**0.024**	**-0.17 (-0.35,-0.00)**	**0.046**

Key: R, radius; T, tibia. No significant interactions by sex.

Table 13.4: Regression analysis of bone microarchitecture on weight at 1 year (all variables expressed as z-scores)

		Men				Women			
		Unadjusted		Adjusted for height and weight		Unadjusted		Adjusted for height and weight	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total	**R**	**0.21 (0.10,0.32)**	**<0.001**	0.09 (-0.03,0.21)	0.126	0.06 (-0.01,0.14)	0.090	0.01 (-0.06,0.08)	0.740
area	**T**	**0.26 (0.14,0.38)**	**<0.001**	0.05 (-0.07,0.16)	0.418	0.10 (-0.00,0.20)	0.052	0.02 (-0.07,0.11)	0.651
Trabecular	**R**	**0.16 (0.03,0.28)**	**0.012**	0.02 (-0.10,0.15)	0.699	0.07 (-0.01,0.16)	0.101	0.02 (-0.07,0.10)	0.705
area	**T**	**0.31 (0.18,0.44)**	**<0.001**	0.09 (-0.03,0.22)	0.136	0.10 (-0.01,0.22)	0.078	0.02 (-0.09,0.12)	0.765
Cortical	**R**	**0.12 (0.00,0.24)**	**0.041**	0.07 (-0.06,0.19)	0.273	0.00 (-0.08,0.08)	0.942	-0.01 (-0.09,0.07)	0.793
area	**T**	0.00 (-0.11,0.12)	0.990	-0.04 (-0.16,0.07)	0.465	0.03 (-0.05,0.10)	0.500	0.03 (-0.04,0.11)	0.372
Cortical	**R**	-0.06 (-0.20,0.09)	0.440	-0.04 (-0.19,0.11)	0.591	-0.06 (-0.19,0.08)	0.414	-0.04 (-0.18,0.10)	0.563
thickness	**T**	**-0.16 (-0.29,-0.03)**	**0.019**	-0.08 (-0.22,0.06)	0.248	-0.03 (-0.14,0.08)	0.587	0.02 (-0.09,0.13)	0.767
Cortical	**R**	**-0.14 (-0.26,-0.01)**	**0.041**	-0.11 (-0.26,0.03)	0.122	0.02 (-0.15,0.20)	0.802	0.04 (-0.14,0.22)	0.694
density	**T**	-0.12 (-0.25,0.00)	0.053	-0.06 (-0.20,0.07)	0.363	0.06 (-0.08,0.21)	0.395	0.09 (-0.06,0.24)	0.235
Cortical	**R**	**0.20 (0.06,0.34)**	**0.005***	**0.18 (0.03,0.33)**	**0.018***	-0.05 (-0.21,0.10)	0.497*	-0.05 (-0.21,0.11)	0.567*
porosity	**T**	0.13 (-0.00,0.27)	0.058	0.06 (-0.09,0.21)	0.443	-0.06 (-0.21,0.09)	0.423	-0.06 (-0.22,0.10)	0.470
Trabecular	**R**	**0.15 (0.03,0.26)**	**0.016***	**0.14 (0.01,0.27)**	**0.038***	-0.09 (-0.24,0.07)	0.276*	-0.09 (-0.25,0.07)	0.278*
density	**T**	**0.14 (0.01,0.26)**	**0.032**	**0.14 (0.00,0.28)**	**0.043**	0.04 (-0.12,0.21)	0.617	0.01 (-0.16,0.18)	0.908
Trabecular	**R**	**0.12 (0.03,0.22)**	**0.013**	0.09 (-0.01,0.20)	0.070	-0.06 (-0.25,0.12)	0.495	-0.10 (-0.28,0.08)	0.286
number	**T**	**0.21 (0.09,0.34)**	**0.001**	0.10 (-0.02,0.21)	0.097	**0.22 (0.06,0.38)**	**0.007**	**0.18 (0.02,0.33)**	**0.023**
Trabecular	**R**	0.10 (-0.04,0.23)	0.156	0.11 (-0.04,0.26)	0.149	-0.05 (-0.20,0.11)	0.537	-0.02 (-0.18,0.14)	0.844
Thickness	**T**	-0.00 (-0.13,0.12)	0.948	0.09 (-0.04,0.23)	0.181	-0.10 (-0.27,0.08)	0.272	-0.10 (-0.28,0.08)	0.269
Trabecular	**R**	-0.06 (-0.14,0.01)	0.084	-0.05 (-0.13,0.03)	0.211	0.05 (-0.15,0.24)	0.655	0.07 (-0.14,0.27)	0.522
separation	**T**	**-0.16 (-0.26,-0.06)**	**0.002**	-0.09 (-0.19,0.01)	0.088	**-0.21 (-0.40,-0.03)**	**0.021**	-0.17 (-0.35,0.01)	0.061

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 13.5: Regression analysis of bone microarchitecture on birth weight and conditional growth in the first year (all variables expressed as z-scores) after adjustment for anthropometry, demographic and lifestyle factors in men and women

		Birth weight				Conditional growth in first year			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
	R	0.09 (-0.02,0.20)	0.122	0.05 (-0.02,0.13)	0.188	0.05 (-0.07,0.17)	0.389	-0.01 (-0.08,0.06)	0.803
Total area	T	**0.15 (0.04,0.25)**	**0.005**	**0.14 (0.05,0.24)**	**0.004**	-0.03 (-0.14,0.09)	0.620	-0.00 (-0.09,0.09)	0.982
Trabecular	R	0.09 (-0.03,0.21)	0.160	0.07 (-0.02,0.16)	0.142	-0.03 (-0.16,0.11)	0.698	-0.00 (-0.09,0.08)	0.932
area	T	**0.13 (0.02,0.24)**	**0.023**	**0.17 (0.06,0.28)**	**0.002**	0.02 (-0.10,0.15)	0.721	-0.01 (-0.11,0.10)	0.894
Cortical	R	0.07 (-0.05,0.19)	0.224	-0.07 (-0.16,0.02)	0.131	0.08 (-0.05,0.21)	0.219	-0.02 (-0.11,0.07)	0.640
area	T	0.09 (-0.02,0.21)	0.102*	-0.06 (-0.15,0.02)	0.133*	-0.06 (-0.18,0.07)	0.362	0.03 (-0.05,0.11)	0.448
Cortical	R	0.08 (-0.07,0.23)	0.279	-0.12 (-0.28,0.03)	0.109	-0.04 (-0.19,0.12)	0.642	-0.05 (-0.20,0.10)	0.505
thickness	T	0.05 (-0.08,0.18)	0.422*	**-0.15 (-0.27,-0.03)**	**0.014***	-0.08 (-0.21,0.06)	0.274	0.01 (-0.10,0.13)	0.805
Cortical	R	0.02 (-0.11,0.16)	0.733	-0.01 (-0.22,0.19)	0.884	-0.08 (-0.23,0.07)	0.279	-0.06 (-0.25,0.13)	0.528
density	T	0.01 (-0.12,0.14)	0.839	-0.10 (-0.26,0.07)	0.239	-0.03 (-0.17,0.11)	0.645	0.06 (-0.09,0.22)	0.419
Cortical	R	0.03 (-0.10,0.16)	0.665	-0.14 (-0.32,0.04)	0.116	**0.15 (0.01,0.29)**	**0.031**	0.03 (-0.14,0.20)	0.739
porosity	T	0.03 (-0.11,0.18)	0.671	-0.05 (-0.23,0.13)	0.601	0.02 (-0.14,0.17)	0.811	-0.05 (-0.22,0.12)	0.560
Trabecular	R	0.01 (-0.11,0.14)	0.829*	**-0.28 (-0.44,-0.12)**	**0.001***	**0.16 (0.03,0.30)**	**0.018***	0.01 (-0.15,0.17)	0.893*
density	T	0.01 (-0.12,0.14)	0.933	**-0.24 (-0.42,-0.06)**	**0.008**	**0.14 (0.01,0.28)**	**0.041**	0.08 (-0.10,0.25)	0.383
Trabecular	R	0.05 (-0.05,0.15)	0.311**	**-0.31 (-0.49,-0.12)**	**0.002****	0.09 (-0.01,0.20)	0.086	0.04 (-0.15,0.23)	0.671
number	T	0.05 (-0.06,0.16)	0.410	0.05 (-0.12,0.22)	0.525	0.06 (-0.05,0.18)	0.288	**0.20 (0.04,0.35)**	**0.014**
Trabecular	R	0.00 (-0.14,0.14)	0.958	**-0.18 (-0.35,-0.00)**	**0.045**	0.13 (-0.02,0.28)	0.098	0.01 (-0.15,0.18)	0.890
Thickness	T	-0.03 (-0.16,0.10)	0.650	**-0.34 (-0.53,-0.15)**	**0.001**	0.12 (-0.02,0.26)	0.084	-0.05 (-0.24,0.14)	0.596
Trabecular	R	-0.04 (-0.12,0.04)	0.321**	**0.35 (0.14,0.56)**	**0.001****	-0.04 (-0.13,0.04)	0.323	-0.09 (-0.30,0.12)	0.415
separation	T	-0.04 (-0.13,0.06)	0.457	-0.01 (-0.21,0.19)	0.924	-0.06 (-0.16,0.05)	0.284	**-0.23 (-0.42,-0.05)**	**0.014**

Key: R, radius; T, tibia.;[1] Adjusted for height, weight, calcium intake, physical activity, smoking, alcohol, social class, bisphosphonate use, number of comorbidities (and in women, time since menopause and HRT use). Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table 13.6: Regression analysis of bone microarchitecture on birth weight and conditional weight at 1 year after adjustment for total area of the respective bone in men and women

		Birth weight				Conditional growth in first year			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Cortical	**R**	0.10 (-0.02,0.22)	0.088	-0.02 (-0.10,0.06)	0.642	0.08 (-0.04,0.20)	0.177	0.01 (-0.06,0.09)	0.743
area	**T**	**0.12 (0.01,0.23)**	**0.032**	0.00 (-0.07,0.08)	0.912	-0.02 (-0.14,0.09)	0.684	0.04 (-0.03,0.11)	0.256
Cortical	**R**	**0.15 (0.02,0.27)**	**0.018**	-0.01 (-0.13,0.11)	0.842	0.01 (-0.12,0.13)	0.903	0.00 (-0.11,0.12)	0.941
thickness	**T**	**0.15 (0.04,0.26)**	**0.007***	-0.01 (-0.11,0.08)	0.800*	-0.08 (-0.19,0.04)	0.187	0.04 (-0.05,0.12)	0.400
Cortical	**R**	0.03 (-0.09,0.15)	0.634	0.08 (-0.08,0.24)	0.318	-0.05 (-0.18,0.07)	0.382	0.06 (-0.09,0.21)	0.442
density	**T**	0.06 (-0.06,0.17)	0.319	-0.01 (-0.16,0.13)	0.854	-0.06 (-0.18,0.06)	0.351	0.12 (-0.02,0.25)	0.082
Cortical	**R**	0.08 (-0.06,0.21)	0.277	-0.10 (-0.25,0.06)	0.236	0.14 (0.00,0.28)	0.050	-0.02 (-0.17,0.13)	0.828
porosity	**T**	0.02 (-0.12,0.16)	0.764	0.02 (-0.14,0.18)	0.848	0.08 (-0.06,0.22)	0.285	-0.06 (-0.21,0.08)	0.399
Trabecular	**R**	0.03 (-0.09,0.14)	0.662	**-0.17 (-0.32,-0.02)**	**0.028**	**0.18 (0.07,0.30)**	**0.002***	0.01 (-0.14,0.15)	0.931*
density	**T**	0.01 (-0.11,0.14)	0.817	-0.11 (-0.28,0.06)	0.193	**0.19 (0.07,0.32)**	**0.003**	0.12 (-0.03,0.27)	0.120
Trabecular	**R**	0.06 (-0.04,0.15)	0.248	-0.16 (-0.35,0.02)	0.084	**0.10 (0.01,0.20)**	**0.038**	-0.00 (-0.18,0.18)	0.997
number	**T**	0.03 (-0.10,0.15)	0.677	0.08 (-0.08,0.25)	0.323	**0.17 (0.05,0.30)**	**0.007**	**0.18 (0.03,0.33)**	**0.022**
Trabecular	**R**	0.01 (-0.12,0.14)	0.892	-0.11 (-0.26,0.03)	0.126	**0.16 (0.03,0.29)**	**0.019**	0.04 (-0.10,0.18)	0.576
Thickness	**T**	0.00 (-0.11,0.12)	0.975	**-0.19 (-0.36,-0.02)**	**0.027**	0.10 (-0.02.0.21)	0.118	0.03 (-0.12,0.19)	0.676
Trabecular	**R**	-0.04 (-0.11,0.03)	0.265*	0.20 (-0.00,0.40)	0.054*	-0.06 (-0.13,0.02)	0.136	-0.04 (-0.23,0.15)	0.685
separation	**T**	-0.02 (-0.13,0.08)	0.659	-0.05 (-0.24,0.15)	0.632	**-0.15 (-0.25,-0.04)**	**0.006**	**-0.20 (-0.37,-0.02)**	**0.026**

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001

In men, there was a positive association between conditional growth and radial cortical porosity and a negative association with tibial cortical thickness although these were fully attenuated after adjustment for adult size. Trabecular number and density were both positively associated with conditional growth in the first year in men whereas this was only true for tibial trabecular number in women(Table 13.3; Figure 13.1). These relationships were robust to adjustment for height and weight in men.

Similarly to conditional growth in the first year, weight at 1 year was positively associated with total area, trabecular area, trabecular density and trabecular number in men (Table 13.4). However, of these, only the relationships with trabecular density were maintained after adjustment for adult size. In women, tibial trabecular number was positively associated with weight at 1 year. This relationship was robust to adjustment for adult size (Table 13.4).

13.5 Results Summary

In men, positive relationships were identified between both birth weight and conditional growth in the first year and bone size (total and trabecular area). Trabecular density and number were both associated with growth in the first year and, after adjustment for bone size, birth weight was also positively related to cortical area and thickness.

As cortical area and thickness, and trabecular density are associated with lean mass and fat mass respectively, an analysis was subsequently performed to assess relationships between early life and body composition to explore whether a common developmental origin might explain part of the associations seen (Table 13.7). Lean mass was positively associated with birth weight and conditional growth in the first year, whereas fat mass was only positively related to the latter.

In women, relationships were found between bone size (total and trabecular area) and birth weight, but not conditional growth in the first year. Although birth weight was negatively related to some parameters (cortical thickness, trabecular density, and trabecular thickness) these relationships were substantially attenuated after adjustment for bone size.

Table 13.7: Regression analysis of body composition (lean mass and fat mass) on birth weight and conditional growth in the first year in men and women

	Men		Women	
	β (95% CI)	p value	β (95% CI)	p value
Birth weight				
Lean mass (kg)	**1.05 (0.27,1.83)**	**0.009**	**1.05 (0.34,1.75)**	**0.004**
Fat mass (kg)	-0.38 (-1.65,0.88)	0.551	1.22 (-0.29,2.73)	0.112
Conditional growth in first year				
Lean mass (kg)	**1.17 (0.41,1.94)**	**0.003**	**0.86 (0.18,1.54)**	**0.013**
Fat mass (kg)	**1.54 (0.32,2.74)**	**0.014**	-0.02 (-1.48,1.44)	0.981

Key: No significant interactions by sex.

13.6 Combined models of early life and body composition

In multiple regression models that included birth weight, conditional growth in the first year, LMI and FMI, it was found that tibial cortical thickness was positively associated with LMI in both men and women (r^2=0.642 and 0.348 respectively) (Table 13.8). Negative relationships were, however, shown with birth weight in women and conditional growth in the first year in men. Using these results, it was possible to create an equation to predict cortical thickness in each sex as shown below.

Tibial cortical thickness in men, µm = 306 - 26.2(birth weight, kg) - 42.2(conditional growth in the first year, z-score) + 51.3(LMI, kg/m^2) + 11.4(FMI, kg/m^2)

Tibial cortical thickness in women, µm = 974 - 92.4(birth weight, kg) + 0.81(conditional growth in the first year, z-score) + 26.1(LMI, kg/m^2) + 4.16(FMI, kg/m^2)

In a similar analysis with tibial trabecular density as the outcome, birth weight and FMI both showed significant associations in women with similar variance explained by growth in early life as to adult body composition. No such relationships were shown in men. In contrast, the majority of variance within the model of tibial total area was explained by early life in both men and women. In women, birth weight alone explained around 8% of the variation.

Table 13.8: Multiple regression analysis of tibial microarchitecture on birth weight, conditional growth in the first year, lean mass index and fat mass index in men and women

	Men			Women		
	β (95% CI)	**R^2**	**p value**	**β (95% CI)**	**R^2**	**p value**
Cortical thickness (µm)						
Birth weight (kg)	-26.2 (-98.6,46.2)	0.0030	0.476	**-92.4 (-150,-35.0)**	**0.0598**	**0.002**
Growth 1st year[1]	**-42.2 (-78.9,-5.52)**	**0.0296**	**0.024**	0.81 (-28.2,29.8)	0.0000	0.956
Lean mass index (kg/m^2)	**51.3 (21.6,81.0)**	**0.0642**	**0.001**	**26.1 (4.59,47.7)**	**0.0348**	**0.018**
Fat mass index (kg/m^2)	11.4 (-3.68,26.6)	0.0130	0.137	4.16 (-5.14,13.5)	0.0049	0.379
Trabecular density (g/cm^3)						
Birth weight (kg)	-4.68 (-14.9,5.52)	0.0048	0.367	**-12.5 (-24.9,-0.08)**	**0.0242**	**0.049**
Growth 1st year[1]	4.71 (-0.45,9.88)	0.0187	0.074	5.70 (-0.58,12.0)	0.0198	0.075
Lean mass index (kg/m^2)	0.56 (-3.63,4.74)	0.0004	0.793	-1.71 (-6.38,2.96)	0.0033	0.470
Fat mass index (kg/m^2)	2.02 (-0.11,4.15)	0.0201	0.063	**2.40 (0.38,4.41)**	**0.0335**	**0.020**
Total area (mm^2)						
Birth weight (kg)	**42.5 (0.47,84.5)**	**0.0230**	**0.048**	**58.1 (27.6,88.6)**	**0.0818**	**<0.001**
Growth 1st year[1]	**30.8 (9.50,52.1)**	**0.0460**	**0.005**	8.58 (-6.82,24.0)	0.0076	0.273
Lean mass index (kg/m^2)	8.15 (-9.10,25.4)	0.0051	0.352	1.57 (-9.88,13.0)	0.0005	0.787
Fat mass index (kg/m^2)	-7.41 (-16.2,1.37)	0.0162	0.097	2.06 (-2.89,7.01)	0.0042	0.412

Key: [1]Conditional growth in the first year (z score). No significant interactions by sex.

14.Results: Relationships between bone microarchitecture and fracture

14.1 Aim

To explore the differences in bone microarchitecture in the radius and tibia by fracture status.

14.2 Summary of methods

Full details of the study methodology can be found in section 9 and the cohort is defined in figure 9.2. Participants that attended the MRC Human Nutrition Research in Cambridge underwent detailed assessment of bone microarchitecture in both the radius and tibia using the Scanco Xtreme HRpQCT scanner. Height and weight were also assessed at the visit. aBMD was measured at both femoral necks using a Lunar Prodigy Advance densitometer (GE Medical Systems Lunar). Morphometric vertebral fractures were diagnosed from a lateral view of the spine taken using the same machine and graded based on the Genant semiquantitative method by two independent reviews(100). Prevalent non-vertebral fractures were determined from the combined results of validated study questionnaires, which included a mannequin on which the fracture site was marked(339). These were completed at three separate time points, administered between 1998 and the present. At baseline (1998-2003) participants were asked whether they had sustained a fracture after the age of 45 years. In 2004-2005 and 2011-2012 they were asked whether they had sustained a fracture since their last visit. From this data, it was ascertained whether each participant had fractured since the age of 45 years.

Additional covariates within the analysis were also obtained from previous questionnaires in the HCS described in section 9.2. Smoking status, alcohol consumption and bisphosphonate use were obtained from data collection in 2004-2005. Calcium intake, physical activity, social class, time since menopause, HRT use and number of comorbidities were obtained from baseline HCS data collection (1998-2003). Number of comorbidities was

scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension.

14.3 Analyses

Study participants were defined as have a prevalent fracture if they had a morphometric vertebral fracture (grade 1-3) or had reported a non-vertebral fracture that had occurred since the age of 45 years. Good agreement was shown between reviewers for the diagnosis of morphometric vertebral fracture (κ=0.65). Continuous variables including height, weight, physical activity, and bone microarchitectural parameters were all assessed for normality. Continuous demographic and lifestyle factors were presented as mean (SD) by fracture status in men and women. Differences between fracture and non-fracture groups were assessed using a Student's t-test. Categorical variables were presented as counts and percentages. Differences in smoking status, alcohol consumption and number of comorbidities by fracture status were assessed using a Fisher's exact test; differences in social class were assessed using a Chi-squared test. The number of fractures at each anatomical site was calculated and presented in men and women.

Radial and tibial bone microarchitecture was presented as mean (SD) by fracture status. Differences between the two groups were assessed using a Student's t-test. Logistic regression was then employed to determine the odds ratio of fracture positivity based on each of the HRpQCT variables, unadjusted and after adjustment for femoral neck aBMD, for age, and for adult size (height and weight), and then for demographic, anthropometric, and lifestyle factors as described previously. As there is some concern over the diagnosis and clinical value of mild (grade 1) vertebral fractures, these analyses were then repeated with only moderate or severe vertebral fractures contributing to fracture status. When logistic regression analyses were carried out in men and women separately, an additional analysis was completed to assess for interaction by sex. Standardised values were used to allow comparison of effect sizes. Statistical significance was assessed as described in section 11.3.

14.4 Results

Three hundred and seventy six individuals underwent HRpQCT assessment. Twenty six of these had not completed the most recent questionnaire and therefore did not have data on their non-vertebral fracture status. A further five individuals did not have vertebral fracture assessments by DXA. Therefore, 345 individuals were included in the analysis. Of these, 45 men and 50 women had suffered fractures since the age of 45 years. Age did not differ by fracture status in men, but women that had fractured were an average of 1.3 years older than women that had not (p=0.005) and their time since menopause was an average of 3.6 years longer. Height, weight, BMI, physical activity, smoking, alcohol consumption and social class did not differ by fracture status in either sex (Table 14.1).

Table 14.1: Demographic characteristics by fracture status in men and women

	Men			Women		
	Fracture (n=45)	No Fracture (n=135)		Fracture (n=51)	No fracture (n=114)	
	Mean (SD)	Mean (SD)	p value	Mean (SD)	Mean (SD)	p value
Age (years)[1]	75.8 (2.4)	76.2 (2.5)	0.364	**77.3 (2.4)**	**76.0 (2.6)**	**0.005**
Height (cm)	174.9 (7.3)	173.2 (6.1)	0.120	160.0 (8.2)	160.1 (5.3)	0.831
Weight (kg)	81.5 (11.5)	83.1 (12.5)	0.446	69.1 (14.1)	72.2 (12.1)	0.147
BMI (kg/m²)	26.6 (3.5)	27.7 (3.8)	0.106	26.8 (5.0)	28.2 (4.5)	0.092
Daily Ca[2*] (mg)	1175 (369)	1249 (281)	0.165	1107 (412)	1146 (381)	0.549
Physical activity	66.7 (13.7)	65.0 (13.3)	0.474	63.1(11.4)	62.3 (14.2)	0.705
Time since menopause (yrs)	NA	NA	NA	**30.3 (6.4)**	**26.7 (6.6)**	**0.002**
	n (%)	**n (%)**	**p value**	**n (%)**	**n (%)**	**p value**
Alcohol						
None	1 (2.2)	7 (5.2)		11 (22.0)	22 (19.5)	
≤Recommend[2]	34 (75.6)	107 (79.3)		39 (78.0)	88 (77.9)	
>Recommend	10 (22.2)	21 (15.6)	0.458	0 (0)	3 (2.7)	0.628
Smoking						
Never	21 (46.7)	51 (37.8)		30 (60.0)	76 (67.9)	
Ex	22 (58.9)	73 (54.1)		16 (32.0)	33 (29.5)	
Current	2 (4.4)	11 (8.1)	0.548	4 (8.0)	3 (2.7)	0.267
Social Status[3]						
I-IIINM	18 (41.9)	56 (43.4)		23 (46.0)	49 (43.0)	
IIIM-V	25 (58.1)	73 (56.6)	0.859	27 (54.0)	65 (57.0)	0.720
Comorbidities						
0	30 (69.8)	72 (56.7)		27 (58.7)	64 (56.6)	
1	11 (25.6)	36 (28.4)		13 (28.3)	37 (32.7)	
2+	2 (4.7)	19 (15.0)	0.162	6 (13.0)	12 (10.6)	0.793
HRT (ever use)[4]						
Yes	NA	NA	NA	19 (38.0)	55 (48.3)	
No				31 (62.0)	59 (51.7)	0.225

Key: [1]At time of scans; [2] Recommended maximum weekly consumption of alcohol (14 units for women, 21 units for men); [3] I-IIINM (I to III non-manual), IIIM-V (III manual to V); [4]Hormone Replacement Therapy as measured at HCS baseline (1998-2003).

In men, the mean(SD) aBMD at the femoral neck on the side with the lowest value was 0.90g/cm^2 in those that had fractured and 0.95g/cm^2 in those that had not (p=0.058). In women, these values were 0.77g/cm^2 and 0.86g/cm^2 respectively (p<0.001). These equate to OR (95% CI) for fracture of 0.69 (0.48,1.02) and 0.38 (0.23,0.62) for every one SD increase in aBMD in men and women respectively.

Table 14.2: Fracture distribution amongst men and women

Fracture site	Men	Women	Total
Vertebral (radiographic)	16	24	40
Distal radius/ulna	5	14	19
Distal tibia/fibula	6	11	17
Humerus	7	5	12
Hand	4	10	14
Foot	7	11	18
Hip	1	1	2
Other	12	18	30
Total	**58**	**94**	**152**

The most common fractures identified were radiographic vertebral fractures (Table 14.2). A total of 16 and 24 were diagnosed in men and women respectively. Of the 16 in men, 6 were classified as mild, 6 were moderate, and 4 severe. Of those in women, 10 were mild, 8 were moderate, and 6 severe. Fractures of the distal radius/ulna and distal tibia/fibula were the next most common and both were more prevalent in women. Only one man and one woman had suffered a hip fracture.

In both men and women, cortical thickness and density were lower in those that had fractured (Table 14.3); however this did not reach statistical significance for cortical density in the radius in either sex. In men, but not women, tibial and radial total and trabecular area were greater in those with a prevalent fracture. As total area was higher but cortical thickness was lower in men with fractures, cortical area did not differ by fracture status. However,

cortical area was lower in women with fractures. Consequently, a significant interaction by sex was shown for tibial cortical area when logistic regression was carried out. In terms of trabecular microarchitecture, trabecular density, number and thickness all tended to be lower in those individuals that had fractured. These differences only reached statistical significance in the radius, and for trabecular number in the tibia, in women. A significant interaction by sex was subsequently shown for radial trabecular thickness. Interestingly, radial cortical porosity was lower in women that had fractured (3.2%) compared with those that had not (3.7%) (p=0.030).

Table 14.4 shows the logistic regression analyses of bone microarchitectural parameters as determinants of fracture status before and after adjustment for femoral neck aBMD. In both men and women, cortical variables (thickness, area and density) were substantially attenuated by adjustment, with the majority of relationships no longer remaining statistically significant at the 5% level. In women, relationships with radial trabecular microarchitecture (density, number, thickness, and separation) were also significantly attenuated by adjustment for aBMD. In contrast, there was only a minor reduction in the size of the odds ratios for fracture for total and trabecular area in men, with significant associations maintained in both the radius and tibia.

When these relationships were adjusted for age, this did not materially affect cortical parameters in men or women. There was minor attenuation of relationships with tibial trabecular microarchitecture in women. Adjustment for height and weight did not significantly attenuate relationships in men or women (results not shown). After full adjustment for demographic, anthropometric, and lifestyle covariates, relationships in men were further attenuated. Consequently, significant associations remained only with tibial total and trabecular area, and, radial trabecular area (Table 14.5). In women, there was little difference found in the results.

When mild vertebral fractures were excluded from the definition of fracture, the fracture status changed in two men to no prevalent fracture but there were no changes in women. When logistic regressions, as shown in table 14.4 and 14.5, were repeated using this new definition in men, this did not materially affect the results.

Table 14.3: Radial and tibial bone microarchitecture by fracture status in men and women

		Men			Women		
		Fracture (n=45) Mean (SD)	No Fracture (n=135) Mean (SD)	p value	Fracture (n=51) Mean (SD)	No fracture (n=114) Mean (SD)	p value
Total area (mm^2)	**R**	**453.7 (75.5)**	**419.1 (75.1)**	**0.009**	286.4 (44.8)	286.3 (43.1)	0.992
	T	**961.4 (157.1)**	**893.3 (138.2)**	**0.006**	717.3 (105.8)	711.6 (103.1)	0.746
Trabecular area (mm^2)	**R**	**382.8 (78.6)**	**341.8 (67.6)**	**0.001**	240.4 (44.8)	236.2 (44.7)	0.588
	T	**822.3 (164.7)**	**750.7 (139.8)**	**0.005**	630.5 (115.3)	613.8 (107.5)	0.368
Cortical area (mm^2)	**R**	68.5 (13.7)	72.2 (14.1)	0.133	**44.3 (8.7)**	**48.6 (8.0)**	**0.003**
	T	137.4 (30.2)	142.9 (27.4)	0.265	**86.1 (15.2)**	**96.6 (15.7)**	**<0.001**
Cortical thickness (µm)	**R**	**762.8 (192.9)**	**846.7 (179.0)**	**0.009**	**633.3 (160.7)**	**705.1 (160.7)**	**0.010**
	T	**1125.7 (272.0)**	**1236.4 (255.5)**	**0.014**	**855.9 (210.2)**	**948.4 (177.3)**	**0.004**
Cortical density ($mgHA/cm^3$)	**R**	893.5 (52.4)	908.8 (49.6)	0.082	895.1 (57.4)	909.3 (54.2)	0.133
	T	**852.1 (69.7)**	**874.0 (60.8)**	**0.045**	**783.3 (59.3)**	**826.5 (58.0)**	**<0.001**
Cortical porosity (%)	**R**	4.1 (1.4)	4.2 (1.5)	0.641	**3.2 (1.3)**	**3.7 (1.3)**	**0.030**
	T	9.7 (3.2)	9.0 (2.6)	0.133	10.2 (2.8)	9.8 (2.4)	0.306
Trabecular density ($mgHA/cm^3$)	**R**	172.9 (43.1)	181.0 (32.1)	0.186	**123.5 (42.0)**	**150.5 (40.5)**	**<0.001**
	T	182.5 (39.1)	190.5 (32.9)	0.181	162.1 (36.0)	174.9 (40.7)	0.056
Trabecular number (cm^{-1})	**R**	23.0 (3.0)	23.6 (2.3)	0.147	**19.4 (4.6)**	**21.3 (3.9)**	**0.008**
	T	24.3 (3.7)	24.5 (3.0)	0.725	**21.9 (3.3)**	**23.0 (3.6)**	**0.049**
Trabecular thickness (µm)	**R**	62.1 (12.3)	63.8 (9.0)	0.333	**52.3 (12.1)**	**58.5 (9.1)**	**<0.001**
	T	62.6 (10.5)	65.0 (9.7)	0.167	61.8 (11.0)	63.5 (12.2)	0.400
Trabecular separation (µm)	**R**	383.1 (91.5)	364.2 (47.8)	0.078	**504.3 (189.7)**	**432.5 (120.7)**	**0.004**
	T	358.2 (67.8)	349.2 (51.0)	0.345	407.7 (88.6)	382.6 (82.4)	0.080

Key: R, radius; T, tibia.

Table 14.4: Logistic regressions with bone microarchitectural parameters (expressed as z-scores) as explanatory variables for fracture unadjusted and after adjustment for femoral neck aBMD

		Men				Women			
		Unadjusted		Adjusted for femoral neck aBMD		Unadjusted		Adjusted for femoral neck aBMD	
		OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Total area	**R**	**1.73 (1.13,2.67)**	**0.013**	**1.60 (1.03,2.49)**	**0.035**	1.00 (0.48,2.08)	0.992	0.96 (0.44,2.08)	0.916
	T	**1.71 (1.15,2.53)**	**0.008**	**1.58 (1.06,2.37)**	**0.025**	1.09 (0.65,1.81)	0.744	1.12 (0.66,1.90)	0.684
Trabecular area	**R**	**1.95 (1.29,2.95)**	**0.002**	**1.82 (1.19,2.78)**	**0.006**	1.19 (0.64,2.23)	0.586	1.06 (0.54,2.07)	0.867
	T	**1.63 (1.15,2.32)**	**0.007**	**1.50 (1.04,2.15)**	**0.029**	1.23 (0.78,1.93)	0.367	1.18 (0.73,1.88)	0.499
Cortical area	**R**	0.72 (0.46,1.11)	0.134	0.77 (0.49,1.21)	0.254	**0.33 (0.15,0.70)**	**0.004**	0.50 (0.22,1.12)	0.093
	T	0.79 (0.52,1.19)	0.265**	0.89 (0.57,1.38)	0.599*	**0.23 (0.10,0.51)**	**<0.001****	**0.36 (0.15,0.88)**	**0.025***
Cortical thickness	**R**	**0.60 (0.40,0.89)**	**0.011**	**0.64 (0.43,0.96)**	**0.030**	**0.56 (0.36,0.88)**	**0.011**	0.72 (0.45,1.16)	0.182
	T	**0.63 (0.43,0.92)**	**0.017**	0.69 (0.46,1.03)	0.068	**0.48 (0.29,0.81)**	**0.005**	0.63 (0.37,1.08)	0.094
Cortical density	**R**	0.73 (0.50,1.04)	0.084	0.77 (0.53,1.12)	0.165	0.78 (0.56,1.08)	0.134	0.93 (0.65,1.32)	0.678
	T	**0.69 (0.48,1.00)**	**0.049**	0.76 (0.52,1.13)	0.174	**0.43 (0.28,0.65)**	**<0.001**	**0.56 (0.36,0.88)**	**0.012**
Cortical porosity	**R**	0.92 (0.65,1.30)	0.639	0.92 (0.64,1.31)	0.630	**0.65 (0.44,0.96)**	**0.032**	**0.58 (0.38,0.90)**	**0.015**
	T	1.27 (0.93,1.75)	0.135	1.24 (0.89,1.73)	0.199	1.20 (0.84,1.71)	0.305	1.04 (0.71,1.53)	0.831
Trabecular density	**R**	0.75 (0.49,1.15)	0.187	0.85 (0.53,1.36)	0.492	**0.49 (0.34,0.73)**	**<0.001**	**0.63 (0.40,0.98)**	**0.043**
	T	0.77 (0.53,1.13)	0.181	0.87 (0.57,1.33)	0.515	0.73 (0.52,1.01)	0.058	0.98 (0.66,1.45)	0.908
Trabecular number	**R**	0.70 (0.43,1.14)	0.151	0.79 (0.47,1.34)	0.388	**0.68 (0.51,0.91)**	**0.010**	0.87 (0.62,1.22)	0.418
	T	0.94 (0.65,1.35)	0.723	1.04 (0.70,1.56)	0.843	0.72 (0.51,1.00)	0.052	0.91 (0.62,1.34)	0.648
Trabecular thickness	**R**	0.83 (0.57,1.21)	0.332*	0.90 (0.60,1.35)	0.622*	**0.50 (0.34,0.75)**	**0.001***	**0.59 (0.38,0.92)**	**0.019***
	T	0.76 (0.52,1.12)	0.167	0.83 (0.56,1.23)	0.347	0.87 (0.64,1.19)	0.398	1.04 (0.74,1.47)	0.821
Trabecular separation	**R**	1.70 (0.89,3.23)	0.107	1.47 (0.74,2.92)	0.277	**1.44 (1.10,1.88)**	**0.008**	1.19 (0.88,1.60)	0.252
	T	1.24 (0.80,1.92)	0.344	1.09 (0.66,1.79)	0.736	1.28 (0.96,1.71)	0.090	1.02 (0.74,1.41)	0.885

Key: OR, odds ratio; CI, confidence interval; R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

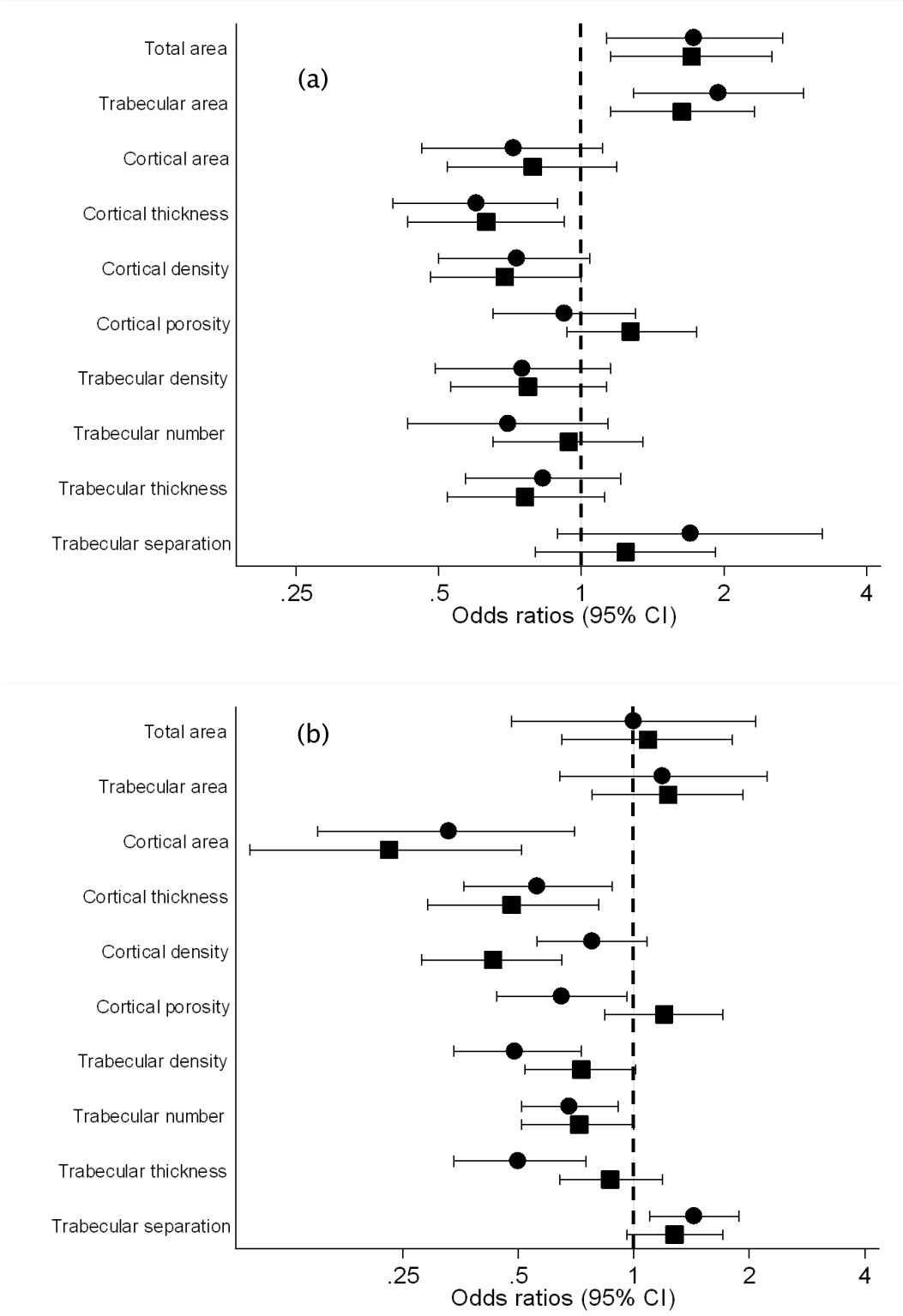

Figure 14.1: Odds ratios and 95% confidence intervals for fracture by standardised bone microarchitecture parameters in (a) men and (b) women

Key: Circle = radius; Square = tibia.

Table 14.5: Logistic regressions with bone microarchitectural parameters (expressed as z-scores) as explanatory variables for fracture after adjustment for femoral neck aBMD, and demographic, anthropometric and lifestyle factors

		Men[1]		Women[1]	
		OR (95% CI)[2]	p value	OR (95% CI)[2]	p value
Total area (mm^2)	R	1.62 (0.96,2.72)	0.070	0.77 (0.27,2.24)	0.637
	T	**2.07 (1.17,3.65)**	**0.012**	1.15 (0.54,2.43)	0.715
Trabecular area (mm^2)	R	**1.85 (1.11,3.08)**	**0.017**	0.94 (0.38,2.33)	0.901
	T	**1.87 (1.12,3.11)**	**0.016**	1.28 (0.65,2.51)	0.470
Cortical area (mm^2)	R	0.74 (0.44,1.25)	0.265	0.40 (0.15,1.09)	0.072
	T	1.00 (0.59,1.69)	0.987*	**0.24 (0.08,0.74)**	**0.013***
Cortical thickness (µm)	R	0.64 (0.41,1.01)	0.055	0.68 (0.39,1.20)	0.183
	T	0.71 (0.44,1.15)	0.159	**0.47 (0.23,0.96)**	**0.040**
Cortical density (mg/cm^3)	R	0.72 (0.46,1.12)	0.148	0.86 (0.57,1.30)	0.484
	T	0.80 (0.51,1.26)	0.341	0.57 (0.35,0.95)	0.031
Cortical porosity (%)	R	0.87 (0.55,1.38)	0.547	**0.54 (0.33,0.90)**	**0.018**
	T	1.22 (0.82,1.81)	0.324	0.92 (0.60,1.41)	0.690
Trabecular density (mg/cm^3)	R	0.73 (0.43,1.23)	0.240	0.62 (0.37,1.05)	0.074
	T	0.76 (0.46,1.24)	0.266	1.10 (0.69,1.76)	0.693
Trabecular number (cm^{-1})	R	0.62 (0.34,1.12)	0.113	0.90 (0.60,1.34)	0.598
	T	1.23 (0.72,2.11)	0.450	1.03 (0.64,1.64)	0.913
Trabecular thickness (µm)	R	0.87 (0.55,1.38)	0.559*	**0.48 (0.28,0.84)**	**0.010***
	T	0.61 (0.37,1.00)	0.051	1.07 (0.72,1.59)	0.751
Trabecular separation (µm)	R	1.92 (0.82,4.50)	0.132	1.16 (0.82,1.64)	0.400
	T	0.99 (0.53,1.87)	0.975	0.92 (0.63,1.34)	0.652

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, alcohol consumption, social class, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use). Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

14.5 Results summary

There were a total of 63 wrist, spine, or hip fractures in the cohort studied. Patterns of differences in bone microarchitecture by fracture status differed slightly in men and women. In men, total area and trabecular area were greater in those that had fractured whereas cortical thickness and density were lower. In women, trabecular density, number, and thickness, and cortical area, thickness, and density, are all lower in those with a prevalent fracture, with relationships stronger in the radius than the tibia.

After adjustment for aBMD at the femoral neck, relationships with cortical parameters (area, thickness and density) and trabecular microarchitecture were attenuated. In men, associations between fracture status and both total and trabecular area were maintained.

15.Discussion

15.1 Main findings

The findings of this project focus on the relationships between bone microarchitecture and participant demography, anthropometry, body composition, and early life. In each case significant associations were demonstrated. Bone microarchitecture in those individuals that had fractured was compared to those that were fracture free; the differences found varied for some parameters by sex.

In both the radius and tibia, men tended to have greater bone size and density. They also had superior trabecular microarchitecture. Sex differences in cortical porosity were site specific. In general, relationships between age and bone microarchitecture appeared stronger in women than men although significant interactions by sex were only found for radial trabecular number and separation. Older women had lower cortical thickness, cortical density, and trabecular density.

In terms of anthropometry, the bones of taller individuals had greater total and trabecular area. Higher BMI was associated with greater cortical area, cortical thickness, trabecular density and trabecular number. As expected, relationships with body weight appeared to be a combination of those demonstrated for height and BMI.

When anthropometry was assessed in more detail using DXA body composition, both LMI and FMI were positively associated with cortical area, cortical thickness, and trabecular number. In the bivariate models, LMI was associated with cortical area and thickness in both the radius and tibia of men and women whereas FMI showed relationships with trabecular number and density. Overall relationships in the tibia were stronger than in the radius, perhaps because of its weight bearing function.

In both men and women, birth weight was positively associated with bone size (total area and trabecular area). Although birth weight was negatively related to some parameters (cortical thickness, trabecular density, and trabecular

thickness) these relationships were substantially attenuated after adjustment for bone size. In men, conditional growth in the first year was positively associated with total area, trabecular area, density and number. No such consistent relationships were found in women.

Clearly, the importance of bone microarchitecture is as a potential marker of bone health and fracture risk. In the cohort studied there were 96 individuals with prevalent fractures (45 men and 51 women). There were significant differences in bone microarchitecture between those that had fractured and those that had not. These patterns varied slightly by sex.

In women, trabecular density, number, and thickness, and cortical area, thickness, and density, were all lower in those with a prior fracture. Overall relationships were stronger in the radius than the tibia. In men, cortical thickness and density were similarly lower in those that had fractured but total area and trabecular area were also greater in this group. After adjustment for aBMD at the femoral neck, relationships with cortical parameters (area, thickness and density) and trabecular microarchitecture were attenuated. However, associations in men between fracture status and both total and trabecular area were maintained.

15.2 Correlations between different bone parameters

The current study showed a negative correlation between total area and both cortical area and thickness. Boutroy and colleagues similarly described associations between various bone parameters and found, in both the radius and tibia, that greater total area was associated with lower cortical thickness (121). We know that in tubular structures, such as a long bone diaphysis, the further away from the neutral axis the material is located, the stronger the structure's bending strength will be. Specifically, doubling the diameter of a tubular structure but maintaining a constant mass, will lead to an eight times increase in bending strength(340, 341). It therefore follows that as bones become larger, and thus significantly stronger, the bending strain exerted for a given stress will be less. This is because stiffness (a structure's resistance to elastic deformation) is dependent not only on the elastic modulus (a material property of the bone tissue) but also its size and shape. Intrinsic cellular

compensatory mechanisms within bone act mainly through cortical compartment size and aim to reach a state that allows maintenance of bone function and limits damage. These mechanisms would be expected to act less strongly on larger bones, due to smaller deformations for a given force, leading to lower cortical thickness and density(338). Compressive strength would not be compromised because the relatively thinner cortex delineates a larger perimeter and therefore cortical area would be maintained.

Larger bones developing thinner cortices would help to prevent them from becoming excessively heavy and potentially impairing locomotion(342). This theory is in keeping with the findings of Bjornerem and colleagues(343). They showed that in women with bones of larger CSA, cortical area was greater in absolute terms, but smaller relative to size, suggesting lower cortical thickness. Those with larger bone CSA also had greater cortical porosity which would diminish overall cortical density(343).

In a similar vein, previous investigators have described bones as either robust (wide relative to length) or slender (narrow relative to length). They have demonstrated that individuals with robust bones had thinner cortices and those with slender bones had thicker cortices(338, 344). Furthermore, they also showed that robustness was established by the age of 2 years and tracked thereafter. This would fit with the hypothesis that bone total area was the driving force established early in life and other indices occur in response to the forces applied.

Interestingly, some researchers have also gone on to hypothesise that given the non-linear relationship between bone size and whole bone stiffness, the bone cells would not be able to surmount this issue to establish functional equivalence. They found that despite evidence of some compensation, slender tibias (narrow relative to length) were up to two or three times less stiff relative to body size compared with robust tibias (wide relative to length)(338). It is suggested that this may be due to intrinsic limitations on cellular activity and biomechanical trade-offs between increasing tissue modulus and avoiding brittleness. These findings are extremely important as they will inform the discussion of other analyses in this project, in particular those pertaining to anthropometry and to early life.

Moving on to trabecular microarchitecture, total area was also negatively correlated with trabecular thickness; a relationship previously shown in an analogous study(121). Again this may be the result of reduced strain and thus reduced modelling, this time in the individual trabeculae.

Cortical thickness was positively associated with cortical density. It is possible that this high correlation is a true association, with both factors representing a state of superior bone health. It is also likely that the partial volume effect is playing a role in this finding. Those with thinner cortices will experience a greater partial volume effect resulting in an artificially low density measurement. This view is supported by the greater correlations found in the radius (r=0.63-0.67) with a thinner cortex than the tibia (0.55-0.56).

15.3 Relationships of bone microarchitecture with age and sex

15.3.1 Bone geometry, age and sex

The findings of the current study add to the available literature in this area as they provide a large number of measurements from men and women in a specific 10 year age band, rather than a wide age range with a consequent smaller number of readings at each age.

The greater bone area (total, cortical, and trabecular) seen in men than women has been well documented(4, 168, 170). Although in younger adults studies have conflicted on whether there is a difference in cortical thickness between men and women(4, 168, 170), in later life it is consistently higher in men, in keeping with what has been shown in this study(4, 170, 171).

Within the age range investigated there were weak associations between age and bone area (total and trabecular) although these did not reach statistical significance in the tibia. In women, cortical thickness was significantly lower in older individuals. This is in keeping with the findings of Vico(172) and Boutroy(121) who demonstrated significant negative correlations between age and cortical thickness in postmenopausal women, and the work of Dalzell who

found that the difference in cortical thickness between men and women increased after the age of 50 years, suggesting that it was thinning more quickly in women(170).

15.3.2 Bone density, age and sex

Trabecular density has been shown to be consistently higher in men in both the radius and tibia throughout adult life(4, 168, 173) and this has been borne out in the current study. Furthermore, it is well established that overall it reduces with age in both men and women(4). Interestingly, Khosla *et al* showed graphically that after the age of 70 years, rates of trabecular bone loss appeared to increase in women, whereas in men, there was stabilisation(4). This is in keeping with what was found in this thesis; trabecular density was negatively associated with age in women around the eighth decade, whereas the relationship in men was weaker.

Gender differences in cortical density are more complicated and are dependent on age. In studies that have examined cohorts of younger adults, cortical density has been found to be similar, if not higher, in women than men in the radius and tibia(4, 170, 171). Invariably, cortical density is subsequently found to reduce with age considerably faster in women than men(4, 170). This accords with the finding in the current study that cortical density was strongly associated with age in women and not men, and may explain why studies of older adults, including the current project, show similar or higher cortical density in men(4, 170).

15.3.3 Trabecular microarchitecture, age and sex

Studies that have examined trabecular microarchitecture from young adulthood through to older age tend to find that both trabecular number and thickness are greater in men than women(170, 173). In a cross-sectional analysis, Khosla *et al* found a greater negative relationship between age and trabecular thickness in males than females(4). However, other studies have not confirmed this(170). In the current cohort, it was found that radial trabecular number was lower with advancing age in women in keeping with previous similar studies(121, 172). The relationship was weaker in the tibia and did not

reach statistical significance. The absence of such associations in men is not necessarily surprising. Although trabecular number has been shown to be lower in older men (mean 71 years) than younger men (mean 27 years), this only reached statistical significance in the tibia and not the radius. Furthermore, the changes that are occurring in older age specifically have not been formally assessed.

15.3.4 Cortical porosity, age and sex

A particularly interesting finding is the site-specific gender differences in cortical porosity. Radial cortical porosity was greater in men ($p<0.001$) whereas in the tibia the reverse was true ($p=0.004$). Burghardt *et al* assessed cortical porosity in 151 subjects aged 20 to 78 years and found higher levels in men than women in both the tibia and radius(171). They noted however that these differences were attenuated after the age of 50 years and MacDonald *et al* found no differences between genders in the eighth decade(168). Our findings of greater tibial cortical porosity in women may reflect the age of the cohort studied. However, given the number of associations assessed this could also be a chance finding.

In this study, radial cortical porosity showed a positive association with age in men but not in women. Other studies have shown increased cortical porosity with age in both sexes(168, 171). These studies did however cover large periods of adult life from the age of 20 years onwards. It is therefore possible that associations between cortical porosity and age differ later in life from those that occur through adult life as a whole. Alternatively, it may be that the relatively small sample size, narrow age range, and imprecision of the variable studied prevented a true association from being demonstrated in women.

15.4 Relationships between lifestyle and bone microarchitecture

15.4.1 Calcium intake

In older individuals, extra-skeletal abnormalities in calcium homeostasis develop which are thought to increase the need for dietary calcium. Studies have shown impaired calcium absorption(345) and in particular attenuation of the response that increases absorption when intake is limited(346). Furthermore, calcium is also lost more readily from the kidneys(347, 348). It is hypothesised that a higher calcium intake is required in later life to offset these problems and prevent the development of secondary hyperparathyroidism with increased bone resorption(349). However, in the current study, no consistent associations were found between dietary calcium intake and bone microarchitecture at either the radius or tibia.

These findings are consistent with a previous pQCT study in 129 women(183) but a larger study in men (n=1172) showed radial and tibial trabecular density to be 2.0% and 1.1% higher for every SD increase in calcium intake(184). These values were 0.3% for cortical density in both the radius and tibia. It is possible that the absence of an association found in the current study was due to a lack of power. Given the number of men that took part, the study only had a power of 27% to show a difference of 2% in trabecular density for each SD change in calcium intake.

15.4.2 Physical activity

In this study there were no consistent associations found between bone microarchitecture and physical activity in either men or women. Physical activity was derived based on the frequency of gardening, housework, climbing stairs and carrying loads. Clearly, these forms of activity are different to those that have previously been shown to influence bone health(194). In particular, they are not high-impact loading exercises which are thought to provide the greatest benefit(186, 187). This may be the main explanation for the absence of associations within the current cohort.

15.4.3 Smoking

In the current study, smoking status was also not found to be consistently associated with bone structure. This was the case both when comparing lifelong non-smokers to current smokers and ex-smokers separately and when comparing lifelong non-smokers to ever smokers. This is in contrast to several published studies which have shown relationships with trabecular density and cortical thickness(184, 197-199). The reason for the lack of associations found in the current study is again most likely to be the result of limited power. For example, the number of current smokers was only 14 in men and 9 in women. Consequently, even in men, the current study only had a 22% power to show a 7% difference in trabecular density, which is the approximate size of the effect shown by the above studies.

15.4.4 Alcohol consumption

Men that drank at least 1 unit of alcohol per week had lower radial cortical thickness and density, and lower radial trabecular thickness and density than those that drank minimal or no alcohol (<1 unit per week), with similar tends seen in women. Although associations with vBMD have been previously assessed, this is the first study to examine relationships with bone geometry and trabecular microarchitecture. In keeping with the current results, Barbour and colleagues also showed a negative association between cortical vBMD and alcohol consumption but a similar inverse relationship for trabecular vBMD did not reach statistical significance(184).

The results of other previous studies have differed from those in the current study as they either found that bone density did not vary with alcohol consumption or that moderate alcohol had a beneficial effect (184, 198, 199, 202, 203). The explanation for these inconsistencies may be the choice of alcohol categories used. Most previous studies compared those that drank alcohol to those that abstained whereas in the current analysis anyone that drank less than 1 unit of alcohol per week was classified in the same group as those that abstained. It appears therefore that this is an important distinction.

15.4.5 Socioeconomic status

The current study found no consistent differences in bone microarchitecture between those of higher (I-IIIn) and lower (IIIm-V) socioeconomic status in men or women. In one previous similar study of 201 men, aged 20-60 years, those with lower socioeconomic status had significantly higher aBMD in both the femoral neck and lumbar spine(350). Although rates of manual labour were much higher in this group, it did not fully account for the differences seen. Similarly, relationships were robust to adjustment for age, weight, calcium intake, family history, activity, and smoking.

In the current study, due to the definition used, social class is more closely related to own occupation in men than women. Consequently, if manual work was influencing bone microarchitecture this would also be more apparent in men. Interestingly, there does appear to be a weak trend towards lower trabecular density, number and thickness in men of higher socioeconomic status after adjustment for other demographic and lifestyle factors.

15.5 Relationships between diseases, medications and bone microarchitecture

In the current study, number of comorbidities was not found to be consistently associated with bone microarchitecture. There are three main explanations for this. The first is that the cohort studied is generally healthy which would limit the ability to study the effects of disease. Secondly, it is also likely to be related to which diseases were included in the score. Although uncontrolled COPD, particularly with hypercapnia, has been shown to be associated with lower bone density(184, 223), evidence is limited to suggest a relationship between bone health and blood pressure(184, 198, 218) or ischaemic heart disease(184, 198). Furthermore, relationships with type II diabetes are complex, although there is some evidence of an effect on the cortex(184, 214, 216, 217). Clearly, a stroke that results in persistent limb paralysis can affect bone microarchitecture mainly through disuse(219-222). However, this is a rare event and would obviously not occur with a TIA. Thirdly, as comorbidities were used purely as a composite score rather than as individual covariates, it is

also possible that one disease might cancel out the effects of another. For example, trabecular density has been found to be lower in individuals with COPD (184, 223) but higher in those with type II diabetes (215). This would reduce the likelihood of a significant associations being identified between comorbidity score and bone microarchitecture.

A strong relationship was found in women between the time since menopause and bone health. Trabecular density was inversely related to the number of years since menopause and this relationship was robust to adjustment for age and other demographic and lifestyle covariates. This is consistent with similar studies using pQCT(226, 227). Interestingly, there was no corresponding increase in bone cross-sectional area akin to that demonstrated by Wapniarz and colleagues(227). Trabecular number and thickness were both negatively associated with time since menopause, in keeping with a previous micro-MRI study(225). Relationships appeared to be stronger for trabecular number than thickness but both were progressively attenuated by adjustment for age and other covariates.

Although HRT has been shown to have short term benefits on bone health, including aBMD, the longer term effects after cessation of therapy have not been well studied(228, 229). Given the age of the Hertfordshire Cohort, it is very likely that those who were taking HRT stopped it several years ago. This may be one explanation for why no difference was found in bone microarchitecture between those that took this medication and those that did not. The other issue here may be confounding by indication. One of the indications for the use of HRT is low bone density. Consequently, although the drug itself may have beneficial effects on bone health, those that took it may have started from a point of relative deficiency.

Similarly, the relationship between bisphosphonate utilisation and poorer trabecular microarchitecture is likely to be due mainly to reverse causality. In order to be started on a bisphosphonate, an individual would need to be deemed to be at substantial risk of future fracture. Most commonly this is due to a low aBMD on DXA or a prior fracture. In the HCS, the results of DXA scans

at baseline would have been reported to each participant's GP and, in some cases, this would have led to the commencement of bisphosphonate therapy. Consequently, there may be a higher number of individuals started on bisphosphonates for this indication. Clearly, if the bisphosphonate has been effective, bone health would be expected to better than if the drug had not been started. However, it appears that this effect is outweighed by the confounding by indication.

15.6 Relationships between bone microarchitecture and adult anthropometry

The current analyses have confirmed that, on average, taller men and women have a greater bone area (total and trabecular) (121, 343). Interestingly, significant interactions by sex were identified with associations of greater magnitude in men than women, with the exception of radial trabecular area. However, in HRpQCT studies of both premenopausal and postmenopausal women, relationships between height and cortical thickness have been conflicting(121, 343). Furthermore, associations in men have not been assessed using this imaging modality. The current study's finding of an inverse relationship in women would be in keeping with the results of Bjornerem and colleagues(343). Replication is required of the findings that taller men tend to have lower tibial cortical thickness, cortical density and trabecular thickness, and greater cortical porosity and trabecular number. However, most of these findings fit with what has already been discussed concerning individuals with larger bones; those with larger bone CSAs tend to have thinner cortices, lower cortical density, thinner trabeculae, and, in men, greater cortical porosity.

Positive associations between BMI and cortical area and thickness were present in both sexes in this study. Similar relationships have been shown in both child and adult pQCT studies when the midshaft was assessed(239, 240). It may be that BMI affects cortical area and thickness as it leads to increased stresses on the bone. This can lead to greater strains which are sensed by osteocytes and promote cortical modelling, particularly in children.

Discussion

In the current study, total and trabecular area did not differ significantly by BMI. In a previous study that tried to address this issue with HRpQCT, in men there was no difference in total area by BMI and cortical parameters were not assessed(241). These results were consistent with our findings. Conversely, in women, total area in the distal tibia was higher in those that were overweight. This difference in outcome may be related to the age of the cohort, as the study by Hoy *et al* involves females with a mean age of 18 years(241). This theory is supported by similar positive associations found between BMI and total area in pQCT studies in children (age 7-10years) but not adults (25-71 years) (239, 240), and may suggest that the periosteal circumference is more plastic in the period of bone development. It is also possible that there is a true gender difference. Further studies are therefore required to investigate this.

The positive relationships between weight and both total and trabecular bone area are likely to be driven mainly through greater height and are entirely consistent with previous HRpQCT studies(121, 172). Cortical thickness was unrelated to weight in both men and women. This may be explained by the competing effects of increased height and greater BMI in heavier individuals. Vico *et al* similarly found cortical thickness did not vary by weight but Boutroy *et al* showed that tibial, but not radial, cortical thickness was lower in women with greater weight(121, 172).

When assessing microarchitecture, we found that BMI was positively associated with trabecular density and trabecular number in both men and women. In pQCT studies, the latter could clearly not be assessed, however positive relationships have been shown with trabecular density(239, 240). When HRpQCT was used previously, similar trends were identified; stronger in men than women. In keeping with our study, no relationship was demonstrated between BMI and trabecular thickness(241).

As with BMI, weight was also positively related to trabecular density and number but not thickness, and this has been demonstrated previously(172). In contrast, in pre- and postmenopausal women, a weak negative association was shown between weight and both radial and tibial trabecular thickness(121). Further work is required to ascertain the reasons for this inconsistency.

Cortical porosity appears to show a sex-specific relationship with weight. In men, strong positive associations are seen but in women they are not. As no other studies have investigated this phenomenon, these represent novel findings which require replication. It is possible to suggest mechanisms by which excess fat might have detrimental effects on cortical bone architecture. However, in the absence of further studies confirming the associations this would be speculative. Exploration of the relationship with fat mass and lean mass individually might shed more light on the likely aetiology of this phenomenon and whether it represents a true relationship.

15.7 Body composition and bone architecture

15.7.1 Body composition in men and women

Marked differences were found between the body composition of men and women. Men had greater total lean mass, LMI and tissue mass (combined total lean mass and fat mass), whereas women had greater total fat mass, FMI and percentage fat mass. The finding that total fat mass is higher in women has been well described(261, 351-357). There is also evidence that the distribution of that fat differs between men and women(358). Similarly, greater lean mass in men than women is invariably found(7, 268, 359-361). It therefore naturally follows that percentage fat mass is higher in women than men.

Correlations between the different compartments of body composition and anthropometry were similar in both sexes. Weight was highly correlated with fat mass and FMI (r=0.82-0.93). LMI was also correlated with FMI: r=0.45 and 0.53 in men and women respectively. Due to the correlation between fat mass and lean mass, as percentage fat increased with greater adiposity so did lean mass.

15.7.2 Relationships between body composition and bone microarchitecture

Looking first at bone geometry, consistent associations were not identified between either component of body composition and total bone area. Those

studies that have identified such a relationship did not adjust for the height of the participants and these findings may therefore purely represent confounding by size(294, 295). There were consistent positive univariate relationships between both LMI and FMI, and cortical area in men and women. Relationships with cortical thickness were similar but weaker. This is consistent with the findings of several previous studies of lean mass(293, 294, 296). However, in studies of associations between fat mass and cortical geometry, the results have not been as uniform(293, 294, 296).

When LMI and FMI were mutually adjusted in a model with bone microarchitecture, associations with cortical area and thickness were only found for LMI. This is consistent with the findings of other investigators(293, 295) and may imply that the associations between FMI and cortical geometry are mediated through the relationships of both with LMI. Correlations have been shown between FMI and LMI as individuals that gain adiposity develop larger muscles to compensate and maintain mobility where possible.

In terms of density measures, there was a tendency towards greater tibial cortical density in women with greater FMI. This relationship was not found in the radius or in men. Other studies have not shown consistent results in this area. Some have found negative associations between cortical vBMD and both lean mass and fat mass(293, 296) whereas others have found positive associations(294, 295). The results of this study do not greatly help to answer this question.

Trabecular density was found to be positively associated with FMI in both men and women. Previous studies in this area were again inconsistent. In postmenopausal women, assessed using HRpQCT, a similar relationship was found(296). However, this was not replicated by the same investigators in their cohort of younger men(296). Some researchers found relationships in the tibia, but not the radius(293, 295) and no associations were identified by Pollock and colleagues in either the radius or tibia(294). Interestingly, when we regressed trabecular density on FMI in a model with LMI the association was again identified. This has not previously been shown and suggests the relationship to be independent of lean mass.

When trabecular microarchitecture was assessed the most prominent relationship was between trabecular number and both LMI and FMI. These associations were stronger for FMI than for LMI and in the tibia than the radius. Consequently, the negative associations between trabecular separation and both LMI and FMI only tended to reach statistical significance in the tibia. The relationship between trabecular number and fat mass has previously been assessed in post-menopausal women and similar positive relationships were found(296). In addition, Ng and colleagues also described a significant positive association with trabecular thickness in women.

Relationships between lean mass and both trabecular and cortical microstructure have not previously been explored. The positive association with trabecular number has therefore not been demonstrated before. Furthermore, relationships between trabecular number and FMI, adjusted for LMI, were also shown which demonstrates that trabecular number appears to be independently related to FMI. Again, this has not been shown previously and therefore represents a novel finding.

15.7.3 Possible mechanisms for the relationship between bone microarchitecture and lean mass

15.7.3.1 The mechanostat hypothesis

Relationships between muscle and bone may be explained in several ways. One of the most well established theories is that of the mechanostat hypothesis (Figure 15.1). The theory that bone adapts primarily to mechanical forces was first postulated by Wolff in 1869 and later refined by Frost(362). It is suggested that skeletal muscle provides a direct mechanical stimulus to bone during a contraction and this generates a bending moment(363). Greater lean mass will also increase the static load on bones which can indirectly affect the magnitude of dynamic forces exerted during locomotion. The ensuing strains are sensed predominantly by osteocytes and this can lead to an appropriate adaptation to that additional strain by a change in bone geometry, density, or microstructure to prevent damage. This mechanotransduction provides a probable mechanism through which muscle and bone are integrally related. The mechanical link between muscles and bones can also be modified

by non-mechanical determinants, such as those covered in the following sections.

15.7.3.2 Genetic factors

An individual's genotype can, either directly or indirectly, impact on both their muscle and bone growth(364-368). Some genes have pleiotropic effects and those that are involved in the determination of both muscle and bone health may lead to a correlation of these phenotypes. Analyses have shown that both lean mass and fat mass have positive genetic correlations with aBMD at several sites, although the correlations tend to be stronger for lean mass(268).

15.7.3.3 Early life environment

As discussed in section 6.2, developmental plasticity is an important mechanism through which events occurring in early life might influence adult phenotypes. There is considerable evidence to demonstrate associations between growth, before and soon after birth, and both bone health(12) and muscle function(369) in later life. Thus, relationships found between these facets of the musculoskeletal system may partly be explained by a common developmental environment.

15.7.3.4 Diet and nutrition

Dietary factors may play a role in both muscle and bone health. There is some evidence that protein intake may affect muscle size and strength, rates of bone loss and fracture(370). Similarly, severe vitamin D deficiency resulting in osteomalacia can cause a reduction in bone density and a myopathy, both of which improve with replacement. Although the evidence for the importance of vitamin D in bone(371) and muscle health(372) at more physiological doses is weaker, supplementation of vitamin D along with calcium is still routinely considered for individuals at increased risk of fracture.

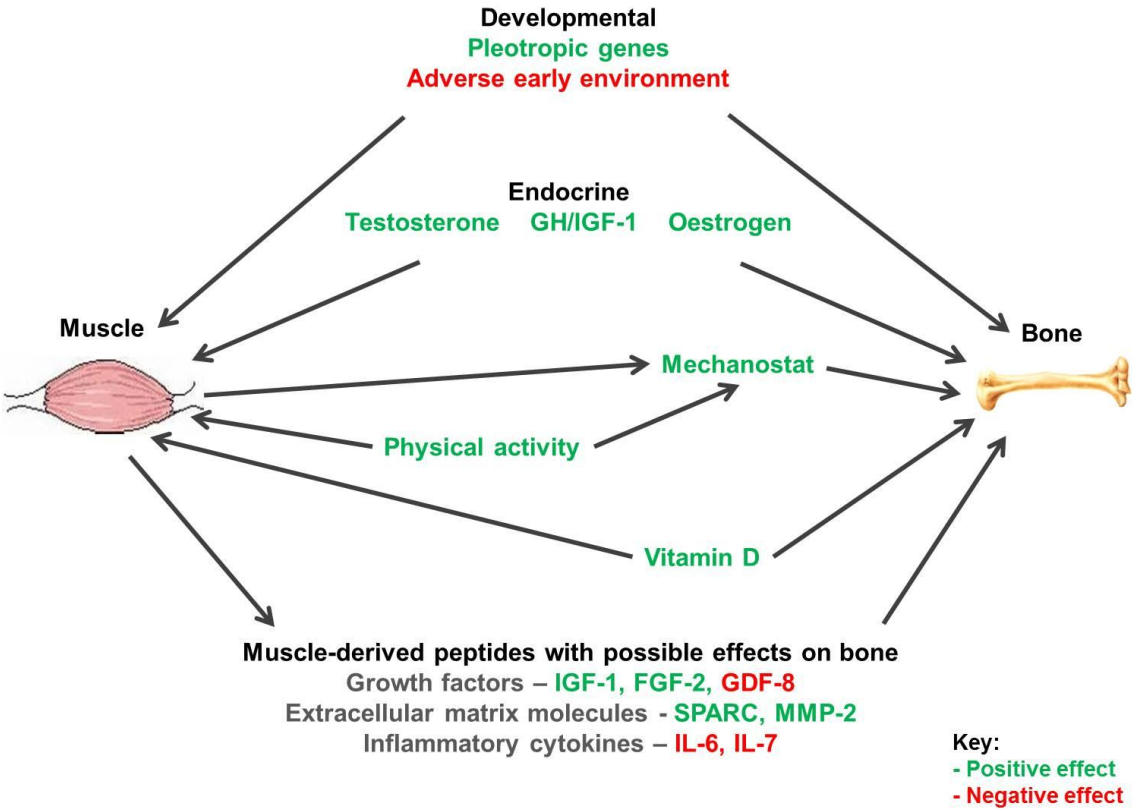

Figure 15.1: Interactions between muscle and bone

15.7.3.5 Physical activity

Both bone and muscle tissues show evidence of plasticity with the ability to adapt when greater stresses are applied to them. Thus, physical exercise can affect both bone structure and muscle size (373-376). For example, a study of pre- and early pubertal girls showed that non-elite gymnasts undertaking high-training rates had greater forearm bone strength, bone content, and lean mass than those that did not, despite lower whole body fat(377). Furthermore, a recent study published in the *British Medical Journal* showed that in children, mean age 6.7 years, LMI was positively correlated with moderate activity and negatively correlated with sedentary activity measured using accelerometers(378). Sedentary activity was also negatively correlated with whole body aBMD and hip BMC(378). This may be related to fewer episodes of mechanical loading during weight-bearing or weaker muscles applying smaller forces to the bones.

15.7.3.6 Hormonal factors

Specific hormones, such as growth hormone, have been found to influence both muscle and bone health(365, 366, 368). Insulin-like growth factor-I (IGF-I) and IGF-II are synthesised by osteoblasts and stimulate proliferation and differentiation of bone cells with effects on collagen production and matrix apposition(379). IGF signalling may also play a regulatory role in skeletal muscle differentiation and growth(380, 381).

Furthermore, sex hormones have been shown to influence bone and muscle health. Oestrogen can enhance bone density and geometry(382-385) and there is evidence that it may also play a role in muscle repair and regeneration(386). Testosterone has a considerable effect on muscle size and strength largely through augmenting protein synthesis(366, 387, 388). It has also been associated with radial bone strength, geometry, and cortical BMC measures in Belgian men over 60 years(385). However, some studies have provided conflicting results regarding the influence of testosterone on bone geometry(382, 383, 389). The potential effects on bone are thought to occur mainly through aromatisation to oestrogens. Further work is required in this area to accurately delineate the role of testosterone in bone health.

15.7.3.7 Co-morbidities

Several specific diseases have been associated with impaired bone and muscle health. The most obvious is that of osteomalacia caused most commonly by vitamin D deficiency. Excessive consumption of alcohol can also have detrimental effects on both muscle (e.g. alcoholic myopathy) and bone(390). More generally, many diseases in older age lead to frailty. This is in part due to the process of catabolism, particularly seen in cancers and inflammatory diseases. Frailty is in turn associated with both low aBMD and sarcopenia.

15.7.4 Possible mechanisms for the relationship between bone microarchitecture and fat mass

15.7.4.1 Mechanostat hypothesis

Unlike lean mass, the physical relationship between fat mass and bone is likely to occur entirely through an increase in the static load applied. As the response of bone is determined by the frequency and amplitude of dynamic loads(391, 392), an increase in fat mass, which should increase the latter, is likely to have a positive effect on bone health particularly in those that are weight bearing. Invariably the frequency of dynamic loading is influenced mainly by the individual's level of physical activity.

Individuals with higher fat mass also tend to have more lean mass. In turn, this can lead to the much greater forces applied to bone through muscle contractions as described above. It is therefore important that we have separated out the effects of fat mass from those of lean mass in order to avoid overestimating its importance.

15.7.4.2 Genetic factors

Fat mass has positive genetic correlations with aBMD in both the hip and lumbar spine(268). Although the associations are not as strong as with lean mass it still provides an explanation by which pleiotropic genes could partly account for the associations between fat and bone.

15.7.4.3 Diet and nutrition

Micronutrient intake, in particular calcium and vitamin D, is important in the maintenance of bone health. Studies of adults with anorexia nervosa show that a significant proportion do not meet the recommended daily intakes of these nutrients(393, 394). This might contribute to an association between lower fat mass and poorer bone health. It has however also been shown that low serum vitamin D levels are prevalent in obese populations(395, 396). This may be due to poor dietary intake of such micronutrients, limited sun exposure, or decreased bioavailability due to deposition in adipose tissue(397).

15.7.4.4 Hormonal factors

There are many endocrine factors that may be involved in the interplay between fat mass and bone health (Figure 15.2). Some of these pathways have been well delineated but in other areas the research is at an early stage and further investigation is required.

Firstly, adipokines are likely to play an important role. The most well described of these is leptin. Produced by adipocytes, its level is proportional to the individual's total body fat mass and is therefore elevated in obesity(398). Leptin is thought to have both peripheral and central actions and to influence several other hormonal systems. Osteoblasts and chondrocytes have leptin receptors. Leptin may increase osteoblast differentiation and proliferation, and through the RANKL-OPG axis, inhibit osteoclastogenesis (284, 399, 400). In this way leptin may increase skeletal mass to support the increased fat mass. In contrast, centrally leptin has been shown to inhibit brainstem serotonergic activity in mice and reduce sympathetic outflow to osteoblastic β-adrenoreceptors, reducing bone formation and increasing resorption(401, 402). Overall, it appears that the peripheral effects predominate, as a recent meta-analysis has shown that leptin tends to be positively associated with higher aBMD in both men and women(403). This relationship is particularly marked in postmenopausal women(403).

Adiponectin, is another adipokine. Its mechanism of action is not fully understood but is likely to relate to direct effects on osteoblasts and osteoclasts(404-406). Although, like leptin, it is produced in fat cells, levels are inversely related to BMI(407). It has been shown that there is a negative association between adiponectin levels and aBMD(403, 408-410). As individuals with higher FMI would be expected to have lower levels of adiponectin which in turn result in higher aBMD, this provides a further explanation for the above bone-fat associations. There is also evidence that osteocalcin, derived from osteoblasts, can stimulate the expression of adiponectin in adipocytes permitting bone to influence fat metabolism (411). Furthermore, there may also be a mechanism by which adiponectin enhances insulin sensitivity (412, 413).

Other adipokines include Dickkopf-1, resistin, and visfatin. Their importance in the determination of bone health has not been fully realized at this time. Adipocytes also produce oestrogen and in postmenopausal women this is the main source. Oestrogen is established to have beneficial effects on bone. It may therefore play a role in the relationship found. However, it might be expected to have a smaller impact if premenopausal women were studied.

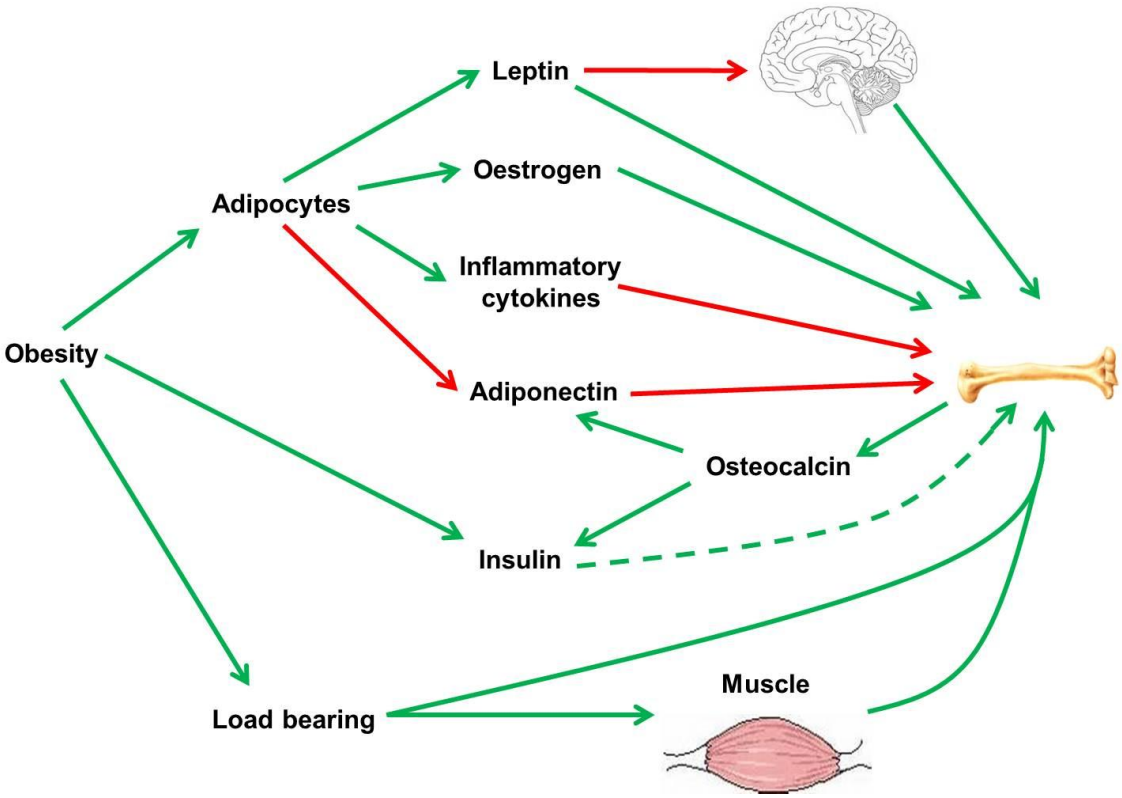

Figure 15.2: Interactions between bone and fat

Insulin, produced by β-cells in the pancreas, is co-secreted with amylin and preptin. Each of these hormones is found at higher levels in obesity. In some individuals a state of insulin resistance develops with a relative deficiency resulting in type II diabetes mellitus. All of these hormones stimulate osteoblast growth; amylin also inhibits osteoclast function and thus bone resorption. Furthermore, Insulin augments production of oestrogen and androgens in the ovaries, inhibits hepatic production of sex-hormone binding globulin, decreases PTH and increases calcitonin, thus reducing bone turnover with potential positive effects on bone health(400, 414). Epidemiological

studies are consistent with these effects showing hypoinsulinaemia to be associated with low aBMD, in part independent of fat mass(415-417). Furthermore, a recent study investigating a large database of non-diabetic women showed positive associations between serum insulin levels and aBMD although the correlations were only significant at the hip(408).

Important hormonal perturbations affecting bone health can also occur in women that exercise heavily or are severely undernourished. The most widely established is the state amenorrhoea which occurs as an adaptive response to a negative energy state and is due to impaired gonadotropin-releasing hormone pulsatility. When compared to menstruating controls of similar age, height, weight, and exercise duration, those with amenorrhoea had lower fat mass and percentage fat, as assessed by DXA, along with lower total body and lumbar spine aBMD(418). Differences were less marked at the hip. They also had lower hormone levels including estrone-1-glucuronide, pregnanediol glucuronide, leptin, and total triiodothyronine(418). Similar patterns were found in amenorrhoeic and eumenorrhoeic women with anorexia nervosa. It can be postulated that the low levels of fat or high levels of exercise, with effects on endocrine homeostasis, might cause deleterious effects on both menstruation and bone health.

As previously discussed, IGF-I has been shown to have anabolic effects on bone(419). In states of severe undernutrition, such as anorexia nervosa, when fat levels are very low, a state of relative GH resistance develops with high levels of GH and low levels of IGF-1(420, 421). The loss of this anabolic effect is associated with a reduction in bone formation markers(422, 423) which can be reversed by recombinant human IGF-1 administration(422, 424).

Serum and urinary cortisol levels have also been found to be higher in individuals with very low BMIs than healthy weight controls. Cortisol can have adverse effects on bone by influencing intestinal calcium absorption, renal tubular calcium excretion, and the modulation of other hormones(425, 426). Consequently, greater serum cortisol levels are associated with lower aBMD(427, 428) and may also partially explain poorer bone health in those with lower fat mass.

In addition, factors secreted by bone cells, such as osteocalcin, can have an effect on metabolism and potentially body composition. In mice, uncarboxylated osteocalcin acts to increase adiponectin expression by adipocytes and to increase insulin production and proliferation of pancreatic β-cells. In a cross-sectional study of elderly Swedish men, total osteocalcin levels were found to be inversely associated with serum glucose(429). These provide possible mechanisms by which bone could affect fat mass.

15.7.5 Importance of fat mass distribution

Up to this point, body fat has been treated as a single entity. In other studies it has however be divided into different types and the results are of interest. The most important distinction appears to be between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) with the former being more strongly linked to adverse outcomes such as cardiovascular mortality. There is also evidence that they may have differing effects on bone health.

Relationships between bone structure and specific distributions of fat were assessed in a cohort of women aged 15-25 years(430). A single-slice CT of the abdomen at the level of the umbilicus was taken to allow VAT and SAT to be determined (cm^2). SAT was positively associated with femoral total area, cortical area, and strength whereas no evidence was found of associations between VAT and femoral bone structure or strength. Bredella and colleagues examined the effects of fat distribution on bone microstructure in a group of 25 obese men (mean age 33.8years)(431). They too found no significant effect of VAT on geometry or on either cortical density, or trabecular number. Those with high VAT did have lower trabecular density, trabecular thickness, and estimated stiffness and fracture load. These results are consistent with the findings of a study in men aged 20-49 years, which found negative associations between VAT and distal radial trabecular density and trabecular thickness(296). In addition, they also showed negative associations with cortical density and thickness, and a positive association with trabecular number. Associations between SAT and distal radial microstructure tended to be similar but weaker than those for total body fat(296).

In an analysis from the ALSPAC study the relationships between fat, both intramuscular and subcutaneous, were assessed in relation to bone morphometry in 1703 males and 2243 females mean(SD) age 17.8(0.4) years. Subcutaneous fat, as assessed by pQCT, was positively associated with tibial peripheral circumference, cortical thickness, and bone strength but not cortical vBMD(432). Conversely, muscle density (the inverse of muscle fat) was negatively associated with cortical thickness, bone strength, and cortical vBMD but not peripheral circumference(432). Thus, cortical thickness and bone strength were shown to be associated with both subcutaneous and intramuscular fat.

There are several theories for the negative associations between VAT and bone health parameters. VAT shows a greater immune cell infiltration than SAT and secretes higher levels of proinflammatory cytokines, such as interleukin-6 and tumour necrosis factor-α. Both of these cytokines stimulate osteoclast production and bone resorption(433). Visceral adiposity is also associated with reduced growth hormone secretion(434, 435) and IGF-1 levels(436), and in obese premenopausal women, levels of IGF-1 correlate positively with aBMD and markers of bone formation(436).

In summary, relationships between SAT and bone microstructure appear to be similar, although weaker, than those found with total fat mass. This includes positive associations with bone size, cortical area and thickness, and trabecular density. In stark contrast, VAT appears to have little effect on femoral geometry and negative associations with trabecular density and thickness. These negative associations between VAT and bone health may be related to the presence of proinflammatory cytokines or attenuation of the GH-IGF-1 axis.

15.8 Early life and bone microarchitecture

15.8.1 Early life and bone size

Clear relationships were demonstrated between birth weight and both tibial and radial bone area (total and trabecular) in men and women. This is consistent with several studies in adults which have shown similar positive

associations in the hip and spine using DXA(12, 301-303, 309) and in the radius and tibia using pQCT(242, 308). It is thought that the effect of growth *in utero* on bone size can be traced back to early childhood with relationships demonstrated with both bone area and BMC(299). Thereafter bone growth tends to track along centile lines including through the pubertal growth spurt(300). This is likely to be the explanation for the enduring effect of early growth on bone size which persists into adulthood.

Conditional growth in the first year and weight at 1 year were both associated with greater tibial and radial bone area in men but not women. Relationships between conditional growth in the first year and bone geometry were attenuated by adjustment for adult size. Individuals that grow more rapidly in infancy are more likely to be taller(437) and it has been demonstrated in this project that taller individuals have bones with greater total area (Chapter 10). Therefore, in men, greater growth in infancy appears to be associated with taller individuals who also have larger bones.

The relationships between birth weight and tibial bone size were attenuated but not removed by adjustment for adult size and demographic and lifestyle factors. This suggests the relationships between birth weight and tibial total and trabecular area were not occurring purely through their association with adult size.

15.8.2 Early life and cortical thickness

Tibial cortical thickness was negatively associated with birth weight in women and conditional growth in the first year in men. It appears that these associations have not previously been assessed in either men or women. Kuh and colleagues looked at relationships between birth weight and both total area and medullary area in the radius(242). They found that the regression coefficients were larger for medullary area than total area which would be consistent with our findings.

Furthermore, a study of 17-21 year old men and women, did show a trend towards a negative association between cortical area and birth weight in men but as cortical area is determined by the combination cortical thickness and

total bone area this may explain why a significant relationship was not found(438). Similar associations were not shown in women.

The finding of lower cortical thickness with higher birth weight initially appears counterintuitive as previous studies have tended to suggest that greater growth in early life is associated with better bone health(12, 308, 309). The answer may be that these relationships are substantially confounded by bone size. It was shown in chapter 10 that tibial total area was negatively correlated with cortical thickness (r=-0.57 in women and r=-0.54 in men). Similar associations have been shown in other studies and a potential biological explanation was provided in section 15.2.

In order to address this issue, associations between early life and bone microarchitecture were adjusted for total area in the corresponding bone. This lead to complete attenuation of all negative associations with cortical thickness, and in fact, a positive relationship was found with birth weight in men. This may suggest that for the size of their bones, men with higher birth weights actually have greater cortical thickness than those with lower birth weights. Such a result has not previously been shown.

15.8.3 Birth weight and trabecular microarchitecture

In women, greater birth weight was associated with lower trabecular density and lower trabecular thickness. These relationships were robust to adjustment for current body size (height and weight). In men, no evidence of such an association was identified, however, the confidence intervals overlap those seen in women and a significant interaction by sex was only found for radial trabecular density. These finding are also consistent with the results of other studies described below.

When Oliver and colleagues previously used participants from the HCS to assess relationships between birth weight and radial bone health by pQCT they showed trends towards inverse associations with trabecular density which reached statistical significance in women after adjustment for BMI and demographic and lifestyle factors(308). Clearly as this study was carried out in

a similar population to the current analyses, the analogous relationship is not surprising but does demonstrate internal consistency.

However, an independent study of young adult Gambian men and women showed trends towards negative associations between birth weight and both cortical and trabecular density in the radius and tibia using pQCT although these only reached statistical significance in men after adjustment for age, height and weight(438). Similar relationships were demonstrated by Kuh and colleagues in the NSHD(242). DXA studies have also shown analogous results(439, 440). In ALSPAC, when aBMD (total body less head) was assessed by DXA at the age of 9 years, it was negatively associated with birth weight(439). This relationship was maintained after adjustment for age at DXA, sex, gestation, parental height and weight, and child's height and weight.

The biological explanation for the inverse association between birth weight and bone density may be similar to that for cortical thickness. It has been shown that total bone area, in both the radius and tibia, is negatively correlated with trabecular density (r=-0.23 radius, -0.23 tibia in women; r=-0.17 radius, -0.19 tibia in men). Similar findings have been demonstrated by other investigators in the radius(121), tibia(338) and hip(440).

As has been discussed, as a bone's size increases its bending strength rises in relation to the fourth power of the radius. Therefore, the strains applied to the bone for a given stress will be less and therefore the stimulus for modelling and remodelling will be smaller. Thus, less bone material will be required to maintain structural integrity. As expected, after adjustment for total bone area, the negative relationship between birth weight and trabecular density is substantially attenuated (Table 13.6).

Using HRpQCT images has allowed the precise microarchitectural changes that underlie relationships with trabecular density to be explored. Strong inverse relationships were found between trabecular thickness and birth weight. This may therefore be the main factor that is driving the inverse association between trabecular density and birth weight. Again, in this and other studies, trabecular thickness has been shown to be negatively associated to total bone area, whereas in contrast, trabecular number is not related to bone size(121).

The inverse association between trabecular thickness and birth weight in women has not previously been demonstrated. It therefore represents a novel finding. Further studies are however required to replicate this finding and determine whether it represents a true sexual dimorphism.

15.8.4 Conditional growth in the first year and trabecular microarchitecture

In men, there was a significant relationship between conditional growth in the first year and both trabecular density and number. A similar trend was shown in the tibia but not the radius in women. As growth in the first year is positively associated with adult size, it might be hypothesized that this may be driving the relationships with trabecular microarchitecture. However, although relationships with trabecular number are attenuated by adjustment for adult height and weight, relationships with trabecular density are not. There is therefore a suggestion that infant growth may have a positive effect on bone health through differences in microarchitecture.

A possible explanation for the association with trabecular density in men might be through relationships with body composition. Table 13.7 clearly shows that fat mass is only associated with conditional growth in the first year in men. As trabecular density is positively associated with fat mass (Chapter 12), this might account for part of the relationship identified. It is also possible that the associations identified occurred due to chance as multiple associations have been tested.

15.8.5 Summary of early life and bone microarchitecture

The most striking findings are the positive associations between birth weight and bone area in both men and women, particularly in the tibia. Although similar relationships are demonstrated with conditional growth in the first year in men these appear to be occurring through associations with greater adult size. Negative associations between growth in early life and cortical thickness, trabecular density and trabecular thickness are consistent with other studies utilising DXA and cross-sectional imaging modalities, and appear to be largely

driven through strong relationships with bone geometry as described above. There is some evidence for an association between conditional growth in the first year and trabecular density. This may be partly driven by body composition, in particular fat mass.

15.8.6 Nutrition *in utero* and bone health

Having determined that an adverse early environment is associated with poorer bone health, investigators have also examined which factors in particular acting at this time produce the deleterious outcomes. To this end, several studies have focused on the role of diet. Due to the obvious ethical constraints, the majority of investigations have been carried out in animals.

Experiments on rats, mice, sheep and pigs have all demonstrated that fetal growth can be slowed by maternal undernutrition. Both protein and calorie restriction of a mother during pregnancy and lactation is associated with smaller offspring(441, 442). In general, the earlier in life that the undernutrition occurs, the more likely it is to have permanent effects on body size(443). If nutrition was reduced sufficiently to cause growth retardation, this led to a permanent reduction in muscle mass(443) and decreased lifespan(444).

Looking at bone outcomes specifically, the feeding of a low protein diet to pregnant rats produces offspring that exhibited a reduction in bone area and BMC, with altered growth plate morphology in adulthood(445). This regime was also found to delay the proliferation and differentiation of bone marrow stromal cells(446) as assessed by fibroblast colony formation at 4 and 8 weeks, which may represent an important candidate mechanism for the development of poorer bone health in later life.

Observational studies in humans have assessed diet at different points in pregnancy within a more physiological range. Mothers in the SWS had their dietary pattern assessed during early and late pregnancy along with body build and lifestyle(447). Dietary pattern was assessed using principal component analysis (PCA) of a validated food frequency questionnaire. A prudent diet was defined as containing high levels of fruit, vegetables, wholemeal bread, rice,

and pasta and low amounts of processed foods. A DXA scan was carried out in the offspring of 198 women. Whole body and lumbar spine BMC and aBMD were higher in children born to mothers with a higher prudent diet score in late pregnancy after adjustment for sex, height, arm circumference, socioeconomic status, maternal smoking, and vitamin D status(448). Associations with prudent diet score in early pregnancy were weaker and not significant.

Furthermore, a study from the Princess Anne Hospital in Southampton has been used to investigate the effects of individual dietary components in pregnancy on childhood bone health(449). The mothers were assessed during early and late pregnancy and samples of umbilical venous blood were also obtained at birth. The body composition of 216 offspring was then assessed at age 9 years. Children born to mothers with a lower vitamin D, as assessed by serum 25-hydroxyvitamin D concentration, had significantly ($p<0.01$) lower whole body BMC at age 9 years. This deficit remained significant after adjustment for childhood weight and bone area. In addition, those offspring with lower concentrations of ionised calcium in umbilical venous serum were found to have a lower childhood bone mass ($r=0.19$, $p=0.02$). These data suggest that fetal calcium level is important for childhood skeletal growth and that vitamin D may act *in utero* to maintain the materno-fetal calcium gradient in a state that is favourable for the fetus.

There have been studies of natural experiments in humans, including the Dutch famine, however bone outcomes were not assessed(450). In one maternity hospital in Motherwell, Scotland between 1952 and 1976, pregnant women were advised to eat a diet rich in meat and low in carbohydrate. It was erroneously felt that this high protein diet would reduce the risk of preeclampsia. Assessment of nutrient intakes in a sample of the women studied at the time showed that the advice given did lead to an increase in the mother's protein intake and to a reduction in energy intake. The result of this was lower weight gain during pregnancy and an offspring birth weight which was on average 0.51 SD below the mean for rest of the Scottish population(451).

In summary, there are several dietary factors in pregnancy which may affect fetal outcomes with potential effects persisting into later life. These therefore provide mechanisms through which an adverse intrauterine environment may occur which promotes longstanding deleterious effects on bone health.

15.8.7 Early growth and fracture

Clearly the associations described above suggest a significant role for early life in the determination of bone health. These findings are consistent with a limited number of studies that have shown a relationship between growth in early life and fracture risk. Some of the first evidence came from a unique Finnish cohort in whom birth and childhood growth data were linked to later hospital discharge records(452). After adjustment for age and sex, investigators found that a lower rate of childhood growth between the ages of 7 and 15 years (height, p=0.006; weight, p=0.01) was associated with a greater risk of hip fracture. Hip fracture risk was also elevated (p=0.05) among adults who were born short but who obtained average height by age 7 years(452).

Further evidence came from the study of a cohort of 6,370 women in whom changes in childhood BMI were determined(453). Participants were assessed at the ages of 1 and 12 years and the change in BMI over this period was calculated. This change was then assigned a z-score based on its position within the cohort as a whole. Those with the lowest z-scores were at greater risk of developing a hip fracture in later life(453). This supports the hypothesis that growth in early life modulates fracture risk.

15.8.8 Multiple linear regression and modelling of bone microarchitecture

It has clearly been demonstrated that both early life and adult body composition show significant relationships with bone microarchitecture. Interestingly, the factors that explain the majority of the variance differ depending on which bone outcome is assessed. For example, in multiple linear regression models, tibial trabecular density was associated with both birth weight and FMI in women; however, tibial total area showed significant

associations with early life factors only. This highlights the importance of assessing bone health in detail in order to accurately delineate the specific sites on which different exposures may act.

When the results of multiple linear regression analyses were used to produce models of tibial cortical thickness, these confirmed independent relationships with both early life and body composition in men and women. The variables included in the models explained almost 10% of the variance in tibial cortical thickness in women. They may therefore be important clinically given the significant role of cortical thickness in the determination of bone strength, as will be discussed in the next section.

15.9 Bone microarchitecture and fracture

15.9.1 Bone geometry and fracture

In both sexes, cortical thickness was lower in those with prevalent fractures. This is in keeping with previous studies in both women(313-315) and men(322). Similar results have also been obtained using CT of the hip. In case-control studies, investigators have demonstrated lower cortical thickness in the femoral head and neck of women with hip fracture than age and sex matched controls(454, 455). Interestingly, Poole and colleagues also showed that those with fracture had discrete patches of significantly thinner bone of up to 30%, which coincided with common sites of fracture initiation(454). Our image analysis techniques do not currently have the capability to delineate discrete areas of thinning. However, we will certainly be revisiting this in the future.

Cortical thickness is directly related to bone strength and thinning reduces resistance to crack propagation. Studies using QCT have consistently established a high level of correlation between cortical thickness and mechanically tested fracture load both at peripheral sites(456, 457) and in the femoral neck(458-460). As thinner cortices cause bones to be weaker, it is not surprising that we find thinner cortices in those with fractures. Confirmation that this is a risk factor which predicts fracture would however require a

longitudinal study. Such a study has been performed in the HCS using conventional pQCT and is currently *in press* in the journal *Bone*. This showed that cortical thickness in the midshaft predicted incident fracture in women but not in men. The absence of a significant association in men may have been due to low fracture numbers limiting study power. In a study of femoral neck QCT and incident hip fracture in the MrOS cohort, cortical volume was also associated with hip fracture risk(461).

There is almost complete attenuation of the relationship between cortical thickness and fracture status after adjustment for aBMD in men and women in our cohort. This has only previously been assessed in women and results were similar. In both studies, relationships with cortical thickness were entirely attenuated by adjustment for aBMD in the radius and, although not completely lost, were substantially reduced in the tibia(313, 315). No such analysis has been carried out in men and thus it is not possible to compare our results in this regard(322).

Cortical area was also lower in women that had fractured, consistent with findings in other studies(313-315). As total bone area did not differ in women with and without fracture, the lower cortical area was driven solely by lower cortical thickness. In contrast, total area was higher in men that had fractured but cortical area did not differ significantly by fracture status. Of note, there was evidence of an interaction by sex showing the relationship between fracture status and tibial cortical area differed significantly in men and women. These findings are consistent with the results of Ostertag and colleagues in men with idiopathic osteoporosis(322). Notably, the relationship between total area and fracture status in men was maintained after adjustment for femoral neck aBMD, suggesting a potential independent effect.

As bone cross-sectional area tends to increase with age in later life, one could hypothesize that this relationship might be due to confounding by age. Similarly, greater body size has been shown to affect bone geometry and also modulates the forces on bones during a fall, influencing fracture risk. However, adjusting for age and adult anthropometry (height and weight) had no effect on the strength of this finding.

Discussion

One might intuitively think that larger bones would invariably be stronger and therefore it would not be expected that those who had fractured would have larger bones. However, it has previously been shown in this thesis that on average larger bones have lower cortical thickness and the possible reasons for this have been discussed in section 15.2. In early adulthood, it is likely that this state is advantageous to the individual providing greater resistance to bending stresses. In later life, however, when remodelling increases and significant bone loss occurs it may become a weakness. A large bone with a thinner cortex will have a greater endosteal surface on which resorption can occur and this could lead to a more substantial loss of bone mass. Consequently, in a wider bone, with a thinner cortex, endocortical resorption is likely to be more deleterious than in an individual with a thicker cortex(462-464). Additionally, as cortices thin further and become more porous they may reduce to a point at which they become fragile and offer less resistance to crack initiation and propagation should a traumatic event occur. The risk of developing discrete patches of significantly thinner bone is also likely to be higher in this group and this has been shown to be associated with fracture status(454).

Although bending strength rises considerably as bone diameter increases, the relationship with buckling ratio is different. Buckling ratio is a measure of the likelihood of a structure to buckle under compression. It is determined by the maximum distance from the centre of mass to the outside of the cortex divided by the cortical thickness. Thus, as the former increases and the latter decreases, the buckling ratio increases along with the likelihood of buckling. The risk of buckling might therefore be expected to be higher in wider bones.

Overall these findings suggest that bone geometry, measured at the distal radius and tibia, does differ by fracture status and thus may play a role in fracture prediction. However, in this study the fractures occurred prior to the measurement of bone microarchitecture and this raises the possibility of reverse causation. For example, it could be hypothesised that following a fracture an individual might be more cautious and consequently take less exercise, thus reducing the loading on their bones with effects on bone microstructure. This would therefore provide an alternative explanation for the

associations found which would similarly apply to the associations found with cortical and trabecular microarchitecture.

Whatever the cause of the relationships, the most consistent finding was the difference in cortical thickness which, when low, is likely to adversely affect the mechanical integrity of the bone. In contrast, it was only in men that total area was greater in those that had fractured and this was robust to adjustment for femoral neck aBMD.

15.9.2 Cortical density, cortical porosity, and fracture

Tibial cortical density was found to be lower in men and women with prevalent fractures. In the radius, similar trends were seen but these did not reach statistical significance. This is consistent with the previous literature which has shown some evidence of associations between cortical density and fracture in both men and women(121, 313, 322). The importance of bone density to bone strength is illustrated in the equation for estimating stress strain index (Appendix 8). Given that the cortex is the region most distant from the centre of gravity of the bone, cortical density is likely to have a greater influence on bending strength than trabecular density.

Interestingly, after adjustment for aBMD, relationships between cortical density and fracture status were attenuated although remained statistically significant in women, suggesting that cortical density may add to the discrimination of those women with and without fractures.

An unexpected finding was the relationship between higher radial cortical porosity and a lower likelihood of fracture in women (OR 0.65, 95% CI 0.44,0.96). Firstly, this is surprising as one might expect that a more porous cortex might predispose to fracture rather than the converse. Furthermore, this is at odds with previous studies that have not shown any evidence of an association between fracture status and either radial or tibial cortical porosity(316, 317). Given the large number of associations tested, it seems most likely that this was a chance finding and does not represent a true relationship. Another possibility is, however, that those individuals that had fractured were more likely to be started on bisphosphonates and it is these

drugs that have caused a reduction in cortical porosity in this group leading to the observed difference.

15.9.3 Trabecular microarchitecture and fracture

In men and women, and in both the radius and tibia, the mean trabecular density, number, and thickness were numerically lower in those with a prevalent fracture suggesting these parameters may be protective. However, the differences only reached statistical significant in the radius in women. Interestingly, the odds ratios found for trabecular density in both men and women were similar in magnitude to those for femoral neck aBMD from DXA.

In keeping with our study, previous investigators have also found radial trabecular density, number, and thickness to be lower in women with a prevalent fracture. In contrast, however, they also identified consistent differences in trabecular density and thickness in the tibia by fracture status(121, 313-315). Prior findings in men were very similar to our results. Specifically, Ostertag and colleagues found no significant difference in trabecular number and thickness between those men with and without fractures(322).

The reason for the consistent lack of an association between trabecular architecture and fracture status in men is uncertain. There is evidence of a difference in the mechanism of trabecular decline between sexes. In women, the sudden decrease in oestrogen following the menopause leads to an increase in remodelling rate with a greater amount of trabecular bone removed in each remodelling event than is replaced(117). This leads to a loss of trabecular plates and their connectivity which causes a considerable decline in strength(465). After the perimenopausal period, trabecular deterioration is more akin to that seen with a general ageing decline. In men, trabecular microarchitectural deterioration occurs to a greater extent by trabecular thinning, due to reduced bone formation, than perforation and loss of connectivity, due to increased bone resorption(384, 466).

The absence of relationships between trabecular architecture and fracture in men in our study may also be related to the less severe nature of disease in

those that fractured compared to women. Although the proportion of men and women that fractured were fairly similar (25% in men and 31% in women), those women that fractured had suffered on average 1.9 fractures compared to just 1.3 in men. This may have reduced the ability to discriminate the fracture and non-fracture groups. There may also be an issue of power. Given the number of men with and without fractures, and the means and SDs of radial trabecular density in each of these groups, there is only 21% power to detect a difference between the two populations. Radial trabecular density was 4.5% lower in those that had fractured; however, it would have had to have been 10.7% lower for the analysis to have had 80% power to show a significant difference.

15.9.4 Importance of bone microarchitecture to fracture

This study provides novel evidence that men with fractures tended to have a greater total bone area without significant differences in trabecular parameters, whereas the bones of women that fractured did not differ in overall size but had deficient trabecular architecture. Interestingly, excluding mild vertebral fractures from the definition of prevalent fracture did not materially affect the results identified.

In both men and women, it was shown that those with a prevalent fracture had lower cortical thickness in both the radius and tibia, and this is consistent with previous studies. It also fits well with our understanding of the determinants of bone strength *ex vivo* and equations utilised in mechanical engineering(456-460). Clearly, this understanding is predicated on the assumption that it is bone health that is determining the likelihood of fracture, and as discussed previously the reverse may be true especially considering the timing of the fractures in relation to the acquisition of scan images. Some similar parameters have previously been shown to contribute to fracture prediction in longitudinal studies when assessed using convention QCT in the hip and peripheral long bone diaphyses but as yet not at the distal radius and tibia using HRpQCT (461).

16.Limitations and strengths

16.1 Limitations

16.1.1 Study Cohort

The Hertfordshire Cohort is a unique group of individuals. All participants were born in Hertfordshire and have either remained there throughout their life or left but returned to live there again in older age. This might suggest that they are inherently different to people that migrate to different geographic locations during their lifetime. However, it has been shown previously that those individuals recruited to the HCS were similar to those within the nationally representative Health Survey for England (HSE)(467). Differences were seen in some demographic and anthropometric parameters but these were generally small. HCS participants were taller with better self-reported general health (SF-36), and were less likely to be at the extremes of socioeconomic status. The proportion of women that were current smokers or heavy drinkers was lower in the HCS(467).

The better self-reported general health, and in women, lower rates of current smoking and heavy drinking, could be evidence of a healthy participant effect. This bias is likely to have been introduced at the initial recruitment stage. Birth weight and weight at 1 year were also compared between those individuals that took part and those that did not (as no further data were available on the group that did not take part). These values were found to be similar, although weight at 1 year was significantly higher in those that participated.

Recruitment of participants to the HCS took place over 10 years ago. In the intervening time period there have been several rounds of follow up with attrition at each point. An assessment was therefore made to determine whether individuals that took part in the current study differed significantly from those initially recruited. This was based on early life, demographic, anthropometric and lifestyle data collected at baseline. It was found that individuals that were recruited to the HCS at baseline but did not take part in the current study were lighter at birth, older and had a higher BMI (table 10.2).

Their levels of physical activity were lower and smoking was more common. Clearly, there appears to be a bias here with healthier individuals at baseline being more likely to continue to participate. There are two main reasons for this. Firstly, those individuals that were less well at baseline are more likely to have died or no longer be fit enough to travel in a taxi to the MRC unit to undergo the required tests. Secondly, those men and women that were most interested in healthcare research may also be more likely both to adopt a healthy lifestyle and to take part in the study.

Interestingly, it was also found that those individuals who did not take part were more likely to abstain from alcohol. It has been shown previously that British men and women of lower educational attainment are more likely to abstain from alcohol(468). Furthermore, it is also possible that the reason for abstinence is ill-health, in which case this finding would still fit with a healthy responder bias.

In order to investigate the source of the selection bias further, an analysis was then completed to ascertain whether the recruitment strategy used in the current study had in itself contributed to the issue. Therefore, individuals that participated in the study were compared to those that were invited but declined to take part (Table 10.3). Those that took part were heavier at birth, on average approximately 8 months younger, 2.4cm taller, had a lower BMI and a higher physical activity score. All of these findings remain consistent with a healthy responder bias. The two groups did not differ by sex, calcium intake, smoker status, alcohol consumption, or social class.

This project investigates internal associations and it is not thought that these are likely to be significantly altered by the small differences between the study and general populations. However, it must still be taken into consideration when interpreting the study results, in particular with respect to generalisability. In this regard, all study participants were born in England and are white Caucasian. This limits generalisability of the findings to people from other geographical regions and of different ethnic origins.

16.1.2 Historical records

The historical records used to identify individuals to take part in the HCS were written by Ethel Burnside and her team of midwives and health visitors before the Second World War. They are clearly an excellent resource but the information documented is limited. For example, although birth weight is available, data on gestation at birth are not. Thus, it is not possible to differentiate a preterm low birth weight baby from one with intrauterine growth restriction. The pathogenesis of these two conditions is likely to be different and it is possible that this might influence the effect on bone health. However, at the time when the cohort was born the majority of significantly preterm babies did not survive and so in most cases low birth weight individuals would be small for dates.

16.1.3 Data from questionnaires

The first limitation of the data collected from questionnaires in the HCS is that it tends to be self-reported rather than objectively measured. With the exception of one round of follow up conducted by postal questionnaire, the remaining questionnaires were administered by trained nurses. The second limitation is that many of the demographic and lifestyle factors used were obtained at earlier time points in data collection; specifically at "baseline" (1998-2003) and "musculoskeletal follow up" (2004-2005). Although further similar information was obtained on participants in 2011, this did not include the entire cohort and so it would have necessitated the exclusion of individuals from the analysis.

Baseline data included calcium intake, physical activity, social class, and in women, time since menopause and use of HRT. Although both calcium intake and physical activity show evidence of tracking and do therefore correlate with future levels(469-471), what we have measured is purely a snapshot of reported levels over 10 years ago. It could be argued that these may be determinants of bone health in their own right; however, it is important to be aware of this limitation.

As social class in the HCS was determined by own current or most recent occupation in men and single women, and of the husband in ever-married

women based on the OPCS Standard Occupational Classification scheme for occupation and social class, it is unlikely to have changed significantly since it was recorded at baseline when participants were mostly in their sixties. However, this is still a potential source of error. In a similar vein, given the age of the cohort at baseline, it was found that all of the women in the study had gone through the menopause. Furthermore, many had started, and even completed, a course of HRT at study baseline and again, because of their ages, it would have been unusual for them to start this treatment subsequently. Therefore, it was felt that HRT use could be reliably coded as ever or never use to be included in the current analysis. Time since menopause was recalculated from age at menopause and current age.

Both smoking status and alcohol consumption, measured in 2004-2005, are subject to change. As with physical activity and calcium intake, previous lifestyle is likely to be predictive of current status and may also have a direct effect on current bone health independent of this. It was felt important to include comorbidities as a covariate in the analyses. The choice of which to diseases to include was, however, restricted by what had been collected. Full information on the presence or absence of individual diseases was only available from baseline (1998-2003). The spectrum of diseases was limited, but did include cardiovascular, respiratory, endocrine and neurological conditions. The lack of a broader range of diseases is a potential limitation.

Non-vertebral fracture status was ascertained at each stage of study follow up; most recently in 2011. As with the demographic and lifestyle factors above, it was self-reported. The accuracy of self-report for fracture is site dependent and has been found to be higher for some common fractures such as the distal forearm(339, 472). When validation of the method was undertaken by Ismail and colleagues using radiographs and medical records, in 11% of cases no evidence of a fracture was found suggesting these were false positives(339). Therefore, although the accuracy of the assessment in the current study is not certain, it is likely to be similar. Unfortunately, the data were not available to distinguish between high and low trauma fractures. This would have been a useful distinction as bone microstructure would be expected to play a greater role in the pathogenesis of low trauma fractures.

16.1.4 Dual energy X-ray absorptiometry

DXA was used in three different modalities in this thesis: i) assessment of aBMD in the femoral neck; ii) determination of body composition; and iii) VFA. All of these techniques have their own significant limitations which must be taken into account when interpreting the findings.

Femoral neck aBMD was used as the current standard of care for the assessment of fracture risk using radiological imaging. Relationships between fracture status and bone microarchitecture were adjusted for femoral neck aBMD to ascertain whether HRpQCT might offer additional information above and beyond DXA. As the technique used to scan the hip was the same as that used in NHS hospitals, any limitations of precision and accuracy found in our study would also be found in clinical practice. Therefore, this makes it an appropriate measurement for HRpQCT to be compared to. The main limitations of DXA scanning per se are discussed in section 3.2.

Although adjusting relationships between fracture status and HRpQCT measurements for femoral neck aBMD allowed consideration to be taken of imaging in routine clinical practice, it did not take into account other risk factors that are also used to predict the likelihood of fracture, such as family history. Furthermore, it could be argued that, as it is possible to assess aBMD in the distal radius, it would be more appropriate to adjust for radial aBMD instead. However, this measurement is only used in specific cases in clinical practice and so does not represent routine care.

The main limitations of body composition assessment by DXA are technical issues. The scanning bed does have a maximum value for weight although it did not result in the exclusion of any individuals from this study. The whole-body field-of-view is also limited. As a result, some larger individuals would not fit completely within this region. In such circumstances, the majority of individuals had one side of the body scanned and then results were doubled to provide the total values(473). Similarly, in this cohort there is a significant proportion with metalwork in situ, such as joint replacements. This problem was addressed where possible in a similar way to that described for large individuals (Appendix 7).

Discussion

Assessment of body composition relies on several assumptions including that fat has a constant attenuation and that lean mass estimates are not affected by hydration. Estimates of fat mass by DXA can also be influenced by "trunk thickness". Interestingly, fat mass estimates have been shown to be more accurate in obese individuals whereas the converse was true for lean mass estimates(474). This issue is a particular problem in the trunk. As a result, some investigators have adopted measurement of appendicular lean mass and fat mass instead of total to avoid this problem. The utilisation of total body measures of body composition in this study allows us to take into consideration central obesity as well as subcutaneous fat in the limbs alone. Assuming that this method was associated with greater error in the assessment of lean mass, this would have reduced the likelihood of finding significant associations. We did however demonstrate consistent relationships with LMI and FMI in both men and women. Despite these limitations, DXA is commonly used for the measurement of body composition as it is simple, widely available, and only associated with a low radiation exposure.

VFA images were assessed using the Genant semiquantitative technique(100) in order to determine whether an individual had suffered a vertebral fracture. These data were then combined with self-reported non-vertebral fracture status to optimise our estimation of whether an individual had ever fractured. The Genant method has several limitations. Firstly, from morphometric data on healthy individuals we know that vertebrae in the mid and lower thoracic region are naturally more wedged than in other areas. It is therefore possible that these may be incorrectly diagnosed as mild vertebral fractures. However, given that the Genant method is a semiquantitative technique, it also requires the individual interpreting the scan to assess the vertebral endplate and other morphological features to determine whether or not there is a true fracture. In the lumbar spine, biconcavity of the vertebral bodies could cause a similar problem. It is therefore difficult to reliably ascertain whether or not a mild fracture is present.

Furthermore, the criteria for a mild fracture are adjacent to those for a moderate fracture and so on. Thus, in borderline cases, it may be difficult to differentiate the two. However, although there is a graded relationship with aBMD depending on fracture severity, individuals with mild fractures still have

a lower than normal aBMD(475). Consequently, the most important issue is determining the presence or absence of fracture. In keeping with the previous literature, in the current study we found good agreement between the two reviewers for whether or not a fracture had occurred (κ=0.65).

16.1.5 High-resolution peripheral quantitative computed tomography

HRpQCT provides detailed information regarding bone structure. However, it does so only at peripheral sites and does not assess the spine and proximal femora, which are important clinical sites of fracture. Therefore, there is some uncertainty regarding whether the readings obtained are representative of bone strength in the axial skeleton. The initial studies that have been carried out have shown a moderate correlation between the two(123, 476).

HRpQCT also has several technical limitations. Firstly, although the Xtreme scanner provides high-resolution images, the voxel size is clearly not zero. As a result, the partial volume effect may influence the results of image analyses. The scanner produces a value for each voxel based on the average density contained within it. Consequently, it is unable to distinguish between a voxel partially filled with high density bone and one completely filled with lower density bone. Therefore, there is a tendency to underestimate the true density of a structure particularly if it has a large surface area for a given volume. This might be one explanation for why thinner cortices are shown to have lower bone density. As women have, on average, thinner cortices it is likely to have a greater effect on their results. Furthermore, the partial volume effect will also have an influence on measurements of trabecular microarchitecture and cortical porosity.

Secondly, accurate segmentation of cortical and trabecular bone is a difficult technical issue. Although we describe these as two separate entities, in reality there is a gradual transition between the two and it is therefore often very hard to delineate exactly when the change occurs from cortical to trabecular bone. As a result of this problem and the limited ability to directly quantify pores, unless the Haversian canals are relatively large, means that assessment of cortical porosity is particularly challenging(477). These factors are all likely to play a role in the high %CV found for cortical porosity, particularly in the

radius(328). This may improve in the future with the development of higher resolution *in vivo* instruments and better methods of image analysis.

Thirdly, due to the high-resolution of the images and the relatively long acquisition time (~3 minutes) there is always the potential for motion artefact which can significantly degrade the images. Specifically, measures of microarchitecture are more sensitive to such effects when compared to geometric and density assessments(329, 478). In this study, attempts were made to minimise this issue by taking appropriate steps to prevent movement by the use of a cast and the maintenance of appropriately calm conditions. If motion artefact was still noticed the scan was then repeated with the aim of obtaining a better quality image. It has been shown, however, that even repeating a scan up to 3 times only obtains better images in around half of individuals(327). This is why there were still some images with significant artefacts that needed to be excluded from the statistical analyses. In general, motion artefact tends to be a greater problem at the radius than the tibia which may explain why measurement precision is greater in the latter(479).

Fourthly, HRpQCT scans were carried out by two separate operators. Although there was a strict protocol followed in image acquisition, including in the positioning of the reference line on the scout view, significant differences were noted by operator between radial trabecular thickness ($p=0.005$) and cortical porosity ($p=0.029$) after adjustment for potential covariates. Given that 20 variables were assessed, we might expect that one significant difference would be found by chance alone and neither would reach statistical significance if a strict Bonferroni correction was applied. It appears less likely that these findings represent a true systematic difference in the way participants were scanned although this possibility needs to be taken into consideration when interpreting the results.

Lastly, although trabecular number and density are directly measured, trabecular thickness and separation are derived using standard stereological relations based on a plate-model geometry(330, 480). These estimations are therefore dependent on several assumptions including those implicit in the plate model. For example, it is assumed that bone tissue has a fixed density within the trabecular region. This is problematic as bone tissue density is

actually affected by various factors including drugs(481). This would be a particular problem in longitudinal studies where the matrix mineral density can change in an individual. Despite these limitations, bone microarchitecture as assessed by HRpQCT correlates well with transiliac bone biopsies(123).

16.1.6 Study design

The chronicity of variable acquisition is important when attempting to attribute causality in any association. In the HCS we are fortunate to have information regarding early life which clearly precedes the collection of HRpQCT data. It is therefore likely that the former is influencing the latter rather than the converse. However, body composition was collected contemporaneously with bone microarchitecture and so in this cross-sectional analysis the direction of causality is more difficult to determine. As a result, inferences are made based on known biological pathways and a general understanding of human physiology.

In the analysis involving fracture status, the non-vertebral fractures had occurred at various time points between the age of 45 years and the time of scanning. Furthermore, it was not possible to determine the date of occurrence of the radiographic vertebral fractures. All fractures did, however, occur prior to HRpQCT scanning. Although we usually think of bone health predicting fracture risk, this temporal relationship raises the possibility that it is fracture status that is influencing bone health. For example, suffering a fracture may influence levels of activity with effects on bone microarchitecture. It is not possible to delineate the direction of causality in this study and this has been taken into consideration when interpreting the findings.

16.1.7 Statistical analyses

The main statistical issue within this project is multiple testing. Although great efforts were made to limit the number of parameters utilised from the HRpQCT scanner, it was necessary to include 10 from both the radius and tibia in order to avoid losing important information provided by this new technique. As relationships were assessed separately in men and women, this meant that

for each area investigated, there were a total of 40 analyses completed. As a result, the likelihood of a p value of less than 0.05 occurring by chance was not insignificant. This had to be taken into account in the interpretation of all results.

Consequently, when trying to ascertain whether a real relationship had been demonstrated, consideration was made of whether there was biological plausibility and whether the results were consistent at different sites (radius and tibia) and in both men and women, although, clearly relationships may be sex and site-specific. The strength of the association is also taken into account along with its consistency with other previous research in the area(482).

16.2 Strengths

There are several strengths to this study. The data available from the Hertfordshire ledgers allows investigation of early life effects without the potential for recall bias. This is not a commonly available resource and is very important to the study. The cohort has also been extensively phenotyped and as a result it is possible to adjust for multiple covariates during the analyses and thus reduces the risk of significant confounding. The narrow age range of participants is both a strength and a weakness. It allows this group to be investigated in detail but limits assessment of age-specific differences and reduces generalisability.

In terms of the imaging modalities, HRpQCT gives the most detailed *in vivo* assessment of bone microarchitecture available in humans. This information permits a more thorough evaluation of the determinants of bone health and allows us to identify the specific structures on which factors, such as birth weight, have their effects. It is also important to emphasize that although we were unable to assess the proximal femur and spine, the distal radius is in itself an important site for fracture. It might be argued that the tibia is not such an important site clinically; however, as a weight-bearing bone it also provides additional value.

DXA is as an appropriate technique for the measurement of body composition and currently remains the gold standard for the assessment of osteopenia and osteoporosis. The use of DXA imaging to obtain information regarding the presence or absence of vertebral fractures is clearly necessary to make an accurate assessment as up to two thirds are occult. This significantly improved our estimation of whether an individual has suffered a fracture which was important when determining the associations between fracture status and bone microarchitecture.

17.Implications for clinical practice

The use of HRpQCT is increasing and it is becoming more prominent within scientific literature. It has grown from the earliest studies, which examined simple sex-specific age-related differences across the adult lifecourse(4, 121), and has now taken on a myriad of roles. Moving from an assessment of aBMD based on DXA alone, the additional structural and microarchitectural information provided by HRpQCT allows researchers to explore in greater detail where various influences on bone specifically occur. This is highlighted by the ability of HRpQCT to discriminate the associations between LMI and cortical geometry from those of FMI and trabecular microarchitecture.

Better understanding of these relationships will drive the development of new hypotheses and there is great potential for this to be expanded to address other research questions. For example, by appreciating how muscle and fat differentially relate to bone we may be able to more fully understand how sarcopenia and obesity might influence bone health, identify which biological mechanisms are likely to have the greatest influence, and develop further studies to explore these mechanisms more fully, as described in section 18.

Furthermore, HRpQCT is starting to be used to glean further insight into the mechanisms by which secondary causes of osteoporosis act and where exactly medications that treat osteoporosis have their effects(235, 236, 483, 484). Theoretically, this could allow stratification of patients to treatments which best remedy the abnormalities in their bone structure. However, in practice this may not improve treatment efficacy and furthermore there are also several issues, including cost, which may prevent its routine use.

Similarly, given the high resolution of the technique, it may become possible to monitor the effectiveness of treatment more precisely. At present, DXA is usually repeated after a minimum of two years and there is uncertainty regarding how accurately the results reflect drug efficacy in terms of fracture risk reduction. HRpQCT may offer an alternative which more precisely delineates whether the drug is working and might allow more informed

treatment decisions to be made. Again, due to practical issues its main use is, however, likely to remain in a research setting.

In the management of osteoporosis, fracture prediction is of great importance to allow targeting of therapy to those at highest risk. The usefulness of HRpQCT for this purpose has not yet been confirmed but research is suggesting that it may provide additional discrimination independent of aBMD in women(315). The current project has again demonstrated the pattern of differences in bone microarchitecture by fracture status in women and has also shown novel findings in men relating to the importance of total and trabecular area. Clinically, this may highlight an important facet of bone strength, in men but not women, which needs to be taken into consideration in future studies. It also provides evidence that HRpQCT may add to fracture prediction in men as well as women. However, a great deal of further work is required to determine whether it provides a clinically meaningful benefit in fracture prediction in longitudinal studies and that this leads to an acceptable and cost effective alternative to current methods.

The findings relating growth in early life to bone microstructure may have public health implications. For example, if the association between conditional growth in the first year and trabecular microarchitecture is replicated in mother-offspring cohorts, such as the Southampton Women's Survey, the next step would be to try to identify a modifiable risk factor which explained some of the relationship. This would then be a target for use in future interventional studies with potential therapeutic implications on a population scale.

18.Further areas of research

Although several studies have investigated changes in bone microarchitecture in men and women over the adult lifecourse, there is little data regarding what is happening in later life specifically. The results of the current study therefore do add to the knowledge base in this area but require replication in similar cohorts. Also, the current analysis is cross-sectional. The completion of longitudinal studies would allow assessment of changes with age in individuals to be demonstrated rather than inferred. This is planned to be carried out in the HCS after a few years have elapsed. Longitudinal data has already been collected on bone structure using conventional pQCT. I plan to initially use this to describe age-related changes in geometry and density in older individuals.

Relationships between body composition and bone microstructure appear to some extent to be age-specific. Although we now have evidence of the associations in a population of older men and women, this requires replication in larger cohorts and similar studies to be carried out in other age groups. Furthermore, although measures of bone microstructure and estimations of muscle mass are important, relationships between direct assessments of muscle function, such as grip strength, and bone strength, as determined by FEA, should also be explored as they are also of clinical significance. Grip strength has recently been measured in most of the study participants and FEA software is now available to us allowing this analysis to be carried out. There is also a need to look in more detail at the importance of fat distribution and to individually assess the importance of intramuscular, visceral and subcutaneous adiposity.

Although there are some theories which in part explain the associations described between bone health and both lean and fat mass, the physiology in this area is by no means fully understood. Further investigation is required to distil out the biological mechanisms that underpin these relationships. It is possible that this will lead to the development of novel therapeutic agents in the treatment of both osteoporosis and sarcopenia. The latter, in particular, is in great demand.

Discussion

Having demonstrated the associations between lean mass and cortical bone health, we are currently undertaking a study to investigate one of the possible mechanisms underlying this association. All participants of the current study will be approached to have accelerometry to assess the mechanical impacts that they each undergo in a week. If this correlates with cortical bone health the next step would be an interventional study which includes high impact loading with the aim of increasing bone, and potentially muscle, health.

Relationships between early growth and bone size have been clearly demonstrated and these may augment bone strength. It has also been shown however that there may also be negative relationships with cortical thickness, trabecular density, and trabecular thickness which would have the opposing effect. It is therefore uncertain whether the overall effect of higher birth weight on bone microarchitecture would be to strengthen or weaken the bones. Assessing the relationships between early life and bone strength, as determined by FEA, would be the next step in addressing this issue.

Furthermore, much of the discussion in this area is predicated on the assumption that relationships between birth weight and bone size occur early and that other associations with bone structure occur as the bone adapts. It would be useful to explore this using a cohort of children with serial cross-sectional imaging and data on growth in early life. It is also possible that early life events could influence the rate of bone loss in later life. Consequently, it is planned to investigate the effects of birth weight and growth in the first year on the rate of change of bone structure as assessed by pQCT.

Having demonstrated cross-sectional relationships between bone microarchitecture and fracture, the next step would be to assess whether HRpQCT has the potential to predict fracture prospectively. This will require a longitudinal study. There are plans to follow up individuals within this study in the future to determine whether or not they have subsequently fractured allowing us to address this question. Assessments of cost effectiveness, feasibility, and acceptability would also be required. Before these are carried out, it would also be possible to use to the FEA software to further investigate the cross-sectional associations between bone strength and stiffness, and fracture status.

Additionally, there are plans to collaborate with engineers at the University of Southampton to perform further analysis of the HRpQCT images to assess whether local areas of cortical thinning relate to fracture risk. By fostering such collaborations it may also be possible to apply newer machine learning models to try to enhance prediction of fracture by HRpQCT outcomes.

19.Conclusions

It has been demonstrated, in this well phenotyped cohort of older men and women from Hertfordshire, that several demographic and lifestyle factors influence bone microarchitecture including age, sex, alcohol consumption, and, in women, the number of years since menopause independent of age. There were also clear relationships between bone microarchitecture and adult anthropometry. Height was predominantly associated with total and trabecular area whereas BMI was associated with cortical area and thickness, and trabecular density and number.

When body composition was assessed in more detail using DXA, differential relationships were shown for LMI and FMI. Although in univariate analyses, associations were similar to BMI, when both components were included in the same model, LMI was associated solely with cortical geometry and FMI with trabecular microarchitecture. These finding are novel and suggest that lean mass and fat mass may be affecting bone independently through different mechanisms.

Evidence is also provided that bone microarchitecture can be influenced early in life, consistent with the developmental origins hypothesis. In both men and women, birth weight was positively associated with both total and trabecular area, and these relationships were robust to adjustment for adult size. Furthermore, in women there were inverse relationships between birth weight and both cortical and trabecular thickness, although these were considerably attenuated after adjustment for bone size. In contrast, in men, there was a positive relationship between growth in the first year and trabecular density which was robust to adjustment for adult size, and demographic and lifestyle factors. Clearly, these latter findings are novel and certainly require replication.

When bone microarchitecture was assessed, in those with and without a fracture, several differences were found. In both men and women, cortical thickness and density were lower in those that had not fractured. Men who

had fractured had greater total and trabecular area. In contrast, women who had fractured had inferior trabecular microarchitecture, including density, number and thickness. Relationships between bone microarchitecture and fracture status for both men and women had not previously been investigated within the same cohort. Therefore, the finding of some significant sex interactions is novel.

The main importance of these relationships is the potential to improve fracture prediction and therefore the targeting of therapy. However, after adjustment for aBMD at the femoral neck, relationships with cortical parameters (area, thickness and density) and trabecular microarchitecture were attenuated, suggesting that these parameters are less likely to add to current fracture prediction tools. In contrast, in men, associations between fracture status and both total and trabecular area were maintained after adjustment and may therefore have a potential role. This finding requires further investigation in prospective longitudinal studies.

Appendices

Appendix 1 – Explanation of Stress Strain Index

The polar SSI predicts the torsional bone strength(485). In contrast to axial SSI, it is not affected by the rotation of the arm within the gantry during scanning. In order to calculate SSI the torsional CSMI is first calculated from the distances of the voxels from the centre of gravity (r_i) and the area of each voxel (a).

$$CSMI = \Sigma r_i^2 * a$$

This is then divided by the maximum distance of any voxel from the centre of gravity (r_{max}) to provide the section modulus or moment of resistance. This value obviously represents the geometric properties of the bone but not its material properties. These are however related to the bone mineral density of the cortex. Therefore, the section modulus is then multiplied by the quotient of the calculated cortical density (CD) divided by normal physiological density (ND, 1200mg/cm^3). Consequently, the equation for calculating SSI is as follows:

$$SSI = \Sigma(r_i^2 * a * CD)/(r_{max} * ND)$$

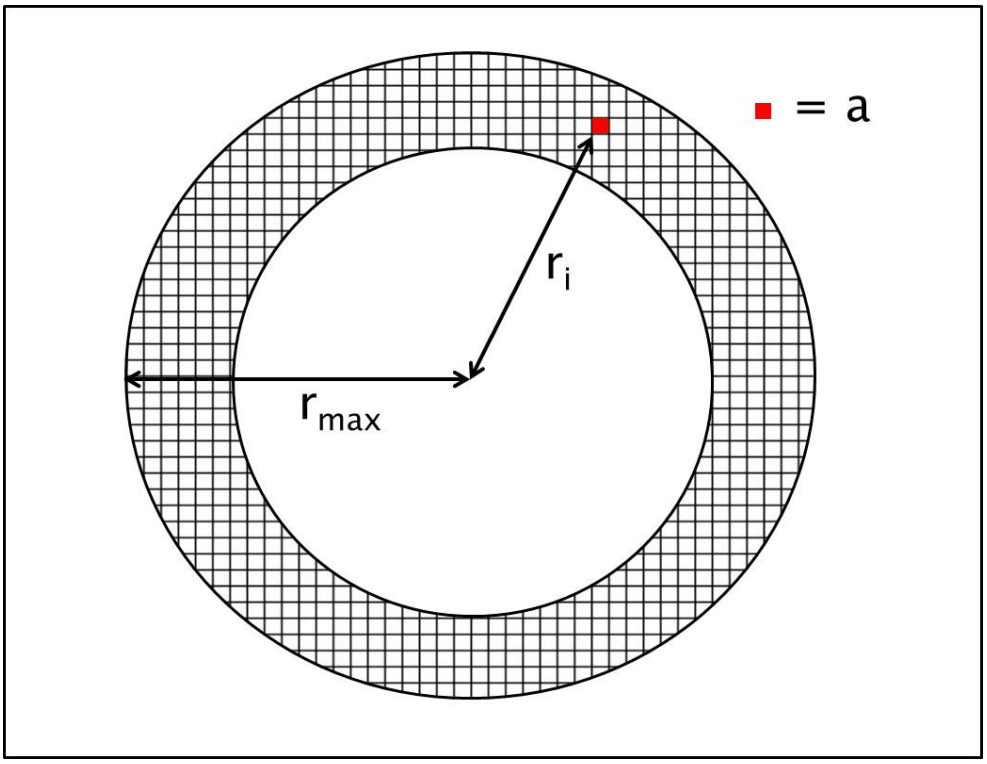

Figure A1: Diagrammatic representation of components of stress strain index

Appendix 2 – Study Invitation Letter

Dear *<<subject title>> <<subject surname>>*

It was around a decade ago that we first invited you to join the Hertfordshire Cohort Study and with your participation we have continued to move forward in our understanding of how events very early on in life can influence a person's risk of developing osteoporosis. We would therefore like to take this opportunity to thank you for the invaluable help that you have given us.

Shortly after your first visit to see us, we carried out a bone density scan (DXA). A few years later you kindly agreed to a repeat bone density scan and a special CT scan of your forearm and shin bones. We now hope to build on this work, and are writing to invite you to participate in our newest project.

We would arrange that on a mutually convenient day, a complimentary taxi would transport you (with 3 other previous participants) to the MRC Human Nutrition Research unit in Cambridge. There we would repeat the above scans to look at potential changes in your bones over time. We would also carry out a new state-of-the-art scan which provides detailed information about the structure of bones at the microscopic level.

Lunch and snacks would be provided and a complimentary taxi would take you home at the end of the session. The whole visit should take about 3-4 hours, excluding travelling time. The results of your DXA scan will be sent to your GP. Further information about exactly what the study would involve is provided on the enclosed participant information sheet.

If you would like to take part, please complete and return the reply slip attached in the enclosed prepaid envelope, and one of our team will contact you shortly to arrange an appropriate date. If you are unable to participate on this occasion, we would be grateful if you could still return the reply slip indicating this. Thank you again for taking the time to consider this invitation.

We hope to see you soon.

Yours sincerely,

Dr Mark Edwards

Serial number_____

Name_____

Telephone no_____

I am willing / unwilling to take part *(please delete as appropriate)*

Appendix 3 – Participant Information Leaflet

What are the determinants of the internal structure of bone?

Chief Investigator: Dr Mark Edwards

Study Site:

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Introduction

You are being invited to take part in a research study. Before you decide whether or not you wish to take part it is important that you understand why the research is being done and what it will involve. Please read this information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

Details about the conduct of the study are on another sheet (Sheet 2 -Study Conduct Information) and this should also be read before deciding whether or not you wish to take part.

What is the purpose of this study?

Osteoporosis is a condition caused by the thinning of bones leading to an increased risk of fracturing. It tends to affect older people and women more than men. Previous studies have shown that some of the risk of osteoporosis is determined very early in life and may be related to your weight at birth and 1 year of age. We aim to find out whether weights at time points in early life also affect the internal structure of bones.

Why have I been chosen?

You have been chosen because you have helped us with a previous related study in which you had two scans to assess your bone health. It would be very useful for us to compare information we have about you from the previous study with information we obtain in this study. We are hoping to find about 500 men and women aged 65-85 years old who are interested in taking part.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given these information sheets to keep and asked to sign a consent form. You are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?

You will be contacted by one of our researchers and an appointment will be made for you to come to the MRC Human Nutrition Research (HNR) in Cambridge on a day that is convenient for you. On that day a complimentary taxi will pick you up and take you home again. The whole session should last about half a day and during your stay, lunch and snacks will be provided.

On your arrival at the MRC HNR we will ask you to sign a consent form. Your height and weight will then be measured before you have 3 scans: a DXA scan, a pQCT scan and a HRpQCT scan.

DXA stands for dual energy x-ray absorptiometry. A DXA scan involves lying on a firm table whilst an x-ray arm passes over you. A dressing gown will be provided for you to wear during the scan. The scan will last a total of about 30 minutes and will measure your body fat, lean tissue and bone density, including in the hips. You may recall having a scan like this before as part of our study. A lateral spine scan will also be performed which requires you to lay on your side for a short time.

PQCT stands for peripheral quantitative computed tomography. A pQCT scan will involve us carefully positioning your lower leg and then your forearm inside a scanner. There will be rests available to help keep the scanned limb completely still during the short procedure. This scan will take approximately 30 minutes and will produce three dimensional images of the bones in your lower leg and forearm from which we can assess their strength. You will also have had this scan in 2005.

HRpQCT stands for high resolution pQCT. This is a similar scan to a pQCT scan although it uses a slightly different machine and produces more detailed pictures. Again your lower leg and then your forearm would be scanned. This scan takes approximately 45 minutes. None of the scans would require your head or body to be enclosed within a tunnel.

The last test is an activity called Jumping Mechanography. The action required for this would be demonstrated to you beforehand and you can then decide whether or not to undertake this part of the study.

You would be asked to stand on a platform and then jump upwards as high as you can. The platform would measure the force you exerted on it. This would be repeated 3 times with at least a one minute break between each repetition.

What do I have to do?

There will not be any dietary or lifestyle restrictions around the time of the study. Your participation in the study will be completed during your one visit to the MRC HNR as described above.

What are the possible benefits of taking part?

The results of your DXA scan (looking at the bone density in your hips) will be sent to you and your GP. You will have had this scan completed previously however it may be of benefit to you to know if any significant changes have occurred over the last few years.

What are the possible disadvantages and risks of taking part?

The DXA, pQCT and HRpQCT all involve a small dose of radiation (approximately 20 μSv). A chest x-ray by comparison is 20μSv and one trans-Atlantic flight would expose you to about 80μSv. We cannot avoid being exposed to some radiation, as natural background radiation (approximately 6 μSv) is all around us in the soil and air. Additional radiation from the scans used will expose you to the equivalent of approximately four days natural background radiation in Cambridge. If a new diagnosis of osteoporosis is made, this could affect your future insurance status (e.g. for life insurance or private medical insurance).

The muscle tests in which you are asked to jump and hop may lead to some aching in the subsequent days. In rare cases, damage to muscles or tendons may occur necessitating treatment, although the risk is not felt to be higher than when jumping for any other reason.

What will happen if anything goes wrong?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given on 'Sheet 2 – study conduct information sheet'. If you wish to make a complaint you can contact Head of Operations at MRC HNR, Ms Polly Page on 01223 426356 or email polly.page@mrc-hnr.cam.ac.uk.

Will my taking part in this study be kept confidential?

Yes. All information that is collected about you during the course of the research will be kept strictly confidential. The details are included in 'Sheet 2 – Study Conduct Information' sheet.

What will happen to the study results?

We will inform your GP of the result of your DXA scan (the bone density at your hips). As the scans from the study will not be processed immediately, your results may not be available for a few months.

The overall results may be presented at scientific meetings or published in a scientific journal. You will not be identified in any of these presentations or publications. A summary of the study results will be available on MRC HNR website. We will be happy to discuss the results with you when the study is completed, and will let you know where you can obtain a copy of the published results. Details are included in 'Sheet 2 – Study Conduct Information'

Will I be reimbursed for my time?

There will be no reimbursement for time however transport to and from the site will be arranged at no cost to you and lunch and snacks will be provided.

Contact for further information

If you have any further questions then please contact Dr Kate Ward at MRC HNR by email kate.ward@mrc-hnr.cam.ac.uk or telephone 01223 437563.

And finally…Thank you for having taken the time to read this information sheet. If you do decide to take part in the study, you will be asked to sign a Consent Form and you will be given a copy of the Consent Form and the Participant Information Sheet to keep.

Appendix 4 – Study Conduct Information Leaflet

This sheet gives information on factors such as confidentiality and data protection, communication with your GP, indemnity and compensation, publication etc. It is important that you read and are happy with this along with 'Participant Information Sheet 1' before agreeing to take part.

What will happen if I don't want to carry on with the study?

If you decide to withdraw from the study, with your consent, samples and data obtained may be kept and used to contribute to study results or, with your consent, for future studies. However, should you request your samples and data to be destroyed along with any other information relating to you, we will ensure that this takes place.

What if there is a problem?

In the unlikely event that something should go wrong during the study, procedures will be stopped and a clinician may see you. Your involvement in the rest of the study may be stopped. Standard procedures are in place at MRC Human Nutrition Research for dealing with serious adverse events should they occur. If you have any other problems, illnesses or concerns during the study you should discuss these with a member of the study team at MRC HNR or Dr Mark Edwards at the MRC Lifecourse Epidemiology Unit in Southampton.

Complaints:

Any complaints you have about this study will be fully investigated. If you have a concern about any aspect of this study, you should speak with Dr Mark Edwards (Tel 02380 777624, email me@mrc.soton.ac.uk) who will do his best to answer your questions. If you remain unhappy and wish to complain formally, you can contact Polly Page, MRC HNR Head of Operations: 01223 426356, polly.page@mrc-hnr.cam.ac.uk or Dr Martina Prude, Head of Research Governance, University of Southampton: 02380 595058, mad4@soton.ac.uk.

Harm:

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation against MRC but you may have to pay your legal costs.

Will my taking part in this study be kept confidential?

Any information that is collected about you during the course of the research will be kept strictly confidential and MRC HNR will be the custodian of the data. Any information about you that leaves MRC HNR will have your name and address removed so that you cannot be recognised from it.

HNR has a standard confidentiality procedure for participants involved in research. This stipulates how personal information is collected, used, stored and disposed of during and following completion of research projects.

Any information that is collected about you during the course of the project will be kept strictly confidential and secure in locked filing cabinets and/or electronic files on computers that have restricted access. Each participant is assigned a unique, linked anonymising code number that is used on all data collected during the research. This code number is used to identify data in place of personal information.

Only the specified research team will have access to personal identifying data information. However, with your agreement, your GP will be notified of your bone density results and copies of these letters will also be provided to you. HNR and LEU maintain a central record of all research projects but this does not include personal information on participants. With your agreement we will store data for 20 years. With your consent, and with the appropriate research ethics approval, retained data may be used for future studies.

What will happen to the study results?

The overall study results may be presented at scientific meetings or published in a scientific journal. You will not be identified in these presentations and publications. A summary of the study results will be available on the MRC HNR

website and we will be happy to discuss the results with you at the end of the study.

Who is organising and funding the study?

This study is being organised by the Nutrition and Bone Health Group at the MRC HNR and the Bone and Joint Research Group at the MRC LEU. The study is funded by Arthritis Research UK.

Who has reviewed the study?

This study has been reviewed by the Scientific Co-ordination Committee of MRC HNR and by the Hertfordshire Local Research Ethics Committee.

Appendix 5 – Consent Form

LREC Reference Number: 11/EE/0196

Name of Lead Investigator: Dr Mark Edwards

Please initial boxes.

1. I confirm that I have read and understand the information sheet dated 1st November 2011 (version 1.2) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

3. I consent to my general practitioner being notified of my participation in this research and to being informed of my results. ☐

4. In the event that they withdraw from the study: *(delete as appropriate)* ☐
   a. I wish the researchers to remove all of my identifiable data collected up to that point.
   b. I am happy for the researchers to retain my identifiable data gathered up to that point.

5. I understand relevant section of my medical notes and data collected during the study may be looked at by individuals from the Medical Research Council, or from regulatory authorities, where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records. ☐

6. I agree to take part in the above study. ☐

7. I am willing to be contacted again in the future about the present study and any potential follow-up from it. I understand that I am under no obligation to undergo any future additional tests and can withdraw this consent at any time by notifying the study team. ☐

Name of Volunteer *(Please print)*	Date	Signature
Name of Research Team Member	Date	Signature

3 copies required: top copy for researcher; one copy for volunteer; one copy to be kept with volunteer's note

Appendix 6 – Ethical approval document

NHS
National Research Ethics Service
NRES Committee East of England - Hertfordshire
Victoria House
Capital Park
Fulbourn
Cambridge
CB21 5XB

Telephone: 01223 597733
Facsimile: 01223 597645

07 July 2011

Dr Mark Edwards
NIHR Academic Clinical Fellow, MRC Lifecourse Epidemiology Unit
MRC Lifecourse Epidemiology Unit
Southampton General Hospital
Southampton
SO16 6YD

Dear Dr Edwards

Study title:	**Understanding the early life and environmental determinants of bone strength and structure using participants from the Hertfordshire Cohort Study.**
REC reference:	**11/EE/0196**
Protocol number:	**8012 1.1**

Thank you for your letter of 28 June 2011, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter from Dr Mark Edwards		10 May 2011
REC application IRAS application Parts A&B 80524/213098/1/991		10 May 2011
Investigator CV - Dr Mark Edwards		
Investigator CV - Dr Elaine Dennison (Academic Supervisor)		29 October 2010
Other: Covering email from Dr Mark Edwards		28 June 2011
Response to Request for Further Information from Dr Mark Edwards		28 June 2011
Letter of invitation to participant - Initial letter of invitation	1.1	08 June 2011
Letter of invitation to participant - Reminder Letter	1.1	08 June 2011
GP/Consultant Information Sheets - DXA scan results to GP	1.1	08 June 2011
Participant Information Sheet: Information Sheet 1 for Study participants 1.1	1.1	08 June 2011
Participant Information Sheet: Volunteer information Sheet 2 - Study conduct information for participants	1.1	08 June 2011
Participant Consent Form	1.1	08 June 2011
Evidence of insurance or indemnity from Mrs Ruth McFadyen		05 May 2011
Evidence of insurance or indemnity MRC Statement on Indemnity		01 October 2008
Letter from Sponsor - Dr Martina Prude, University of Southampton		05 May 2011
Other: Letter from Funder - Arthritis Research UK		12 November 2010
Protocol	1.1	08 June 2011

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

11/EE/0196	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Steve Eckersall
Chair
Email: Anna.Bradnam@eoe.nhs.uk

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Cc: Dr Martina Prude (Sponsor Contact)
Head of Research Governance
Room 4055 Building 37
University of Southampton
Hampshire
SO17 1BJ

Mr Kevin Pearson (R&D Contact)
Medical Research Council Centre
Hills Road
Cambridge
CB2 0QH

This Research Ethics Committee is an advisory committee to East of England Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Appendix 7 - Sponsorship documentation

UNIVERSITY OF Southampton

Dr Mark Edwards
School of Medicine
Medical Research Council
Lifecourse Epidemiology Unit
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

05 May 2011

Dear Dr Edwards

RGO Ref: 8012

Project Title **Understanding the Early Life and Environmental Determinants of Bone Strength and Structure Using Participants from the Herefordshire Cohort Study**

I am writing to confirm that the University of Southampton is prepared to act as Research Sponsor for this study under the terms of the Department of Health Research Governance Framework for Health and Social Care (2nd edition 2005).
http://www.dh.gov.uk/en/Aboutus/Researchanddevelopment/Researchgovernance/DH_400211

I would like to take this opportunity to remind you of your responsibilities under the terms of the Research Governance Framework Medicines for Human Use Act 2004 if conducting a clinical trial.

We encourage you to become fully conversant with the terms of the Research Governance Framework by referring to the Department of Health document which can be accessed at:
http://www.legislation.gov.uk/uksi/2004/1031/contents/made
http://www.legislation.gov.uk/uksi/2006/1928/contents/mad

The University of Southampton fulfils the role of Research Sponsor in ensuring management, monitoring and reporting arrangements for research. I understand that you will be acting as the Principal Investigator responsible for the daily management for this study, and that you will be providing regular reports on the progress of the study to the Research Governance Office on this basis.

Please also familiarise yourself with the Terms and Conditions of Sponsorship on our website:
http://www.soton.ac.uk/corporateservices/rgo/media/TCSpons%20(CTIMP)%20V2%2022011.do
http://www.soton.ac.uk/corporateservices/rgo/media/TCSpons%20(Non%20CTIMP)%20V2%20022011.doc

In this regard if your project involves NHS patients or resources please also be reminded that you may need a Research Passport to apply for an honorary research contract of employment. Information can be found on our website:
http://www.soton.ac.uk/corporateservices/rgo/respassport/about.htm

(...continued overleaf)

UNIVERSITY OF Southampton

Please send us a copy of your NHS REC and Trust approval letters when available.

Please do not hesitate to contact me should you require any additional information or support. May I also take this opportunity to wish you every success with your research.

Yours sincerely

M Prude.

Dr Martina Prude
Head of Research Governance
Tel: 023 8059 5058
email: rgoinfo@soton.ac.uk

Corporate Services, University of Southampton, Highfield Campus, Southampton SO17 1BJ United Kingdom
Tel: +44 (0) 23 8059 4684 Fax: +44 (0) 23 8059 5781 www.southampton.ac.uk

UNIVERSITY OF Southampton

Dr Mark Edwards
School of Medicine
Medical Research Council
Lifecourse Epidemiology Unit
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

RGO REF - 8012

05 May 2011

Dear Dr Edwards

Professional Indemnity and Clinical Trials Insurance

Project Title **Understanding the Early Life and Environmental Determinants of Bone Strength and Structure Using Participants from the Herefordshire Cohort Study**

Participant Type:	No Of Participants:	Participant Age Group:	Notes:
Healthy volunteers	500	Adults	

Thank you for forwarding the completed questionnaire and attached papers.

Having taken note of the information provided, I can confirm that this project will be covered under the terms and conditions of the above policy, subject to written informed consent being obtained from the participating volunteers.

I would also advise that it is a condition of the University's insurance that any incidents that could eventually result in a claim are reported immediately. Serious adverse events, suspected unexpected serious adverse reactions and similar fall into this category. For studies hosted by or sponsored by SUHT the Research and Development Office will copy the SAE and SUSAR reports they receive to the University Insurance Office. For all other studies such events must be reported to me at the same time as they are reported under the Protocol. Failure to do this could invalidate the insurance.

Insurance will only be activated when we have received a copy of the Ethics Committee approval and you must not begin your project prior to this. Please forward a copy of the Ethics Committee approval letter as soon as it is to hand to complete the insurance placement.

If there are any changes to the above details, please advise us as failure to do so may invalidate the insurance.

Yours sincerely

Mrs Ruth McFadyen
Insurance Services Manager

Tel: 023 8059 2417
email: hrm@soton.ac.uk

cc: File

Finance Department, University of Southampton, Highfield Campus, Southampton SO17 1BJ United Kingdom
Tel: +44 (0) 23 8059 5000 Fax: +44 (0) 23 8059 2195 www.southampton.ac.uk

Appendix 8 – Body composition artefact protocol

Artefact	Management of data
Arthroplasty/Metal/ Artificial Limb	
Arthroplasty/metal – right hip joint	Remove right trunk, right leg and right total – replace with left trunk, left leg and left total. In total, remove right total and double the left total.
Arthroplasty/metal – left hip joint	Remove left trunk, left leg and left total – replace with right trunk, right leg and right total. In total, remove left total and double the right total.
Arthroplasty/metal – both hip joints	Remove data for trunk (right and left), legs (right and left) and total (right and left) – will only have data for arms.
Arthroplasty/metal/ artificial limb – right leg	Remove right leg and replace with left leg data. Remove right leg from total and right total and replace with left leg data.
Arthroplasty/metal/ artificial limb – left leg	Remove left leg and replace with right leg data. Remove left leg from total and left total and replace with right leg data.
Arthroplasty/metal/ artificial limb – both legs	Remove both legs – will only have data for trunk and arms.
Arthroplasty – right shoulder	Remove right arm and replace with left arm data. Remove right arm from total and right total and replace with left arm data
Arthroplasty – left shoulder	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data
Pacemaker/defib on left side	Remove left trunk and replace with right trunk. Remove left trunk from total and left total and replace with right trunk data.
Participant outside scan area	
Outside scan width on right side – arm	Remove right arm and replace with left arm data. Remove right arm from total and right total and replace with left arm data.
Outside scan width on left side – arm	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data.
Outside scan width on both sides – arms	Remove both arms – will only have data for trunk and legs.
Outside scan width on right side (half body replacement)	Remove right arm, right leg, right trunk and right total– replace with left arm, left leg, left trunk and left total. In total, remove right total and double the left total
Outside scan width on left side (half body replacement)	Remove left arm, left leg, left trunk and left total– replace with right arm, right leg, right trunk and right total. In total, remove left total and double the right total

Combinations of artefacts	
Outside scan width on left side and metal in right hip	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove right trunk, right leg and right total – replace with left trunk, left leg and left total. In total, remove right total and double the left total.
Outside scan width on left side and metal in left hip	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove left trunk, left leg and left total – replace with right trunk, right leg and right total. In total, remove left total and double the right total.
Outside scan width on left side and metal in right leg	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove right leg and replace with left leg data. Remove right leg from total and right total and replace with left leg data.
Outside scan width on left side and metal in left leg	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove left leg and replace with right leg data. Remove left leg from total and left total and replace with right leg data.
Outside scan width on left side and metal in both legs	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove both legs – will only have data for trunk and arms.
Outside scan width on left side and pacemaker	Remove left arm and replace with right arm data. Remove left arm from total and left total and replace with right arm data. Remove left trunk and replace with right data. Remove left trunk from total and left total and replace with right trunk data.
Outside scan width on right side and metal in right hip	Remove right arm and replace with left arm data. Remove right arm from total and right total and replace with left arm data. Remove right trunk, right leg and right total – replace with left trunk, left leg and left total. In total, remove right total and double the left total.
Pacemaker and metal in left leg	Remove left trunk and replace with right data. Remove left trunk from total and left total and replace with right trunk data. Remove left leg and replace with right leg data. Remove left leg from total and left total and replace with right leg data.
Metal in both hips and metal in right leg	Remove data for trunk (right and left), legs (right and left) and total (right and left) – will only have data for arms.
Outside scan width on both sides and metal in right leg	Remove both arms – will only have data for trunk and legs. Remove right leg and replace with left leg data. Remove right leg from total and right total and replace with left leg data.
Right hip replacement and right shoulder replacement	Remove right trunk, right leg and right total – replace with left trunk, left leg and left total. In total, remove right total and double the left total. Remove right arm and replace with left arm data. Remove right arm from total and right total and replace with left arm data.

Appendix 9 – Additional tables

Table A 1: Regression analysis of bone microarchitecture on daily dietary calcium intake in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[1]	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	3.06 (-0.54,6.65)	0.096	**3.76 (0.14,7.38)**	**0.042**	0.97 (-0.75,2.69)	0.267	0.67 (-0.98,2.31)	0.424
	T	0.37 (-6.45,7.20)	0.914	-0.96 (-6.87,4.96)	0.750	1.91 (-3.14,6.95)	0.456	1.63 (-2.88,6.14)	0.476
Trabecular area (mm^2)	R	2.80 (-0.66,6.27)	0.112	3.51 (-0.03,7.05)	0.052	1.28 (-0.49,3.05)	0.157	0.90 (-0.83,2.63)	0.306
	T	1.38 (-5.63,8.39)	0.698	0.36 (-5.60,6.33)	0.905	1.74 (-3.60,7.09)	0.521	1.19 (-3.63,6.00)	0.627
Cortical area (mm^2)	R	-0.06 (-0.72,0.60)	0.859	-0.19 (-0.89,0.51)	0.593	**-0.33 (-0.67,-0.00)**	**0.047**	-0.27 (-0.62,0.07)	0.121
	T	-0.54 (-1.84,0.76)	0.412	-0.77 (-2.08,0.55)	0.252	-0.10 (-0.90,0.70)	0.806	0.14 (-0.65,0.93)	0.730
Cortical thickness (μm)	R	-5.01 (-14.13,4.11)	0.28	-6.52 (-16.32,3.27)	0.190	**-6.70 (-13.12,-0.27)**	**0.041**	-5.21 (-12.08,1.66)	0.136
	T	-3.56 (-15.88,8.76)	0.569	-4.49 (-16.73,7.75)	0.470	-1.07 (-10.50,8.37)	0.824	1.18 (-8.20,10.55)	0.805
Cortical density (mg/cm^3)	R	-1.25 (-3.62,1.12)	0.300	-1.42 (-3.97,1.14)	0.276	-1.63 (-3.94,0.69)	0.168	-1.30 (-3.80,1.21)	0.309
	T	0.21 (-2.71,3.13)	0.886	0.15 (-2.96,3.26)	0.925	0.78 (-2.36,3.92)	0.623	1.51 (-1.75,4.77)	0.361
Cortical porosity (%)	R	-0.02 (-0.09,0.04)	0.473	-0.03 (-0.09,0.04)	0.446	0.01 (-0.05,0.06)	0.798	0.01 (-0.05,0.07)	0.731
	T	-0.04 (-0.17,0.09)	0.56	-0.05 (-0.18,0.09)	0.508	-0.02 (-0.15,0.11)	0.788	-0.03 (-0.17,0.11)	0.701
Trabecular density (mg/cm^3)	R	-0.12 (-1.88,1.64)	0.891	-0.21 (-2.12,1.69)	0.824	-0.45 (-2.12,1.23)	0.601	0.07 (-1.64,1.78)	0.936
	T	-0.13 (-1.78,1.51)	0.875	-0.77 (-2.48,0.94)	0.376	0.93 (-1.02,2.89)	0.347	1.50 (-0.48,3.49)	0.137
Trabecular number (cm^{-1})	R	-0.01 (-0.13,0.11)	0.881	-0.04 (-0.17,0.08)	0.506	-0.03 (-0.19,0.14)	0.744	0.01 (-0.15,0.18)	0.857
	T	0.03 (-0.12,0.18)	0.658	-0.00 (-0.13,0.13)	0.991	0.04 (-0.14,0.21)	0.670	0.07 (-0.10,0.24)	0.416
Trabecular thickness (μm)	R	-0.04 (-0.53,0.45)	0.875	0.03 (-0.50,0.56)	0.904	-0.15 (-0.56,0.27)	0.492	-0.06 (-0.49,0.38)	0.795
	T	-0.17 (-0.63,0.30)	0.480	-0.31 (-0.79,0.17)	0.209	0.26 (-0.32,0.85)	0.378	0.38 (-0.24,1.00)	0.231
Trabecular separation (μm)	R	-0.19 (-3.08,2.71)	0.899	0.50 (-2.70,3.70)	0.758	1.11 (-4.66,6.89)	0.704	-0.03 (-5.95,5.89)	0.992
	T	-0.77 (-3.39,1.85)	0.563	0.05 (-2.41,2.50)	0.971	-1.07 (-5.31,3.16)	0.617	-1.93 (-6.18,2.32)	0.371

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, physical activity, smoking status, alcohol consumption, social class, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use). [2]Regression coefficients per 100mg change in dietary calcium. No significant interactions by sex.

Table A 2: Regression analysis of bone microarchitecture on physical activity in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[1]	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	0.31 (-0.49,1.11)	0.445	0.18 (-0.60,0.97)	0.644	-0.16 (-0.64,0.31)	0.495	0.03 (-0.42,0.48)	0.884
	T	-0.81 (-2.30,0.68)	0.284	-0.11 (-1.39,1.17)	0.870	0.01 (-1.11,1.14)	0.979	0.38 (-0.62,1.37)	0.457
Trabecular area (mm^2)	R	0.09 (-0.68,0.86)	0.819	0.01 (-0.76,0.78)	0.986	-0.17 (-0.66,0.32)	0.499	0.04 (-0.43,0.51)	0.865
	T	-0.99 (-2.52,0.54)	0.202	-0.19 (-1.48,1.10)	0.772	0.21 (-0.99,1.40)	0.734	0.58 (-0.49,1.64)	0.286
Cortical area (mm^2)	R	**0.15 (0.01,0.30)**	**0.038**	0.15 (-0.00,0.30)	0.056	0.01 (-0.08,0.10)	0.878	-0.00 (-0.10,0.09)	0.952
	T	0.19 (-0.10,0.47)	0.194*	0.06 (-0.23,0.34)	0.696	**-0.19 (-0.36,-0.01)**	**0.038***	**-0.18 (-0.35,-0.00)**	**0.046**
Cortical thickness (μm)	R	1.56 (-0.46,3.57)	0.129	1.76 (-0.37,3.89)	0.105	0.76 (-1.02,2.55)	0.399	0.20 (-1.68,2.08)	0.835
	T	2.23 (-0.46,4.91)	0.103*	0.83 (-1.82,3.48)	0.536	-1.51 (-3.60,0.58)	0.155*	-1.92 (-3.99,0.14)	0.068
Cortical density (mg/cm^3)	R	-0.03 (-0.56,0.50)	0.918	0.19 (-0.37,0.74)	0.506	-0.01 (-0.65,0.63)	0.969	-0.20 (-0.89,0.49)	0.565
	T	0.37 (-0.27,1.01)	0.252	0.30 (-0.37,0.98)	0.371	-0.17 (-0.87,0.53)	0.628	-0.36 (-1.08,0.36)	0.327
Cortical porosity (%)	R	0.01 (-0.01,0.02)	0.363	0.00 (-0.01,0.02)	0.838	-0.00 (-0.02,0.01)	0.683	-0.00 (-0.02,0.01)	0.612
	T	-0.01 (-0.04,0.02)	0.612	-0.01 (-0.04,0.01)	0.325	-0.02 (-0.05,0.01)	0.141	-0.02 (-0.05,0.01)	0.312
Trabecular density (mg/cm^3)	R	0.22 (-0.17,0.61)	0.261	0.22 (-0.20,0.63)	0.299	0.13 (-0.33,0.59)	0.581	0.20 (-0.26,0.67)	0.389
	T	0.23 (-0.13,0.59)	0.212	0.21 (-0.16,0.58)	0.271	-0.16 (-0.60,0.27)	0.456	-0.12 (-0.56,0.32)	0.599
Trabecular number (cm^{-1})	R	0.01 (-0.02,0.03)	0.594	0.01 (-0.02,0.03)	0.626	0.02 (-0.03,0.06)	0.512	0.03 (-0.02,0.07)	0.222
	T	**0.03 (0.00,0.07)**	**0.044****	0.02 (-0.01,0.05)	0.118	-0.03 (-0.07,0.01)	0.091**	-0.02 (-0.06,0.01)	0.218
Trabecular thickness (μm)	R	0.05 (-0.06,0.16)	0.352	0.05 (-0.07,0.16)	0.408	0.00 (-0.11,0.12)	0.967	-0.00 (-0.12,0.12)	0.964
	T	-0.00 (-0.11,0.10)	0.937	0.01 (-0.09,0.12)	0.778	0.02 (-0.11,0.15)	0.773	0.00 (-0.13,0.14)	0.945
Trabecular separation (μm)	R	-0.12 (-0.77,0.52)	0.703	-0.14 (-0.84,0.55)	0.686	-0.55 (-2.14,1.04)	0.494	-0.87 (-2.49,0.75)	0.293
	T	**-0.61 (-1.18,-0.04)**	**0.036***	-0.47 (-1.00,0.06)	0.084	0.60 (-0.34,1.54)	0.206*	0.40 (-0.54,1.34)	0.399

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, smoking status, alcohol consumption, social class, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use). [2]Regression coefficients per unit change in physical activity score. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table A 3: Regression analysis of bone microarchitecture comparing ex-smokers and current smokers to lifelong non-smokers in men and women

		Ex-smokers vs never smokers				Current smokers vs never smokers			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	7.94 (-14.82,30.71)	0.492	14.03 (-0.41,28.47)	0.057	9.29 (-34.69,53.27)	0.677	2.96 (-26.82,32.74)	0.845
	T	-1.62 (-44.18,40.94)	0.940	**46.44 (13.48,79.39)**	**0.006**	18.47 (-64.32,101.26)	0.660	-7.03 (-74.69,60.64)	0.838
Trabecular area (mm^2)	R	4.04 (-17.85,25.93)	0.716	13.12 (-1.79,28.04)	0.084	5.58 (-36.72,47.87)	0.795	-0.86 (-31.62,29.90)	0.956
	T	2.43 (-41.24,46.10)	0.913	**45.05 (10.10,80.01)**	**0.012**	4.11 (-81.04,89.25)	0.924	-5.19 (-76.96,66.57)	0.887
Cortical area (mm^2)	R	0.60 (-3.55,4.74)	0.777	0.91 (-1.87,3.69)	0.519	5.98 (-2.02,13.99)	0.142	4.35 (-1.39,10.09)	0.137
	T	1.54 (-6.50,9.57)	0.707	2.27 (-3.11,7.64)	0.407	**17.91 (2.29,33.54)**	**0.025**	-1.91 (-12.95,9.13)	0.733
Cortical thickness (μm)	R	3.35 (-54.06,60.75)	0.909	0.10 (-54.45,54.65)	0.997	49.67 (-61.22,160.57)	0.378	54.94 (-57.57,167.44)	0.336
	T	13.73 (-62.68,90.15)	0.723	-23.01 (-86.14,40.12)	0.473	125.43 (-23.23,274.1)	0.098	-11.77 (-141.4,117.9)	0.858
Cortical density (mg/cm^3)	R	-7.18 (-22.02,7.65)	0.341	-0.28 (-19.95,19.39)	0.977	-26.47 (-55.12,2.19)	0.070	-7.12 (-47.69,33.44)	0.729
	T	1.09 (-17.04,19.22)	0.906	4.30 (-17.03,25.64)	0.691	-24.10 (-59.38,11.17)	0.179	-9.89 (-53.70,33.92)	0.656
Cortical porosity (%)	R	0.33 (-0.09,0.75)	0.123	0.11 (-0.35,0.56)	0.646	0.79 (-0.02,1.61)	0.056	**0.98 (0.04,1.92)**	**0.042**
	T	0.02 (-0.77,0.80)	0.963	-0.38 (-1.24,0.49)	0.392	**2.03 (0.50,3.56)**	**0.009**	-0.07 (-1.85,1.71)	0.935
Trabecular density (mg/cm^3)	R	0.91 (-10.13,11.95)	0.871	6.38 (-7.55,20.32)	0.367	-4.99 (-26.31,16.34)	0.645	8.09 (-20.65,36.83)	0.579
	T	-5.85 (-16.06,4.36)	0.260	-2.92 (-15.97,10.14)	0.660	1.14 (-18.77,21.04)	0.910	-12.69 (-39.50,14.11)	0.351
Trabecular number (cm^{-1})	R	-0.01 (-0.75,0.72)	0.972	1.11 (-0.25,2.46)	0.108	0.79 (-0.63,2.22)	0.272	0.96 (-1.83,3.76)	0.496
	T	-0.01 (-0.92,0.91)	0.988*	0.39 (-0.79,1.57)	0.518	**1.79 (0.00,3.58)**	**0.049***	-1.53 (-3.96,0.89)	0.213
Trabecular thickness (μm)	R	0.62 (-2.43,3.66)	0.691	-0.28 (-3.80,3.24)	0.875	-3.68 (-9.57,2.20)	0.219	-0.29 (-7.54,6.97)	0.938
	T	-2.04 (-4.91,0.84)	0.164	-1.47 (-5.40,2.45)	0.460	-3.64 (-9.23,1.96)	0.201	-1.13 (-9.20,6.93)	0.782
Trabecular separation (μm)	R	-2.29 (-20.48,15.91)	0.804	-29.04 (-77.20,19.12)	0.236	-11.75 (-46.90,23.40)	0.510	-6.83 (-106.14,92.49)	0.892
	T	1.51 (-14.69,17.72)	0.854	4.06 (-24.67,32.79)	0.781	-23.89 (-55.48,7.71)	0.138	31.84 (-27.14,90.82)	0.288

Key: R, radius; T, tibia. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table A 4: Regression analysis of bone microarchitecture comparing ever smokers to lifelong non-smokers in men and women

		Men				Women			
		Unadjusted		Adjusted		Unadjusted		Adjusted	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	8.11 (-13.95,30.17)	0.469	**26.27 (3.73,48.82)**	**0.023**	12.40 (-1.28,26.07)	0.075	10.52 (-3.30,24.35)	0.135
	T	0.83 (-40.47,42.12)	0.969	21.66 (-14.98,58.29)	0.245	**38.42 (7.10,69.74)**	**0.016**	**41.86 (11.42,72.30)**	**0.007**
Trabecular area (mm^2)	R	4.23 (-16.99,25.45)	0.695	**23.53 (1.50,45.55)**	**0.036**	11.06 (-3.07,25.19)	0.124	9.40 (-5.10,23.89)	0.202
	T	2.63 (-39.74,45.00)	0.903	30.35 (-6.63,67.33)	0.107	**37.52 (4.35,70.68)**	**0.027**	**44.05 (11.56,76.54)**	**0.008**
Cortical area (mm^2)	R	1.26 (-2.77,5.30)	0.537	-0.54 (-4.88,3.80)	0.807	1.42 (-1.22,4.06)	0.291	1.11 (-1.79,4.01)	0.452
	T	3.53 (-4.35,11.41)	0.378	-2.55 (-10.72,5.63)	0.539	1.64 (-3.45,6.72)	0.525	-2.02 (-7.34,3.30)	0.455
Cortical thickness (μm)	R	9.09 (-46.65,64.82)	0.748	-34.22 (-95.20,26.75)	0.269	8.19 (-43.52,59.90)	0.755	10.26 (-47.28,67.80)	0.725
	T	27.33 (-47.21,101.87)	0.470	-38.86 (-115.0,37.28)	0.315	-21.32 (-80.93,38.29)	0.481	-49.25 (-112.6,14.04)	0.126
Cortical density (mg/cm^3)	R	-9.57 (-24.02,4.87)	0.193	-13.04 (-28.94,2.85)	0.107	-1.29 (-19.90,17.31)	0.891	3.29 (-17.76,24.34)	0.758
	T	-1.98 (-19.65,15.70)	0.826	-9.56 (-28.90,9.78)	0.331	2.18 (-17.99,22.34)	0.832	4.15 (-17.95,26.24)	0.711
Cortical porosity (%)	R	0.39 (-0.02,0.80)	0.063	0.33 (-0.08,0.74)	0.118	0.24 (-0.20,0.67)	0.289	0.22 (-0.28,0.73)	0.383
	T	0.26 (-0.51,1.04)	0.504	0.41 (-0.44,1.25)	0.343	-0.33 (-1.15,0.49)	0.426	-0.79 (-1.73,0.14)	0.096
Trabecular density (mg/cm^3)	R	0.18 (-10.53,10.89)	0.974	-1.46 (-13.31,10.40)	0.809	6.64 (-6.54,19.81)	0.322	2.26 (-12.04,16.55)	0.755
	T	-5.01 (-14.92,4.91)	0.321	-9.35 (-19.96,1.26)	0.084	-4.38 (-16.73,7.96)	0.484	-8.79 (-22.21,4.64)	0.198
Trabecular number (cm^{-1})	R	0.09 (-0.63,0.80)	0.811	-0.15 (-0.93,0.63)	0.705	1.09 (-0.19,2.36)	0.096	0.44 (-0.92,1.81)	0.522
	T	0.21 (-0.69,1.11)	0.645	-0.33 (-1.15,0.49)	0.424	0.10 (-1.02,1.22)	0.862	-0.50 (-1.63,0.64)	0.386
Trabecular thickness (μm)	R	0.08 (-2.89,3.05)	0.956	0.16 (-3.15,3.47)	0.924	-0.28 (-3.61,3.04)	0.867	-0.76 (-4.41,2.90)	0.683
	T	-2.23 (-5.02,0.56)	0.116	-2.40 (-5.39,0.60)	0.116	-1.42 (-5.13,2.28)	0.450	-1.45 (-5.64,2.74)	0.496
Trabecular separation (μm)	R	-3.46 (-21.11,14.19)	0.699	0.20 (-19.71,20.12)	0.984	-25.76 (-71.32,19.80)	0.266	-2.29 (-51.86,47.28)	0.927
	T	-1.55 (-17.38,14.27)	0.847	6.38 (-8.80,21.57)	0.408	8.23 (-18.96,35.41)	0.551	21.72 (-6.96,50.40)	0.137

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, alcohol consumption, social class, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use. No significant interactions by sex.

Table A 5: Regression analysis of bone microarchitecture comparing social class I–IIIn with social class IIIm–V in men and women

		Men				Women			
		Unadjusted		Adjusted[1]		Unadjusted		Adjusted[1]	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	1.88 (-20.85,24.61)	0.871	**27.53 (5.08,49.97)**	**0.017***	-8.48 (-21.60,4.64)	0.204	-6.74 (-19.67,6.18)	0.304*
	T	**-41.97 (-83.13,-0.81)**	**0.046**	5.96 (-30.52,42.44)	0.747	5.31 (-25.60,36.22)	0.735	5.95 (-22.27,34.17)	0.678
Trabecular area (mm^2)	R	-1.74 (-23.52,20.05)	0.875	**22.53 (0.59,44.48)**	**0.044**	-9.27 (-22.79,4.25)	0.178	-7.37 (-20.94,6.20)	0.285
	T	**-43.81 (-85.99,-1.64)**	**0.042**	6.85 (-29.92,43.63)	0.713	5.43 (-27.31,38.17)	0.744	4.16 (-25.96,34.29)	0.785
Cortical area (mm^2)	R	0.41 (-3.71,4.52)	0.845	0.61 (-3.71,4.94)	0.780	0.85 (-1.70,3.39)	0.513	0.63 (-2.07,3.34)	0.645
	T	2.86 (-5.08,10.81)	0.478	2.43 (-5.68,10.54)	0.555	0.73 (-4.16,5.62)	0.769	2.16 (-2.77,7.09)	0.388
Cortical thickness (µm)	R	-1.91 (-58.55,54.72)	0.947	-35.69 (-96.42,25.05)	0.248	35.25 (-14.12,84.62)	0.161	27.17 (-26.77,81.11)	0.321
	T	72.96 (-1.02,146.94)	0.053	27.53 (-47.93,102.99)	0.472	-0.08 (-57.80,57.65)	0.998	16.40 (-42.25,75.06)	0.581
Cortical density (mg/cm^3)	R	-1.50 (-16.12,13.12)	0.840	-8.47 (-24.32,7.38)	0.293	-6.39 (-24.16,11.38)	0.479	-7.64 (-27.34,12.05)	0.444
	T	12.23 (-5.45,29.92)	0.174	9.28 (-9.87,28.43)	0.340	-2.89 (-22.10,16.33)	0.767	-2.16 (-22.56,18.25)	0.835
Cortical porosity (%)	R	0.01 (-0.39,0.41)	0.963	0.11 (-0.30,0.52)	0.600	0.30 (-0.12,0.73)	0.156	0.23 (-0.24,0.70)	0.333
	T	-0.32 (-1.10,0.46)	0.419	-0.35 (-1.18,0.48)	0.403	0.17 (-0.61,0.95)	0.670	0.36 (-0.51,1.22)	0.418
Trabecular density (mg/cm^3)	R	-7.92 (-18.74,2.91)	0.151	-9.68 (-21.49,2.13)	0.107	2.06 (-10.75,14.88)	0.751	-1.47 (-14.87,11.93)	0.829
	T	-9.70 (-19.70,0.31)	0.057	**-13.01 (-23.6,-2.45)**	**0.016**	-2.71 (-14.68,9.27)	0.656	-2.68 (-15.13,9.76)	0.671
Trabecular number (cm^{-1})	R	-0.03 (-0.74,0.68)	0.931	-0.10 (-0.88,0.68)	0.802	0.50 (-0.75,1.75)	0.432	0.11 (-1.17,1.39)	0.868
	T	-0.86 (-1.75,0.03)	0.059	**-0.99 (-1.81,-0.18)**	**0.018**	-0.05 (-1.12,1.02)	0.930	-0.10 (-1.15,0.95)	0.847
Trabecular thickness (µm)	R	-2.91 (-5.91,0.09)	0.057	**-3.44 (-6.72,-0.16)**	**0.040**	-0.57 (-3.76,2.61)	0.723	-1.05 (-4.47,2.38)	0.547
	T	-1.05 (-3.88,1.79)	0.467	-1.88 (-4.86,1.10)	0.215	-0.46 (-4.06,3.14)	0.801	-0.26 (-4.15,3.62)	0.894
Trabecular separation (µm)	R	6.58 (-11.29,24.46)	0.468	9.22 (-10.64,29.07)	0.361	-15.44 (-59.51,28.62)	0.490	0.12 (-46.35,46.58)	0.996
	T	**18.12 (2.30,33.94)**	**0.025**	**20.43 (5.30,35.56)**	**0.008**	-1.36 (-27.30,24.58)	0.918	-2.04 (-28.62,24.54)	0.880

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, alcohol consumption, bisphosphonate use, comorbidity score (and in women, time since menopause and HRT use). [2]Positive regression coefficients reflect higher values in social class I–IIIn. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table A 6: Regression analysis of bone microarchitecture comparing those with 1 or 2+ comorbidities to those with no comorbidities in men and women

		1 vs 0 comorbidities[1]				2+ vs 0 comorbidities[1]			
		Men		Women		Men		Women	
		β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value	β (95% CI)[2]	p value
Total area (mm^2)	R	-10.90 (-36.95,15.16)	0.410	5.94 (-8.66,20.55)	0.423	-21.76 (-56.43,12.91)	0.217	5.10 (-16.46,26.67)	0.641
	T	-6.34 (-54.77,42.09)	0.796	7.91 (-27.02,42.83)	0.655	-34.98 (-97.96,28.01)	0.275	-14.13 (-66.71,38.45)	0.596
Trabecular area (mm^2)	R	-23.31 (-48.14,1.51)	0.065	3.81 (-11.28,18.90)	0.619	-24.08 (-57.11,8.95)	0.152	1.60 (-20.67,23.87)	0.888
	T	-3.27 (-52.36,45.82)	0.896	5.43 (-31.50,42.36)	0.772	-35.27 (-99.56,29.01)	0.280	-22.12 (-77.72,33.47)	0.433
Cortical area (mm^2)	R	**4.77 (0.12,9.42)**	**0.044**	2.42 (-0.41,5.25)	0.093	1.54 (-4.64,7.73)	0.623	3.98 (-0.20,8.15)	0.062
	T	6.28 (-2.99,15.55)	0.183	2.56 (-2.88,8.00)	0.354	-0.75 (-12.80,11.30)	0.903	7.75 (-0.44,15.94)	0.064
Cortical thickness (μm)	R	57.56 (-7.00,122.13)	0.080	34.98 (-20.36,90.33)	0.214	52.58 (-33.34,138.50)	0.229	55.42 (-26.28,137.11)	0.182
	T	39.79 (-47.31,126.89)	0.369	14.58 (-49.95,79.10)	0.656	3.85 (-109.42,117.12)	0.947	60.36 (-36.78,157.50)	0.222
Cortical density (mg/cm^3)	R	9.01 (-7.95,25.96)	0.296	5.68 (-14.21,25.58)	0.573	0.85 (-21.71,23.42)	0.941	-5.37 (-34.73,24.00)	0.719
	T	-8.91 (-29.79,11.97)	0.401	12.82 (-8.68,34.32)	0.241	-11.33 (-38.49,15.82)	0.411	20.30 (-12.07,52.67)	0.217
Cortical porosity (%)	R	0.25 (-0.22,0.72)	0.290	0.00 (-0.47,0.47)	0.995	0.15 (-0.48,0.77)	0.644	0.63 (-0.07,1.32)	0.077
	T	**1.04 (0.12,1.95)**	**0.027***	-0.36 (-1.23,0.52)	0.423*	0.55 (-0.64,1.75)	0.361	0.34 (-0.98,1.66)	0.613
Trabecular density (mg/cm^3)	R	7.45 (-4.87,19.78)	0.234	-5.82 (-20.16,8.52)	0.424	0.28 (-16.12,16.67)	0.974	13.40 (-7.77,34.56)	0.213
	T	1.93 (-9.38,13.23)	0.737	-0.21 (-13.58,13.16)	0.975	-9.31 (-24.12,5.50)	0.216*	18.39 (-1.74,38.52)	0.073*
Trabecular number (cm^{-1})	R	0.10 (-0.73,0.94)	0.806	-0.67 (-2.07,0.73)	0.346	-0.37 (-1.48,0.74)	0.514	0.23 (-1.84,2.30)	0.825
	T	0.81 (-0.24,1.86)	0.128	0.08 (-1.11,1.27)	0.900	0.22 (-1.15,1.59)	0.751	1.16 (-0.63,2.95)	0.204
Trabecular thickness (μm)	R	2.21 (-1.21,5.64)	0.204	-0.28 (-3.80,3.23)	0.874	1.68 (-2.87,6.24)	0.467	4.06 (-1.13,9.25)	0.124
	T	-1.47 (-4.70,1.77)	0.373	0.44 (-3.57,4.46)	0.828	-3.74 (-7.98,0.49)	0.083*	4.63 (-1.42,10.68)	0.132*
Trabecular separation (μm)	R	3.45 (-17.19,24.08)	0.742	27.45 (-22.14,77.04)	0.276	7.90 (-19.56,35.35)	0.571	2.59 (-70.61,75.79)	0.944
	T	-9.10 (-27.15,8.94)	0.321	1.43 (-27.68,30.54)	0.923	3.98 (-19.64,27.61)	0.740	-28.01 (-71.84,15.82)	0.209

Key: R, radius; T, tibia. [1]Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table A 7: Regression analysis of bone microarchitecture comparing those with 1 or 2+ comorbidities to those with no comorbidities after adjustment[1] in men and women

		1 vs 0 comorbidities[2]				2 vs 0 comorbidities[2]			
		Men		Women		Men		Women	
		β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Total area (mm²)	R	-20.52 (-46.60,5.57)	0.122	3.21 (-11.45,17.86)	0.666	-16.67 (-49.19,15.86)	0.313	-6.99 (-29.80,15.81)	0.545
	T	-35.00 (-77.73,7.73)	0.108	5.43 (-26.49,37.35)	0.737	-32.10 (-84.62,20.43)	0.229	-40.45 (-90.78,9.89)	0.114
Trabecular area (mm²)	R	**-33.19 (-58.50,-7.87)**	**0.011***	-0.16 (-15.56,15.23)	0.983*	-17.51 (-49.07,14.06)	0.275	-10.89 (-34.85,13.07)	0.371
	T	-25.07 (-68.21,18.07)	0.253	0.48 (-33.66,34.62)	0.978	-30.44 (-83.47,22.58)	0.259	-45.28 (-99.12,8.56)	0.099
Cortical area (mm²)	R	2.55 (-2.48,7.58)	0.318	**3.64 (0.57,6.70)**	**0.020**	0.27 (-6.00,6.54)	0.931	3.79 (-0.98,8.55)	0.118
	T	1.61 (-7.90,11.11)	0.739	4.13 (-1.47,9.73)	0.147	-3.76 (-15.44,7.92)	0.526	2.78 (-6.05,11.61)	0.535
Cortical thickness (μm)	R	44.74 (-25.90,115.39)	0.213	**64.09 (2.87,125.31)**	**0.040**	34.09 (-53.99,122.17)	0.446	91.84 (-3.44,187.12)	0.059
	T	27.15 (-61.26,115.55)	0.545	35.30 (-31.51,102.11)	0.298	-20.74 (-129.4,87.93)	0.707	57.44 (-47.92,162.80)	0.283
Cortical density (mg/cm³)	R	11.70 (-6.68,30.09)	0.211	18.19 (-4.08,40.46)	0.109	0.15 (-22.78,23.07)	0.99	7.59 (-27.08,42.25)	0.666
	T	-11.16 (-33.63,11.31)	0.328	**25.75 (2.58,48.91)**	**0.030**	-14.62 (-42.24,13.00)	0.297	25.25 (-11.28,61.78)	0.174
Cortical porosity (%)	R	0.08 (-0.39,0.56)	0.734	-0.08 (-0.60,0.45)	0.774	-0.03 (-0.62,0.57)	0.932	**0.84 (0.02,1.66)**	**0.046**
	T	**0.98 (0.01,1.95)**	**0.047***	-0.62 (-1.60,0.36)	0.214*	0.51 (-0.68,1.69)	0.402	0.31 (-1.23,1.85)	0.693
Trabecular density (mg/cm³)	R	5.24 (-8.50,18.98)	0.453	-4.32 (-19.47,10.82)	0.573	0.77 (-16.36,17.90)	0.929	11.86 (-11.72,35.43)	0.322
	T	2.24 (-10.11,14.59)	0.721	4.20 (-9.95,18.36)	0.558	-10.55 (-25.73,4.63)	0.172	18.55 (-3.77,40.88)	0.103
Trabecular number (cm⁻¹)	R	-0.07 (-0.98,0.83)	0.872	-0.46 (-1.91,1.00)	0.537	-0.58 (-1.71,0.55)	0.310	-0.66 (-2.92,1.61)	0.568
	T	0.32 (-0.63,1.28)	0.505	0.38 (-0.81,1.58)	0.526	-0.17 (-1.35,1.00)	0.773	0.54 (-1.34,2.43)	0.570
Trabecular thickness (μm)	R	1.99 (-1.83,5.81)	0.306	-0.01 (-3.87,3.85)	0.997	2.32 (-2.44,7.09)	0.337	**6.11 (0.10,12.12)**	**0.046**
	T	-0.28 (-3.78,3.21)	0.874	1.50 (-2.91,5.92)	0.502	-3.30 (-7.60,0.99)	0.131*	**7.17 (0.20,14.13)**	**0.044***
Trabecular separation (μm)	R	6.64 (-16.49,29.78)	0.571	18.55 (-34.22,71.32)	0.488	12.14 (-16.70,40.99)	0.407	19.91 (-62.22,102.05)	0.633
	T	-2.89 (-20.61,14.83)	0.748	-3.85 (-34.13,26.42)	0.802	9.56 (-12.22,31.34)	0.387	-14.56 (-62.30,33.18)	0.548

Key: R, radius; T, tibia.[1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, alcohol consumption, social class, bisphosphonate use (and in women, time since menopause and HRT use). [2]Number of comorbidities was scored out of five with one point for the presence of each of chronic bronchitis, diabetes mellitus, ischaemic heart disease, cerebrovascular disease (stroke or TIA), and hypertension. Interactions by sex: *p<0.05, **p<0.01, ***p<0.001.

Table A 8: Regression analysis of bone microarchitecture comparing those women who have ever used HRT to those that have not.

		Unadjusted		Adjusted[1]	
		β (95% CI)	p value	β (95% CI)	p value
Total area (mm^2)	R	-2.29 (-15.45,10.87)	0.732	-4.25 (-17.68,9.17)	0.532
	T	9.77 (-21.07,40.61)	0.533	13.35 (-16.00,42.71)	0.370
Trabecular area (mm^2)	R	-3.02 (-16.58,10.54)	0.661	-3.78 (-17.87,10.31)	0.597
	T	9.33 (-23.34,42.00)	0.574	15.72 (-15.61,47.06)	0.323
Cortical area (mm^2)	R	1.06 (-1.48,3.60)	0.412	-0.26 (-3.06,2.55)	0.858
	T	1.33 (-3.55,6.21)	0.592	-1.06 (-6.18,4.07)	0.684
Cortical thickness (µm)	R	17.66 (-31.85,67.16)	0.482	-3.71 (-59.72,52.31)	0.896
	T	-13.98 (-71.59,43.62)	0.632	-37.25 (-98.26,23.76)	0.230
Cortical density (mg/cm^3)	R	5.20 (-12.55,22.96)	0.564	-0.00 (-20.46,20.45)	1.000
	T	0.81 (-18.39,20.01)	0.934	-1.33 (-22.56,19.90)	0.901
Cortical porosity (%)	R	0.05 (-0.37,0.48)	0.798	-0.03 (-0.51,0.46)	0.912
	T	-0.26 (-1.04,0.52)	0.511	-0.61 (-1.52,0.29)	0.179
Trabecular density (mg/cm^3)	R	9.12 (-3.60,21.84)	0.159	5.32 (-8.60,19.24)	0.451
	T	6.30 (-5.63,18.23)	0.299	1.69 (-11.26,14.63)	0.797
Trabecular number (cm^{-1})	R	0.92 (-0.32,2.16)	0.146	0.45 (-0.88,1.78)	0.504
	T	0.45 (-0.62,1.51)	0.408	0.09 (-1.00,1.18)	0.867
Trabecular thickness (µm)	R	1.09 (-2.09,4.27)	0.500	0.55 (-3.00,4.11)	0.759
	T	0.69 (-2.91,4.28)	0.706	-0.35 (-4.39,3.70)	0.866
Trabecular separation (µm)	R	-14.96 (-58.97,29.04)	0.503	3.80 (-44.45,52.05)	0.876
	T	-13.69 (-39.51,12.13)	0.297	-4.42 (-32.06,23.23)	0.753

Key: R, radius; T, tibia. [1]Adjusted for age, height, weight, dietary calcium intake, physical activity, smoking status, alcohol consumption, social class, bisphosphonate use, comorbidity score, and time since menopause.

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