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Title

Bone microstructure and self-perception of fracture risk among women participating in the UK arm of the GLOW study

by

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ABSTRACT

Osteoporotic fractures are associated with considerable morbidity, mortality and socioeconomic cost. A significant contribution to fracture risk is bone fragility which can be assessed using a novel imaging technique, high-resolution peripheral quantitative computed tomography (HR-pQCT), that provides data on bone geometry, density and microarchitecture, and has previously been used in a study of women aged 55-80 recruited through primary care to the Southampton, UK based arm of the Global Longitudinal Study of Osteoporosis in Women (GLOW) Study.

Self-perception of risk of a condition requires an individual to compare their own health status to others, and has been considered in the osteoporosis literature. Self-perception of fracture risk (SPR) has been previously reported to be underestimated in postmenopausal women worldwide, suggesting that there might be a disconnect between SPR and actual fracture risk, and has also been previously captured in the GLOW Study.

The aims of this thesis are to use the GLOW study to: (a) examine relationships between HR-pQCT parameters and fracture (b) determine associations between HR-pQCT parameters and adiposity (c) consider associations between SPR and bone health; and (d) consider the determinants of SPR.

492 participants from the UK arm of GLOW underwent HR-pQCT of the non-dominant distal radius and tibia and dual energy x-ray absorptiometry (DXA) to estimate body composition and femoral neck areal bone mineral density (aBMD). Information on demographics, lifestyle, fracture, SPR and comorbidities was obtained from study questionnaires.

Microstructural parameters of the bone evaluated by HR-pQCT appear to be different at skeletal regions containing predominantly trabecular bone between healthy postmenopausal women and those who had fractured. There was a trend suggesting favourable cortical and trabecular microarchitecture with increased BMI category at both radius and tibia, although women with the highest BMI appeared to have less favourable bone microarchitecture taking into account their body size. Higher SPR bands were related to a decrease in areal BMD at the femoral neck and lower tibial trabecular volumetric density. Finally, we demonstrated that SPR in this group captured an aspect

of fracture risk not currently measured using FRAX, and translated to improved antiosteoporosis medication (AOM) uptake.

This study highlights novel associations between SPR, body composition and bone microarchitecture. It has demonstrated that patterns of bone microarchitecture differ in postmenopausal women by fracture status, and that SPR may contribute to fracture prediction, beyond conventional fracture algorithm variables.

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Contributions

Prof. Dennison wrote the substantial amendment protocol for the GLOW study and acquired ethical approval. I acquired Research and Development approvals for the study and was responsible for site file maintenance and general study management. I wrote the participant information sheets, consent forms and other supporting documentation. I took part in booking, co-ordinating participant travel and carried out participant visits. I regularly took informed consent. 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.

Acknowledgements

1. Introduction to the Subject Area

1.1 Osteoporosis

Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in the fragility of bone. [1] (Figure 1.1). The World Health Organisation (WHO) in 1994 defined osteoporosis based on bone mineral density measurements of less than 2.5 standard deviations (SD) below the young normal mean as assessed by Dual-energy X-ray absorptiometry (DXA) [2].
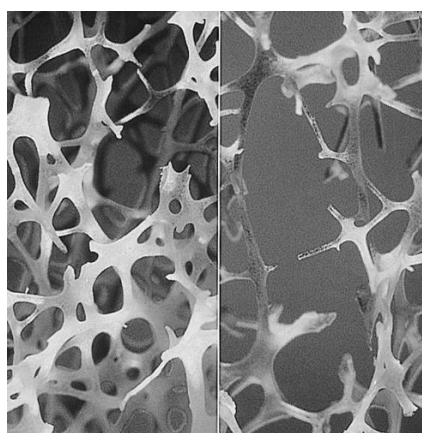
Figure 1.1 Normal bone and bone with microstructure deterioration.

Adapted from Wikimedia
https://commons.wikimedia.org/wiki/File:Bone_normal_and_degraded_micro_structure.jpg

1.1.1 Epidemiology of osteoporosis

Osteoporosis represents a major public health problem through its association with low trauma fractures that can lead to increased mortality, disability, and long-term decrease in function [3–5]. These fractures come at a great personal cost to the individuals affected, both physically and psychologically, a significant burden on society in general and a huge impact economically [3,6,7].

The 2004 US Surgeon General's report estimated that 10 million Americans over the age of 50 have osteoporosis, leading to 1.5 million fragility fractures each year [6], with another 34 million Americans at risk of the disease. Economically, the cost to the US was around $17.9 billion per annum. In the EU, a report estimated that, in 2010, 6.6% of men and 22.1% of women aged over 50 years had osteoporosis, and that there were 3.5

million fragility fractures [7]. The annual direct costs attributable to fracture treatment in the EU equated to approximately €24 billion [7].However, when indirect costs such as long-term care and fracture prevention therapies were taken into account, this figure rose to €37 billion per year [7]. With aging of the population, the economic cost of osteoporosis and fractures are projected to increase in the EU from €37.4 billion in 2010 to €46.8 billion by 2025 and, in the US, from $17 billion in 2005 to $25.3 billion by 2025 [7]. Osteoporotic fracture is a well-established health problem in the West; however, its prevalence is increasingly recognized in Asian countries.

The most commonly affected sites for osteoporosis related fractures have traditionally been considered to be the vertebrae, femoral neck, and distal radius-ulna [8], and these fractures are more common in women in people 65 years old and above (Figure 1.2). Hip fracture has been recognized as the most serious consequence of osteoporosis because of its complications, which include chronic pain, disability, reduced quality of life, and premature death[9,10]. Hip fracture incidence rises exponentially with age leading to around 90% fractures occurring in those 50 years and older [11]. In this age group, there is a female to male incidence ratio of around two to one [12]. This higher incidence, along with the fact that they live longer, means that women account for three quarters of the hip fracture burden [11,13].
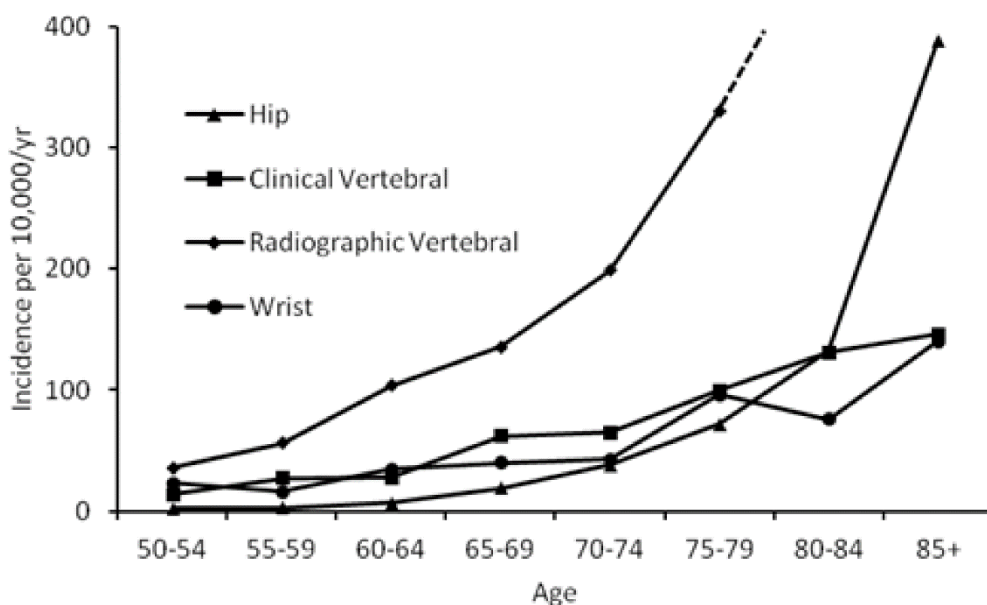
Figure 1.2 Hip, clinical vertebral, radiographic vertebral and wrist fracture incidence in women by age.

Adapted with permission from Litwic et al, Geographic differences in fractures among women [12].

There is a significant variation in the risk of hip fracture worldwide. Age-standardised rates varied approximately 10-fold in both men and women. Age- and sex-standardised rates are high in Northern (Iceland, Ireland, Denmark, Norway and Sweden) and Central Europe with an annual incidence >250/100,000. Other high-risk countries include Iran, Oman, Argentina and Taiwan. Regions of moderate risk with an incidence of 150–250/100,000 are Oceania, the Russian Federation, Western and South-East Europe and the countries of North America. Generally low risk regions included Latin America, (with the exception of Argentina), Africa, Saudi Arabia, India and China with annual incidence <100/100,000 [14] (Figure 1.3).
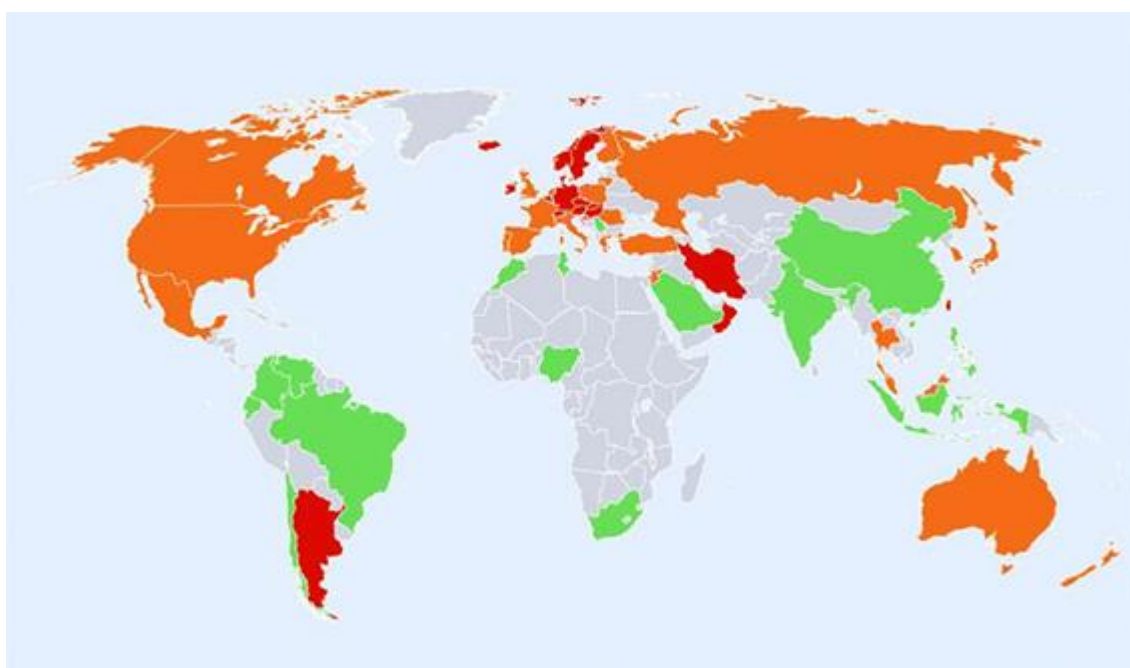
Figure 1.3 Hip fracture rates for men and women combined in different countries of the world categorised by risk.

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 [14]

Similar to the hip fracture incidence, the 10-year probability of a major osteoporotic fracture varies significantly in different countries [14]. Differences in fracture rates worldwide are partly attributable to ethnic differences in susceptibility to fracture. Studies in the USA and UK have demonstrated that the lowest frequencies of hip fracture are observed in black individuals [15,16]. Hip fracture rates in women of mixed and Asian ethnicity are lower than those observed in white women, but higher than in black women [15,16]. Differences in hip fracture rates by ethnicity are thought to be due to

variance in skeletal size and microarchitecture, peak bone mineral density and skeletal loss, as well as differences in proximal femoral geometry [17,18]. African-American women have higher areal BMD, greater bone area, increased trabecular thickness, cortical area and cortical thickness and reduced cortical porosity compared to Caucasian women [1719].

Vertebral fractures are one of the most common fractures associated with skeletal fragility that can lead to decreased quality of life and functional limitation due to back pain [20]. They usually occur due to compressive loading of the spine with lifting or changing position. The lifetime risk of a clinically diagnosed vertebral fracture after the age of 50 is estimated to be 9% in men and 15% in women [21]. The prevalence of vertebral fractures increases with age for both sexes [22]. However, only one-third of vertebral fractures are recognized clinically at the time of their occurrence due to the absence of symptoms [23]. While far fewer data are available describing geographic variation in vertebral than hip fracture, findings from study among Europeans showed difference in prevalence of vertebral fracture between countries, with the highest rates again in Scandinavia compared with other European regions [24].

Fractures of the distal forearm almost invariably occur following a fall onto an outstretched hand. There is a more marked gender disparity in distal forearm fractures than is seen with either hip or vertebral fractures; with low and stable rates between the ages of 20 and 80 years in men; and a rapid rise in incidence between the ages of 45 and 60 in women followed by a plateau thereafter. Overall the age-adjusted rate of distal forearm fractures is four times higher in women than men [25].

1.1.2 **Morbidity**

Osteoporotic fractures are associated with significant morbidity. Following a fracture, patients are at risk of their physical, psychological and socioeconomic status being adversely affected. There are many reports describing the personal burden of hip fracture[9,26]. Approximately 20% of patients with hip fractures develop a postoperative complication, with chest infections (9%) and heart failure (5%) being the most common [27]. Fourteen percent of 50-55 year old patients require nursing home care following discharge from hospital and this rises to more than 50% in those over the age of 90 years [28].

As compared with hip fracture, there are limited reports describing the epidemiology and impact of vertebral fracture rates, which may result in an underestimation of the burden that they impose [29]. The major burden faced by patients with vertebral fracture is back pain, but they may also experience functional difficulties, kyphosis, and height loss [28]. In one study, participants with radiologically identified vertebral fracture at baseline had repeat radiographs performed 3 years later; women who had suffered a further fracture during this period experienced substantial levels of disability with impairment in key physical functions of independent living [30].

Wrist fractures may impact on some activities such as writing, washing and dressing. Overall relatively few patients are completely disabled, but about half of the wrist fracture patients report only fair or poor functional outcomes at 6 months post fracture [28 31]. In addition, complications such as reflex sympathetic dystrophy, neuropathies, post-traumatic arthritis, limitation of motion, and physical deformity are not infrequently seen [28].

The impact of osteoporotic fracture is not restricted to physical burden and may also result in psychological consequences such as low self-esteem, impaired body image and mood changes [32].

1.1.3 **Mortality**

People who sustain hip or vertebral fractures have increased mortality rates compared to those without fractures. Hip fracture has a mortality of 10% at one month and 30% at one year [33]. The elevated risk has been shown to persist for up to 10 years [34]. Hip fracture mortality increases with age, and is greater for those with poor pre-fracture functional status and coexisting illnesses [35]. The risk of death is greatest immediately after the fracture and decreases gradually over time. In only 25% does the cause of death occur directly due to the fracture itself or resulting complications such as infection, thrombo-embolic disease or surgery [9]; in the remaining majority it is attributable to coexisting morbidity due to underlying diseases.

There is excess mortality also after vertebral fracture. In the UK General Practice Research Database (GPRD) study, survival at 1 and 5 years post vertebral fracture were

86.5% and 56.5% respectively. These were markedly lower than the expected levels of 93.6% and 69.9% respectively [25]. 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 [36]. Although the prevalence of fragility fractures is higher in women, it is usually men that have greater rates of fracture-associated mortality. Interestingly, the patterns of mortality predicted by fracture in the thoracic spine differ between men and women, with a significant prediction for respiratory mortality in men and injury mortality in women [37].

In contrast, there is little or no risk of death associated with isolated wrist fractures. Most of the studies investigating mortality in distal radius fracture patients have not detected any differences compared to the general population [38,39]. However, there are studies that report an increased mortality in that group; Johnell et al. [40] observed that the mortality rate at one year and five years follow-up at 6% and 26 % respectively. Rozental et al. [41] assessed a longer follow-up period of seven years and reported the mortality rate at 21%.

1.2 Bone physiology

1.2.1 Skeleton

Human skeleton consists of cortical and trabecular bone (Figure 1.4). Cortical bone is dense and solid and surrounds the marrow space, whereas trabecular bone is porous and is composed of special complexes of thin rods and plates of bone tissue. Cortical bone accounts for approximately 80% of the total bone mass in the adult skeleton. The ratio of cortical to trabecular bone vary at different bone sites. Short, flat and irregular bones are composed of trabecular bone surrounded by a thin layer of cortical bone, for example, the vertebra is composed of cortical to trabecular bone in a ratio of 25:75. A long bone has two main parts: the diaphysis and the epiphysis. The metaphysis is the narrow portion of a long bone between the epiphysis and the diaphysis; it contains the growth plate, and as it grows it ossifies. The diaphysis is the midsection of a long bone and is primarily composed of cortical bone; for example, in the radial diaphysis, the cortical to trabecular bone ratio is 95:5. The diaphysis expands at each end to form an epiphysis. The epiphysis contains trabecular bone and have an outer shell of cortical bone; in the femoral head cortical to trabecular bone ratio is 50:50 [42]. Cortical bone has

an outer periosteal surface, important for appositional growth and fracture repair, and an inner endosteal surface with a high remodelling activity. Cortical bone is composed of osteons. Cortical osteons (Haversian systems) are the structural unit of cortical bone aligned parallel to the long axis of the bone and consisting of concentric bone layers called lamellae, which surround an osteonic canal (Figure 1.4). 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 [43]. 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. In trabecular bone, as in cortical bone, the collagen fibres are orientated in an ordered manner. 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 [44].
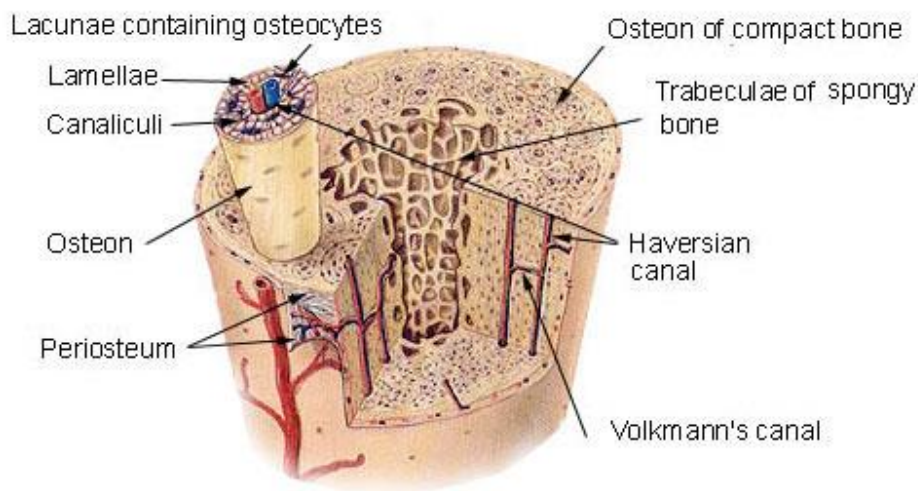
Figure 1.4 Cortical and trabecular bone structure [45] . Image reused from Wikipedia
http://en.wikipedia.org/wiki/File:Illu_compact_spongy_bone.jpg

1.2.2   Bone cells responsible for bone formation and resorption

Bone is composed of 50 to 70% mineral, 20 to 40% organic matrix, 5 to 10% water, and <3% lipids. Osteoblasts, osteocytes and osteoclasts are the three main types of bone

cells. Osteoblasts produce the bone matrix. Bone organic matrix is mostly composed of type I collagen and provides elasticity and flexibility. The mineral content of bone is formed from calcium and phosphate deposited as calcium- phosphate salts, which undergo mineralisation to hydroxyapatite [Ca10(PO4)6(OH)2]. It provides mechanical rigidity and load-bearing strength to bone. Osteoblasts are bone forming cells and may become embedded within bone mineral as mature osteocytes (comprising 90–95% of the cells within bone) or remain on the surface as bone-lining cells. Osteoclasts are multinucleated cells responsible for bone resorption. Osteoblasts and osteoclasts form bone remodelling units. Osteocytes, terminally differentiated osteoblasts, play a key role in the regulation of modelling and remodelling. The arrangement of the osteocytes around Haversian canals acts as a mechanosensory system and allows communication both directly between neighbouring osteocytes and through the release of endocrine, paracrine and autocrine signalling factors to other bone cells.

1.2.3   **Changes in bone structure across the life course**

Bone undergoes growth, modelling, and remodelling during life. Modelling is the process by which bones change their shape as a result of physiologic processes, such as growth and repair, and mechanical loading. Bone formation typically exceeds bone resorption on the periosteal surface, so bones normally widen with aging. In contrast, bone resorption typically exceeds bone formation on the endosteal surface, leading to expansion of marrow space with aging.

Bone remodelling is the process by which existing bone is renewed. The balance of formation and resorption has a critical influence on bone mass and strength. The remodelling process carried out by osteoclasts and osteoblasts involves continuous resorption of old bone and formation of new bone to prevent accumulation of bone microdamage and maintain calcium and phosphate homeostasis. Remodelling continues through the human life span. There is a positive balance during childhood until achievement of peak bone mass in early adulthood , with a subsequent period of stability and then a negative balance in older age, with osteoclast activity greater than osteoblast activity, leading to bone loss [46]. At birth, bone mass appears to be similar in males and females with no evidence to suggest a significant gender difference [47]. Most sex differences in bone width, mass and strength develop during puberty. In boys, diaphyseal bone width increases due to periosteal apposition [48] with a corresponding

increase in endosteal resorption, but to a lesser extent, leading to increase in cortical thickness. In girls, periosteal apposition occurs to a lesser extent and medullary size remains stable or even reduces due to endosteal bone formation. In girls bone width becomes smaller than in boys, but cortical thickness and vBMD remain comparable [48,49]. Men have a larger bone size than women in adulthood, which is consistent with their larger body size [50]. In premenopausal women, there is significant endosteal resorption and periosteal apposition within long bones, with apposition occurring at a lower rate than resorption leading to loss in cortical thickness [51]. Bone remodelling accelerates in perimenopausal and early postmenopausal women. The oestrogen deficiency that ensues at this point is thought to be the main cause. Structural changes with ageing include thinning of the cortex, increasing intracortical porosity, and reducing trabecular thickness [52]. Similar changes with age occur in men although the effect of progressive oestrogen deficiency is far less marked [53]. Cortical bone porosity depends on the proportion of actively remodelling to inactive cortical osteons. Cortical bone porosity is usually <5%, but healthy aging adults during increased cortical remodelling experience thinning of the cortex and an increase in cortical porosity.

1.3 Measurement of bone health using DXA and HR-pQCT

1.3.1 DXA

There are different techniques to measure bone health. DXA is recognized as the gold-standard method to measure BMD with acceptable accuracy errors, good precision and reproducibility. The assessment of BMD by DXA is the measurement used in the WHO definitions of osteopenia and osteoporosis [2].

In DXA scanning, two beams of x-rays of different energies, one high the other low, by alternating the voltage of the x-ray tube (kV switching), are directed from a radiation source towards a radiation detector. The participant is placed on a table in the path of the radiation beam. As the beam passes through the body, some photons are absorbed and some scatter (Compton scattering) but the remaining photons pass through the body and are detected by a linear array of x-ray detectors. 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 bone mineral content (BMC) within the beam paths, bone area (BA), the projected area of the bone onto the image plane, typically in cm^2 and

areal bone mineral density (aBMD). aBMD is the mineral mass of bone per unit image area in g/cm^2 [54]. 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 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. BMC is the mineral mass component of bone in the form of hydroxyapatite. It is typically measured in grams and does not include the mass of any of the organic components of bone (marrow, collagen, etc.). Calculation of BMC using DXA also assumes a constant ratio of fat mass to lean mass in the overlying soft tissue. Because DXA scanners use two X-ray energies in the presence of three types of tissue (bone mineral, lean tissue and adipose tissue), DXA is affected by the inhomogeneous distribution of adipose tissue in the human body [55]. In pixels containing both bone and soft tissue, the composition of the soft tissue element of the pixel is estimated from pixels containing no bone situated adjacent to the bone. By assuming the same percentage of fat to lean mass within the soft tissue portion, error can be induced. For the same reason, bone marrow fat may also affect measurements of BMD by DXA.

Errors in determining BMC, bone area and proximal femur geometry can arise using fan beam DXA due to magnification effects due to proximity to the x-ray source [56]. In obese individuals with increased posterior soft tissue thickness, the distance of the skeleton from the x-ray source is decreased leading to magnification effects due to proximity to the x-ray source as the observed width of scanned bone increases [56,57]. Computational input is required to correct magnification errors in BMC, area and geometry outcomes. Measurements of BMD are confounded by greater soft tissue thickness, which absorbs the x-ray beam such that the resulting attenuation is increased. Studies involving fat layering of phantoms have shown aBMD by DXA to be increased with increasing fat layering relative to baseline measurements [58–60].

DXA scanners allow measurement of several skeletal sites but clinical assessments in adults are usually limited to the lumbar spine, proximal femur and forearm [61]. The standard protocol is to scan two sites with recommendations from the International Society of Clinical Densitometry (ISCD) to measure aBMD at the posteroanterior

lumbar spine and the proximal femur (neck or total hip) [62]. If one of these sites is not available, then the forearm is used. Areas that should not be used for diagnosis as they overestimate the prevalence of osteoporosis are the lateral spine and Ward's triangle. In patients in whom the hip and spine are difficult to measure or interpret, standard practice is to measure aBMD in the non-dominant radius at a point 33% of the way from distal to proximal. The one-third radius region is useful as a site containing entirely cortical bone.

1.3.2   **High-resolution peripheral QCT (HR-pQCT)**

HR-pQCT is an imaging technique that uses computerized processing of X-ray attenuation for the acquisition of sectional images, and involves the same principles as CT, but enables high resolution scanning of the distal appendicular skeleton. A 360° rotating x- ray tube generates x-rays which are passed from the radiation source through a section of the distal radius or distal tibia. A two-dimensional (2D) array of detectors measure the transmitted radiation. This generates an attenuation profile, which is then reconstructed into an image by computing the spatial distribution of the attenuation onto a blank matrix. From the series of 2D parallel image slices, it is possible to produce a three-dimensional (3D) high-quality model. The standard scan protocol and analysis produces 82 μm isotropic voxels. A voxel is a volume element that represents a value on a regular grid in 3D space. At each site, 110 computerized tomography slices are obtained and used to reproduce a 9.02 mm (radial or tibial length) 3D image.

The HR-pQCT single-scan effective dose is estimated to be 3 μSv [63]. It is recommended that no more than three measures are made at a single site during an appointment due to the recommended radiation dose limit being 50 μSv/year [64].

There is currently only one manufacturer of HR-pQCT, that uses a dedicated imaging system to assess trabecular and cortical structure at distal peripheral sites, XtremeCT, Scanco Medical AG, Bruttisellen, Switzerland. When assessments are made of the distal radius and tibia and analysed using the standard, manufacturer-recommended method, several standard variables are created (Table 1.1).

Volumetric bone mineral density (vBMD, mg HA/cm^3) can be determined for the whole bone, trabecular bone (Tb.vBMD, mg HA/cm^3), and cortical bone (Ct.vBMD, mg

HA/cm^3) from a pre-calibration step. The scanner is calibrated using a phantom with five hydroxyapatite-resin compartments of densities from 0 mgHA/cm^3 (a soft tissue equivalent with no mineral content) to 800 mgHA/cm^3. Image slices are taken of the phantom and the mean attenuation for each of the compartments calculated. From this pre-calibration data the attenuation values of the scan can be converted into measures of BMD (mgHA/cm^3). The bone volume fraction (BV/TV, %) is determined from the trabecular vBMD assuming the density of fully mineralized bone is 1200 mg HA/cm^3.

Trabecular microarchitecture is assessed in terms of trabecular thickness (Tb.Th, mm), number (Tb.N, 1/mm), and separation (Tb.Sp, mm) although only Tb.N is measured directly. Average Tb.Th and Tb.Sp are calculated using semi-derived methods (Tb.Th = (BV/TV)/Tb.N and Tb.Sp = (1-BV/TV)/Tb.N) [65]. It is because the voxel size of the scanner is close to the average thickness of a human trabecular structure [65].

Segmentation of the cortical and trabecular bone compartments is necessary for density and structural analysis. Delineation of the cortical and trabecular compartments is done automatically using a filter and threshold-based algorithm, to identify voxels that belong to cortical bone. However, this method is insufficient for extraction of the cortex when it is thin and/or highly porous or when the trabecular structure is rich and well connected to the cortex. An alternate algorithm, "extended cortical analysis" - uses a dual-threshold segmentation technique [66,67]. It provides a more robust extraction of the cortical and trabecular compartments and involves a two-step algorithm to automatically identify the periosteal and then the endocortical surface. Although the dual-threshold technique improves the segmentation of the cortical and trabecular compartments, errors can persist. Therefore, a manual correction should be applied as necessary. This minimises accuracy error that can lead to skewed results ie cortical thickness. Once the compartments are defined, the extended cortical analysis allows for the assessment of cortical porosity and cortical tissue mineral density, as well as a direct measure of cortical thickness (Ct.Th, mm).

Cortical volume is defined as the total cortical volume (including pores). Cortical bone volume is determined as the total volume of mineralised cortical bone within the cortex (excluding pores). Assessments of cortical porosity (Ct.Po, %), ratio of the pore volume relative to the total volume of the cortical compartment, are limited by the resolution of HR-pQCT, as Haversian canals range in size from 30-350um, but only 5–8% of the total

pore volume is estimated to consist of the pores smaller than 90 μm [68]. There are two methods to assess Ct.Po - the threshold-based approach and the density based approach. Both are well correlated with the gold standard, but cannot be directly compared due to methodologic differences. The threshold-based approach [67] is implemented in the XtremeCT analysis software provided by Scanco with Ct.Po calculated as the ratio of the total pore volume within the cortical compartment to the sum of the cortical volume. It enables segmentation of the individual pores from the scan and allows pore structure to be measured. However, this technique captures only pores within the limits of scan resolution, underestimating Ct.Po by 3-11%. In contrast the density based approach approach [69], implemented in the StrAx1.0 software, estimates of the ratio of void space present in each voxel, and taking the mean across all voxels to fully mineralized bone in cortical compartment; assuming that fully mineralized bone has density between 1000 and 1200 mg HA/cm3. This method aims to capture pores with diameters below the spatial resolution of the scanner; but, it relies on the assumption of a fixed bone tissue mineral density overestimating Ct.Po by 6-21% due to misclassifying image noise and artifacts as void space. It is important to be aware of the technical aspects of the acquisition and analysis of HR-pQCT imaging prior to interpretation of results.

Other geometric measurements include total area (Tot.Ar, mm^2) mean surface area of the cortical and trabecular compartments; cortical area (Ct.Ar, mm^2) mean surface area of the cortical compartment; and trabecular area (Tb.Ar, mm^2) mean surface area of the trabecular compartment. Further software has been produced that allows assessment of bone mechanical properties using finite element analysis (FEA).

Table 1.1 Standard HR-pQCT parameters.

HR-pQCT parameter	Abbreviation	Description	Standard unit
Bone volume ratio	BV/TV	Ratio of bone volume to total volume in region of interest derived by dividing Tb.vBMD by an assumed 100% mineralisation of 1200 mgHA/cm3	%

Trabecular thickness	Tb.Th	Mean thickness of trabeculae	mm
Trabecular separation	Tb.Sp	Mean distance between trabeculae	mm
Trabecular number	Tb.N	Mean number of trabeculae per mm	per mm
Cortical thickness	Ct.Th	Mean thickness between the periosteal and endosteal surfaces	mm
Total bone mineral density	Tot.vBMD	Average mineral density within the periosteal surface	mg HA/cm^3
Cortical bone mineral density	Ct.vBMD	Average mineral density within the cortical compartment	mg HA/cm^3
Trabecular bone mineral density	Tb.vBMD	Average mineral density within the trabecular compartment	mg HA/cm^3
Total bone area	Tot.Ar	Measure of total cross-sectional area within the periosteal surface	mm^2

Unlike DXA, HR-pQCT enables the distinction between cortical and trabecular bone compartments, allowing the study of bone microstructure. HR-pQCT enables high resolution imaging of the non-weight bearing distal radius which is a common fracture site and of the weight bearing tibia. There is less soft tissue present at distal sites and therefore HR-pQCT is likely to be less affected by soft tissue confounding than DXA scanning.

HR-pQCT only assesses bone at the distal radius and tibia. However, studies that have examined the relationship between HR-pQCT and DXA have found that skeletal stiffness, density and microarchitecture at peripheral sites assessed by HR-pQCT, was significantly associated with measurements of the axial skeleton [70,71].

1.4   Risk factors for osteoporosis and fracture

There are many factors that influence fracture risk, either through bone mineral density or through independent mechanisms. Risk factors for osteoporosis include low BMD, age, gender, a previous personal history of fracture, a family history of hip fracture, smoking, alcohol use and certain diseases associated with osteoporosis e.g. rheumatoid arthritis, diabetes, hyperthyroidism or premature menopause (<45 years), glucocorticoid therapy and low body mass index. WHO supported an initiative which uses risk factors that can be incorporated into the Fracture Risk Assessment (FRAX) tool, with or without bone mineral density (BMD) measurement, to estimate a 10-year probability of either hip fracture or major osteoporotic fracture. Risk factors included in fracture prediction tool FRAX are presented in table 1.2 and will be discussed in this chapter.

Table 1.2 Risk factors for osteoporosis

Risk factors for osteoporosis
Age *
Maternal history of hip fracture*
Previous personal history of fragility fracture*
Body mass index (≤ 19 kg/m^2)*
Glucocorticoid treatment*
Current smoking*
Alcohol intake of 3 or more units daily*
Rheumatoid arthritis*
Untreated hypogonadism
Premature menopause
Endocrine disorders (e.g. Type I diabetes, hyperthyroidism)
Gastrointestinal disease
Malabsorption
Chronic liver disease

* Risk factors independent of BMD

1.4.1 Age and sex

Overall, 61% of osteoporotic fractures occur in women, with a female to male ratio of 1.6:1 [72]. Nearly 75% of hip, spine and distal forearm fractures occur in people 65 years old or over [73] and these are more common in women, with rates in this age group reaching around twice those in men [74].

As discussed above, hip fracture incidence rises exponentially with age leading to around 90% fractures occurring in those 50 years and older [11], and with a female to male incidence ratio of about 2:1 [14]. Similarly, the incidence of vertebral fractures increases with age in both men and women, and also varies by gender, with females having a 4 to 5 times higher risk of fracture than men [75]. In contrast, the incidence of fractures of the distal forearm rises in women during the perimenopausal period but stabilizes thereafter; in men the incidence is low and remains stable between the ages of 20 and 80 years.

1.4.2   BMI

The association between BMI and fracture risk is complex, differs across skeletal sites, and is modified by the interaction between BMI and BMD [76]. The results of a meta-analysis of 60,000 men and women from 12 prospective, population based cohorts have shown that total fractures, osteoporotic fractures and hip fractures were all inversely correlated to BMI in both men and women [77]. In that study fracture risk was markedly higher at the lower values of BMI, particularly with a BMI of 20 kg/m^2 or less. The magnitude was greater for hip fracture than for any osteoporotic fracture or any fracture; and was largely independent of age and sex, but dependent on BMD (Figure 1.5). After adjustment for BMD, BMI was not predictive of fracture risk except for hip fracture at a BMI of 20kg/m^2 or less [77]. These findings were very consistent with a larger and more recent meta-analysis [76].
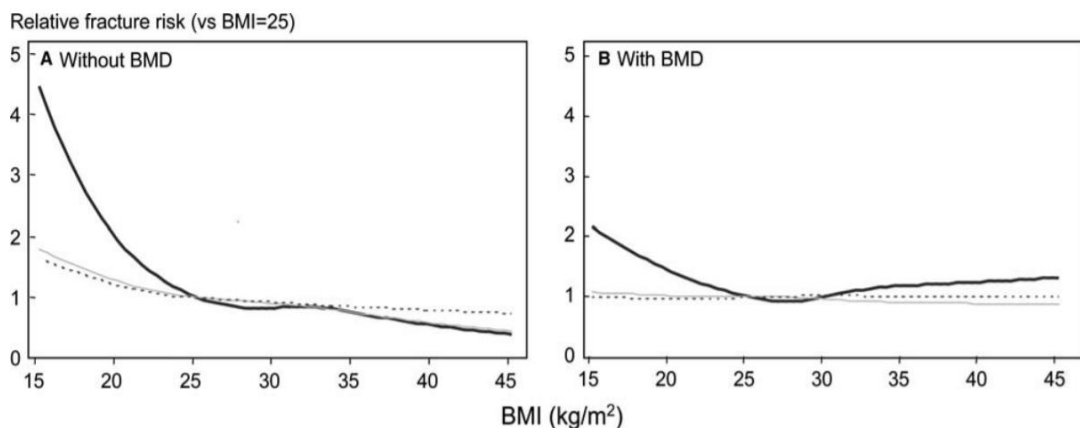
Figure 1.5 Relative fracture risk at various levels of BMI (kg/m2) for men and women combined.

The reference is a BMI=25, (A) adjusted for current age and time since start of follow-up, and (B) additionally adjusted for BMD. The bold solid line describes hip fracture, the solid line any osteoporotic fracture, and the dotted line any fracture (BMI body mass index, BMD bone mineral density)

The relationship between BMI and fracture risk is nonlinear. It's been reported that change of 5 units from a BMI of 25 kg/m2 to a BMI of 20 kg/m^2 , corresponds to a doubling of the hip fracture risk, and 27% difference in fracture risk for any osteoporotic fracture, whereas change from a BMI of 25 kg/m2 to a BMI of 30 kg/m^2 corresponded to 17% reduction in hip fracture risk, and 11% difference in fracture risk for any osteoporotic fracture [77].

Obesity was believed to be protective against fracture because of the higher bone mineral density (BMD) associated with obesity and the protective effect of soft tissue padding during falls [78]. However, fracture risk in obesity is not lower at all skeletal sites. The relationship between adiposity and bone microstructure will be discussed further in chapter 1.7.3.

1.4.3   Parental history of fracture

There is a strong familial predisposition to the risk of osteoporotic fractures. Cummings and colleagues reported that the risk of hip fracture doubled amongst women with a maternal history of hip fracture, especially before the age of 80 [79]. Also, the risk of hip or wrist fracture is increased in women with a family history of these type of fractures respectively [80]. In agreement with observational studies, a meta-analysis reported an increased risk of any fracture, osteoporotic fracture and hip fracture in men and women with parental history of fracture [81]. The risk ratio (RR) in that study for any fracture was 1.17 (95% CI = 1.07–1.28), for any osteoporotic fracture was 1.18 (95% CI = 1.06–1.31), and for hip fracture was 1.49 (95% CI = 1.17–1.89). The increased fracture risk was independent od BMD.

1.4.4   Previous fracture

Previous osteoporotic fracture is a well-documented risk factor for future fracture. Postmenopausal women with a prior fracture have a doubled overall risk of future fractures when compared to women without prior fracture [82]. The strongest associations are for vertebral fractures with a 4-fold increase in risk among women of sustaining a subsequent vertebral fracture, with the further increase in fracture risk with the number of prior vertebral fractures [82].

 Previous fracture is associated with a significantly increased risk of an osteoporotic fracture in men and women at all ages, above the age of 50, with and without adjustment for BMD (Figure 1.6) [83]. Early prevention of future fracture among individuals with a fracture after age 50 should be a part of post-fracture management, as the absolute risk for fractures is highest in the first year after a clinical fracture [84,85].
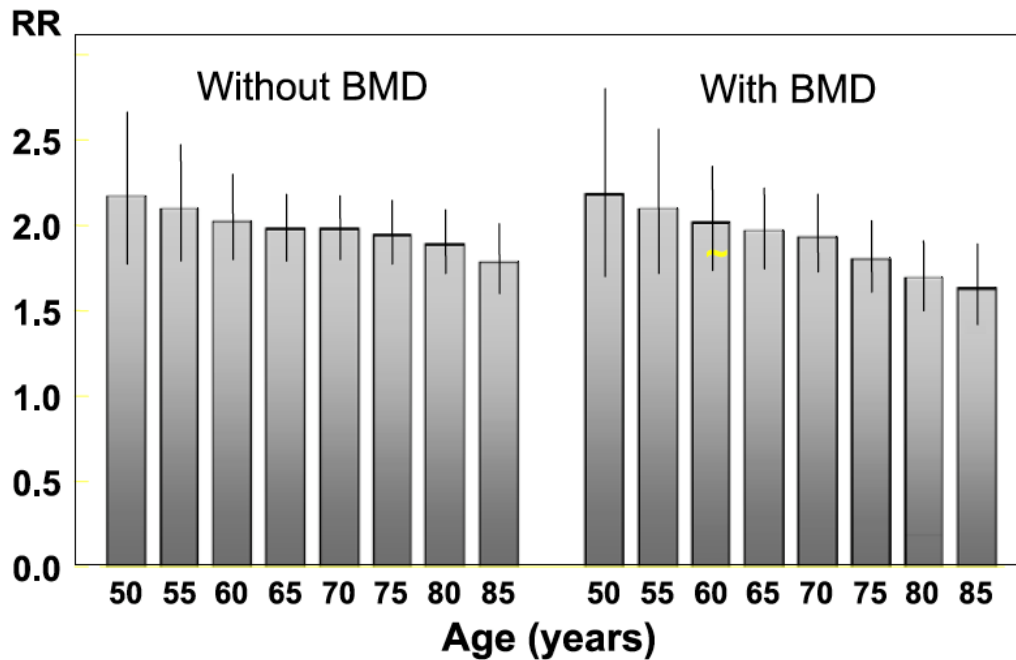
Figure 1.6 Risk ratio for an osteoporotic fracture in men and women with a prior history of fracture with and without adjustment for BMD.

Reproduced with permission from Kanis et al. A meta-analysis of previous fracture and subsequent fracture risk [83].

1.4.5   **Smoking and alcohol**

Cigarette smoking and heavy alcohol intake are considered risk factors for osteoporosis and osteoporotic fractures. Findings from the Framingham Study confirmed that heavy alcohol consumption was associated with an increased risk of hip fracture for women and for men [86]. Kanis and colleagues reported a nonlinear effect of alcohol intake and increase in osteoporotic and hip fractures risk, with no significant increase in risk observed at intakes of 2 units or less daily, but above this threshold, alcohol intake was associated with an increased risk of any fracture (RR=1.23), any osteoporotic fracture (RR=1.38), or hip fracture (RR=1.68)[87]. These findings were in agreement with a more recent meta-analysis reporting light alcohol consumption is inversely associated with hip fracture risk, but heavy alcohol consumption associated with an elevated hip fracture risk [88].

A history of smoking carries an increased risk for future fractures. Current smokers compared to non-smokers have an increased risk of any fracture (RR=1.25), any

osteoporotic fracture (RR=1.29) and hip fracture (RR=1.84) [89]. Similarly, there is an increased risk of fracture in ex-smokers compared with individuals with no smoking history, but the risk lower than for current smokers [89].

1.4.6 Glucocorticosteroids

The increased risk of fracture observed in patients using oral glucocorticosteroids (GC) is related to GC dose. The risk of both hip and vertebral fractures is twice as high in patients using high doses of GS (<7.5mg prednisolone or equivalent) than in those using low doses (<2.5mg prednisolone or equivalent) [90]. The fracture risk among GC users is highest for vertebral fracture with a RR of 1.55 for low daily doses, 2.59 for daily doses of 2.5-7.5 mg and 5.18 for high daily doses compared to no GC users; for hip fracture the RR is 0.99, rising to 1.77 and 2.27, respectively [91]. All fracture risks decline shortly after cessation of oral GC treatment toward baseline [91]. The risk of fractures is more strongly related to daily dose than to cumulative doses in GC users. Moreover, there is an increased fracture incidence among patients initiating oral GC therapy, of vertebral fracture, compared to chronic GC users [92]. For non-vertebral fracture, the annual rate is reported to be similar among GC initiators and among chronic GC users. This suggests that bone loss affects both axial and appendicular sites, but oral GCs have a predominant effect at the spine leading to vertebral fragility.

1.4.7 Rheumatoid arthritis

Individuals with Rheumatoid arthritis (RA) are considered to be at increased risk of hip fracture [93–95]. A study by van Staa and colleagues used data from the British General Practice Research Database to demonstrate that patients with RA have an increased risk of fractures at the hip, pelvis, vertebrae, humerus, and tibia/fibula [96]. The reason for this increased fracture risk in RA patients is multifactorial, but a recent study by Clynes and colleagues has demonstrated that individuals with RA have an increased risk of fracture even after adjustment for both eBMD and falls, suggesting that some other aspect of bone quality may be impaired in the condition [97].

1.4.8 Secondary osteoporosis due to comorbidities

Several diseases have been shown to be a secondary causes of osteoporosis. These include type I diabetes mellitus, osteogenesis imperfecta in adults, untreated long-

standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition or malabsorption, and chronic liver disease.

Type I and type II diabetes mellitus (DM) are both associated with an increased risk of fractures [98–100]. The mechanism through which this occurs is likely to be different, as BMD is decreased in patients with type I DM and increased in patients with type II DM. The risk is higher with type I than with type II DM [99,100]. Vestergaard in the meta-analysis reported an increase of hip fracture risk in type I DM (RR = 6.94; 95% CI: 3.25–14.78), and RR = 1.38 (95% CI: 1.25–1.53) of hip fracture in type II DM compared to subjects without diabetes [100]. The risk for fractures increases with the duration of the disease and the use of insulin [98100].

Individuals with osteogenesis imperfecta (OI), a hereditary, connective tissue disorder caused by mutations in the genes of collagen type 1, have an increased risk of fractures throughout their life compared to the general population [101,102]. The relative risk of fractures is largest in the childhood and adolescent years and declines with age in patients with OI compared to the general population [102]. Though the relative risk declines with age, the absolute rates of fractures are the highest during the childhood and adolescent years with an incidence rate (IR) 233.9 per 1000 person years, lower during adulthood (IR 84.5 per 1000 person years), and increase again, as in general population, in women 55 years and older (IR 111.9 per 1000 person years) (Figure 1.7) [102].
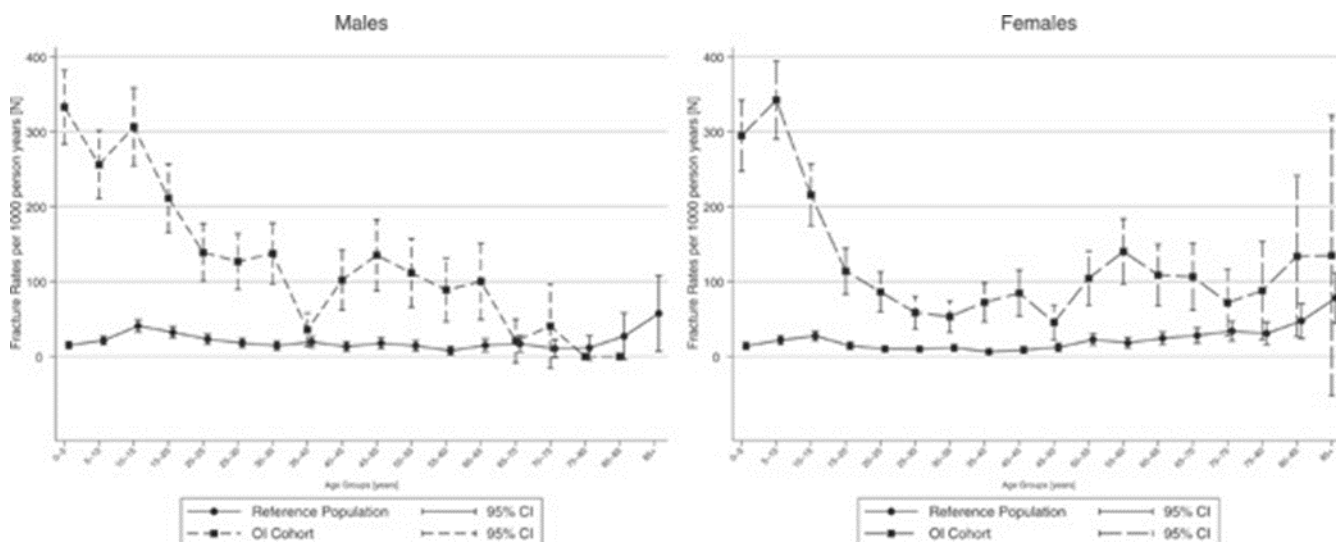
Figure 1.7 Fracture rate in the OI cohort and the reference population by age, for males and females separately.

Reproduced with permission from Folkestad et al. Fracture Rates and Fracture Sites in Patients With Osteogenesis Imperfecta: A Nationwide Register-Based Cohort Study [102].

Untreated hyperthyroidism has been associated with a decreased bone mineral density and an increased fracture risk [103,104]. While bone loss has been documented at all skeletal sites, there is preferential involvement of cortical bone, and therefore an additional distal forearm BMD scan is suggested [105]. While the cause of the hyperthyroidism may not matter, the severity is an important factor, as a TSH value below 0.01 mU/L has been associated with a 2- and 3.5-fold increased risk of hip and spine fractures, respectively [106]. Moreover, variation of serum TSH values within the reference range in post-menopausal women is associated with changes in BMD and fracture risk [107].

Hypogonadism is an important risk factor for osteoporosis in both sexes. Premature menopause is also associated with low BMD and increased risk of fractures [108,109]. Similarly, in men, primary and secondary hypogonadism leads to the decreased BMD and increased fracture risk [110].

Malabsorption occurs in many small-bowel disorders and affected nutrients include calcium, phosphorous, and vitamin D. Decreased absorption of calcium and vitamin D, contribute to a net negative calcium balance and compensatory elevation in PTH leading to increased bone turnover and decreased BMD. Also, malnutrition, particularly protein undernutrition, may contribute to the occurrence of osteoporotic fracture, by lowering

bone mass and altering muscle strength [111]. The effect of chronic liver disease on bone metabolism is mainly ascribed to osteoblast function impairment, together with low vitamin D levels, hypogonadism, and malnutrition [112].

1.4.9 **aBMD**

BMD is included as a potential variable to include in the FRAX algorithm. Many well controlled prospective studies with DXA indicate that the risk of fracture increases by a factor of 1.4–2.6 for each SD reduction in aBMD [113]. Accuracy of site specific measurements show the higher gradients of risk for their respective sites. For example, measurements at the hip predict hip fracture with better accuracy than do measurements at lumbar spine or forearm, with the highest gradient of risk found at the hip to predict hip fracture at 2.6. However, as many as half of all fractures in postmenopausal women occur in individuals with aBMD values above the WHO threshold for osteoporosis [114,115]. The low sensitivity is one of the reasons why widespread population based screening is not widely recommended in women at the time of the menopause. As a result, other methods of bone assessment, including HR-pQCT, have been explored in relation to fracture prediction. It will be further discussed in chapter 1.6.

1.4.10 **Falls**

Falls are not currently included in the FRAX algorithm, but it is recognised that falls from standing height or less are the most common mechanism of fragility fractures [116]. However, the number of falls is much greater than the number of consequent fractures with only 5% to 10% of falls in older adults leading to skeletal injury [116]. Prior falls are considered to be a risk factor for future fracture[117]. Furthermore, the findings from the Study of Osteoporotic Fractures in Men suggest that the predictive value of past falls for future fracture risk is independent of that captured by FRAX with or without BMD[118]. Therefore, in clinical practice patients with frequent falls should be recognized to be at higher fracture risk than estimated by FRAX [117]. Fracture assessment tools including FRAX will be discussed in the next section.

1.5 **Fracture risk assessment**

At present, there is no universally accepted policy for population screening in Europe to identify patients with osteoporosis or those at high risk of fracture.

Whereas BMD provides the cornerstone for the diagnosis of osteoporosis, based on the clear link between lower BMD and increased fracture risk [113], it has been widely recognised that low BMD is a risk factor for fragility fracture rather than as a disease in itself. The use of clinical risk factors in addition to BMD measurement has been demonstrated to increase the accuracy of hip and major osteoporotic fracture risk assessment [119] and a number of tools have been developed to calculate an individual's risk of fracture. These include the Garvan fracture risk calculator [120], QFracture™ [121] and FRAX® [122]. Fracture risk assessment tools are based either on clinical risk factors and BMD measurement (FRAX, Garvan) or on clinical risk factors alone (QFracture). Comparative features of three fracture risk assessment algorithms are presented in Table 1.3.

Table 1.3 Characteristics of FRAX, QFracture and Garvan fracture risk calculators

		FRAX	QFracture	Garvan
Clinical risk factors and bone parameters as input variable				
	Age and Sex	+	+	+
	Height	+	+	+
	Weight	**+**	**+**	**+**
	Current Smoking	+	+[1]	-
	Alcohol status	**+**	+[1]	-
	Parental hip fracture	**+**	**+**	-
	Rheumatoid arthritis	+	+	-
	Glucocorticoid use	+	+	-
	Falls	-	+	+[2]
	Prior fracture	+	+	+[3]
	Ethnicity	+[4]	+[5]	-
	Other	+[6]	+[7]	-
	BMD	+	-	+
	Trabecular bone score assessment	+	-	-
Mortality risk accounted for		+	-	-
Output		probability	risk	risk

[1] Smoking status (non-smoker, ex-smoker, light, moderate, heavy), alcohol use different doses per day available

[2,3] Number of falls/prior fractures

[4] 68 population specific calculators available, four for the USA and three for Singapore

[5] 9 ethnic backgrounds available

[6] Secondary osteoporosis

[7] Diabetes, living in a care home, dementia, cancer, asthma, COPD, Cardiovascular disease, chronic liver disease, chronic kidney disease, Parkinson's disease, SLE, malabsorption, endocrinopathy, epilepsy, antidepressant use, HRT use

1.5.1 **FRAX**

As discussed above, FRAX® is a computer-based algorithm (http://www.shef.ac.uk/FRAX) that calculates the 10-year probability of a major fracture (hip, clinical spine, humerus or wrist fracture) and the 10-year probability of hip fracture for individuals between the ages of 40–90 years [122]. It has been developed across a large number of population-based cohorts worldwide from Europe, North

America, Asia, and Australia including nearly 45,000 individuals, and is the most comprehensively evaluated risk assessment tool currently available.

Fracture risk is calculated based on information readily available from standard clinical sources that includes many of the factors described above, including age, body mass index, prior fragility fracture, parental history of hip fracture, current tobacco smoking, ever use of long-term oral glucocorticoids, rheumatoid arthritis, other causes of secondary osteoporosis and alcohol consumption. Femoral neck BMD can be added if available to enhance fracture risk prediction [119]. Fracture probability varies markedly in different regions of the world [14]. Thus, the FRAX® models need to be calibrated to those countries where the epidemiology of fracture and death is known. The freely available internet-based calculator for sixty-eight country (population)-specific FRAX in 34 languages is available to account for geographical variations in fracture incidence and mortality. In healthcare settings where trabecular bone score is available, this can also be incorporated into the fracture risk calculation.

The FRAX assessment does not include all clinical risk factors and takes no account of dose responses for several risk factors, as there is a limitation of data available globally in population-based cohorts. For example, the lack of standardized documentation of falls events across the 23 cohorts used in the development and validation of the FRAX tool has meant that the use of prior falls as a clinical risk factor was not possible. Similarly, details of glucocorticoid exposure (e.g., dose and duration) were not available in the original FRAX cohorts, so that the relationship again assumes an average exposure; this will lead to an underestimation of fracture risk for recipients of higher daily doses of steroids and overestimation for low daily doses [90]. Subsequently, an adjustment to the calculated fracture risk has been proposed based on the relative fracture risks according to steroid dose [123]. Although, FRAX has not been validated in patients who have received anti-osteoporosis treatment, there is evidence that it may still provide a useful guide in terms of continuation or cessation of therapy [124].

Since its availability in 2008, FRAX estimates of fracture probability have been incorporated into clinical guidelines of several national societies including the NOF [125]. Although there are different models for fracture risk thresholds at which treatment is

recommended (fixed and age-dependent) it is recognized that setting of intervention thresholds need to be country-specific [125].

1.5.2 QFracture and Garvan fracture risk calculator

Two further risk assessment tools have been developed from single cohorts: the Garvan Fracture Risk Calculator (https://www.garvan.org.au/bone-fracture-risk) [120] and QFracture (http://www.qfracture.org) [121]. The Garvan calculator was derived using the Australian Dubbo cohort of around 2000 individuals, and includes men and women age 60 years or more [120]. It yields absolute fracture risk as a percentage over 5 or 10 years for osteoporotic fracture or hip fracture. Like the FRAX tool, it is based on age, sex, prior fracture and bone mineral density. It differs from FRAX by including a history of falls (categorised as 0, 1, 2 and >2 in the previous year) and the number of previous fragility fractures (categorised as 0, 1, 2 and >2), but does not include other FRAX variables.

The QFracture™ tool is based on routinely collected data, in primary care in the UK, of around 2 million men and women aged 30–85 years. It takes into account multiple clinical risk factors, 30 in total [121]. Unlike FRAX, it also includes a history of falls (yes/no, over an unspecified time frame) and excludes BMD [121].

The Garvan calculator and QFracture generate cumulative fracture risk, as opposed to FRAX, which yields probability of fracture adjusted for the competing hazard of death. There are differences in the calibration of these instruments and the outputs cannot be used interchangeably.

1.6 Relationships between bone density, microarchitecture and fracture

1.6.1 Introduction

Measurements of DXA-derived aBMD of the hip or spine and use of prediction tools including clinical risk factors, such as FRAX, are considered the gold-standard for assessing fracture risk [113,119]. Marshall and colleagues reported that aBMD at all measurement sites predicted the occurrence of osteoporotic fractures with relative risks (RR) of around 1.5 for every 1 SD below the age-adjusted mean; stronger relationships

were found between spine aBMD and vertebral fractures (RR 1.9-2.8), and hip aBMD and hip fractures (RR 2.0-3.5) [113]. The use of clinical risk factors in addition to aBMD measurement has been demonstrated to increase the accuracy for assessing fracture risk [119].

However, it is widely recognised that the majority of fractures occur in individuals not diagnosed with osteoporosis by BMD testing and/or in those with few clinical risk factors, and thus low fracture probability by FRAX [115]. As many as half of all fractures in postmenopausal women occur in individuals with aBMD values above the WHO threshold for osteoporosis, considered low risk for osteoporotic fracture [114,115]. As a result, other methods of bone assessment, including HR-pQCT, have been explored in relation to fracture prediction.

1.6.2   HR-pQCT differences by fracture status

Several studies have investigated the differences in bone microarchitecture by fracture status in postmenopausal women. Until recently, these were predominantly case-control studies that have compared those with fracture and those without and demonstrated associations between cortical and trabecular bone measures at the distal radius and tibia and fracture. In terms of cortical parameters, reduced cortical area, thickness and vBMD were reported in those with a fracture at radius and tibia [126–130]. Cortical porosity generally did not differ by fracture status; however, Sundh and colleagues, in studies of women with prevalent hip fracture [131] and in older men with any fracture [132] reported cortical porosity be higher when compared to controls.

Differences have also been described in many HR-pQCT–derived trabecular parameters between fractured individuals and controls with lower trabecular density, number and thickness in those with a prevalent fracture [126,128,129,133].

However, the retrospective design of the studies raising uncertainty about whether the fracture-associated differences observed by HR-pQCT are a cause or consequence of the fracture event.

More recently, differences in HR-pQCT measures between cortical and trabecular bone measures at the distal radius and tibia and fracture have been reported by a few prospective studies [134–138]; and are consistent with those from retrospective studies. In

postmenopausal women with fracture, total vBMD and trabecular vBMD were significantly lower at both sites radius and tibia compared those without fracture in all studies [134–138].

Women with incident fractures had significantly lower cortical parameters (area and thickness at the radius [135,136] and tibia [136,137], vBMD at the radius[135] and tibia[136]) and trabecular parameters (lower trabecular number at the radius[135,137] and tibia[136,137] and higher trabecular separation at the radius[135] and tibia[136]) compared with control women.

Samelson and colleagues reported findings from a large prospective multi-national cohort study investigating 7,254 individuals (4,768 women, 2,486 men) [138]. In agreement with other studies, those individuals who sustained an incident fracture had worse bone measures for nearly all parameters compared to those who did not fracture. A recent meta-analysis [139] reported that fracture associated differences increased with age and were consistently larger in the radius than in the tibia, especially for trabecular measures: trabecular vBMD ($p = 0.02$), trabecular thickness ($p = 0.15$) and trabecular number ($p = 0.04$). Furthermore, it demonstrated that fracture-associated differences in total vBMD and trabecular vBMD at both sites radius and tibia, as well as cortical. vBMD and cortical thickness can be reliably detected in individual patients using the XtremeCT scanner. The findings supported the use of HR-pQCT for fracture prediction in a clinical setting where available.

1.6.3   HR-pQCT differences by fracture status independent of aBMD

In clinical practice the imaging modality of choice for assessing fracture risk is aBMD by DXA and many studies using HR-pQCT have adjusted for aBMD in their analyses. In cross-sectional studies, at the radius, HR-pQCT analyses were adjusted for aBMD at the ultradistal radius, and most relationships were maintained, but the difference in trabecular and cortical thickness by fracture status were no longer significant [128,140]. For analyses at the tibia, adjustment for FN aBMD, did not significantly affect the study findings [128,140].

In agreement with previous studies, Biver and colleagues [135], undertook a prospective study that reported that trabecular and cortical vBMD and microstructure predicted incident fractures, independently of femoral neck aBMD. However, the associations

were markedly attenuated after adjustment for ultra-distal radius aBMD. The associations with fractures were of lower magnitude at the tibia than at the radius. In addition a large prospective multi-national cohort study found that trabecular and cortical bone density and microstructure measured at the peripheral skeleton predicted incident fractures independently of femoral neck BMD [138]. Although most relationships were maintained, the difference in tibial cortical thickness and trabecular thickness, and radial cortical vBMD were no longer associated with fracture after adjustment for femoral neck aBMD. When analyses have been adjusted for DXA radius aBMD (rather than femoral neck aBMD), radius trabecular number and trabecular vBMD were reported to be independently associated with fracture risk.

In general, differences in vBMD and bone microarchitecture by fracture status are maintained after adjustment for aBMD suggesting that HR-pQCT can improve the prediction of fractures beyond central DXA, but relationships are attenuated after adjustment for aBMD measured by DXA at the radius.

1.6.4 HR-pQCT differences by fracture status independent of FRAX

Researchers have investigated if HR-pQCT differences by fracture status are independent of FRAX. In one prospective study of postmenopausal women, Biver and colleagues [135], reported that trabecular and cortical vBMD and microstructure predicted incident low trauma clinical fractures and major osteoporotic fractures, independently of FRAX-BMD. The associations with fractures were of lower magnitude at the tibia than at the radius. Combination of FRAX with trabecular and cortical vBMD and microstructure resulted in better fracture prediction than FRAX alone. However, it did not improve the fracture risk prediction above the results from microstructure parameters alone.

Another study reported HR-pQCT differences by fracture status with significantly lower total and trabecular vBMD at both sites, cortical parameters (area and thickness at the radius, vBMD at the tibia), trabecular number in women with incident fracture compared to control [136]. Less favourable bone microarchitecture and density was still significantly associated with an increased risk of fracture after adjusting for FRAX probabilities.

In agreement with previous studies, Samelson and colleagues [138], reported that trabecular and cortical bone density and microstructure predicted incident fractures independently of FRAX. After adjustment for FRAX score, the associations were attenuated but remained significant for bone parameters. In general, differences in vBMD and bone microarchitecture by fracture status are maintained after adjustment for FRAX suggesting that HR-pQCT improve the prediction of fractures beyond FRAX.

1.6.5 HR-pQCT differences by fracture status in osteopenic women

HR-pQCT parameters were also assessed in osteopenic postmenopausal women with fracture and those without [141,142]. Total vBMD was lower in fracture subjects [141,142]. Similar to subjects with osteoporosis, cortical parameters in osteopenic postmenopausal women with fracture were less preferential i.e. lower cortical vBMD, cortical thickness and smaller cortical area at radius and tibia than controls [141]. Cortical porosity has not been found to differ by fracture status in osteopenic individuals [141].

In terms of trabecular parameters, trabecular vBMD was lower in fracture subjects at radius, but not at tibia; and significantly greater trabecular area at both sites [141]. There was a trend toward lower trabecular number and thickness and greater trabecular separation in fracture subjects observed at radius, but no significant difference in women with fracture and those without at tibia [141].

1.6.6 Clusters of HR-pQCT parameters and bone phenotypes

Studies evaluating HR-pQCT differences by fracture status have suggested that specific components of bone structure, such as cortical thickness and trabecular microarchitecture, are deficient in fracture cases. Edwards and colleagues explored different bone phenotypes, combining multiple outcomes related to bone strength, and their relationships to fracture, used statistical cluster analysis, taking into account all parameters measured by HR-pQCT [143]. Two high risk bone phenotypes were identified; the first was characterised by low cortical thickness and density and, in men only, a higher total and trabecular area whereas the second showed low trabecular density and number. The first cluster contained a much smaller proportion of osteoporotic individuals as defined by DXA despite having a similar proportion with prevalent fractures. Individuals in the second cluster had low femoral neck areal BMD when compared to the reference cluster. A considerable proportion of individuals in this

cluster also fell into the osteoporotic range as defined by DXA. This is an isolated study and further research was required to replicate the findings in different cohort. We have attempted to replicate these findings in the GLOW Study in Chapter 4.1.

1.7   Adiposity and bone

1.7.1   Introduction

Obesity is a state of imbalance between calories ingested and calories expended leading to excessive or abnormal fat accumulation that presents a risk to health [144]. Obesity increases the likelihood of various diseases including ischaemic heart disease, type 2 diabetes, obstructive sleep apnoea, certain types of cancer, and osteoarthritis [145]. Although several classifications and definitions for degrees of obesity are accepted, the most widely accepted classifications are those from the World Health Organization (WHO), based on body mass index (BMI): Class 1: BMI of 30 to < 35, Class 2: BMI of 35 to < 40, Class 3: BMI of 40 or higher. The levels of obesity are rising and have been doing so at a rapid rate [146] (Figure 1.8). It is estimated that around 600 million adults, approximately 11% of all men and 15% of all women, worldwide are obese [147,148]. Based on current trends, it has been predicted that 20% of the population will be obese by 2025 in the 33 of the 53 countries in the WHO European Region [147]. In England and Scotland the prevalence of obesity is predicted to increase to >30% by 2025 and to more than half of the UK adult population by 2050 [147,149]. The health and economic burden of increasing BMI is substantial. A high BMI accounts for 4.4 million deaths and 134.0 million disability-adjusted life-years (DALYs)[147]. The estimated economic cost of obesity-related diseases is approximately 7% of health care budgets across the European Union [150]. The Foresight report estimated that overweight and obesity will cost the National Health Service £10 billion per year by 2050 [149].

Obese men

West Africa
Central Africa
Southern Africa
East Africa
Middle East and north Africa
Central Asia
South Asia
Southeast Asia
East Asia
High-income Asia Pacific
Melanesia
Polynesia and Micronesia
Caribbean
Andean Latin America
Central Latin America
Southern Latin America
High-income English-speaking countries
Northwestern Europe
Southwestern Europe
Central Europe
Eastern Europe

Obese women

Severely obese men

Severely obese women

Figure 1.8 Trends in the number of obese and severely obese people by region [146].

1.7.2 **Adiposity imaging**

While BMI is widely used as an index of the degree of obesity, it cannot be used to distinguish body fat from lean mass. It is possible to estimate the amount of fat tissue within an individual using DXA. The differential attenuation of the two X-ray energies is used to estimate soft tissue composition (i.e. fat and lean body mass) as well as BMC. Adiposity can be measured in regions where no bone is present**.** Adipose tissue is made up of lipid (85%), proteins, minerals (3%) and water (12%) [54]. It is important to note that DXA, specifically measures the mass of total lipid, not adipose tissue. Therefore, DXA fat mass (FM) is defined as all lipid mass, including phospholipids, organ, marrow and subcutaneous adipose. FM is measured in either g or kg. Total body

adipose tissue can be divided broadly as subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). There is also non-visceral internal adipose tissue consisting of intra and peri muscular adipose tissue and other non-visceral adipose tissue. DXA cannot measure overlapping components of adipose tissue, but it could be used to monitor select components of adipose tissue. DXA can also provide FM values for individual parts of the body such as the android/gynoid region, arms, legs and trunk.

There are other modalities for FM imaging, and all have pros and cons, such as ultrasound (no radiation, but low accuracy), computed tomography (accurate, measures specific body fat compartments, but is expensive and has higher radiation) or magnetic resonance imaging (accurate, measures specific body fat compartments, no radiation, but is expensive and has prolonged scan time) [151]. DXA is relatively simple to perform, less expensive and more accessible than MRI or CT. Radiation exposure is much less than CT. Although subcutaneous and visceral fat cannot be clearly separated by DXA, abdominal fat mass determined by DXA correlates well with visceral fat as determined by other methods such as computed tomography and magnetic resonance imaging [152,153].

1.7.3 **Obesity and bone density and structure**

Extensive epidemiological studies have reported that elevated body weight or body mass index are positively associated with aBMD of the lumbar spine, femoral neck, distal radius, proximal femur and leg [127,154–156] and that a decrease in body weight leads to bone loss [157].

A number of cross-sectional population-based studies have investigated the relationships between the components of body mass and aBMD. Recent meta-analysis of 20,226 individuals in 44 studies found that, while LM and FM were both associated with aBMD in men and women. While, overall in this analysis the variation in LM accounted for 21% of differences in whole body aBMD, and the variation in FM for approximately 8% of differences in aBMD, in postmenopausal women the magnitude of correlation between FM and aBMD was equivalent to that between LM and aBMD [158].

The generally accepted explanation of this relationship is that a larger body mass induces greater mechanical loading on bone, with a consequent increase in aBMD [77,156]. However, the higher aBMD in an obese individual may not be due to similar bone

microarchitecture and may not confer greater strength than a lower aBMD in a lighter person.

More recently studies have begun to use HR-pQCT to assess bone microarchitecture in obesity. Evans and colleagues in a cross-sectional case-control study designed to look at bone microarchitecture and obesity in men and women in younger and older adulthood demonstrated that both obese men and women had higher vBMD at the distal radius (p < 0.001 older group) and distal tibia (p < 0.001 both ages) when compared to normal weight individuals [159]. Obese adults had higher cortical thickness, cortical BMD, lower cortical porosity, higher trabecular BMD, and higher trabecular number than normal adults [159]. There was no difference in bone size between obese and normal adults. Differences in HR-pQCT measurements between obese and normal adults were seen more consistently in the older than the younger group [159]. Similarly, in a study of young obese men, BMI was positively associated with trabecular number and inversely associated with trabecular separation [160]. Only one study has investigated associations between obesity and measures of bone micro-architecture in elderly women reporting that higher total vBMD was observed in obesity; due to greater cortical thickness, cortical area and cortical vBMD and greater trabecular vBMD due to greater trabecular number with a lower trabecular separation [127]. At the tibia, positive trend was observed in cortical thickness in obesity, but it was not significant [127]. In the obese group cortical porosity was 21% lower at the tibia, whilst no differences in cortical porosity were observed at the radius. Total area and trabecular area were no different in obesity. However, the increase of all parameters in obese women was lower relative to the excess of weight for BMI [127]. Although some positive relationships have been shown between adiposity and bone geometry, the specific compartments affected have varied, relationships have been inconsistent, and studies have been performed mainly in obese children, adolescents and young adults [161–165]. Importantly it is not known whether associations between BMI and bone microarchitecture are the same between different classes of obesity (overweight, Class I, Class II/III, morbid obesity) at both weight-bearing and non-weight-bearing skeletal sites.

Fat distribution may affect associations between adiposity and bone density and microarchitecture [166–168]. Cohen and colleagues reported that greater central adiposity was associated with lower trabecular bone volume and bone formation on bone biopsy in premenopausal women [166]. The inverse relationship between central obesity and

trabecular bone volume remained significant after controlling for age and BMI. In another study involving healthy Korean subjects, the visceral fat area measured by abdominal CT is inversely associated with aBMD, whereas the subcutaneous fat area does not show any significant association [167]. Ng and colleagues reported differences in the association between subcutaneous and visceral adipose compartments and bone density and microstructural parameters, differences which were also age- and gender-dependent [168]. Interestingly, total body fat and SAT were positively correlated with vBMD, but there was no correlation between VAT and femoral neck and lumbar spine vBMD in postmenopausal women [168].

1.7.4   **Obesity and fracture**

Many epidemiological studies have reported that low body weight and low BMI are risk factors for fragility fracture. The results of a meta-analysis of 60,000 men and women from 12 prospective, population based cohorts showed that total fractures, osteoporotic fractures and hip fractures were all inversely correlated to BMI in both men and women [77] Obesity was believed to be protective against fracture because of the higher bone mineral density (BMD) associated with obesity and the protective effect of soft tissue padding during falls [78]. However, fracture risk in obesity is not lower at all skeletal sites. Accumulating evidence indicates that the relationship between BMI and fracture varies according to fracture site with lower rates of hip and pelvis in obese individuals, [169,170], whereas higher risk of some non-spine fractures including proximal humerus, upper leg, and ankle fracture in obese individuals [169,171–173] .

 A large number of low-trauma fractures occur in overweight and obese men and women, and the prevalence of low-trauma fractures is similar in obese and non-obese women [174]. In the GLOW study, fracture prevalence in obese women at baseline was 222 per 1000 compared with a similar 227 per 1000 in non-obese women [169]. At two year follow up, fracture incidence was 61.7 per 1000, again similar to the rate of 66.0 per 1000 in non-obese women [169]. Furthermore, in GLOW study relationships between weight, height and BMI and individual fracture sites were demonstrated with inverse associations between BMI and hip, wrist and spine fracture; positive association between BMI and ankle fracture. Finally, the burden of fracture on healthcare systems in obese populations was determined in the GLOW study [175]. It reported that obese women with fracture undergo a longer period of hospitalization for treatment and have

poorer functional status and health-related quality of life HRQL than non-obese women. Hence, despite protective effects of obesity against hip and vertebral fracture, fractures elsewhere in the skeleton make a significant contribution to the burden of fracture on healthcare systems [175].

Using data from Fracture Liaison Service (U.K.), investigating 4288 post-menopausal women over 50 years of age with a low trauma fracture (30 % of women had a BMI ≥ 30 Kg/ m$^{2)}$), Ong and colleagues reported that obese patients when compared with the non-obese, were more likely to fracture their ankle (OR 1.48) and upper arm (OR 1.48), but were less likely to fracture their wrist (OR 0.65) [176].

Another study carried out on a large Spanish population of women aged 50 years or over has confirmed that association between obesity and fracture in postmenopausal women is site-dependent: with obesity protective against hip and pelvis fractures but associated with increased risk of proximal humerus fractures [171].

Johansson et al performed a meta-analysis of data from prospective cohorts from more than 25 countries in 398 610 women aged 20–105 years (mean 63), followed for an average of 5.7 years [76]. In this study 19% of osteoporotic fractures and 13% of hip fractures occurred in obese women. It showed that low BMI was protective against lower leg fractures and that high BMI was a risk factor for upper arm fracture; ankle fractures were excluded, as were not considered to be osteoporotic.

Whether obesity is protective or a risk factor for fracture at some fracture sites remains uncertain, with inconsistent reports at the forearm for example [173,177,178]. Clinically diagnosed fractures of the vertebrae have been reported to be lower in obese women [169,179]. However, recent report from the Newcastle thousand families study suggested that obesity appears to be a risk factor for prevalent vertebral fracture in men and women [180]. The predictive value of FRAX® tool was assessed for obese and non-obese women in participants in the Study of Osteoporotic Fractures [181]. As expected, there is a lower predicted risk of hip and major osteoporotic fracture in obese women vs. non-obese women, as BMI is an inputted value into the FRAX® tool. Overall it suggested that FRAX with and without BMD is of similar value in predicting hip and major osteoporotic fractures in obese women vs. non-obese women. However, the sensitivity

for hip and major osteoporotic fractures was lower in obese women but the specificity was higher when compared to non-obese women.

In GLOW cohort results suggest that obesity is a risk factor for certain fractures, particularly those of the ankle; obese women were more likely than others to have experienced previous ankle or lower leg fractures [169]. Even after adjusting for maternal hip fracture, current oestrogen use, current cortisone use, current smoking, fair/poor health, age, osteoarthritis, and Parkinson's disease, incident ankle fractures remained more common [169]. Lower leg fractures were not statistically significant, these fractures appeared similar to ankle and upper leg fractures with respect to rates in obese versus non-obese women [169]. Given reports of excess ankle fracture risk among women of very high BMI in GLOW, we have investigated relationships between adiposity and bone microarchitecture among UK arm of GLOW study in chapter 4.2.

1.8   Risk perception and bone

1.8.1   Introduction

In health decision-making, individuals are expected to navigate choices involving weighing risk for consequences with benefits of action. The Health Belief Model (HBM) has been widely used to predict and explain health-related behaviours [182,183]. This model postulates that health behaviours are more likely to occur if individuals regard themselves as susceptible to the condition, believe the condition would have potentially serious consequences and/or believe that a particular course of action available to them would reduce the susceptibility or severity or lead to other positive outcomes [183–185]. In addition, cues to action (trigger that prompts the desire to make a health change), and self-efficacy (belief in one's ability to improve health by taking action) are also important variables in explaining health behaviours [183,185]. Other models of health behaviour include protection motivation theory [186], the precaution adoption process mode [187], and the transtheoretical model of change [188]. Evidence supporting these models suggests that feeling personally vulnerable to a health threat motivates people to take action to reduce the threat.

1.8.2 Accuracy of risk perception

Risk perception has been described as an individual's subjective perception of the likelihood of developing a disease [189]. Patients' perception of their actual risk of disease is important regardless of actual risk for developing these diseases [190]. When individuals believe themselves to be at lower risk for outcomes than their actual risk; this phenomenon is termed "unrealistic optimism" [191], whereas if one believes to be at higher risk for outcomes than one's actual risk it is 'unrealistic pessimism''. The risk perception have implications for how patients view health protective actions they may take to reduce their risks [192]. There is evidence that change in risk perception, has a role in health decision-making and can result in health behaviour change [193]. For those at high risk, an accurate understanding of risk can help identify and adopt relevant lifestyle changes and adherence to preventive interventions (e.g., pharmacologic treatment) that can lead to a better health-related quality of life [190]. For those at low or average risk, accurate risk perception can help patients reduce anxiety and avoid unnecessary intervention [190]. However, risk perceptions may also have implications for overall well-being. Among individuals with high disease risk perceptions, subsequent diagnosis is associated with poorer well-being; among those with low risk perceptions, subsequent diagnosis is unrelated to well-being [194].

1.8.3 Formation of risk perception

There is evidence that a number of factors contribute to risk perception formation [195]. These include incorporating numeric information about a threat, personal experiences, salience of available examples (such as family history of a disease, or increased media coverage of a disease) and emotions (for example anger, fear). Risk perceptions are specific to a threat and do not reflect a general sense of optimism or pessimism.

1.8.4 Types of risk perception

There are three types of risk perceptions: deliberative, affective, and experimental [195]. In deliberative risk perception an individual relies on a number of reason-based strategies to obtain an estimate of the likelihood of the negative outcome (e.g., percentage likelihood of disease). In contrast, affective risk perception refers to emotions associated with risk. Affect influences optimal judgment and decision-making. Experimental risk perception integrates deliberative and affective information to form rapid judgments, not concentrating on the process through which the perception is derived (intuition).

Recognising the type of risk perception could help develop effective interventions to be implemented to facilitate health behaviour change.

However, complex interactions between different types of risk perceptions need to be considered [195]. Combination of risk perceptions could result in optimal - or non-optimal – decisions; for example, individuals who are worried about an outcome (affective) and perceive themselves to be at high risk for that outcome (deliberative) are less motivated or less likely to engage in preventive interventions. Therefore, identifying type of risk perception is insufficient, as interactions and associations among different components are substantial.

1.8.5 Osteoporosis risk perception

Studies have evaluated osteoporosis risk perceptions among peri- and postmenopausal women [184,196,197]. While women largely perceive osteoporosis to be a serious health concern, there is low general belief in personal susceptibility to osteoporosis. Nayak and colleagues investigated osteoporosis related beliefs among women and men aged 60 years and over; they reported that 44.6 per cent of respondents agreed or strongly agreed that they were at risk for osteoporosis, and only 26.3 per cent agreed or strongly agreed that they were likely to develop it. This is in agreement with other studies of osteoporosis beliefs [196–198].

Despite the large impact of osteoporosis on the health and function of older women, women are more concerned about other diseases, such as cancer, heart disease, or neurological diseases [196]. In a qualitative study examining attitudes and perceptions toward osteoporosis prevention among postmenopausal women, osteoporosis was not perceived as a serious disease [199]. The age of the women did not affect their perceptions of the seriousness of osteoporosis [199]. Osteoporosis is typically asymptomatic until a fracture occurs and is identified clinically. As a society, we are most sympathetic toward diseases of which we can see the signs and symptoms and it could be challenging to communicate the seriousness of the 'silent disease' [199]. While osteoporotic fractures can be debilitating and result in mortality, disability, and long-term decrease in function [3–5], these are often considered to be natural and expected changes associated with the aging body [199] .

Furthermore, research suggests that women's knowledge about osteoporosis and its risk factors and protective factors is minimal [200]. Limited knowledge of osteoporosis risk factors, could be one of the reasons for underestimating the risk of the disease. Another, may be the selective attention to or consideration of factors that influence their risk. This is consistent with studies suggesting that, when thinking about risk, people tend to cite factors that decrease rather than increase risk [197,201,202].

Gerend and colleagues examined women's explanations for their risk [197]. Interestingly, women who rated their risk as low attributed their risk protective personal actions (e.g. taking calcium, exercising), whereas women who rated their risk as high focused on personal family history [197]. This is in agreement with other studies on self-serving attributional bias, which demonstrated that people tend to attribute their successes to their own efforts and attribute their failures to external factors [203204].

Osteoporosis beliefs are related to osteoporosis prevention behaviour [205]. In study on health beliefs and attitudes toward the prevention of osteoporosis, only those women who reported actively worrying about developing osteoporosis were more likely to be engaged in significant osteoporosis preventive behaviours [196]. It is in agreement with other studies showing that perceived susceptibility to a health condition is a strong predictor of health-related behaviour concerning that condition, such as screening participation [184,206]. In turn, participation in osteoporosis screening and gaining the knowledge on personal bone density has been shown to increase perception of osteoporosis risk and influence women's decisions about osteoporosis preventive behaviours, such as calcium intake, and use of osteoporosis preventing medication [205].

Risk perception is complex and multidimensional in nature. Increasing women's awareness of osteoporosis should be a priority for future osteoporosis prevention campaigns. Moreover, targeting osteoporosis beliefs with patient education, interventions to address misconceptions women may hold about their risk for developing the disease would be important way for those women to recognize their increased vulnerability and adopt specific behavioural strategies that can reduce their risk of developing osteoporosis.

1.8.6   **Fracture risk perception**

As discussed above, a number of tools have been developed to calculate an individual's risk of fracture. Despite this self-perceived risk of osteoporotic fractures has been reported to be underestimated in postmenopausal women worldwide [197,207,208]. Rothmann et al observed that women participating in the Risk-Stratified Osteoporosis Strategy Evaluation (ROSE) study underestimated their fracture risk compared to the risk estimated by FRAX [208]. Similarly, findings from GLOW showed that women at increased fracture risk generally perceive their risk to be lower or about the same as women of the same age [207]. In that study, 20% of women rated themselves at increased risk of fracture compared with about 35% who indicated they considered themselves at lower risk than their peers. Among women who reported individual or multiple risks for fracture that put them at higher fracture risk than their peers, fewer than 50% recognized the increased risk. Having a diagnosis of either osteoporosis or osteopenia was most likely to raise a woman's perception of risk. However, even women who had multiple FRAX risk factors, a diagnosis of osteoporosis, and current use of an osteoporosis medication, underestimated their fracture risk with only 62% thinking to be at increased risk.

Individuals need to recognize and understand the risks that predispose them to fracture in order to be motivated to both seek medical care and adhere to recommendations made if effective prevention strategies are to be successful. The perception of personal fracture risk has been shown to modify an individual's behaviour related to their bone health [189]. Heightened self-perceived risks of osteoporosis and fracture significantly increases the likelihood of seeking medical advice hence, increasing the chances, in appropriate individuals, of being given a diagnosis of osteoporosis – a well-known predictor of treatment initiation [189]. Moreover, heightened self-perceived risks of fracture is known to be associated with BMD testing [184,189]. Although the positive effect of risk perception on BMD testing has been previously described, the relationship between the results of bone microarchitecture parameters and fracture risk perception has not been investigated. This will be investigated in Chapter 4.4.

The lack of accurate fracture risk perception has adverse implications for successful fracture preventive behaviours. Motivation for patients to seek and follow treatment is related to perceived susceptibility to a disease [209] Osteoporosis treatment initiation and

compliance is suboptimal. A systematic review on interventions to improve osteoporosis treatment in fragility fracture patients has shown that an average of 22 % of eligible patients initiated pharmacological treatment across 57 studies with 64 intervention groups [210].

Barcenilla-Wong et al. also reported that elevated self-perceived risk of fracture increases the likelihood of taking anti-osteoporosis medications (AOM) prospectively [189]. Similarly, Beaton and colleagues reported that patients' perceived need for osteoporosis treatment and understanding of osteoporosis improved initiation of osteoporosis medication [211]. It is in agreement with other studies, which indicate that patient perception of benefits of osteoporosis medication, was linked with taking medication [212,213]. Some patients perceive medications as not necessary if they are otherwise healthy and feel calcium and vitamin D supplementation are sufficient [213]. Patients may be more adherent if they fear the consequences of fracture, but this requires that they perceive themselves to be at-risk, which may require education [213].

There is also evidence that experiencing a consequence of inadequate treatment (fracture) or having a relevant clinical test (BMD measurement) were associated with restarting osteoporosis therapy [214].

Patient perceptions of fracture risk are central in the path to initiation of anti-osteoporotic pharmacotherapy[211]. Interventions to facilitate accurate patient perceptions of osteoporosis and fracture risk status could prove helpful in improving management of patient bone health. We explore these themes in Chapter 4.3.

2. Objectives

2.1 Objectives

The overall objectives of this thesis are to use the UK arm of a multinational cohort, the GLOW study, described in the next chapter, and who have been extensively phenotyped including HR-pQCT to investigate the following:

1. To investigate whether certain bone microarchitecture clusters associate with heightened fracture risk, as has been suggested by one previous study [143].
2. To investigate relationships between adiposity and bone microarchitecture.
3. To investigate whether self-perception of fracture risk (SPR) is associated with incident fracture independent of traditional fracture risk prediction tools, and assess if SPR is related to uptake and persistence of anti-osteoporosis medication.
4. To investigate the determinants of SPR, and relationships between SPR and subsequent bone density and microarchitecture.

3. Methods

3.1 Background of the Global Longitudinal Study of Osteoporosis in Women

Global Longitudinal Study of Osteoporosis in Women (GLOW) is a multinational, observational, longitudinal, prospective cohort study designed to improve our understanding of the patient experience, risk and prevention of osteoporosis-related fractures among female residents who were 55 years of age and older at the time of cohort inception [215]. The study was conducted in 10 countries involving 723 physicians and 60,393 women. Subjects were evaluated over a five year period, completed self-administered annual questionnaires and reported the diagnosis and treatment of osteoporosis as well as new fractures. Practices typical of each region were identified through primary care networks organised for administrative, research or educational purposes. The collection of data in a similar manner in ten countries aimed to allow for comparisons of patient experience with prevention and treatment and provide insights into the distribution of risk among older women on an international basis.

3.1.1 Study sites selection

GLOW was conducted in 3 continents: Australia, Europe, and North America; in ten countries (Australia, Belgium, Canada, France, Germany, Italy, Netherlands, Spain, UK, and USA) at 17 study sites (Table 3.1). The site selection was based on the ability of the local investigators to consistently administer the survey methodology, on the availability of a wide spectrum of osteoporosis treatment options and bone densitometry, and the existence of prior studies in those regions. The number of sites to those chosen for this study was restricted by practical considerations concerning the number of survey translations and number of countries in which the survey process could be supervised. Each study site obtained ethics committee approval to conduct the study in the specific location.

Table 3.1 GLOW study sites

Continent	Country	Site
Australia	Australia	Sydney
Europe	Belgium	Leuven
	France	Lyon
		Paris
	Germany	Essen
	Italy	Verona
	Netherlands	Amsterdam
	Spain	Barcelona
	United Kingdom	Southampton
North America	Canada	Hamilton, Ontario
	United States of America	Birmingham, Alabama
		Cincinnati, Ohio
		Los Angeles, California
		Pittsburgh, Pennsylvania
		Rockland County, New York
		Seattle, Washington
		Worcester, Massachusetts

3.1.2 **Physician sample selection**

Clinical investigators at the sites constituted the GLOW Scientific Advisory Board with responsibility for study management. Practices typical of each region were recruited with the assistance of members of the GLOW Scientific Advisory Committee through primary care networks organized for administrative, research, or educational purposes or by identifying all physicians in a geographic area. Primary care physicians were defined as physicians who spent the majority of their time providing primary health care to patients and depending on the country in which the study site was located, this included internal medicine physicians (IM), family practitioners (FP), and general practitioners (GP). In total 723 physicians participated in the GLOW study; 51 GP in Australia, 339 GP in Europe and 35 GP, 103 FP and 195 IM in North America. The number of physicians ranged from 14 to 72 per site (median 40) [215].

3.1.3 **Participant selection**

Subjects were recruited through the offices of their primary care physicians. Each practice listed all women aged ≥55 years who had consulted their primary care physician within the past 24 months. Random sampling was age-stratified to ensure two thirds were aged ≥65 years. In each participating practice, all eligible women 65 and over and a random sample of half that number under age 65 will were recruited. Patients were excluded if they were institutionalized, not able to complete the study survey by themselves due to cognitive impairment, language barriers or were too ill.

A total of 60,393 women were enrolled between October 2006 and February 2008. Approximately 25,000 came from eight sites in Europe (Amsterdam 2,856; Barcelona 2,910; Essen 3,465; Leuven 3,692; Lyon 3,366; Paris 1,714; Southampton 4,079; and Verona 3,252) 28,000 from seven sites in the USA (Birmingham, Alabama 5,061; Cincinnati, Ohio 3,128; Los Angeles, California 3,102; Pittsburgh, Pennsylvania 4,233; Rockland County, New York 3,500; Seattle, Washington 4,055; Worcester, Massachusetts 5,091), and almost 7,000 from two sites in Canada and Australia (Hamilton, Ontario 3,985; Sydney 2,904).

3.2 **Sample selection**

The UK has been represented by Southampton, which has access to local DXA and HR-pQCT scanning facilities. Between 2006 and 2008 4,079 women were enrolled to the Southampton arm of the GLOW Study at baseline. Follow-up questionnaire was returned by 3,619 participants at year 1, 3395 participants at year 2 and by 3,149 women at year 3. Then there was a year when no data was collected. Letters were sent to all participants during the dormant year. Subsequently year 5 a follow-up questionnaire was returned by 2899 participants.

After completion of the year 5 follow-up questionnaire, a subgroup of participants with baseline data and at least one follow-up questionnaire were re-invited by letter to attend their local study centre for DXA (lumbar spine, left hip, total body) and HR-pQCT (distal forearm and tibia) scans in order to measure bone density and detailed bone micro-architecture. The number of participants re-invited was based on a pragmatic approach to achieve a sample of 500.

The power calculation was based on the following assumptions: the probability of having a previous fracture from the age of 45 years among any of the 10 bones

(clavicle, arm, wrist, spine, rib, hip, pelvis, ankle, upper leg, lower leg) for a participant with a hip BMD value equal to the sample mean was 0.184 (estimated in the GLOW sample using logistic regression), 5% significance level and 80% power. Under these assumptions, the minimum detectable effect of a reduction in hip BMD of one SD below the sample mean was an increase in probability of previous fracture from 0.184 to 0.2344. This was reassuring as it corresponded to a detectable odds ratio of 1.36 which is much smaller than the actual effect size on the risk of fracture for an SD decrease in BMD; in GLOW, the actual odds ratio for this in relation to previous fracture since 45 years is 1.59.

Recruitment to the current study started in 2014. Between 2014 and 2017 1367 women were sent letters of invitation (Appendix 1) from the local GLOW centre along with a patient information sheet (Appendix 2). The potential participants were asked to return a reply slip, in a prepaid envelope, indicating whether or not they wished to be included. About 7-10 days after the initial contact, all sample members received a postcard reminder/thank you. Approximately 3-4 weeks after initial contact, a second letter of invitation to have bone scans performed were mailed to all sample members who had not yet either responded or declined participation. At that point 568 participants were willing to take part in the study, 176 declined a bone scan and 625 had not responded. 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 Osteoporosis Centre at Southampton and a taxi was booked to transport them to and from the unit. A total of 520 individuals agreed to take part in the study. Participant recruitment is summarised in the flowchart (Figure 3.1).

```
┌─────────────────────────────────────────────────────────────┐
│  After completion of the year 5 follow-up questionnaire,    │
│  participants of the UK arm of the GLOW study were          │
│  re-invited by letter to attend their local study centre    │
│  for DXA and HRpQCT scans                                   │
│                                                             │
│                        n=1367                               │
└─────────────────────────────────────────────────────────────┘
                            ↓
┌─────────────────────────────────────────────────────────────┐
│  Invitation for Bone Scan was sent from MRC Lifecourse      │
│  Epidemiology Unit in Southampton along with a patient      │
│  information sheet, reply slip and postage-paid return      │
│  envelope                                                   │
│                                                             │
│                        n=1367                               │
└─────────────────────────────────────────────────────────────┘
                            ↓
┌─────────────────────────────────────────────────────────────┐
│  Seven to ten days after invitation is mailed, postcard     │
│  reminder/thank you is sent to all sample members who       │
│  have not actively declined participation in the study      │
│                                                             │
│                        n=1367                               │
└─────────────────────────────────────────────────────────────┘
                            ↓
┌─────────────────────────────────────────────────────────────┐
│  Approximately 3-4 weeks after initial contact, a           │
│  replacement questionnaire packet is mailed to all sample   │
│  members who had not yet either responded or declined       │
│  participation                                              │
│                                                             │
│  n willing to take part = 568   n declined = 176            │
│                                 n not responded = 625       │
└─────────────────────────────────────────────────────────────┘
                            ↓
┌─────────────────────────────────────────────────────────────┐
│  Participants, who agreed to take part and attended for     │
│  HRpQCT and DXA                                             │
│                                                             │
│                        n=520                                │
└─────────────────────────────────────────────────────────────┘
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Figure 3.1 Participant recruitment flowchart.

Recruitment of participants to the UK arm of the GLOW cohort took place over 7 years ago. 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 demographic, anthropometric and lifestyle data collected at baseline. It was found that individuals that were recruited to the UK arm of the GLOW study at baseline but did not take part in the current study were older and had a higher BMI (table 3.2).

Table 3.2 Baseline participant characteristics of GLOW women who had scans (DXA/HR-pQCT) and women who did not.

Participant characteristic [N(%)]	Women without scans (n=3556)	Women with scans (n=523)	P-value
Age (years)**	69.4 (62.5, 77.0)	62.0 (58.7, 66.9)	<0.001
Self-reported height (cm)*	161.5 (6.9)	162.6 (6.2)	0.001
Self-reported weight (kg)*	68.3 (12.9)	67.8 (11.7)	0.395
BMI (kg/m^2) from self-reported values*	26.2 (4.8)	25.6 (4.4)	0.017
Current smoker	261 (7.5%)	28 (5.4%)	0.095
Alcohol consumption (drinks per week)			
None	1193 (34.2%)	110 (21.2%)	
1-6	1432 (41.0%)	219 (42.2%)	
7-13	655 (18.8%)	138 (26.6%)	<0.001
14-20	187 (5.4%)	41 (7.9%)	
>20	26 (0.7%)	11 (2.1%)	
Physically active compared to others of similar age			
Not at all	133 (3.8%)	7 (1.4%)	
A little	650 (18.7%)	72 (13.9%)	<0.001
Somewhat	1690 (48.7%)	258 (49.9%)	
Very	994 (28.7%)	180 (34.8%)	
Educational attainment			
Below GCSE	1526 (42.9%)	130 (24.9%)	
GCSE	1030 (29.0%)	181 (34.6%)	<0.001
A-level	473 (13.3%)	63 (12.0%)	
Degree	527 (14.8%)	149 (28.5%)	
Current use of anti-osteoporotic medications	332 (10.0%)	35 (6.9%)	0.028
Falls in previous 12 months			
None	2128 (61.2%)	336 (65.4%)	
Once	822 (23.6%)	112 (21.8%)	0.168
2 times or more	528 (15.2%)	66 (12.8%)	
Fracture since 45 years	735 (21.9%)	73 (14.5%)	<0.001
Number of comorbidities			
0	662 (23.0%)	130 (28.0%)	
1	827 (28.7%)	140 (30.2%)	
2	698 (24.2%)	121 (26.1%)	0.002
3	397 (13.8%)	43 (9.3%)	
4 or more	295 (10.2%)	30 (6.5%)	

*Mean (SD); **Median (lower quartile, upper quartile)

P-values for differences in medians were calculated using the Wilcoxon rank-sum test; t-tests were used for means and chi-squared tests were used for the categorical variables.

Participants were considered to be taking AOM if they reported current use of alendronate, calcitonin, etidronate, ibandronate, pamidronate, raloxifene, risedronate, strontium ranelate, teriparatide, tibolone or zoledronic acid

Number of comorbidities was calculated out of the following (ever told by doctor): hypertension; heart disease; high cholesterol; asthma; chronic bronchitis/emphysema; osteoporosis; osteoarthritis/degenerative joint disease; rheumatoid arthritis; stroke; ulcerative colitis/Crohn's disease; celiac disease; Parkinson's disease; multiple sclerosis; cancer; and type 1 diabetes.

Their levels of physical activity were lower, smoking was more common, and they were more likely to abstain from alcohol. Women who took part in the current study had a higher recorded educational attainment and lower comorbidity score.

3.3 Informed Consent

On arrival to the Osteoporosis Centre at Southampton, written informed consent was obtained (Appendix 3). 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. A copy of the completed consent form was given to them.

3.4 Questionnaires

Questionnaires were mailed by the Study Centre and self- administered. Information were collected for the following domains: demographic information, medical history, risk factors for osteoporosis-related fracture, perception about fracture risk and osteoporosis, medication use, health care utilization, physical activity and physical function and quality of life (Appendix 4). Instrument development was described in detail in the publication by Hooven et al. [215], but in summary, where possible items from published validated instruments were used and questions that had not been used previously were tested in a sample of women in the study age group. Follow-up questionnaires were administered annually after 1, 2 and 3 years. Then there was a year when data were not collected. The final questionnaire was administered 5 years after the baseline data were collected. In addition to repeating questions about risk factors for osteoporosis-related fracture, perception about fracture risk and osteoporosis, medications, quality of life, and functional status, the follow-up surveys included questions about persistence with medication, reasons for non-adherence, and any incident fractures and requested information about site of fracture and detail about

fracture-associated treatment. Of note, denosumab was not included in the AOM list. It may be that baseline surveys were mailed between October 2006 and February 2008 and at that time denosumab was not widely used. After 5 years of follow-up, patients in the UK centre only were invited to attend for a DXA and HR-pQCT bone scan.

3.4.1 Self-perceived risk of fracture

One of the domains covered in the self-administered questionnaires was the perception about fracture risk and osteoporosis. The questions related to this domain included level of concern about osteoporosis; talked with doctor about osteoporosis; patient told she has osteoporosis or osteopenia; talked with doctor about fall prevention; ever had bone density test; perception of fracture risk and perception of osteoporosis risk. Self-reports of personal risk factors included: current weight and height, parental hip fracture, two or more falls in the past 12 months, current use of cortisone or prednisolone, diagnosis of rheumatoid arthritis, personal history of fracture (clavicle, arm, wrist, spine, rib, hip, pelvis, upper leg, lower leg, and ankle) since age 45 years, current cigarette smoking and consumption of three or more units of alcohol daily. Women rated their SPR, using a five point scale ('much lower', 'little lower', 'about the same', 'little higher' and 'much higher'), compared with women of the same age.

3.5 Anthropometric measurements

Height and weight was measured by 2 highly experienced practitioners, using the same stadiometer and scale, with a standardised procedure for acquisition; minimising inter-operator error.

Height was measured using a free standing Marsden stadiometer . The subject removed their shoes and they were asked to stand as tall and straight as possible with their feet together and their arms held loosely at the side. The headpiece was rested gently on the participant's head and the height was measured to the nearest 0.1cm.

Weight was measured to the nearest 0.1 kg on the day of scanning using a Marsden MPPS-250 (Marsden Weighing Machine Group Limited, Rotherham, UK) digital floor scale. 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.

3.6   **Dual energy x-ray absorptiometry (DXA)**

Participants were scanned in the posterior-anterior (PA) projection, using a Hologic Horizon W; software version Apex 5.5.3.1 (Vertec Scientific, Reading, UK). Scans of the whole body, lumbar spine and left hip were performed.

3.6.1   **Preparation**

Before the procedure, participants were asked to remove all metal jewellery, glasses and anything else that could significantly attenuate the x-ray beam. A dressing gown was provided for them to wear, if their clothes contained metal fasteners. Pillows were used as necessary ensuring as comfortable position as possible during the scan. Participants were asked to remain still and not to talk during the procedure.

3.6.2   **Femur scan**

Participants adopted a supine, straight and central position on the scan table, with their spine straight on the table pad, within the scan limit borders marked on the mattress. A foot positioner, provided by the manufacturers, should be placed under the patient's legs and aligned with the participant's midline. Their medial foot edge should be placed against the foot positioner pyramid. This position allowed internal rotation of the femur by approximately 20° and brought the femoral neck parallel to the plane of the scan table. A Velcro strap can be used to hold the foot in position. The participant's arms were placed on the chest, away from the scan field (Figure 3.2).

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 the femoral shaft was not straight or the leg wasn't sufficiently rotated, then the scan was aborted and repeated. If the subject had a hip replacement, the left side was not scanned. The right hip was scanned instead. The contralateral side was assessed using the same process. Imaging took approximately 1 minute.

Following the scan, image quality was assessed. Rotating the hip prior to initiation should have lead, to the lesser trochanter appearing as a small protuberance only. 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 just touch the edge of the greater trochanter, ideally with an equal amount of space in the box either side of the bone. If the neck box overlay any of the ischium, the ischium was deleted in accordance with the Hologic recommendation.

Figure 3.2 Positioning for femur DXA scan.

3.6.3 Lumbar spine

The participant remained in a supine, straight and central position. They were asked to put legs elevated over the spine scan positioning block with the hips and knees flexed to 90 degrees and the knees and feet together (Figure 3.3). The anterior superior iliac spine was equidistant from the table to prevent rotation of the spine. An express scan ensured correct positioning. An express scan ensured correct positioning. The operator ensured the scan image was straight and central within the scan field and extended from mid-L5 to mid-T12 so as to image the full L1 to L4 region. There were equal areas of soft tissue at either side of the spine. The fast array mode was used for the final scan. The image

was then analysed. The global region of interest was positioned with the top border within the T12-L1 intervertebral space and the bottom border within the L4-L5 intervertebral space, angled to accommodate the shape of the vertebrae. Right and left borders were not altered. The bone map was then identified with vertebral lines placed within the L1-2, L2-3 and L3-4 intervertebral spaces.

Figure 3.3 Positioning for lumbar spine DXA scan.

3.6.4 **Whole body scan**

The subject was positioned lying supine on the bed within the black rectangle in a straight and central position, with the head placed towards the top of the table. Feet were rotated inwards slightly leaving a gap between the toes. If movement was a problem, thin tape was placed around the toes to support the legs in position The operator ensured the body was within the scan line limits indicated on the scan table, with the anterior superior iliac spines equidistant from the table top to prevent rotation of the pelvis and the feet within the scan limit border. Participant's arms were placed by their sides, with palms downwards and slightly separated from the thighs (Figure 3.4). Sub-region defining lines were positioned in accordance with the Hologic QDR User's Guide instructions.

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/hip replacement), the whole body scan was not performed.

Figure 3.4 Positioning for whole body DXA scan.

3.6.5 **Quality assurance and quality control**

All scans were performed by three highly trained operators with standardised protocols for acquisition and analysis; minimising inter-operator error. Quality assurance (QA) testing was performed on a daily basis using a spine phantom. The same phantom was used weekly to perform body composition QA and radiographic uniformity.

3.7 **High-resolution peripheral quantitative computed tomography**

Scans were acquired using the Switzerland XtremeCT I, (Scanco Medical, Basserdorf) in the high resolution mode (image matrix= 1536x1536). A stack of 110 parallel HR-pQCT slices were acquired over a scan length of 150mm, diameter of 125mm and stack height of 9.8mm with an isotropic voxel size of 82 μm. Each participant underwent a HR-pQCT scan of the non-dominant distal radius and tibia, with the dominant limb used if the participant had sustained prior fracture of the non-dominant limb. The

participant had been asked which hand they wrote with and the alternate side was considered to be the non-dominant one. The effective radiation dose for each site was 3uSv. All measurements were taken following the manufacturer's protocols.

3.7.1   Preparation

Prior to scanning, the instrument was pre-calibrated with the participant located a safe distance away. Before the procedure, participants were asked to remove all clothing and jewellery that could significantly attenuate the x-ray beam. The height of the chair was adjusted ensuring as comfortable position as possible during the scan. Participants were asked to remain still and not to talk during the procedure to prevent motion artefact.

3.7.2   Radius HR-pQCT

With the participant seated, the operator placed the hand and lower arm into the forearm cast and used an appropriately sized arm pad to stabilise the arm within the cast (Figure 3.5). The chair was positioned so that the arm rest of the chair was level with the gantry opening and the arm was placed into the device and secured (Figure 3.6). A scout scan was performed to determine the measurement area. A reference line was placed on the notch on the articular surface of the distal radius on the scout image to indicate the position of the first measurement slice (9.5 mm from the reference line) (Figure 3.7). The participant was instructed to remain motionless and the scan was performed. Upon completion, the cast was removed, and the scan quality evaluated. The operator visually inspected random slices to check consistent quality. The operator drew a contour around the cortical perimeter on the first image, before running the automatic contouring detection program which iterated the contouring process through the slice stack. Images were then analysed using the 'Evaluation 3D' option.

Figure 3.5 Cast for radial HR-pQCT scan.
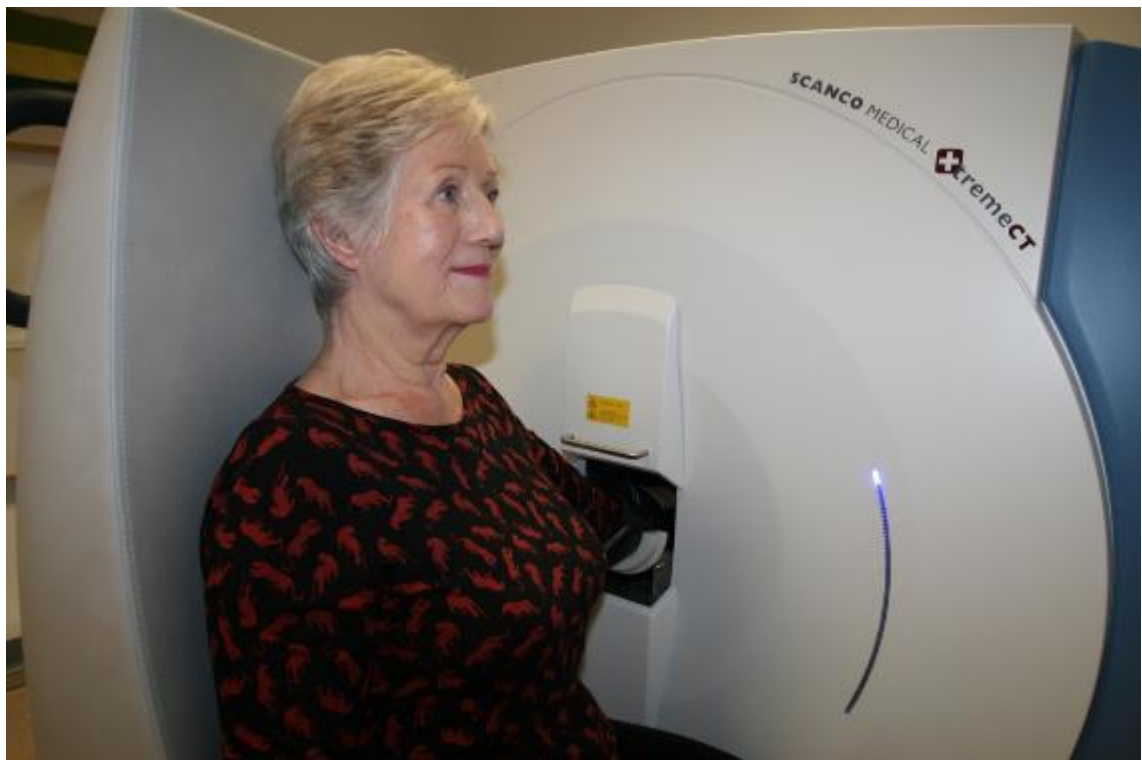
Figure 3.6 Positioning of the participant for the distal radius HR-pQCT scan.

Figure 3.7 Scout view for radial HR-pQCT scan picture to be changed.

3.7.3 **Tibial HR-pQCT**

With the participant seated, the operator placed the foot and lower leg into the tibia cast and used an appropriately sized foot pad to secure the leg within the cast (Figure 3.8). The foot holder was clipped into position by pressing down on the levers inside the gantry. With the levers lowered, the bolt was inserted into the positioning mechanism and released the levers to lock foot holder in place. The leg was slid in as far as possible and the underside of the foot holder was positioned on top of the positioning screws (Fig 3.9). A scout scan was performed to determine the measurement area. A reference line was placed on distal margin of the tibial plafond on the scout image. The first slice of the region of interest was 22.5mm proximal to the reference line at the tibia. (Figure 3.10). The participant was instructed to remain motionless and the scan was performed. Upon completion, the cast was removed, and the scan quality evaluated. The operator visually inspected random slices to check consistent quality. The operator drew a contour around the cortical perimeter on the first image, before running the automatic contouring detection program which iterated the contouring process through the slice stack. Images were then analysed using the 'Evaluation 3D' option.

Figure 3.8 Cast for tibial HR-pQCT scan.

Figure 3.9 Positioning of the participant for the distal tibia HR-pQCT scan.
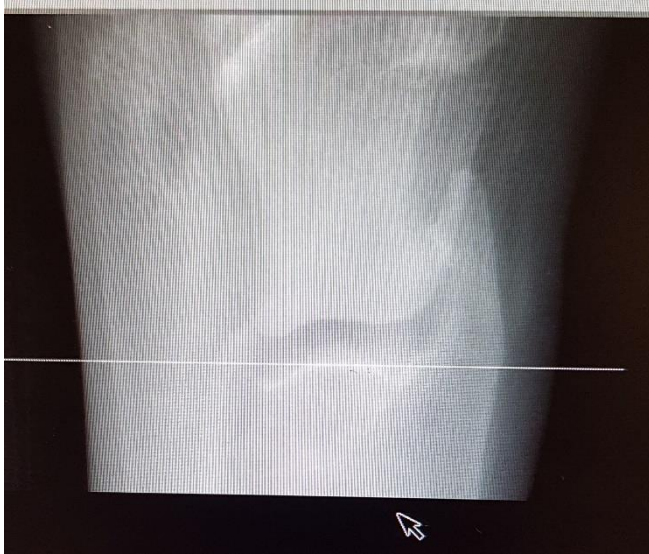
Figure 3.10 Scout view for tibial HR-pQCT scan.

3.7.4   **Motion artefact**

Scanned images should be inspected visually for motion artifacts. To determine what degree of motion is acceptable, several grading scales have been developed, where the most commonly used and recommended, by a joint working group between the International Osteoporosis Foundation, American Society of Bone and Mineral Research, and European Calcified Tissue Society in recently published guidelines, is a 5-level motion grading scale [216]. Scoring should be done consistently by the same operator where possible, as even with a standardized scoring system, motion scoring remains subjective, and operator agreement has shown to remain only moderate, even with intensive training [217–219].

The images acquired where checked to determine the quality/ degree of motion artefacts and graded on a scale from 1 to 5. Grade 1 was used for perfect scans with no motion artefacts visible, Grade 2 for good scans with very slight artefacts, horizontal streaks at upper and lower end of radius/tibia just a little visible, Grade 3 for acceptable scans with horizontal streaks visible, but the cortex 'fitted together', Grade 4 represented poor scans with large horizontal streaks where the cortex hardly fitted together and the trabeculae were smeared while Grade 5 represented unacceptable scans. The first and last slice of each scan was selected to determine quality, as in the more proximal slices the cortex is thicker and so it was easier to check integrity.

3.7.5 **HR-pQCT Outcomes**

High-resolution peripheral QCT (HR-pQCT) enables high resolution scanning of the distal appendicular skeleton. A 360° rotating x- ray tube generates x-rays which are passed through a section of the distal radius or distal tibia, and detected by a static 2D detector array. This generates an attenuation profile, which is reconstructed into an image by computing the spatial distribution of the attenuation onto a blank matrix. This simultaneous acquisition of a series of 2D parallel image slices, is then computed into a high resolution 3D image (isotropic resolution = 82 μm).

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 3.11). 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. 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.
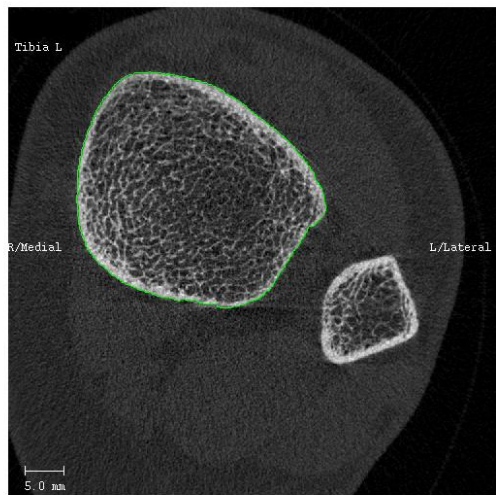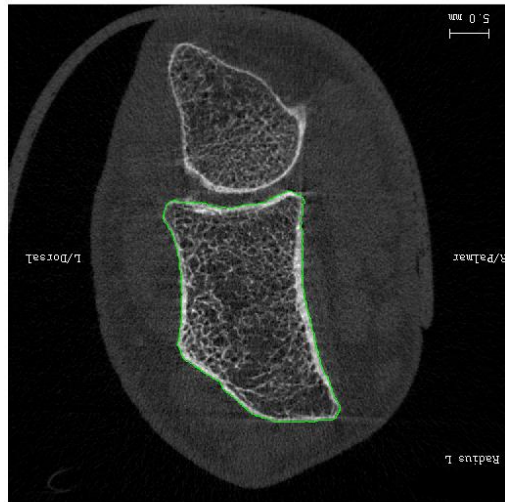
Figure 3.11 High-resolution image of distal radius and tibia with line delineating outer margin of cortex.

3.7.5.1   Bone density

Total, cortical and trabecular densities are determined from a pre-calibration step. Total volumetric density (total vBMD) (mgHA/cm3) was defined as total mineral mass divided by the total bone volume, cortical volumetric density (cortical vBMD) (mgHA/cm3) as cortical mineral mass divided by the cortical volume and trabecular volumetric density (trabecular vBMD) (mgHA/cm3) as trabecular mineral mass divided by the volume inside the cortical bone. The scanner was calibrated using a phantom with five hydroxyapatite-resin compartments of densities 0 mgHA/cm3 (a soft tissue equivalent with no mineral content), 100 mgHA/cm3, 200 mgHA/cm3, 400 mgHA/cm3 and 800 mgHA/cm3 (Figure 3.11). Image slices are taken of the phantom and the mean attenuation for each of the compartments calculated. From this pre-calibration data the

attenuation values of the scan were used to calculate values for cortical vBMD, trabecular vBMD and total vBMD. The trabecular region was divided into inner (60%) and outer trabecular (40%) regions.
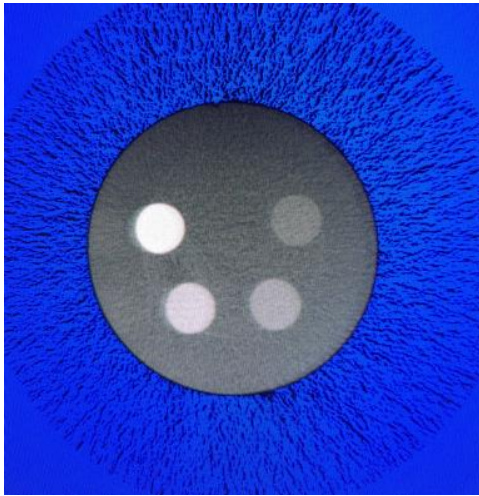
Figure 3.12 Scan image of the HR-pQCT phantom.

3.7.5.2   Trabecular microarchitecture

HR-pQCT with a high isotropic resolution (82μm) allows direct assessment of the inter-trabecular distances. Trabecular microarchitectural parameters were derived from the standard image analysis. A 3D ridge extraction method picks out ridges (i.e. trabeculae) and draws spheres between them. It then calculates the mean sphere diameter and from this calculates trabecular number (Tb.N) (mm-1) defined as mean number of trabeculae per mm within the trabecular compartment (ref 330). Trabecular thickness (Tb.Th) (mm) defined as mean thickness of trabeculae within the trabecular compartment was derived from trabecular number and trabecular vBMD using plate-model assumptions. Trabecular separation (Tb.Sp) (mm) was defined as mean distance between trabeculae within the trabecular compartment.

3.7.5.3   Cortical bone

Cortical microstructure, including porosity was analysed using the method of Burghardt and colleagues [220]. 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. Cortical porosity (Ct.Po) (%) was defined as the percentage of cortical area occupied by pores. Cortical thickness (Ct.Th) (mm2) was defined as the mean values for thickness between the periosteal and endosteal surfaces over the 110 slices.

3.7.5.4   Bone geometry

Total area (Tot.Ar) (mm2), cortical area (Ct.Ar) (mm2), and trabecular area (Tb.Ar) (mm2) were the mean values for respectively surface area of the cortical and trabecular compartments, cortical compartment, trabecular compartment over the 110 slices.

3.8   Data management and statistical analyses

Demographic variables from baseline and follow-up in year 1,2,3 and 5 data in the GLOW were merged with the DXA and HR-pQCT parameters and current anthropometric data. Age was calculated using visit date and date of birth. Each DXA and HR-pQCT variable was viewed as a histogram using the study population with subsequent inspection of the data normality. Any outlying values were assessed. The statistical analyses performed will be explained at the start of each results section.

3.9   Ethical approval and research governance

Ethical approval was obtained from the South East London Research Ethics Committee on 2nd January 2014 (Appendix 5).

4. Results

This thesis contains 4 manuscripts. Three have been published in peer review journals [221–223], the fourth has been submitted for publication in Osteoporosis International, in April 2020. They will be presented in turn as subsections of this chapter.

4.1 First manuscript – Bone Phenotype Assessed by HR-pQCT and Associations with Fracture Risk in the GLOW Study

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Abstract

The epidemiology and pathogenesis of fractures in postmenopausal women has previously been investigated in the Global Longitudinal study of Osteoporosis in Women (GLOW). To date, however, relationships between bone imaging outcomes and fracture have not been studied in this cohort. We examined relationships between high-resolution peripheral quantitative computed tomography (HR-pQCT) parameters and fracture in the UK arm of GLOW, performing a cluster analysis to assess if our findings were similar to observations reported from older participants of the Hertfordshire Cohort Study (HCS), and extended the analysis to include tibial measurements. We recorded fracture events and performed HR-pQCT of the distal radius and tibia and dual-energy X-ray absorptiometry (DXA) of the hip in 321 women, mean age 70.6 (SD 5.4) years, identifying four clusters at each site. We saw differing relationships at the radius and tibia. Two radial clusters (3 and 4) had a significantly lower hip areal bone mineral density ($p\backslash0.001$) compared to Cluster 1; only

individuals in Cluster 4 had a significantly higher risk of fracture (p = 0.005). At the

tibia, clusters 1, 3 and 4 had lower hip areal bone mineral density (p\0.001) compared

to Cluster 2; individuals in Cluster 3 had a significantly higher risk of fracture (p =

0.009). In GLOW our findings at the radius were very similar to those previously

reported in the HCS, suggesting that combining variables derived from HR-pQCT may

give useful information regarding fracture risk in populations where this modality is

available. Further data relating to tibial HR-pQCT-phenotype and fractures are provided

in this paper, and would benefit from validation in other studies. Differences observed

may reflect age differences in the two cohorts.

Introduction

Osteoporosis is a disease characterised by loss of bone mass and structural deterioration,

resulting in increased bone fragility and propensity to fracture. It is a major public

health problem, with a high impact on quality of life and high rates of morbidity.

Worldwide, there are nearly nine million osteoporotic fractures each year [72]. The burden

of fragility fractures will grow with ageing of the population; the US Surgeon General's

report of 2004, consistent with data from the UK, suggested that almost one in two

women and one in five men will experience a fracture in their remaining lifetime from

the age of 50 years [25]. The economic cost of osteoporosis and fractures are projected to

increase in the EU from €37.4 billion in 2010 to €46.8 billion by 2025 and, in the US,

from $17 billion in 2005 to $25.3 billion by 2025 [7,224].

In clinical practice, the definition of osteoporosis relies on measurements of areal bone

mineral density (aBMD) by dual-energy X-ray absorptiometry (DXA) [225]. While aBMD

is a significant predictor of fracture risk, it is limited because of its two-dimensional

nature, which is affected by the size and position of the subject and cannot distinguish

between cortical and trabecular compartments. Epidemiological data indicate that a

significant proportion of fractures occur in women who would not be classified as

osteoporotic according to current aBMD criteria, highlighting the limitations of this

approach and the need for other assessment methods to determine underlying causes of

bone fragility [115,172] Recent advances in imaging permit the assessment of bone

microstructure in vivo using high-resolution peripheral quantitative computed

tomography (HR-pQCT). This imaging modality has been utilized in research settings

to examine factors, including skeletal properties of cortical bone and trabecular microarchitecture, that may contribute to fracture risk [128,140,143,226,227].

So far, most studies investigating aetiology of fracture have analysed specific components of bone structure assessing differences in single outcomes between fracture and non-fractured cases [128,140,226,227]. However, cluster analysis allows us to use the data derived from such scans to define bone phenotypes taking into account all parameters derived from HR-pQCT scans. A recent study of older men and women, however, demonstrated that two separate phenotypes were associated with high fracture rates, using such mathematical cluster analysis of bone size, volumetric density (vBMD) and microarchitecture from HR-pQCT [143]. In the first phenotype, cortical parameters differed with mean cortical thickness and cortical vBMD lower than the sample mean, whereas the second phenotype was characterised by deficiencies in predominantly trabecular bone with lower values than the sample mean. Replication of these findings in an unrelated cohort was a key conclusion of this study and was the rationale of undertaking this current work in the Global Longitudinal study of Osteoporosis in Women (GLOW) study. The epidemiology and pathogenesis of fractures in postmenopausal women has been widely investigated in GLOW—a prospective, multinational, observational, population-based study of postmenopausal women who were 55 years of age and older [169,207,227–232]. However, relationships between bone imaging outcomes and fracture rates have not previously been examined in this cohort. Women who participated in the UK component of the GLOW underwent DXA and HR-pQCT of the distal radius and tibia. Extensive phenotyping of HR-pQCT images allowed the assessment of relationships between individual HR-pQCT parameters and fracture, and a cluster analysis which we undertook to assess if the findings were similar to observations reported in older participants of the Hertfordshire Cohort Study, and extended to the tibial site.

Materials and methods

Study participants

GLOW is a prospective, observational cohort study conducted through general physician practices in 10 countries. Study design and recruitment have been described in detail previously [215]. In brief, practices, representative of each region, were recruited through primary care networks and provided the names of women aged 55 years and

older who had been seen by their physician in the past 24 months. The primary aim of GLOW was to characterise the descriptive epidemiology and health impact of osteoporosis-related fractures among women who were 55 years of age and older worldwide. Globally, GLOW enrolled over 60,000 women through over 700 physicians in 10 countries, and conducted annual follow-up for up to 5 years through annual patient questionnaires. In Southampton only, participants with baseline data and at least one follow-up questionnaire were invited, after completion of 5 years of follow-up, for a follow-up study which included DXA and HR-pQCT. Participants were scanned between April 2014 and September 2016. Patients, who were institutionalized or were not able to complete the study survey by themselves due to cognitive impairment, language barriers, institutionalization, or were too ill to complete the survey or attend for the scans were excluded.

Questionnaires

Information was collected using self-administered questionnaires and included details regarding smoking status, alcohol consumption, education level, use of anti-osteoporotic medication (AOM), years since menopause and use of oestrogen or hormone replacement therapy (HRT). Participants were also asked to rate how physically active they were compared to other women of the same age out of the following possible responses: 'very active', 'somewhat active', 'a little' and 'not at all'. Subjects were considered to be taking anti-osteoporosis medication if, from baseline to the 5-year follow-up, they reported current use of alendronate, calcitonin, etidronate, ibandronate, pamidronate, raloxifene, risedronate, strontium ranelate, teriparatide, tibolone or zoledronic acid. Fracture history was ascertained at baseline and further information on fractures was obtained after 1-, 2-, 3- and 5-year follow-up. Fracture location included the following: clavicle, upper arm, wrist, spine, rib, hip, pelvis, ankle, upper leg and lower leg. Fractures that were reported at baseline, or accrued over 5 years of follow-up were included; hence the fractured subjects were those with prevalent fracture at the time of scan.

Anthropometry and Dual-Energy X-ray Absorptiometry (DXA)

Height was measured to the nearest 0.1 cm using a Marsden stadiometer; weight was measured to the nearest 0.1 kg on the day of scanning using a Marsden MPPS-250 (Marsden Weighing Machine Group Limited, Rotherham, UK) digital floor scale.

Total hip areal bone mineral density (aBMD, g/cm^2) was measured at both sides using DXA Hologic Horizon W; software version Apex 5.5.3.1 (Vertec Scientific, Reading, UK); the total effective dose equivalent of the hip scans was 4.7 microsieverts.

Assessment of Bone by HR-pQCT

Each participant underwent a HR-pQCT scan of the non-dominant distal radius and tibia using XtremeCT I, (Scanco Medical, Basserdorf, Switzerland); if there was a history of fracture on the non-dominant limb, the non-fractured limb was measured. A stack of 110 parallel HR-pQCT slices were acquired with an isotropic voxel size of 82 μm. Methods used to process the HR-pQCT data have been described previously [226]. The standard evaluation and cortical porosity scripts were run to obtain estimates of total area, trabecular area, cortical area, cortical volumetric density, trabecular volumetric density, trabecular number, trabecular thickness, trabecular separation, cortical porosity and cortical thickness [220]. Of participants with radius scans, 93 of 442 participants had grade 5 scans and were excluded; of participants with tibial scans, 15 of 447 had grade 5 scans and were excluded. The main analysis sample consisted of 321 individuals with complete data on fracture history and the radial HR-pQCT parameters; analysis of the tibial HR-pQCT parameters was based on a subset of 306/321 participants who also had complete data on the tibial HR-pQCT parameters.

Statistical analysis

Linear regression was used to examine the relationships between individual HR-pQCT parameters and fracture history. Unadjusted and fully adjusted associations, accounting for age at time of HR-pQCT scan, height, BMI, physical activity, smoking status, alcohol consumption, education, time since last period, use of AOM and oestrogen/HRT, were examined.

The k-means partitioning method of cluster analysis was used to produce clusters of the HR-pQCT parameters for the tibia and radius separately. The number of clusters selected was based on the stability of the clustering, and on the potential for identifying contrasting phenotypes [143]. The means and standard deviations (SD) of the standardized HR-pQCT parameters, and fracture proportion were then determined for each cluster. Poisson regression with robust variance estimation was used to determine the likelihood of fracture in each cluster compared to the lowest risk cluster. Mean total hip aBMD in each cluster was compared to the cluster with the lowest fracture risk. Data were analysed using Stata, version 14.0.

Results

The characteristics of the study population are shown in Table 1 The mean (SD) age of the 321 participants studied was 70.6 (5.4) years at the time of the radius scan. Overall, 63 (19.6%) women reported a fracture among at least one of the fracture locations. The most common fracture site was at the wrist with 25 fractures (32.5% of all fractures among the 10 fracture locations), followed by ankle (15 fractures), rib (11 fractures), lower leg (11 fractures), upper arm (6 fractures), spine (4 fractures), hip (2 fractures), clavicle (2 fractures), pelvis (1 fractures), and upper leg (0 fractures). Less than 6% of women were smokers; and a vast majority (91%) did not exceed the recommended limits of alcohol intake.

Table 1 Participants characteristics

Participant characteristic	Mean (SD)
Age at baseline (years)	63.0 (5.4)
Age at radius scan (years)	70.6 (5.4)
Age at tibia scan (years)*	70.5 (5.3)
Height (cm)	160.5 (6.0)
Weight (kg)	68.8 (12.4)
BMI (kg/m^2)	26.7 (4.8)
Total hip bone mineral density	0.84 (0.11)
	N(%)
Physically active compared to others: Not at all	0 (0.0%)
A little	39 (12.2%)
Somewhat	164 (51.2%)
Very	117 (36.6%)
Current smoker	18 (5.6%)
Alcoholic drinks per week: None	64 (20.0%)
1-6	133 (41.6%)
7-13	95 (29.7%)
14-20	22 (6.9%)
>20	6 (1.9%)
Education: Below GCSE	78 (24.3%)
CSE O level / GCSE	108 (33.6%)
A Level	35 (10.9%)
Degree	100 (31.2%)
Use of anti-osteoporotic medication	37 (12.0%)
Ever used oestrogen / hormone replacement therapy	160 (50.6%)
Years since last menstrual period: <10	100 (32.1%)
10-19	130 (41.7%)
20-29	65 (20.8%)
>29	17 (5.4%)

*n=306 tibia, 321 radius
Participants were asked how physically active they were compared to other
women of the same age

HR-pQCT parameters and fracture status

The associations between fracture history and individual radius and tibia HR-pQCT
parameters are presented in Table 2. History of fracture was associated with lower radial
cortical porosity (p=0.012), trabecular density (p=0.001) and trabecular number
(p<0.001), and higher trabecular separation (p<0.001). These associations were robust

to adjustment. At the tibia, history of fracture was associated with lower trabecular density (p=0.002) and number (p<0.001), and higher trabecular separation (p<0.001); associations regarding trabecular number and trabecular separation were robust to adjustment.

Table 2 Standard deviation difference in mean HR-pQCT parameters (95%CI) for individuals who experienced a fracture since age 45 compared to those who did not

HR-pQCT parameter	Unadjusted		Adjusted*	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Radius				
Total area	0.12 (-0.16,0.39)	0.412	0.02 (-0.24,0.28)	0.869
Trabecular area	0.14 (-0.14,0.42)	0.317	0.03 (-0.23,0.29)	0.848
Cortical area	-0.11 (-0.38,0.17)	0.451	0.02 (-0.25,0.29)	0.890
Cortical thickness	-0.19 (-0.47,0.08)	0.170	-0.05 (-0.32,0.22)	0.710
Cortical volumetric density	-0.04 (-0.32,0.23)	0.752	0.08 (-0.19,0.34)	0.581
Cortical porosity	-0.35 (-0.63,-0.08)	0.012	-0.31 (-0.60,-0.03)	0.033
Trabecular volumetric density	-0.45 (-0.73,-0.18)	0.001	-0.35 (-0.63,-0.07)	0.016
Trabecular number	-0.66 (-0.92,-0.39)	<0.001	-0.59 (-0.86,-0.32)	<0.001
Trabecular thickness	0.08 (-0.20,0.35)	0.587	0.17 (-0.13,0.47)	0.270
Trabecular separation	0.59 (0.32,0.86)	<0.001	0.50 (0.23,0.78)	<0.001
Tibia				
Total area	0.25 (-0.03,0.53)	0.078	0.09 (-0.13,0.32)	0.417
Trabecular area	0.27 (-0.01,0.55)	0.062	0.10 (-0.13,0.33)	0.380
Cortical area	-0.15 (-0.43,0.13)	0.284	-0.03 (-0.29,0.23)	0.809
Cortical thickness	-0.24 (-0.52,0.04)	0.089	-0.13 (-0.39,0.14)	0.354
Cortical volumetric density	-0.18 (-0.46,0.10)	0.203	-0.05 (-0.31,0.21)	0.720
Cortical porosity	-0.03 (-0.31,0.25)	0.844	-0.11 (-0.39,0.18)	0.458
Trabecular volumetric density	-0.43 (-0.71,-0.16)	0.002	-0.28 (-0.57,0.01)	0.060
Trabecular number	-0.49 (-0.77,-0.22)	<0.001	-0.42 (-0.69,-0.15)	0.003
Trabecular thickness	-0.03 (-0.31,0.25)	0.838	0.11 (-0.19,0.41)	0.463
Trabecular separation	0.50 (0.22,0.78)	<0.001	0.40 (0.13,0.67)	0.004

*Adjusted for age at time of HR-pQCT scan, height, BMI, physical activity, smoking status, alcohol consumption, education, time since last period, use of antiosteoporotic medication, and use of oestrogen/hormone replacement therapy
vBMD: volumetric bone mineral density

Cluster analysis of radial HR-pQCT parameters

Four clusters were obtained. The summary statistics of the standardised HR-pQCT parameters, hip aBMD and fracture prevalence according to the different clusters are illustrated in Table 3 and Figure 1.

Table 3 Mean (SD) parameters by cluster analysis group (4 clusters of radial HR-pQCT parameters obtained)

Parameter	Cluster 1 (n=84)	Cluster 2 (n=80)	Cluster 3 (n=71)	Cluster 4 (n=86)
HR-pQCT (Standardised)				
Total area	-0.69 (0.84)	-0.07 (0.75)	0.89 (0.86)	0.01 (0.90)
Trabecular area	-0.86 (0.79)	-0.09 (0.68)	**1.01 (0.76)**	0.08 (0.80)
Cortical area	0.97 (0.69)	0.17 (0.70)	**-1.18 (0.65)**	-0.13 (0.60)
Cortical thickness	**1.04 (0.68)**	0.17 (0.63)	**-1.22 (0.59)**	-0.17 (0.54)
Cortical volumetric density	**1.08 (0.58)**	-0.05 (0.60)	**-1.27 (0.55)**	0.04 (0.60)
Cortical porosity	-0.49 (0.75)	0.71 (0.78)	0.35 (1.00)	-0.48 (0.88)
Trabecular volumetric density	0.39 (0.62)	**1.02 (0.63)**	-0.47 (0.66)	-0.95 (0.65)
Trabecular number	0.27 (0.66)	0.96 (0.68)	-0.29 (0.79)	-0.92 (0.74)
Trabecular thickness	0.36 (0.85)	0.63 (0.73)	-0.51 (0.84)	-0.52 (1.00)
Trabecular separation	-0.26 (0.65)	**-1.03 (0.75)**	0.35 (0.71)	0.92 (0.63)
DXA				
Total hip aBMD	0.89 (0.11)	0.89 (0.10)	0.78 (0.09)	0.78 (0.10)
P-value	*reference*	*0.943*	*<0.001*	*<0.001*
Fracture history				
Any fracture[+]	11 (13.1%)	13 (16.3%)	11 (15.5%)	28 (32.6%)
RR (95% CI) of fracture	1.00 (*reference*)	1.24 (0.59, 2.61)	1.18 (0.55, 2.57)	2.49 (1.32, 4.67)
P-value	*reference*	*0.569*	*0.671*	*0.005*

P-values calculated using a Poisson regression model with a robust variance estimator. P-values for differences in hip aBMD were calculated using linear regression. P-values are for differences compared to Cluster 1 (lowest risk)

[+] N(%)
RR: Relative risk   CI: Confidence interval
Bold if mean > 1SD from sample mean
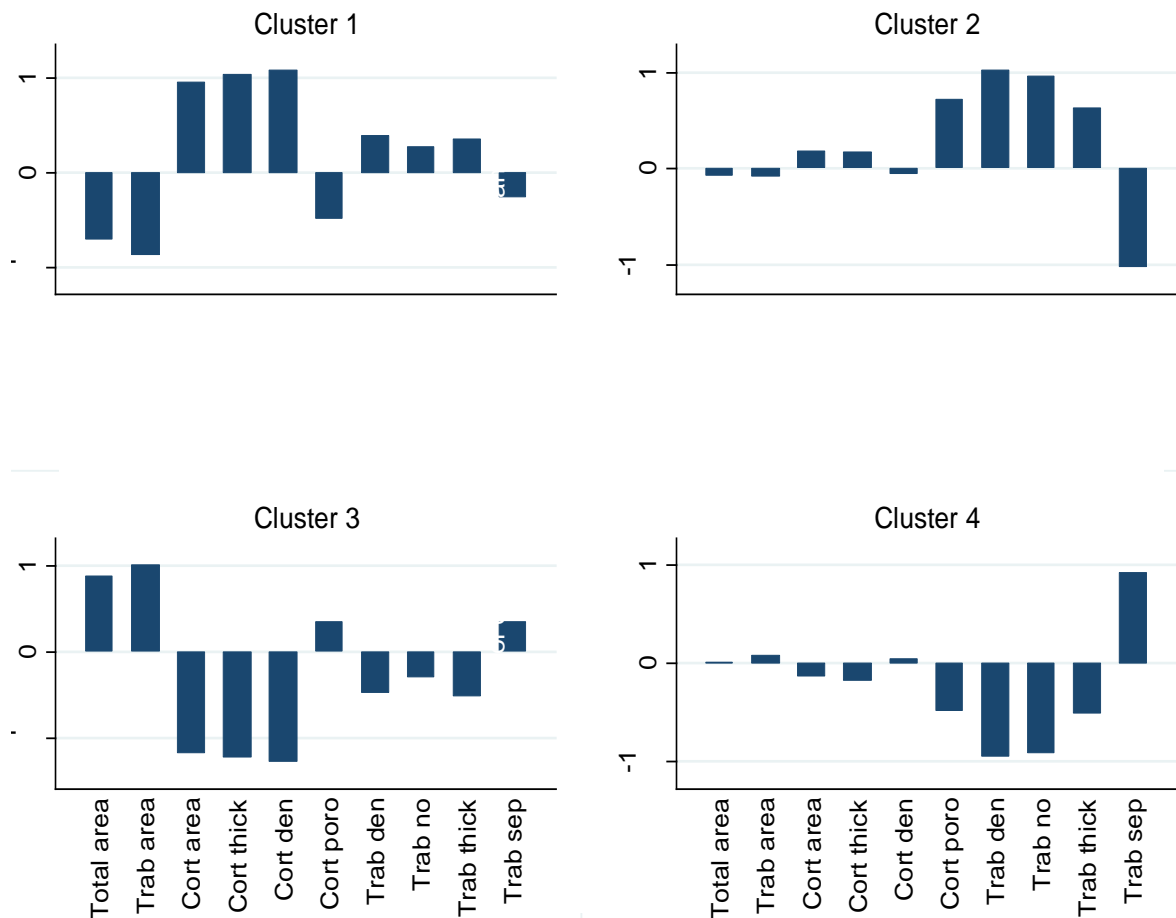
Figure 1 Means of standardized radial HR-pQCT parameters according to each cluster.

In Cluster 4, there was a trend towards lower trabecular density and number and higher trabecular separation compared to the analysis sample (differences in means >0.9 SDs). In this cluster, hip aBMD was significantly lower (p<0.001) and individuals had a significantly higher risk of fracture (relative risk [95% CI] compared to Cluster 1: 2.49 [1.32, 4.67], (p=0.005). In contrast to a trabecular deficiency pattern in Cluster 4, Cluster 3 showed differences predominantly in cortical parameters with trend towards lower cortical area, cortical thickness and cortical density, and higher trabecular area compared to the measured sample (differences in means exceeded one SD). Hip aBMD in this cluster was significantly lower, but there was no significant difference in fracture risk compared to Cluster 1.

Similarly, in Cluster 1, differences were predominantly in cortical parameters, but here with a trend towards higher cortical area, cortical thickness and cortical density

compared to the measured sample (differences in means >0.95 SDs). As expected, total hip aBMD was the highest and fracture risk was the lowest in this cluster.

Cluster 2 had higher trabecular density and lower trabecular separation, but there were no other HR-pQCT parameter with means that differed by more than one SD compared to the sample mean. There was no significant difference in hip aBMD or fracture risk in this cluster. Adjustment for hip aBMD throughout did not remove previously observed associations, except that the associations for trabecular density of the radius were attenuated when additionally adjusted for aBMD.

Cluster analysis of tibial HR-pQCT parameters

Four clusters were obtained among the 306 participants with complete data for the tibia parameters. The summary statistics of the standardised HR-pQCT parameters, hip aBMD and fracture prevalence according to the different clusters are illustrated in Table 4.

Fracture risk was lowest and hip aBMD was highest in Cluster 2. This cluster had lower trabecular area and higher cortical area, thickness and density compared to the analysis sample (differences in means exceeded one SD). Cluster 3 had the highest risk of fracture and the lowest hip aBMD; this cluster was characterised by higher total and trabecular area and lower trabecular density compared to the analysis sample. For the other clusters, none of the tibia parameters differed from the analysis sample by more than one SD.

Table 4. Mean (SD) parameters by cluster analysis group (4 clusters of tibial HR-pQCT parameters obtained)

Parameter	Cluster 1 (n=83)	Cluster 2 (n=63)	Cluster 3 (n=77)	Cluster 4 (n=83)
HR-pQCT (Standardised)				
Total area	-0.62 (0.71)	-0.82 (0.77)	**1.01 (0.66)**	0.31 (0.64)
Trabecular area	-0.56 (0.67)	**-1.00 (0.71)**	**1.04 (0.60)**	0.35 (0.58)
Cortical area	0.05 (0.60)	**1.20 (0.70)**	-0.67 (0.87)	-0.34 (0.80)
Cortical thickness	0.11 (0.71)	**1.29 (0.66)**	-0.78 (0.73)	-0.36 (0.63)
Cortical volumetric density	0.23 (0.58)	**1.19 (0.65)**	-0.67 (0.82)	-0.51 (0.78)
Cortical porosity	0.00 (0.94)	-0.77 (0.84)	0.01 (0.85)	0.59 (0.91)
Trabecular volumetric density	-0.28 (0.71)	0.60 (0.78)	**-1.03 (0.65)**	0.78 (0.63)
Trabecular number	-0.75 (0.68)	0.69 (0.61)	-0.52 (0.88)	0.71 (0.74)
Trabecular thickness	0.50 (0.88)	0.05 (0.85)	-0.89 (0.79)	0.28 (0.85)
Trabecular separation	0.72 (0.64)	-0.71 (0.65)	0.63 (0.80)	-0.76 (0.72)
DXA				
Total hip aBMD	0.79 (0.09)	0.94 (0.1)	0.78 (0.1)	0.86 (0.09)
P-value	*<0.001*	*reference*	*<0.001*	*<0.001*
Fracture history				
Any fracture[+]	19 (22.9%)	7 (11.1%)	24 (31.2%)	11 (13.3%)
RR (95% CI) of fracture	2.06 (0.92, 4.60)	1.00 (*reference*)	2.81 (1.29, 6.09)	1.19 (0.49, 2.91)
P-value	*0.078*	*reference*	*0.009*	*0.698*

P-values calculated using a Poisson regression model with a robust variance estimator. P-values for differences in hip aBMD were calculated using linear regression. P-values are for differences compared to Cluster 1 (lowest risk)

[+] N(%)
RR: Relative risk   CI: Confidence interval
Bold if mean > 1SD from sample mean

Discussion

This study demonstrated that microstructural parameters of the bone evaluated by HR-pQCT are different between healthy participants and fracture participants at skeletal regions containing predominantly trabecular bone. Trabecular parameters assessed by HR-pQCT provided additional skeletal information to that captured from the standard areal bone mineral density (BMD) measurements by DXA. A cluster analysis of the radial and tibial HR-pQCT parameters derived one cluster with a significantly higher fracture risk. Individuals in this cluster had lower trabecular density and number, and consequently higher trabecular separation compared to the wider sample. In this cluster, hip aBMD was significantly lower.

An aim of this study was to attempt to replicate findings from the Hertfordshire Cohort Study [143]. We showed that various indices of bone microarchitecture of the radius, most notably cortical porosity, trabecular density, trabecular number and trabecular separation, appeared to be compromised among postmenopausal UK women with a previous history of fracture. These results are in agreement with findings from Hertfordshire [143] and another published study [140] suggesting that alterations of trabecular architecture are likely to play an important role in skeletal fragility associated with osteoporosis. In this study, the results for trabecular parameters described above remained robust to adjustments for demographic and lifestyle factors indicating that results are not due to confounding. Interestingly, and perhaps unexpectedly, history of fracture was associated with lower cortical porosity. Fracture cases had higher cortical area, consistent with findings from other cohorts, however they also had higher cortical vBMD which is probably due to the lower porosity. This observation has now been made in both the Hertfordshire and GLOW cohorts, and warrants further investigation.

We did see differences in relationships at the radius and tibia which require validation in other samples. This may reflect technical differences in acquisition at the two sites, or differences due to the weight bearing/ non-weight bearing nature of the two sites. Fractures in this group were more typically reported at the distal radius, which may also be relevant.

Cluster analysis of the radial HR-pQCT parameters demonstrated one phenotype associated with higher risks of fracture. The altered parameters in this cluster included lower trabecular density and number and higher trabecular separation. This is consistent with the previous study on cluster analysis of bone microarchitecture from HR-pQCT and fracture risk [143]. Similarly, hip aBMD was low in this cluster when compared to the reference cluster in both studies. Interestingly, there was one more very similar phenotype derived by cluster analysis in both studies. It was characterised by higher trabecular area and lower cortical area, thickness and density. In this study, this cluster was not associated with higher fracture risk which is in contrast to the previously published study, where the participants were recruited from the Hertfordshire Cohort Study (HCS). Participants of the HCS were older, of mean age with and without a fracture 77.2 (2.4) and 76.0 (2.6) respectively, compared to participants in our study (mean age of 70.6 (5.4) at time of scan). In the HCS, there were also differences in

phenotype between genders where one cluster associated with high rates of fracture was characterised by low cortical thickness and density in men and women, but in men only, a cluster characterised by higher total and trabecular area was associated with increased fracture risk. Moreover, this cluster in men was not associated with low femoral neck areal BMD. In GLOW only females were recruited but higher trabecular and total area (in addition to lower trabecular density) were the characteristics of Cluster 3 significantly associated with fracture risk, suggesting a consistency of phenotype. This study has some limitations. As it is a cross-sectional study, causality cannot be determined since it is not possible to know whether bone microarchitecture changes preceded the fracture. Well-designed prospective studies providing longitudinal data are therefore very important. Although it is reported that cluster analysis models can be very unstable, which could affect the generalizability of the findings in this study, the results were largely consistent to a study by Edwards et al [143].

In conclusion, this study indicates a phenotype with a significantly higher fracture risk, using cluster analysis of radial and tibial HR-pQCT parameters. This approach may have clinical utility in patients where such scans are available, as it allows the incorporation of a large number of variables acquired during a scan to be combined into a bone phenotype that may be more useful for a clinician and patient alike. While our observations were generally in accord with those found in the Hertfordshire Cohort Study, we did note some differences that may reflect the demographic differences between the two groups, particularly age. Given the number of cohorts where HR-pQCT data are available, we would welcome attempts at similar analyses. Ultimately our study adds to the growing body of evidence demonstrating distinct phenotypes of bone fragility, which may have implications for targeted prevention and treatment of osteoporosis in the future. Further research is required to examine the identified phenotype and its ability to predict future fracture.

4.2 Second manuscript – Adiposity and bone microarchitecture in the GLOW Study

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Abstract

Purpose: To examine relationships between bone microarchitecture and fat mass with areal bone mineral density (aBMD) and microarchitecture according to BMI obesity categories in the UK arm of the Global Longitudinal Study of Osteoporosis in Women.

Methods: 491 women completed questionnaires detailing medical history; underwent anthropometric assessment; high-resolution peripheral quantitative computed tomography (HR-pQCT) scans of the radius and tibia; and DXA scans of whole body, proximal femur and lumbar spine. Fat mass index (FMI) residuals (independent of lean mass index) were derived. Linear regression was used to examine HR-pQCT and DXA aBMD parameters (raw values and values normalised for body weight) according to BMI category (unadjusted) and according to FMI residuals (with and without adjustment for anthropometric, demographic and lifestyle covariates).

Results: Mean (SD) age was 70.9 (5.4) years; 35.0% were overweight, 14.5% class 1 obese, and 7.7% class 2/3 obese. There were significant increasing trends according to BMI category in aBMD of whole body, hip, femoral neck and lumbar spine ($p \leq 0.001$); cortical parameters: area ($p < 0.001$), thickness ($p < 0.001$); volumetric density ($p < 0.03$) and trabecular: number ($p < 0.001$), volumetric density ($p < 0.04$) and trabecular

separation (p<0.001 for decreasing trend) at radius and tibia. When normalised for body weight, all HR-pQCT and DXA aBMD parameters decreased as BMI increased (p<0.001). FMI residuals were associated with bone size and trabecular architecture at the radius and tibia, and tibial cortical microarchitecture.

Conclusions: Significant trends in HR-pQCT parameters suggested favourable bone microarchitecture at the radius and tibia with increasing BMI but these were not proportionate to increased weight.

Introduction

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue leading to increased bone fragility [1]. Osteoporotic fractures are associated with considerable morbidity, mortality and socioeconomic cost [7]. As the worldwide population is aging, the prevalence of osteoporosis is escalating and becoming a major public health issue [233], with data from US and UK suggesting that almost one in two women and one in five men will experience a fracture in their remaining lifetime from the age of 50 years [6,25]. Economically, the cost of osteoporosis and fractures are projected to increase in the EU from €37.4 billion in 2010 to €46.8 billion by 2025 and, in the US, from $17 billion in 2005 to $25.3 billion by 2025 [7,224].

While low body mass index (BMI) is well recognised as an important risk factor for fractures in postmenopausal women, the interaction of obesity with bone metabolism and microarchitecture is complex and not fully understood. BMI is incorporated in the fracture risk assessment tool (FRAX), and higher BMI is associated with lower future fracture risk. Higher BMI was traditionally considered protective against fracture through a direct effect of increased loading through body weight on bone mineral density, and because of reduced impact of falls as a result of increased soft-tissue padding [77]. However, accumulating evidence indicates that the relationship between BMI and fracture varies according to fracture site with lower rates of hip and pelvis fractures in obese individuals [169,170], in contrast to a higher risk of some non-spine fractures including those of the proximal humerus, upper leg, and ankle, perhaps because bone mineral density, although higher in more adipose patients, does not show a rise commensurate with body size [127,169,171–173]

The greater risk of lower limb fractures with obesity might therefore reflect biomechanical factors, but could also result from differences in bone structure. It is widely accepted that bone density is not the sole determinant of bone strength [234]; additional factors including bone geometry and bone micro-architecture may also be important. High-resolution peripheral quantitative computed tomography (HR-pQCT) allows study of cortical and trabecular compartments of the bone and is not as affected by overlying soft tissue, providing a more reliable way to assess bone structure in obesity. Results of studies undertaken to date using this technology have been inconsistent, and studies have been performed mainly in obese children and adolescents [235]. One cross-sectional case-control study demonstrated that both obese men and women had higher volumetric BMD at the distal radius and distal tibia when compared to normal weight individuals [159]. Only one modest sized study has investigated associations between obesity and measures of bone micro-architecture in elderly French women, where the reported prevalence of obesity was relatively low, finding that that obese postmenopausal women had higher volumetric BMD and higher values of cortical and trabecular architecture compared with normal weight postmenopausal women [127]. However, the increase of all parameters in obese women was lower relative to the excess of weight for BMI. Importantly it is not known whether associations between BMI and bone microarchitecture are the same between different classes of obesity (overweight, Class I, Class II/III, morbid obesity) at both weight bearing and non-weight-bearing skeletal sites in other populations.

The aim of this study was therefore to examine the relationships of bone microarchitecture with fat mass and to evaluate bone density, microarchitecture and geometry according to BMI categories of obesity in the UK arm of the GLOW study.

Methods

Study participants

GLOW is a prospective, observational cohort study conducted through general physician practices in 10 countries. Study design and recruitment have been described in detail previously[215]. In brief, practices, representative of each region, were recruited

through primary care networks and provided the names of women aged 55 years and older who had been seen by their physician in the past 24 months. The primary aim of GLOW was to characterize the descriptive epidemiology and health impact of osteoporosis-related fractures among women who were 55 years of age and older worldwide. Globally, GLOW enrolled over 60,000 women through over 700 physicians in 10 countries, and conducted annual follow-up for up to 5 years. In Southampton only, participants with baseline data and at least one follow-up questionnaire were invited, after completion of 5 years of follow-up, for a follow-up study which included DXA and HR-pQCT. Participants were scanned between April 2014 and December 2017. Patients who were institutionalized or were not able to complete the study survey by themselves due to cognitive impairment, language barriers, institutionalization, or were too ill to complete the survey or attend for the scans were excluded.

Questionnaires

Information was collected using self-administered questionnaires and included details regarding smoking status, alcohol consumption, education level, medical diagnoses (participants were asked if a doctor or health provider had ever told them that they had any of the listed morbidities including type 2 diabetes mellitus and hypertension) use of anti-osteoporotic medication (AOM), years since menopause and use of oestrogen or hormone replacement therapy (HRT). Information on possible confounders was taken from the questionnaires where it was available closest in time to the scan date. Participants were also asked to rate how physically active they were compared to other women of the same age out of the following responses: 'very active'; 'somewhat active'; 'a little'; and 'not at all'. Participants were considered to be taking AOM if, from baseline to the 5 year-follow-up, they reported current use of alendronate, calcitonin, etidronate, ibandronate, pamidronate, raloxifene, risedronate, strontium ranelate, teriparatide, tibolone or zoledronic acid.

Assessment of bone by HR-pQCT

Participants underwent a HR-pQCT scan of the non-dominant distal radius and tibia using XtremeCT I, (Scanco Medical, Basserdorf, Switzerland) on the same day as the DXA scan; if there was a history of fracture on the non-dominant limb, the non-

fractured limb was measured. A stack of 104 parallel HR-pQCT slices were acquired with an isotropic voxel size of 82 μm. Each scan was assessed for motion artefact, and if present a second scan was performed. The quality of the measurements was assessed by using a 5-point scale recommended by the manufacturer (1, excellent; 2, good; 3, acceptable; 4, poor; 5, unacceptable) [218]. Grade 5 images were excluded due to excessive motion artefact. Initial image analysis was carried out using the standard manufacturer's method and Image Processing Language (IPL, Version 6.1, ScancoMedical). For this analysis, the standard evaluation and cortical porosity scripts were run to obtain estimates of the following parameters at the radius and tibia: total area and trabecular area, volumetric density, number, thickness and separation; cortical area, thickness, volumetric density and pores diameter; and cortical porosity [220].

Anthropometry and DXA

Height was measured to the nearest 0.1 cm using a Marsden stadiometer on the day of scanning; weight was measured to the nearest 0.1 kg using a Marsden MPPS-250 (Marsden Weighing Machine Group Limited, Rotherham, UK) digital floor scale. BMI was calculated by dividing body weight by height2 (kg/m2). BMI categories were defined as underweight (BMI < 18.5), normal (18.5 ≤ BMI < 25), overweight (25 ≤ BMI < 30), class 1 obese (30 ≤ BMI < 35), Class 2/3 obese (BMI ≥ 35), morbid obesity (BMI ≥ 35 and either hypertension or type 2 diabetes or both conditions). DXA Hologic Horizon W (software version Apex 5.5.3.1 [Vertec Scientific, Reading, UK]) was used to measure whole body fat and fat free mass, from which lean mass is estimated, as well as areal bone mineral density (aBMD, g/cm2) of the whole body, hip, femoral neck and lumbar spine.

Derived measures

Lean mass index (LMI, kg/m2) and fat mass index (FMI kg/m2) were derived by dividing the corresponding measures by height2 (m2). To obtain a measure of fat mass that was independent of lean mass, standardised FMI residuals were obtained from a linear regression model with FMI as the outcome and LMI as the predictor.

Statistical analysis

The analysis sample comprised 491 individuals with non-missing values for BMI category or FMI residual and HR-pQCT of at least one site, radius or tibia, scanned.

Skewed parameters were transformed prior to standardising. Mean (SD) z-scores for the HR-pQCT parameters of the tibia and radius and DXA aBMD parameters were examined according to BMI category using linear regression; test for linear trends according to BMI category were also performed. To investigate whether increases in bone parameters in higher BMI groups were in proportion to participant's greater weight, these steps were repeated after dividing the bone parameters by body weight.

Linear regression was used to examine the association between FMI residuals and HR-pQCT parameters. The following models were implemented: unadjusted; adjusted for age at time of HR-pQCT scan, physical activity, smoking status, alcohol consumption, education, time since menopause and use of AOM and oestrogen/hormone replacement (pill/skin patch); and additionally adjusted for total hip BMD. Analyses were conducted using Stata 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC); $p < 0.05$ was regarded as statistically significant.

Results

Participant characteristics

Baseline participant characteristics of the study sample are presented in Table 1. Mean (SD) age at scan was 70.9 (5.4) years. Mean (SD) BMI was 26.8 (5.0) kg; 35.0% were overweight ($25 \leq$ BMI < 30), 14.5 % were class 1 obese ($30 \leq$ BMI < 35) and 7.7% were class 2/3 obese (BMI ≥ 35). Only 4% were Class 2/3 obese and had hypertension or type 2 diabetes (data not shown). Mean (SD) values for whole body fat mass and FMI were 29.5 (9.1) kg and 11.5 (3.6) kg/m2 respectively.

Table 1. Participants characteristics of the analysis sample (n=491).

Participant characteristic	Mean (SD) / N(%)	Obs
Age at scan (years)	70.9 (5.4)	491
Height (cm)	160.3 (6.2)	491
Weight (kg)	68.6 (12.7)	491
BMI (kg/m^2)	26.8 (5.0)	491
BMI categories: Underweight (BMI < 18.5)	10 (2.0%)	
Normal (18.5 ≤ BMI < 25)	200 (40.7%)	
Overweight (25 ≤ BMI < 30)	172 (35.0%)	491
Class 1 obese (30 ≤ BMI < 35)	71 (14.5%)	
Class 2/3 obese (BMI ≥ 35)	38 (7.7%)	
Whole body fat mass (kg)	29.5 (9.1)	409
Fat mass index (kg/m^2)	11.5 (3.6)	409
Whole body total aBMD (g/cm^2)	1.01 (0.10)	412
Total hip aBMD (g/cm^2)	0.84 (0.11)	466
Femoral neck aBMD (g/cm^2)	0.69 (0.10)	459
Total lumbar spine aBMD (g/cm^2)	0.92 (0.15)	486
Physically active*: Not at all / a little	65 (13.4%)	
Somewhat	249 (51.4%)	484
Very	170 (35.1%)	
Current smoker	29 (6.0%)	483
Alcoholic drinks per week: None	122 (25.3%)	
1-6	194 (40.2%)	483
7-13	113 (23.4%)	
>13	54 (11.2%)	
Education: Below GCSE	124 (25.3%)	
CSE O level / GCSE	165 (33.6%)	491
A Level	61 (12.4%)	
Degree	141 (28.7%)	
Use of anti-osteoporotic medication	78 (17.1%)	455
Ever used oestrogen / hormone replacement therapy	243 (50.8%)	478
Years since last menstrual period: <10	150 (31.4%)	
10-19	212 (44.4%)	477
20-29	89 (18.7%)	
>29	26 (5.5%)	
Hypertension**	206 (42.9%)	480
Type 2 diabetes**	26 (5.4%)	483

Obs: Number of non-missing observations

*Asked how physically active compared to other women of the same age

**Ever told by health professional

FMI residuals in relation to HR-pQCT parameters

Associations between FMI residuals and HR-pQCT parameters are presented in Table 2. At the radius, FMI residuals were positively associated with trabecular number and negatively associated with total area, trabecular area and trabecular separation in unadjusted and adjusted models; associations regarding total and trabecular area were robust when additionally adjusted for total hip aBMD. FMI residuals were positively associated with cortical thickness in unadjusted analysis only.

Table 2. Standard deviation difference in mean HR-pQCT parameters (95%CI) per standard deviation increase in fat mass index residuals.

HR-pQCT parameter	Unadjusted		Adjusted*		Additionally adjusted for hip BMD	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Radius						
Total area	**-0.23 (-0.34,-0.13)**	**<0.001**	**-0.21 (-0.34,-0.09)**	**0.001**	**-0.21 (-0.34,-0.08)**	**0.002**
Trabecular area	**-0.23 (-0.34,-0.12)**	**<0.001**	**-0.21 (-0.33,-0.08)**	**0.002**	**-0.19 (-0.32,-0.06)**	**0.004**
Cortical area	0.04 (-0.07,0.15)	0.432	0.04 (-0.08,0.17)	0.497	-0.01 (-0.13,0.10)	0.815
Cortical thickness	**0.11 (0.00,0.22)**	**0.046**	0.09 (-0.04,0.22)	0.159	0.04 (-0.08,0.16)	0.533
Cortical volumetric density	0.09 (-0.02,0.20)	0.120	0.09 (-0.04,0.22)	0.158	0.04 (-0.08,0.16)	0.491
Cortical porosity	-0.02 (-0.12,0.09)	0.763	-0.05 (-0.18,0.07)	0.396	-0.07 (-0.20,0.06)	0.284
Cortical pores diameter	-0.09 (-0.19,0.02)	0.110	-0.09 (-0.22,0.03)	0.155	**-0.13 (-0.27,-0.00)**	**0.045**
Trabecular volumetric density	0.10 (-0.01,0.21)	0.079	0.08 (-0.04,0.20)	0.200	0.01 (-0.10,0.11)	0.883
Trabecular number	**0.15 (0.04,0.26)**	**0.006**	**0.15 (0.03,0.28)**	**0.016**	0.09 (-0.02,0.21)	0.098
Trabecular thickness	-0.01 (-0.12,0.10)	0.819	-0.01 (-0.14,0.12)	0.922	-0.07 (-0.20,0.06)	0.273
Trabecular separation	**-0.16 (-0.26,-0.05)**	**0.005**	**-0.15 (-0.27,-0.02)**	**0.019**	-0.08 (-0.19,0.03)	0.145
Tibia						
Total area	**-0.15 (-0.24,-0.05)**	**0.003**	**-0.14 (-0.25,-0.02)**	**0.020**	**-0.16 (-0.28,-0.04)**	**0.010**
Trabecular area	**-0.16 (-0.26,-0.06)**	**0.001**	**-0.15 (-0.27,-0.04)**	**0.008**	**-0.16 (-0.28,-0.04)**	**0.007**
Cortical area	**0.15 (0.05,0.24)**	**0.002**	**0.16 (0.06,0.26)**	**0.002**	**0.10 (0.01,0.20)**	**0.032**
Cortical thickness	**0.15 (0.05,0.24)**	**0.003**	**0.14 (0.03,0.25)**	**0.010**	0.10 (-0.01,0.20)	0.067
Cortical volumetric density	**0.17 (0.08,0.26)**	**<0.001**	**0.22 (0.12,0.32)**	**<0.001**	**0.16 (0.07,0.26)**	**0.001**
Cortical porosity	**-0.12 (-0.22,-0.03)**	**0.013**	**-0.17 (-0.28,-0.06)**	**0.002**	**-0.15 (-0.26,-0.04)**	**0.009**
Cortical pores diameter	**-0.13 (-0.23,-0.03)**	**0.009**	**-0.13 (-0.24,-0.01)**	**0.027**	**-0.14 (-0.26,-0.02)**	**0.022**
Trabecular volumetric density	0.02 (-0.07,0.12)	0.646	0.01 (-0.11,0.12)	0.892	-0.07 (-0.17,0.03)	0.173
Trabecular number	**0.13 (0.03,0.23)**	**0.009**	**0.13 (0.02,0.24)**	**0.024**	0.06 (-0.05,0.16)	0.290
Trabecular thickness	**-0.10 (-0.19,-0.00)**	**0.045**	-0.11 (-0.22,0.01)	0.066	**-0.13 (-0.25,-0.02)**	**0.027**
Trabecular separation	**-0.12 (-0.22,-0.02)**	**0.019**	-0.11 (-0.22,0.00)	0.053	-0.03 (-0.13,0.07)	0.537

P: P-value; CI: Confidence interval

*Adjusted for age at time of HR-pQCT scan, physical activity, smoking status, alcohol consumption, education, time since menopause and use of anti-osteoporotic medications and oestrogen/ hormone replacement (pill/skin patch)

Higher fat mass index residuals indicate greater fat mass index than expected, given lean mass index

Estimates were obtained from linear regression models

Significant associations (p<0.05) are highlighted in bold

At the tibia, FMI residuals were positively associated with cortical area (p<0.04) and volumetric density (p≤0.001) and negatively associated with total and trabecular area (p<0.03), cortical porosity (p<0.02) and cortical pores diameter (p<0.03); these

associations were robust in all models. FMI residuals were positively associated with cortical thickness (p<0.02) and trabecular number (p<0.03) in unadjusted and adjusted analysis but not after adjustment for total hip aBMD.

DXA aBMD parameters in relation to BMI category

The DXA aBMD parameters according to BMI category are presented in Table 3. All DXA aBMD parameters increased with increasing BMI category (p-values for trend ≤0.001). However, this trend was reversed for all DXA aBMD parameters after normalizing values for body weight (p-values for trend <0.001) (Table 4).

HR-pQCT parameters in relation to BMI category

The HR-pQCT parameters at radius and tibia according to BMI category are presented in Table 3. There were significant trends in cortical and trabecular parameters at both radius and tibia according to BMI category. At the radius there was significant increase in cortical area (p<0.001), thickness (p<0.001) and cortical volumetric density (p<0.03) and trabecular number (p<0.001), trabecular volumetric density (p<0.003) and decrease in trabecular separation (p<0.001) as BMI category increased. At the tibia there was significant increase in cortical area (p<0.001), thickness (p<0.001), volumetric density (p<0.001), and trabecular microarchitecture: trabecular number (p<0.001), and trabecular volumetric density (p<0.04) as well as decrease in cortical pores diameter (p<0.001), trabecular thickness (p<0.01) and trabecular separation (p<0.001) parameters as BMI category increased. However, at tibia this pattern was reversed in morbid obesity with a less favourable profile for some of the tibial parameters (compared to other class 2/3 obese participants without hypertension or type 2 diabetes), mainly of the trabecular compartment: lower trabecular number (p<0.01), higher trabecular separation (p<0.01) and lower trabecular volumetric density (p<0.03); and higher cortical pores diameter (p<0.05); these parameters did not differ significantly between participants with morbid obesity and those with normal BMI. When normalised for body weight, all HR-pQCT parameters decreased as BMI category increased (p<0.001) (Table 4)

Table 3: Mean (SD) standardised HR-pQCT and DXA aBMD parameters according to BMI category.

HR-pQCT radius parameter	Underweight (n=4)	Normal (n=160)	Overweight (n=139)	Class 1 (n=54)	Class 2/3 (n=27)	P-values for trend	P-value*
Total area	0.01 (1.24)	0.07 (0.99)	-0.08 (1.09)	0.04 (0.89)	-0.05 (0.76)	0.522	0.741
Trabecular area	0.18 (1.11)	0.10 (0.98)	-0.09 (1.10)	-0.00 (0.88)	-0.15 (0.79)	0.153	0.718
Cortical area	-1.07 (1.20)	-0.20 (0.94)	0.05 (1.00)	0.23 (1.08)	0.60 (0.74)	**<0.001**	0.673
Cortical thickness	-1.02 (0.86)	-0.18 (0.95)	0.06 (1.04)	0.21 (1.00)	0.46 (0.76)	**<0.001**	0.739
Cortical volumetric density	-0.55 (0.65)	-0.11 (0.94)	0.06 (1.05)	0.05 (1.12)	0.32 (0.76)	**0.020**	0.872
Cortical porosity	0.04 (0.88)	0.03 (0.98)	-0.06 (1.08)	0.03 (0.96)	0.08 (0.81)	0.948	0.774
Cortical pores diameter	0.52 (0.84)	0.07 (1.07)	-0.00 (0.94)	-0.13 (0.97)	-0.17 (0.96)	0.088	0.711
Trabecular volumetric density	-0.64 (0.48)	-0.09 (1.01)	-0.04 (0.96)	0.16 (1.05)	0.53 (0.96)	**0.002**	0.552
Trabecular number	-0.88 (0.76)	-0.16 (0.95)	-0.07 (0.96)	0.36 (1.00)	0.68 (1.06)	**<0.001**	0.218
Trabecular thickness	-0.05 (1.12)	0.07 (0.95)	-0.02 (1.08)	-0.22 (0.98)	0.14 (0.94)	0.399	0.959
Trabecular separation	0.87 (0.64)	0.15 (0.95)	0.08 (0.93)	-0.34 (1.07)	-0.69 (1.08)	**<0.001**	0.248
HR-pQCT tibia parameter	Underweight (n=10)	Normal (n=196)	Overweight (n=166)	Class 1 (n=69)	Class 2/3 (n=36)	P-values for trend	P-value*
Total area	0.53 (1.10)	0.04 (0.99)	-0.11 (1.00)	0.05 (1.02)	0.07 (0.97)	0.666	0.925
Trabecular area	0.64 (1.04)	0.07 (0.98)	-0.11 (1.00)	-0.00 (1.02)	-0.05 (1.00)	0.141	0.865
Cortical area	-0.97 (1.13)	-0.24 (0.92)	0.02 (0.93)	0.39 (1.03)	0.74 (0.95)	**<0.001**	0.111
Cortical thickness	-1.02 (1.06)	-0.20 (0.95)	0.05 (0.96)	0.27 (0.98)	0.58 (1.03)	**<0.001**	0.421
Cortical volumetric density	-0.65 (1.34)	-0.15 (0.92)	0.08 (0.99)	0.20 (1.06)	0.26 (1.08)	**<0.001**	0.521
Cortical porosity	0.02 (0.68)	0.12 (0.97)	-0.08 (0.98)	-0.14 (0.99)	-0.04 (1.27)	0.076	0.898
Cortical pores diameter	0.24 (0.82)	0.15 (0.95)	0.04 (1.01)	-0.38 (1.04)	-0.29 (0.98)	**<0.001**	**0.046**
Trabecular volumetric density	-0.48 (0.90)	-0.06 (1.07)	0.01 (0.97)	0.15 (0.91)	0.15 (0.91)	**0.037**	**0.028**
Trabecular number	-0.17 (1.07)	-0.23 (1.03)	0.00 (0.92)	0.40 (0.88)	0.51 (1.02)	**<0.001**	**0.009**
Trabecular thickness	-0.54 (0.88)	0.15 (1.01)	0.02 (1.04)	-0.24 (0.87)	-0.31 (0.85)	**0.006**	0.784
Trabecular separation	0.24 (1.04)	0.21 (1.04)	0.00 (0.91)	-0.37 (0.92)	-0.46 (1.02)	**<0.001**	**0.007**
DXA aBMD parameter	Underweight (n=10)	Normal (n=199)	Overweight (n=172)	Class 1 (n=71)	Class 2/3 (n=38)	P-values for trend	P-value*
Whole body total aBMD	-0.21 (1.24)	-0.12 (1.04)	-0.04 (0.94)	0.37 (1.00)	0.27 (0.80)	**0.001**	0.548
Total hip aBMD	-0.86 (0.95)	-0.27 (0.91)	0.01 (0.96)	0.44 (0.99)	0.72 (0.98)	**<0.001**	0.777
Femoral neck aBMD	-0.39 (1.06)	-0.19 (0.96)	-0.02 (0.99)	0.37 (0.99)	0.55 (0.91)	**<0.001**	0.130
Total lumbar spine aBMD	-0.42 (1.10)	-0.19 (0.98)	-0.00 (0.95)	0.34 (0.98)	0.50 (1.02)	**<0.001**	0.692

BMI categories were defined as follows: Underweight (BMI<18.5); Normal (18.5≤BMI<25); Overweight (25≤BMI<30); Class 1 (30≤BMI<35); Class 2/3 (BM≥35)
*P-values for difference in parameter between participants with morbid obesity (Class 2/3 obese with hypertension or diabetes) and those with Class 2/3 obesity but without hypertension or diabetes. Significant p-values (p<0.05) are highlighted in bold.

Table 4: Mean (SD) standardised HR-pQCT and DXA aBMD parameters (divided by body weight before standardising) according to BMI category.

HR-pQCT radius parameter	Underweight (n=4)	Normal (n=160)	Overweight (n=139)	Class 1 (n=54)	Class 2/3 (n=27)	P-values for trend
Total area	1.54 (1.00)	0.64 (0.76)	-0.19 (0.74)	-0.78 (0.61)	-1.52 (0.54)	**<0.001**
Trabecular area	1.45 (0.95)	0.59 (0.81)	-0.18 (0.81)	-0.69 (0.66)	-1.39 (0.57)	**<0.001**
Cortical area	0.46 (1.11)	0.38 (0.87)	-0.06 (0.95)	-0.55 (0.92)	-0.87 (0.89)	**<0.001**
Cortical thickness	0.33 (0.73)	0.33 (0.89)	-0.04 (0.99)	-0.47 (0.87)	-0.84 (0.87)	**<0.001**
Cortical volumetric density	1.71 (0.34)	0.67 (0.72)	-0.14 (0.67)	-0.94 (0.55)	-1.56 (0.62)	**<0.001**
Cortical porosity	0.80 (0.84)	0.32 (0.94)	-0.12 (1.01)	-0.36 (0.89)	-0.62 (0.69)	**<0.001**
Cortical pores diameter	1.87 (0.49)	0.68 (0.72)	-0.14 (0.62)	-0.92 (0.57)	-1.69 (0.49)	**<0.001**
Trabecular volumetric density	0.42 (0.63)	0.31 (1.04)	-0.11 (0.91)	-0.39 (0.82)	-0.53 (0.78)	**<0.001**
Trabecular number	0.64 (1.13)	0.45 (0.98)	-0.17 (0.89)	-0.48 (0.69)	-0.86 (0.70)	**<0.001**
Trabecular thickness	1.28 (0.66)	0.57 (0.75)	-0.10 (0.84)	-0.89 (0.70)	-1.25 (0.79)	**<0.001**
Trabecular separation	1.42 (0.42)	0.49 (0.74)	-0.01 (0.83)	-0.76 (0.80)	-1.49 (0.84)	**<0.001**
HR-pQCT tibia parameter	Underweight (n=10)	Normal (n=196)	Overweight (n=166)	Class 1 (n=69)	Class 2/3 (n=36)	P-values for trend
Total area	1.96 (0.65)	0.66 (0.69)	-0.22 (0.64)	-0.83 (0.59)	-1.54 (0.64)	**<0.001**
Trabecular area	1.92 (0.72)	0.60 (0.74)	-0.21 (0.70)	-0.76 (0.67)	-1.41 (0.70)	**<0.001**
Cortical area	0.35 (1.29)	0.33 (0.98)	-0.07 (0.91)	-0.43 (0.86)	-0.74 (0.77)	**<0.001**
Cortical thickness	0.19 (1.22)	0.33 (0.96)	-0.04 (0.91)	-0.47 (0.86)	-0.76 (0.89)	**<0.001**
Cortical volumetric density	1.54 (0.76)	0.64 (0.74)	-0.13 (0.65)	-0.91 (0.57)	-1.55 (0.60)	**<0.001**
Cortical porosity	0.92 (0.70)	0.45 (0.90)	-0.14 (0.85)	-0.59 (0.83)	-0.90 (0.95)	**<0.001**
Cortical pores diameter	1.70 (0.64)	0.65 (0.69)	-0.12 (0.62)	-0.98 (0.58)	-1.58 (0.60)	**<0.001**
Trabecular volumetric density	0.80 (1.04)	0.40 (0.99)	-0.09 (0.86)	-0.52 (0.70)	-1.00 (0.73)	**<0.001**
Trabecular number	1.44 (1.00)	0.42 (0.95)	-0.11 (0.79)	-0.53 (0.69)	-1.14 (0.75)	**<0.001**
Trabecular thickness	0.90 (0.83)	0.58 (0.80)	-0.10 (0.77)	-0.82 (0.64)	-1.38 (0.68)	**<0.001**
Trabecular separation	1.18 (0.76)	0.56 (0.79)	-0.08 (0.73)	-0.84 (0.69)	-1.37 (0.80)	**<0.001**
DXA aBMD parameter	Underweight (n=10)	Normal (n=199)	Overweight (n=172)	Class 1 (n=71)	Class 2/3 (n=38)	P-values for trend
Whole body total aBMD	1.66 (0.65)	0.66 (0.72)	-0.20 (0.65)	-0.91 (0.54)	-1.65 (0.61)	**<0.001**
Total hip aBMD	1.22 (0.90)	0.56 (0.80)	-0.13 (0.80)	-0.70 (0.68)	-1.31 (0.76)	**<0.001**
Femoral neck aBMD	1.41 (0.82)	0.54 (0.78)	-0.16 (0.81)	-0.70 (0.70)	-1.31 (0.80)	**<0.001**
Total lumbar spine aBMD	1.18 (0.95)	0.49 (0.83)	-0.11 (0.81)	-0.62 (0.78)	-1.22 (0.92)	**<0.001**

BMI categories were defined as follows: Underweight (BMI<18.5); Normal (18.5≤BMI<25); Overweight (25≤BMI<30); Class 1 (30≤BMI<35); Class 2/3 (BM≥35)
*P-values for difference in parameter between participants with morbid obesity (Class 2/3 obese with hypertension or diabetes) and those with Class 2/3 obesity but without hypertension or diabetes. Significant p-values (p<0.05) are highlighted in bold.

Discussion

In this study, we found that in postmenopausal women there were significant trends in HR-pQCT parameters suggesting favourable bone microarchitecture at both radius and tibia with an increase of BMI category. However, when normalised for body weight, all HR-pQCT and DXA aBMD parameters decreased as BMI increased, illustrating that, for parameters where higher values are indicative of better bone health, these improvements were not proportional to the increase in body weight. We observed different FMI patterns at the radius and tibia; at the radius FMI residuals were associated with parameters of bone size and trabecular architecture, whereas at the tibia, FMI residuals were associated with the cortical compartment parameters, bone size and trabecular architecture. However, there appeared to be a less favourable tibial profile among women with morbid obesity, which may contribute to the higher lower limb fracture rates observed in this group.

Excess body weight due to obesity has traditionally been considered to have a positive effect on bone with a well-described association of high BMD with obesity [155,172]. Similarly, in our study, an increase of aBMD assessed by DXA was observed with increased BMI. However, a higher aBMD in people with a higher BMI may represent appropriate adjustment of the skeleton to increased body weight, but not relate to greater bone strength [236]. Lower rates of hip, pelvis, and wrist fractures in obese individuals may result from the protective effects of increased soft-tissue padding and differences in fall characteristics [169,170,235], whereas a higher risk for ankle, upper leg and humerus fractures might reflect biomechanical factors, but could also represent relative reduced parameters at highest BMIs [169,173]. We found significant increase in cortical area, thickness, cortical volumetric density and trabecular number and decrease in trabecular separation parameters at the radius as BMI category increased. At the tibia there was significant increase in cortical area, thickness, volumetric density and trabecular microarchitecture: trabecular number, trabecular volumetric density and decrease in trabecular separation and thickness parameters as BMI category increased. However, at tibia this pattern was reversed in morbid obesity with a fall in some tibial parameters (compared to participants without hypertension or diabetes who were class 2/3 obese) mainly of the trabecular compartment i.e. trabecular volumetric density (due to lower trabecular number and higher trabecular separation). We did see differences in

relationships at the radius and tibia which require validation in other samples. This may reflect technical differences in acquisition at the two sites, or differences due to the weight bearing/non-weight bearing nature of the two sites. Fracture risk in the GLOW global cohort was reported to be increased at the ankle and upper leg in obese women, which may also be relevant [169].

In previous work Sornay-Rendu and colleagues reported greater volumetric BMD at the distal radius and distal tibia resulting from greater trabecular volumetric density and trabecular thickness and greater cortical volumetric density (due to lower cortical pores) in obese postmenopausal women, compared to a non-obese control group [127]. Evans et al. compared bone density and microarchitecture in younger (age 25-40) and older (age 55-75) obese men and women to a non-obese control group [159]. Greater differences in BMD and HR-pQCT measurements between obese and normal adults were observed in the older adults than the younger adults with greater volumetric BMD at the distal radius and distal tibia in obese, compared to non-obese individuals in the older age group. In the younger group, obese adults had greater volumetric BMD than normal BMI adults at the tibia only. Older obese individuals had favourable cortical and trabecular compartment parameters with thicker cortices, higher cortical volumetric density, higher trabecular volumetric density, and higher trabecular number than normal weight adults at both sites scanned, whereas in the younger group the higher volumetric BMD in obesity was due to greater trabecular density, due to higher trabecular number and lower trabecular separation at radius and tibia. Those results suggest that obesity may protect against age-related bone loss, and also increase peak bone mass. However, no BMI categories of obesity were distinguished in these studies. Sukumar et al performed a study of 211 women of a wider age range (25-71) and BMI classified into 3 categories (normal weight, obese-class 1 and obese-class 2/3) measuring bone parameters by pQCT. In contrast to our findings, they reported that women with Class 2/3 obesity had reduced cortical but increased trabecular volumetric density at tibia measured by pQCT [237]. However, in that study, the negative association between BMI and cortical volumetric BMD was significant only in the premenopausal ($p<0.0001$) and not in the postmenopausal ($p=0.1$) women. It is possible that cortical volumetric BMD does not decline as dramatically in obese compared to in leaner women with aging.

We observed different FMI patterns at the radius and tibia. At the distal radius FMI residuals were associated with parameters of bone size and trabecular architecture, whereas at the distal tibia, FMI residuals were associated with cortical compartment parameters and bone size. The existing literature has shown some positive relationships (among women and after accounting for LMI) between adiposity and bone geometry, however the specific compartments affected have varied and studies are few [161]. Edwards and colleagues reported positive relationships between FMI and trabecular number and cortical area in tibia and only trabecular number in the radius. [238] Interestingly, in the current study, at the distal tibia, FMI residuals were associated more strongly with cortical compartment parameters and bone size, in contrast to the study by Edwards et al., that indicated a stronger association with the trabecular compartment. In that study participants were recruited from the Hertfordshire Cohort Study (HCS). Participants of the HCS were older, of mean age (mean [SD] age of 76.4 (2.6) compared to 70.9 (5.4) for GLOW participants.

Morbid obesity has been associated with an excessive increase of leptin levels [239]. Associations between leptin and BMD are complex, with human and murine studies yielding conflicting results and leptin exerting positive and negative effects on bone metabolism, depending on whether it acts directly on bone cells or indirectly (via the hypothalamus and autonomic nervous system), respectively [240–247]. Circulating leptin levels may be affected by inflammatory cytokines [240]]. Obesity is considered to be a low grade pro-inflammatory state, associated with greater concentrations of pro-inflammatory cytokines, which are inversely associated with BMD and positively associated with bone resorption [244–247]. It has been suggested that subcutaneous adipose tissue-derived IL-6 could be associated with the impairment of insulin sensitivity in the skeletal muscle of morbidly obese subjects [239]. In obesity, adipose tissue becomes inflamed, both via increased production of inflammatory cytokines by mature adipocytes and through infiltration of adipose tissue by macrophages [245]. It has been suggested that most adipokines, in morbidly obese humans, are derived from non-fat cells [246,247]. We observed that the trend of favourable bone microarchitecture at both radius and tibia with an increase of BMI category is reversed at the level of morbid obesity. Blood samples were not available in our study to test cytokines levels, though such research would be valuable.

There are limitations to our study. These are observational data that demonstrate trends and associations, but not causality between obesity, fat mass and bone microarchitecture. In addition, our study populations of postmenopausal women were UK community-dwelling subjects and our findings need to be tested in other populations. BMI may be considered a suboptimal measure of obesity, as body fat distribution could affect bone density and microarchitecture. Finally, the numbers of individuals at the extreme BMI categories are small. Larger studies of obese women are required.

In conclusion, we have observed a significant trend suggesting favourable cortical and trabecular microarchitecture with increased BMI category in postmenopausal women at both radius and tibia. At tibia this pattern was reversed in morbid obesity with a less favourable tibial parameters mainly of the trabecular compartment. Furthermore, for bone parameters where higher values indicate better bone health, improvements in these parameters with increased BMI category were not proportion to the increase in body weight. There were different FMI patterns at the radius and tibia; with radius FMI residuals associated with parameters of bone size and trabecular architecture, whereas at the tibia, FMI residuals were associated with cortical compartment and parameters of bone size. Understanding better the relationships between obesity, fat mass and bone microarchitecture, and impact of morbidity, may give insights into targeted interventions for prevention of osteoporotic fractures later in life.

4.3 Third manuscript – Self-perception of fracture risk: 'what can it tell us?'

Authors and affiliations

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Abstract

Purpose—This study aimed to assess how well self-perception of fracture risk, and fracture risk as estimated by the fracture prediction tool FRAX, and related to fracture

incidence and uptake and persistence of anti-osteoporosis medication among women participating in the Global Longitudinal study of Osteoporosis in Women (GLOW).

Methods—GLOW is an international cohort study involving 723 physician practices across 10 countries in Europe, North America and Australia. 60393 women aged ≥55 years completed baseline questionnaires detailing medical history, including co-morbidities, fractures and self-perceived fracture risk (SPR). Annual follow-up included self-reported incident fractures and anti-osteoporosis medication (AOM) use. We calculated FRAX risk without bone mineral density measurement.

Results—Of the 39241 women with at least one year of follow-up data, 2132 (5.4%) sustained an incident major osteoporotic fracture over 5 years of follow-up. Within each SPR category, risk of fracture increased as the FRAX categorisation of risk increased. In GLOW only 11% of women with a lower baseline SPR were taking AOM at baseline, compared with 46% of women with a higher SPR. AOM use tended to increase in the years after a reported fracture. However, women with lower SPR who fractured still reported lower AOM rates than women with or without a fracture but a higher SPR.

Conclusions—These results suggest that SPR captures some aspect of fracture risk not currently measured using conventional fracture prediction tools and is also associated with improved medication uptake.

Introduction

Osteoporosis-related fractures confer a significant healthcare burden. Approximately one in two women and one in four men over age 50 will have an osteoporosis-related fracture in their lifetime [25]. In addition to the personal impact on millions of people around the world, fractures caused by osteoporosis represent a major and growing socioeconomic burden. In 2005 in the United States alone, there were 297,000 hip fractures, 547,000 vertebral fractures, 397,000 wrist fractures, 135,000 pelvic fractures, and 675,000 fractures at other sites costing nearly $17 billion [224]. The cost of osteoporotic fracture in the UK approaches £3 billion annually and, across the EU, the estimated total economic cost of the approximately 3.5 million fragility fractures in 2010 was €37 billion [7]. As the population ages, costs are expected to escalate.

Therapeutic options can significantly reduce the risk of osteoporosis-related fractures. However, suboptimal use of anti–osteoporosis medications (AOM) and low adherence among women who have started AOM are recognised problems, similar to adherence problems reported for many non-communicable diseases such as ischaemic heart disease, chronic obstructive pulmonary disease and osteoporosis [248–250]. More than one third of people do not comply with prescribed treatment regimens [251]. A more recent study of patients, who received AOM within 1 year after fracture, reported that persistence with AOM was 75% and 45.3% after 1 and 5 years respectively [252].

It is possible that empowering patients through improved understanding of their disease and adequate appreciation of fracture risk may be beneficial, as increased self-awareness might lead to greater healthcare engagement and treatment. Although, it has been demonstrated that a person's perception of osteoporosis is associated with improved medication adherence [249], it has been reported that people with an increased fracture risk commonly underestimate their actual risk, suggesting that there might be a disconnect between self-perception of fracture risk and actual fracture risk [207].

In a study to consider self-perception of fracture risk further, we aimed to determine how well a person's fracture risk perception aligned with fracture probability as assessed by FRAX in a large, multinational cohort study, and also assess whether incident fracture was associated with altered (i) use of AOM (ii) self-reported adherence to AOM.

Methods

Study design

GLOW is an observational cohort study conducted in physicians' practices at 17 sites in 10 countries (Austria, Belgium, Canada, France, Germany, Italy, Netherlands, Spain, UK and USA). Details of the study design have been previously described [215]. In brief, typical practices of each region were recruited through primary care networks. Each practice provided a list of women aged 55 years or older, who within the past 24 months had consulted their primary care physician. Sampling was age-stratified to ensure that

two thirds of women were 65 years of age or older, excluding those who were unable to complete the study survey due to cognitive impairment, language barriers, institutionalisation or illness. Each study site obtained ethics committee approval to conduct the study in the specific location.

Questionnaires

Self-administered questionnaires covered domains that included: demographic characteristics and risk factors, perception about fracture risk relative to women of the same age (ranked as much lower', 'little lower', 'about the same', 'little higher' and 'much higher', on a 5 point scale), medication use, medical diagnosis, healthcare use and access, physical activity and physical and emotional health status including self - rated health. Self-reports of personal risk factors included: current weight and height, parental hip fracture, falls in the past 12 months, current use of cortisone or prednisolone, diagnosis of rheumatoid arthritis, personal history of fracture (clavicle, arm, wrist, spine, rib, hip, pelvis, upper leg, lower leg, and ankle) since age 45 years, current cigarette smoking and consumption of three or more units of alcohol daily. Follow up questionnaires were sent annually for 5 years. These asked about any incident fractures and requested information about site of fracture and any hospital treatment received.

FRAX

FRAX scores were calculated for women from responses on their baseline survey, without inclusion of bone mineral density measurement. The FRAX tool with or without the use of BMD is a well-validated instrument and enhances fracture risk prediction [250]. Women were classified as 'high risk', 'medium risk' and 'low risk' if their FRAX 10 year probability of major osteoporotic fracture and hip fracture was both $\geq 20\%$ and $\geq 3\%$, either $\geq 20\%$ or $\geq 3\%$, or both $\leq 20\%$ and $\geq 3\%$ respectively.

Medication

Women were considered to be taking anti-osteoporosis medications (AOM) if they reported current use of alendronate, etidronate, ibandronate, risedronate, pamidronate,

zoledronate, strontium ranelate, calcitonin, PTH [1-84], teriparatide, raloxifene, or tibolone.

Analysis

Data from women who completed a baseline questionnaire and at least one year of follow-up were included in the analysis. Women who reported any incident major fracture (hip, spine, upper arm, shoulder, or wrist) that occurred between baseline and their last year of consecutive follow-up-- between 1 and 5 years after baseline-- were classified as incident fracture positive. If a woman reported more than one incident fracture, the date of her earliest fracture was used. For this analysis SPR was defined as 'higher' when women rated their SPR, using a five point scale as 'little higher' and 'much higher'; and as 'lower' when rated as 'much lower' or 'little lower', compared with women of the same age. A Cox proportional hazards model predicting 5-year incident fracture based on SPR category, FRAX risk category, and an interaction term of SPR with FRAX was used to calculate unadjusted hazard ratios (HRs) and 95% confidence intervals (CI) for each SPR and FRAX risk category combination. A separate Cox proportional hazards model predicting 5-year incident fracture based on SPR and FRAX was used to determine if the two were independently significant predictors of fracture. Finally, as a sensitivity analysis, two additional Cox proportional hazards model predicting 5-year incident fracture were run. The first added number of falls reported in the past 12 months on the baseline GLOW survey to a model with SPR and FRAX, and was used to determine if the relationship between SPR, FRAX, and incident fracture would remain after adjusting for history of falls. The second added a variable for the country of the GLOW respondent to a model with SPR and FRAX, to determine if the relationship between SPR, FRAX, and incident fracture would remain after adjusting for geographic region. Associations were considered significant if the p-value was <0.05. All calculations were done using SAS version 9.4.

Results

A total of 60,393 patients from 723 physicians' practices enrolled in the study between October 2006 and February 2008. Approximately 25000 participants were recruited in Europe, 28000 in USA, and almost 7000 in Canada and Australia. There were 39,241

(65%) women with at least one year of follow-up data. The mean age was 68 years and mean weight 70kg. History of maternal hip fracture was reported by 14% participants and personal history of a fracture of the wrist, spine, or hip was 11% at baseline. The reported prevalence of common comorbid conditions was: 11% asthma, 0.8% rheumatoid arthritis, 50% hypercholesterolaemia and 49% hypertension; 19% of women said their health status was "fair" or "poor". Fifty six percent of women expressed "some" concern about osteoporosis and 21% said they were "very concerned" about the condition. When women rated their SPR (their own risk of fracture compared with women their own age), 36% rated their risk as lower and 17% as higher. The remaining 46% considered their risk "the same." Women who rated their SPR as higher also reported more falls than women who rated their SPR as lower; among women with lower SPR only 11% reported two or more falls in the prior year, compared to 23% of women with higher SPR (p<0.0001). The number of reported comorbidities was also significantly associated with SPR (p<0.0001). Similar to falls, women who rated their SPR higher reported more comorbidities than women rating their SPR as lower with figures of 20% and 17% for 3 comorbidities, 11% and 6.6% for 4 comorbidities, and 7.5% and 3.1% for ≥ 5 comorbidities respectively.

Incident major fracture (hip, wrist, spine, shoulder, arm) was reported by 2132 (5.4%) women over 5-years of follow-up. Table 1 shows the associations between SPR and FRAX derived fracture risk. Within each SPR category, risk of incident fracture increased as FRAX categorisation of risk increased. The highest risk of fracture was seen in women with both a high SPR and high FRAX risk. In a Cox model containing both FRAX risk and SPR both variables were highly significant (p<0.0001), suggesting that a woman's own perception of fracture risk is an additional predictor of fracture beyond that calculated by FRAX. In the model, compared to women with lower SPR, women with SPR of "about the same" as other women their age had a slightly increased fracture hazard ratio (95% CI) of 1.15 (1.04 – 1.27). Women with SPR "much or a little higher" as other women their age had almost twice the rate of fracture, with a hazard ratio (95% CI) of 1.88 (1.68 – 2.11). In the same model, compared to women with low FRAX risk, women with medium FRAX risk had a fracture hazard ratio (95% CI) of 1.53 (1.37 – 1.70), and women with high FRAX risk had a fracture hazard ratio (95% CI) of 2.81 (2.52 – 3.12). FRAX was a stronger predictor of a fracture than SPR (Type 3

Wald chi-square values of 366.34 and 134.40 respectively) but both variables had a highly significant, independent association with fracture.

Table 1 Fracture number (and HR for incident fracture) according to self-perceived fracture risk (SPR) and FRAX stratification

SPR		Low FRAX risk[*]	Medium FRAX risk[*]	High FRAX risk[*]
Much or little lower	n (%) annual fracture incidence HR (95% CI)	6581 (17) 1.3% 1.00	5189 (13) 1.8% 1.35 (1.12 – 1.64)	2468 (6.3) 3.3% 2.66 (2.18 – 3.23)
About the same	n (%) annual fracture incidence HR (95% CI)	8566 (22) 1.4% 1.00	6047 (15) 2.2% 1.62 (1.38 – 1.90)	3561 (9.1) 3.7% 2.76 (2.35 – 3.25)
Much or a little higher	n (%) annual fracture incidence HR (95% CI)	2164 (5.5) 2.1% 1.00	2418 (6.2) 3.6% 1.65 (1.30 – 2.08)	2247 (5.7) 6.3% 3.10 (2.49 – 3.86)

*Difference between fracture rates according to FRAX risk p<0.0001

Woman who have fallen consider themselves at higher risk for fracture (p < 0.0001). It is possible that women reporting a high SPR may do so because they perceive their risk of falls to be higher than women of the same age. We attempted to investigate this issue In a separate Cox model that included SPR, FRAX, and the number of falls in the year prior to the survey's baseline, the falls variable was a significant predictor of a subsequent fracture with a hazard ratio (95% CI) of 1.24 (1.12-1.37) for one fall and 1.66 (1.48-1.85) for ≥ 2 falls, but the results for SPR and FRAX both remained highly significant. Moreover, the Type 3 Wald chi-square values for SPR (107.13) and FRAX (345.58) were higher than that of the falls variable (81.68) and the HRs for SPR and FRAX did not change appreciably. This indicates that both have a significant, independent contribution towards predicting fracture, but that SPR was stronger.

At baseline, 7579 (20%) of the cohort reported taking an AOM. There were 565 incident fractures reported at year 1, 531 at year 2, 494 at year 3, and 534 at year 5. Of

14238 women in GLOW with a lower baseline SPR, 1527 (11%) were taking an AOM at baseline, while of 6829 women with a higher SPR 3042 (46%) were taking an AOM at baseline. AOM use tended to increase in the year following an incident fracture in women with lower or higher SPR. For example, among women with a lower baseline SPR 11% were taking AOM at baseline and 23% at year 1 if the woman reported an incident fracture, while the corresponding figures for those with higher baseline SPR were 46% and 55% respectively. However, among women with lower SPR who reported an incident fracture, use of AOM was consistently lower than that of women with higher SPR regardless of their fracture status (p<0.0001) (Figure 1).
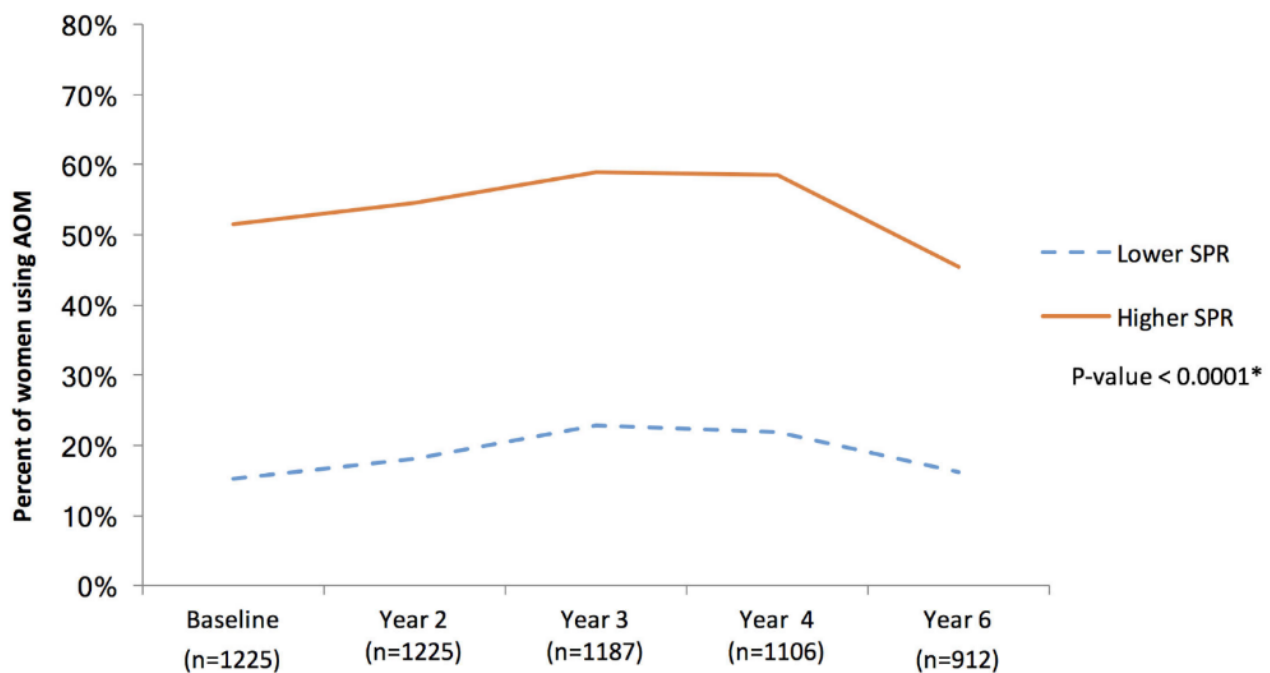
Figure 1 Anti – osteoporosis medications (AOM) use in women who sustained any major fracture over 5 year follow up period in the GLOW study by self-perceived fracture risk (SPR).

*p-value <0.0001 for each comparison between lower and higher SPR.

Results presented throughout did not include country of origin. Although we repeated analyses and in a Cox model that included SPR, FRAX, and the country of the GLOW survey respondent, the country variable was significant (p<0.0001), but again without substantial change in effect size or direction.

Discussion

We have demonstrated that SPR of fracture does capture some aspect of fracture risk not currently measured using the conventional fracture prediction tool FRAX, and also translates to improved medication uptake. Self-perception of risk of a condition is a difficult concept, as it requires an individual to compare their own health status to others. In previous work, SPR of osteoporosis and osteoporotic fractures has previously been reported to be underestimated in postmenopausal women worldwide. Rothmann et al, observed that women participating in the Risk-Stratified Osteoporosis Strategy Evaluation (ROSE) study underestimated their fracture risk compared to the risk estimated by FRAX, although it demonstrated that women did have some understanding of the importance of some risk factors such as prior fracture, parental history and falls [208]. Women at increased fracture risk generally perceive their risk to be lower or about the same as women of the same age, as has been shown previously in GLOW [207232]. Our data suggest that SPR offers a further contribution to fracture prediction, independent of fracture prediction by FRAX.

An increased number of falls present in women with higher SPR in this study; a variable not captured by FRAX, is a possible partial explanation for the independent addition of SPR to fracture prediction algorithms. Falls increase fracture risk among older adults and multiple falls are a marker of physical frailty. However, even in our model including falls, SPR remained a significant independent predictor of fracture. Interestingly, fear of falling, a self-perceived concept, has been found to be predictive of future falls [253]. Polypharmacy may be another explanation for our findings. Due to the age range of our study group, most individuals suffered from at least one chronic condition. Some comorbidities increase falls risk, which could lead to increased fracture risk; for example neurological diseases have been shown to have the highest fracture rates among participants of the GLOW study [232]. In this study higher number of comorbidities was associated with higher SPR. The presence of chronic disease may imply a need to take prescribed drugs that might increase the risk of falling. Several types of drugs are associated with a significant risk of falls. Use of antidepressants has been reported to have the strongest association with falls, but also other classes of medication among other including antihypertensives, nonsteroidal anti-inflammatory

drugs and antipsychotics were found to have positive association with falling [254]. Furthermore, drug adherence may be lower in frail patients with cognitive impairment.

SPR was also associated with self-reported AOM uptake. In this large, international observational study women with higher SPR were more likely to report AOM use than women with lower SPR. AOM use was higher in the year after an incident fracture for women in both groups with lower or higher SPR, but the absolute rate of AOM use was always higher among women with higher SPR. Whether changing the SPR from lower to higher would lead to improve in uptake or adherence to AOM requires further investigation.

It is well documented that prescription rates of AOM following osteoporotic fracture are low and adherence to medication is poor. Previous studies suggest that just 17% of treatment naive women with a new fracture began AOM in the first year of follow-up and between 26 - 70% of women prescribed oral bisphosphonates continued to take them at 1 year [252,255]. This is consistent with our findings, in which a high proportion of women with incident fracture did not start AOM.

Established predictors of treatment initiation include a diagnosis of osteoporosis and low measured bone density [255]. In the current study, SPR of fracture also predicted osteoporosis treatment. Other studies also found that patient health beliefs predicted treatment. For example, patient beliefs in the benefits of medications, and distrust of medications were reported to differentiate between initiators and non-initiators of osteoporosis medication [256,257]. It is well recognised that a well-informed, empowered patient should be at the heart of the chronic disease management model. In cases of osteoporosis, it may be that improved understanding of the disease, its management and appreciation of fracture risk among patients would help to tackle under-use of AOM, and we hope to address this in future work, in a randomised controlled trial setting.

Our study has some limitations. Educational and cultural differences may influence SPR of fracture. While we performed a sensitivity analysis for country, in this cohort data regarding ethnicity were not available apart from in women from USA and Canada. Furthermore, although some educational data were collected, it is difficult to draw conclusions due to difference in the educational systems between the participating countries. Finally, information on AOM use was based upon self-reported questionnaire

and not verified by pharmacy records. It has been reported, however, that agreement between self-report and pharmacy data is high [258].

In conclusion, our data suggest that self-reported risk of fracture does capture an aspect of fracture risk not currently measured using the conventional fracture predictions tool FRAX, and that greater self-reported risk also translates to improved osteoporosis medication uptake. These observations suggest that education interventions may help to improve medication uptake and adherence, and that a woman's perception of her own risk of fragility fracture should be considered when counselling her regarding management of osteoporosis.

4.4 Fourth manuscript - Self-perceived fracture risk in the Global Longitudinal Study of Osteoporosis in Women: its correlates and relationship with bone microarchitecture

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Abstract

Purpose: To examine correlates of self-perceived fracture risk (SPR) and relationships between SPR and subsequent bone density and microarchitecture in the UK arm of the Global Longitudinal Study of Osteoporosis in Women.

Methods: 3912 women completed baseline questionnaires detailing medical history and SPR; 492 underwent HR-pQCT scans of the radius and tibia and DXA scans of total body, hip, femoral neck and lumbar spine a median of 7.5 years later. Correlates of SPR were examined and a cluster analysis of potential predictors of SPR performed. SPR in

relation to HR-pQCT and aBMD parameters was examined using linear regression with and without adjustment for anthropometric, demographic and lifestyle covariates.

Results: Mean (SD) baseline age was 69 (9.0) years; 56.6% reported a similar SPR; 28.6% lower SPR; 14.9% higher SPR compared to women of similar age. In mutually-adjusted analysis, higher SPR was associated (p<0.05) with: lower physical activity and educational attainment; use of anti–osteoporosis medications (AOM) and calcium supplements; greater number of falls in the previous year; history of fracture since aged 45; family history of hip fracture; and increased comorbidity. Higher SPR, history of fracture, and use of AOM, calcium and vitamin D clustered together. Even after adjustments that included AOM use, higher SPR was associated with: lower radial trabecular volumetric density and number, and higher trabecular separation; lower tibial cortical area and trabecular volumetric density; and lower aBMD at the femoral neck.

Conclusions: Despite greater AOM use, women with higher baseline SPR had poorer subsequent bone health.

Introduction

Osteoporosis, a disease characterized by low bone mass and structural deterioration, is classified as a public health problem due to its association with an increased risk for fragility fractures and, consequently has a high impact on quality of life and high rates of morbidity [72]. Worldwide, there are nearly nine million osteoporotic fractures each year, with reports suggesting that one in two women and one in five men will experience a fracture in their remaining lifetime from the age of 50 years [25,72]. With aging of the population, the economic cost of osteoporosis and fractures is projected to increase in the EU from €37.4 billion in 2010 to €46.8 billion by 2025 and, in the US, from $17 billion in 2005 to $25.3 billion by 2025 [7,224].

Patient and healthcare provider awareness of individual fracture risk is essential for accurate planning and successful implementation of prevention strategies. A number of web-based tools have been developed to improve the identification of individuals at high fracture risk. Clinical risk factors such as age, weight and skeletal properties are included in fracture prediction algorithms, with the most commonly used globally being

FRAX. Recently, it has been reported in the Global Longitudinal Study of Osteoporosis in Women (GLOW) that self-perception of fracture risk (SPR) may also capture aspects of fracture risk not measured using current risk prediction tools, and has been associated with fracture risk independently of FRAX [221].

Self-perception of risk of a condition is a difficult concept, as it requires an individual to compare their own health status to others. There is evidence that self-perception of risk of osteoporosis and osteoporotic fractures is underestimated in postmenopausal women worldwide [259], and that self-perceived risks of osteoporosis and fracture affect certain behaviours such as seeking medical advice, anti–osteoporosis medication use and BMD screening, which might lead to greater healthcare engagement, treatment and altered bone health [189,221]. Furthermore, findings from GLOW cohort suggest that increased self-perceived fracture risk is strongly associated with incident fracture rate [232]. However, very little is known about what determines self-perceived fracture risk (SPR).

To address this, we have used data from the UK arm of the Global Longitudinal Study of Osteoporosis in Women (GLOW) to: identify correlates of SPR; examine how these correlates interrelate by performing a cluster analysis; and relate SPR to subsequent bone density and microarchitecture.

Methods

Study participants

GLOW is a prospective, observational cohort study conducted through general physician practices in 10 countries. Study design and recruitment have been described in detail previously [215]. In brief, practices, representative of each region, were recruited through primary care networks and provided the names of women aged 55 years and older who had been seen by their physician in the past 24 months. The primary aim of GLOW was to characterise the descriptive epidemiology and health impact of osteoporosis-related fractures among women who were 55 years of age and older worldwide. Globally, GLOW enrolled over 60,000 women through over 700 physicians in 10 countries, and conducted annual follow-up for up to 5 years. In Southampton only, a subgroup of participants with baseline data and at least one follow-up questionnaire

were invited, after completion of 5 years of follow-up, for a follow-up study which included dual energy x-ray absorptiometry (DXA) and high resolution peripheral quantitative computed tomography (HR-pQCT) scans. Participants were scanned between April 2014 and December 2017. Patients, who were institutionalized or were not able to complete the study survey by themselves due to cognitive impairment, language barriers, institutionalization, or were too ill to complete the survey or attend for the scans were excluded.

Baseline questionnaires

To ascertain self-perceived fracture risk (SPR), participants were asked to rate their risk of fracturing/breaking a bone, compared to other women of the same age, out of the following responses: 'much lower'; 'a little lower'; 'about the same'; 'a little higher'; and 'much higher'. Fracture history since age 45 years was ascertained at the following locations: clavicle, upper arm, wrist, spine, rib, hip, pelvis, ankle, upper leg and lower leg. Family history of hip fracture was obtained by asking participants whether their mother or father had ever broken or fractured their hip. Information on the number of falls during the previous 12 months was also collected.

Further information ascertained from questionnaires included: age; self-reported height and weight; smoking status; alcohol consumption; physical activity; educational attainment; current use of anti-osteoporotic medication (AOM), calcium supplements and Vitamin D supplements (or multivitamin with Vitamin D); current/previous use of oestrogen or hormone replacement therapy (HRT); and years since menopause. Participants were considered to be taking AOM if they reported current use of alendronate, calcitonin, etidronate, ibandronate, pamidronate, raloxifene, risedronate, strontium ranelate, teriparatide, tibolone or zoledronic acid. Participants were asked whether a doctor or health provider had ever told them that they had the following conditions: hypertension; heart disease; high cholesterol; asthma; chronic bronchitis/emphysema; osteoporosis; osteoarthritis/degenerative joint disease; rheumatoid arthritis; stroke; ulcerative colitis/Crohn's disease; celiac disease; Parkinson's disease; multiple sclerosis; cancer; and type 1 diabetes.

Anthropometry and DXA

In a subgroup of participants that underwent DXA at a median (lower quartile, upper quartile) of 7.5 (7.1, 8.9) years after the baseline questionnaire, height was measured to the nearest 0.1 cm using a Marsden stadiometer on the day of scanning; weight was measured to the nearest 0.1 kg using a Marsden MPPS-250 (Marsden Weighing Machine Group Limited, Rotherham, UK) digital floor scale. Areal bone mineral density (aBMD, g/cm^2) of the total body, hip, femoral neck and lumbar spine was measured using a DXA Hologic Horizon W (software version Apex 5.5.3.1 [Vertec Scientific, Reading, UK]).

Assessment of bone by HR-pQCT

This subgroup of participants also underwent a HR-pQCT scan of the non-dominant distal radius and tibia using XtremeCT (Scanco Medical, Basserdorf, Switzerland) on the same day as the DXA scan; if there was a history of fracture on the non-dominant limb, the non-fractured limb was measured. A stack of 104 parallel HR-pQCT slices were acquired with an isotropic voxel size of 82 μm. Methods used to process the HR-pQCT data have been described previously [226]. For this analysis, the standard evaluation and cortical porosity scripts were run to obtain estimates of the following parameters at the radius and tibia: total area and trabecular area, volumetric density, number, thickness and separation; cortical area, thickness, volumetric density and pores diameter; and cortical porosity [220].

Derived variables

Self-reported body mass index (BMI) at baseline was calculated from the self-reported measures of height and weight. Self-reported height and weight were correlated (r=0.32, p<0.001); a sex-specific standardised residual of weight-adjusted-for-height at baseline was derived as a marker of adiposity for inclusion in regression models. Variables for BMI and weight-for-height residual were also calculated at follow-up from measured height and weight among the subgroup that underwent DXA and HR-pQCT. The total number of comorbidities at baseline, excluding osteoporosis, was used as a marker for overall morbidity. FRAX scores for 10-year probability of major osteoporotic fracture (MOF) and hip fracture were calculated for women from their baseline survey responses, without inclusion of bone mineral density measurements.

Statistical analysis: cross-sectional correlates of SPR at baseline

Participant characteristics of the 3912 women with data on SPR at baseline were described using summary statistics (Table 1). Ordinal logistic regression was used to examine univariate associations between participant characteristics and SPR. Characteristics significantly associated ($p<0.05$) with SPR were then included in a mutually-adjusted model; FRAX scores were not included in mutually-adjusted analyses as the inclusion of these variables and participant characteristics which are components of FRAX may result in multicollinearity. Sensitivity analyses were performed among the following groups; have osteoporosis; current use of AOM; have osteoporosis or current use of AOM.

Statistical analysis: cluster analysis of potential predictors of SPR

A cluster analysis of the participant characteristics in Table 1 (excluding SPR and only using self-reported height and weight-for-height residual as measures of anthropometry) was performed among the 2582 participants with complete data on these characteristics; a flow diagram for the various samples of participants used for analysis is presented in Figure 1. This used the TwoStep Cluster Analysis procedure in SPSS (version 25) which is suitable for a mixture of categorical and continuous variables [260]. This procedure involves grouping observations into clusters based on the distance measure and then applying a hierarchical clustering algorithm to these clusters; the cluster solution with the lowest Bayesian information criterion (BIC) is selected as optimal. The change in log-likelihood from merging two clusters as opposed to keeping them separate was used as the distance measure. Goodness-of-fit of the cluster solution was determined using the silhouette coefficient, a measure of how similar participants are within clusters compared to how similar they are between clusters, which ranges from -1 to 1 (<0.2: poor; 0.2-0.5: fair; >0.5: good). Participant characteristics were then compared between the clusters using descriptive statistics.

Baseline samples

Sample for examining SPR correlates
Participants with data on SPR *(n=3912)*

Cluster analysis sample
Complete baseline data on SPR and participant characteristics included in the cluster analysis *(n=2582)*

Data on at least one radial HR-pQCT parameter *(n=384)*

Data on all radial parameters *(n=375)*

Excluded from radial analysis due to problems with scans or poor scan quality *(n=103)*

Data on at least one tibial HR-pQCT parameter *(n=477)*

Data on all tibial parameters *(n=468)*

Excluded from tibial analysis due to problems with scans or poor scan quality *(n=19)*

Subgroup with data on SPR at baseline who underwent DXA and HR-pQCT a median of 7.5 years after baseline

Sample for examining baseline SPR in relation to HR-pQCT and DXA parameters
Data on at least one radial or tibial parameter *(n=492)*

Number of observations for DXA aBMD parameters:
Whole body total *(n=410)*
Total hip *(n=464)*
Femoral neck *(n=457)*
Total lumbar spine *(n=471)*

SPR: Self-perception of fracture risk; DXA: Dual-energy X-ray absorptiometry; aBMD: Areal bone mineral density; HR-pQCT: High-resolution peripheral quantitative computed tomography

Figure 1 Flow diagram for the analytical samples of participants.

Statistical analysis: SPR in relation to DXA aBMD and HR-pQCT parameters

The sample for this subgroup analysis comprised 492 individuals with data on SPR and at least one of the HR-pQCT parameters outlined above. Of these 492 participants, 384 and 477 had data on at least one radial and tibial HR-pQCT parameter respectively; the number of participants with available data for the DXA aBMD parameters ranged from 410 to 471, depending on the parameter (Figure 1). Participant characteristics of this whole subgroup were described using summary statistics. Linear regression was used to

examine SPR in relation to the HR-pQCT parameters of the tibia and radius and the aBMD parameters. Unadjusted and adjusted associations, accounting for age at time of scan, follow-up time, measured height at follow-up, weight-for-height residual from measured values at follow-up, physical activity, smoking status, alcohol consumption, education, time since last menstrual cycle, use of AOM, calcium and vitamin D supplements, and oestrogen/HRT, were examined. SPR was treated as an ordinal variable with five levels. Apart from the cluster analysis, all analyses were conducted using Stata, version 15.0.

Results

Participant characteristics

Baseline participant characteristics of the baseline analysis sample (n=3912) are presented in Table 1. Mean (SD) age was 69.0 (9.0) years. Overall, 2213 (56.6%) reported a similar SPR compared to other women of the same age; 1118 (28.6%) reported a lower risk and 581 (14.9%) reported a higher risk. Median (lower quartile, upper quartile) FRAX probabilities for 10-year MOF and hip fracture are presented in Table 1. MOF FRAX probabilities for women with lower, similar and higher SPR were 10.4 (7.1, 16.0), 10.7 (7.1, 17.2) and 15.6 (9.1, 22.8) respectively; corresponding FRAX probabilities for hip fracture were 2.1 (1.1, 5.4), 2.1 (1.0, 5.5) and 3.7 (1.6, 8.8) (data not shown).

Table 1 Baseline participant characteristics of the analysis sample (n=3912).

Participant characteristic	N (%)	Missing values
Age (years)*	69.0 (9.0)	0
Self-reported height (cm)*	161.7 (6.8)	193
Self-reported weight (kg)*	68.3 (12.8)	215
BMI (kg/m²)*	26.1 (4.7)	354
Current smoker	273 (7.1%)	48
Self-perceived fracture risk		
Much lower	472 (12.1%)	
A little lower	646 (16.5%)	
About the same	2213 (56.6%)	0
A little higher	442 (11.3%)	
Much higher	139 (3.6%)	
Alcohol consumption		
None	1242 (32.0%)	
1-6	1598 (41.2%)	
7-13	779 (20.1%)	34
14-20	222 (5.7%)	
>20	37 (1.0%)	
Physically active compared to others		
Not at all	135 (3.5%)	
A little	694 (18.0%)	56
Somewhat	1893 (49.1%)	
Very	1134 (29.4%)	
Educational attainment		
Below GCSE	1540 (39.4%)	
GCSE	1185 (30.3%)	0
A-level	522 (13.3%)	
Degree	665 (17.0%)	
Current use of anti-osteoporotic medication	348 (9.4%)	222
Ever used oestrogen/hormone replacement therapy	1328 (34.6%)	71
Currently taking calcium	736 (19.3%)	97
Currently taking Vit D/multivitamin with Vit D	695 (18.2%)	103
Years since menopause		
Less than 10 years	677 (17.8%)	
10-19 years	1195 (31.3%)	98
20-29 years	1050 (27.5%)	
30 or more years	892 (23.4%)	
Falls in previous 12 months		
None	2394 (61.9%)	
Once	902 (23.3%)	44
2 times or more	572 (14.8%)	
Fracture since 45 years	763 (20.5%)	182
Family history of hip fracture	490 (14.3%)	489
FRAX 10-year probability (MOF)[†]	10.9 (7.3, 17.6)	1359
FRAX 10-year probability (hip fracture)[†]	2.2 (1.1, 5.9)	1359
Osteoporosis	413 (10.9%)	138
Number of comorbidities		
0	840 (24.9%)	
1	1002 (29.7%)	
2	826 (24.5%)	543
3	445 (13.2%)	
4 or more	256 (7.6%)	

*Mean (SD); Median (lower quartile, upper quartile); MOF: Major osteoporotic fracture

Participant characteristics for the subgroup analysis sample (n=492) who underwent bone assessments are presented in Table 2. Mean (SD) age at scan was 70.9 (5.4) years respectively, resulting in a median (lower quartile, upper quartile) follow-up time of 7.5 (7.1, 8.9) years. Overall, 283 (57.5%) reported a similar SPR compared to other women of the same age; 140 (28.5%) reported a lower risk and 69 (14.0%) reported a higher risk

Table 2 Baseline characteristics of subgroup who participated in bone phenotyping study (n= 492).

Participant characteristic	N(%)	Non-missing values
Age of SPR ascertainment*	63.0 (5.4)	492
Age at scan (years)*	70.9 (5.4)	489
Height (cm)*	160.3 (6.2)	482
Weight (kg)*	68.7 (12.7)	482
BMI (kg/m^2)*	26.8 (5.0)	482
Whole body total aBMD (g/cm^2)*	1.01 (0.10)	410
Total hip aBMD (g/cm^2)*	0.84 (0.11)	464
Femoral neck aBMD (g/cm^2)*	0.69 (0.10)	457
Total lumbar spine aBMD (g/cm^2)*	0.92 (0.15)	471
Any fracture since 45 years	69 (14.4%)	478
Family history of hip fracture	63 (14.3%)	442
SPR compared to others		
Much lower	42 (8.5%)	
A little lower	98 (19.9%)	
About the same	283 (57.5%)	492
A little higher	58 (11.8%)	
Much higher	11 (2.2%)	
How active compared to others		
Not at all	7 (1.4%)	
A little	69 (14.2%)	487
Somewhat	241 (49.5%)	
Very	170 (34.9%)	
Current smoker	28 (5.7%)	487
Alcoholic drinks per week:		
None	101 (20.6%)	
1-6	208 (42.4%)	
7-13	131 (26.7%)	490
14-20	39 (8.0%)	
>20	11 (2.2%)	
Education:		
Below GCSE	120 (24.4%)	
CSE O level / GCSE	170 (34.6%)	492
A Level	61 (12.4%)	
Degree	141 (28.7%)	
Use of anti-osteoporotic medication	31 (6.5%)	478
Currently taking calcium	101 (20.9%)	484
Currently taking Vit D / multivitamin with Vit D	112 (23.2%)	482
Ever used oestrogen / hormone replacement therapy	238 (48.6%)	490
Years since last menstrual cycle:		
<10	153 (31.9%)	
10-19	212 (44.3%)	479
20-29	89 (18.6%)	
>29	25 (5.2%)	

*Mean (SD)

SPR: Self-perceived fracture risk; DXA: Dual-energy X-ray absorptiometry; aBMD: Areal bone mineral density;

Associations between baseline participant characteristics and SPR

Cross-sectional associations between baseline participant characteristics and SPR are presented in Table 3. In univariate analyses, the following were associated ($p<0.05$) with higher SPR: shorter self-reported height; lower alcohol consumption, physical activity and educational attainment; current use of AOM and calcium supplements; longer time since menopause; greater number of falls in the previous 12 months; history of fracture since aged 45 years; family history of hip fracture; higher FRAX scores for MOF and hip fracture; and increased comorbidity. Apart from associations regarding self-reported height and alcohol consumption, all were significant ($p<0.05$) in mutually-adjusted analysis (FRAX variables were not included in the mutually-adjusted model); however, the direction was reversed for time since menopause such that greater time was associated with reduced SPR.

In sensitivity analyses among participants with osteoporosis, currently taking AOM and with either of these conditions, many associations were not significant, perhaps due to the reduction in sample size. However, the following characteristics associated with SPR in the main analysis were also significant ($p<0.05$) or had a trend towards significance ($p\leq0.071$) in sensitivity analyses (Supplementary Table 1): physical activity; currently taking calcium; and having a fracture since 45

Table 3: Odds ratios for having a higher category of self-perceived fracture risk for the presence versus absence of each characteristic.

Characteristic	Univariate		Mutually-adjusted	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age*	1.02 (0.96,1.08)	0.584		
Self-reported height*	**0.92 (0.86,0.97)**	**0.006**	0.95 (0.87,1.02)	0.152
Weight-for-height residual*	1.02 (0.96,1.09)	0.527		
Current smoker	1.11 (0.88,1.41)	0.388		
Alcohol consumption**	**0.94 (0.88,1.00)**	**0.049**	1.04 (0.95,1.13)	0.399
Physically active compared to others of similar age**	**0.52 (0.48,0.57)**	**<0.001**	**0.52 (0.47,0.58)**	**<0.001**
Educational attainment**	**0.90 (0.85,0.95)**	**<0.001**	**0.87 (0.81,0.93)**	**<0.001**
Current use of anti-osteoporotic medication	**8.99 (7.15,11.29)**	**<0.001**	**6.10 (4.48,8.32)**	**<0.001**
Ever used oestrogen/hormone replacement therapy	1.06 (0.94,1.21)	0.345		
Currently taking calcium supplements	**3.04 (2.58,3.59)**	**<0.001**	**1.64 (1.32,2.03)**	**<0.001**
Currently taking Vit D/multivitamin with Vit D	1.09 (0.93,1.28)	0.300		
Years since menopause**	**1.06 (1.00,1.13)**	**0.040**	**0.82 (0.76,0.89)**	**<0.001**
Falls in previous 12 months**	**1.44 (1.32,1.57)**	**<0.001**	**1.23 (1.10,1.37)**	**<0.001**
Fracture since 45 years	**3.49 (2.96,4.12)**	**<0.001**	**2.63 (2.13,3.24)**	**<0.001**
Family history of hip fracture	**1.34 (1.12,1.62)**	**0.002**	**1.40 (1.13,1.74)**	**0.002**
FRAX 10-year probability (MOF)*	**1.26 (1.16,1.36)**	**<0.001**		
FRAX 10-year probability (hip fracture)*	**1.18 (1.09,1.27)**	**<0.001**		
Number of comorbidities**	**1.20 (1.13,1.27)**	**<0.001**	**1.08 (1.01,1.15)**	**0.033**

Ordinal logistic regression models were used with the 5-level variable for self-perceived fracture risk as the outcome

*Odds ratio per standard deviation increase   **Odds ratio per higher category of characteristic

Supplementary Table 1: Odds ratios (OR) for having a higher category of self-perceived fracture risk for the presence versus absence of each characteristic among participants with an osteoporosis diagnoses and currently using anti-osteoporotic medications (AOM).

Characteristic	Osteoporosis diagnosis OR (95% CI)	P-value	Using AOM OR (95% CI)	P-value	Osteoporosis or using AOM OR (95% CI)	P-value
Age*	0.91 (0.75,1.11)	0.350	0.78 (0.63,0.97)	0.022	0.88 (0.74,1.04)	0.136
Self-reported height*	0.98 (0.81,1.19)	0.875	1.01 (0.82,1.23)	0.952	0.96 (0.81,1.13)	0.588
Weight-for-height residual*	0.89 (0.74,1.06)	0.189	0.86 (0.70,1.05)	0.140	0.90 (0.77,1.06)	0.220
Current smoker	0.95 (0.48,1.90)	0.887	1.89 (0.77,4.64)	0.165	1.01 (0.53,1.90)	0.981
Alcohol consumption**	1.07 (0.87,1.31)	0.545	0.93 (0.75,1.14)	0.474	0.95 (0.80,1.13)	0.579
Physically active compared to others of similar age**	0.70 (0.56,0.86)	0.001	0.67 (0.53,0.84)	0.001	0.67 (0.56,0.81)	<0.001
Educational attainment**	1.06 (0.90,1.25)	0.465	1.09 (0.92,1.30)	0.323	1.09 (0.94,1.26)	0.249
Current use of anti-osteoporotic medication	2.60 (1.79,3.79)	<0.001	N/A	N/A	N/A	N/A
Ever used oestrogen/hormone replacement therapy	1.41 (0.96,2.09)	0.083	1.49 (0.98,2.27)	0.065	1.35 (0.96,1.89)	0.086
Currently taking calcium supplements	1.48 (1.02,2.13)	0.038	1.49 (0.98,2.27)	0.062	1.42 (1.03,1.95)	0.033
Currently taking Vit D/multivitamin with Vit D	1.57 (1.04,2.36)	0.032	1.49 (0.95,2.32)	0.080	1.48 (1.03,2.13)	0.033
Years since menopause**	0.96 (0.79,1.17)	0.682	0.99 (0.80,1.22)	0.908	0.99 (0.83,1.17)	0.895
Falls in previous 12 months**	1.10 (0.89,1.36)	0.370	1.10 (0.86,1.39)	0.455	1.19 (0.99,1.44)	0.066
Fracture since 45 years	1.85 (1.28,2.66)	0.001	1.43 (0.97,2.12)	0.071	1.79 (1.30,2.47)	<0.001
Family history of hip fracture	1.09 (0.68,1.77)	0.717	1.03 (0.61,1.73)	0.922	1.18 (0.77,1.82)	0.453
FRAX 10-year probability (MOF)*	1.16 (0.91,1.47)	0.227	0.98 (0.77,1.25)	0.879	1.08 (0.88,1.32)	0.466
FRAX 10-year probability (hip fracture)*	1.11 (0.87,1.41)	0.420	0.92 (0.72,1.18)	0.518	1.02 (0.83,1.26)	0.858
Number of comorbidities**	0.89 (0.76,1.04)	0.129	1.00 (0.84,1.19)	0.965	0.94 (0.82,1.08)	0.396

Ordinal logistic regression models were used with the 5-level variable for self-perceived fracture risk as the outcome. All characteristics were ascertained at baseline
*Odds ratio per standard deviation increase   **Odds ratio per higher category of characteristic

Cluster analysis of participant characteristics

The four-cluster solution was optimal according to the BIC criterion; the number of participants in each cluster ranged from 459-904.

Descriptive statistics for the participant characteristics according to each cluster are shown in Table 4. Compared to the other clusters, Cluster 1 had a greater proportion of women with the following characteristics: current use of AOM (35.4% vs ≤4.5% in other clusters), calcium supplements (97.3% vs ≤1.1%) and Vitamin D supplements (51.5% vs ≤13.1%); and a fracture since age 45 years (33.7% vs ≤27.5%). Although not used in the cluster analysis algorithm, the proportion with higher SPR was also much higher in Cluster 1 (32.9%) compared to other clusters (≤11.2%).

The silhouette coefficient of 0.1 indicated that the clustering was not substantial. However, the results show that higher SPR and the risk factors for this variable tend to cluster together.

Table 4: Participant characteristics according to each cluster.

Participant characteristic	Cluster1 (n=489)	Cluster2 (n=904)	Cluster3 (n=730)	Cluster4 (n=459)
Age (years)*	69.3 (8.6)	65.1 (4.9)	76.0 (7.0)	59.1 (2.9)
Self-reported height (cm)*	161.7 (6.8)	163.0 (6.3)	160.3 (6.5)	163.7 (6.0)
Self-reported weight (kg)*	64.8 (10.9)	70.0 (12.7)	68.4 (13.2)	69.0 (13.0)
BMI(kg/m^2)*	24.8 (4.0)	26.4 (4.6)	26.6 (5.0)	25.7 (4.7)
Self-perceived fracture risk				
Lower	96 (19.6%)	284 (31.4%)	217 (29.7%)	149 (32.5%)
Similar	232 (47.4%)	538 (59.5%)	431 (59.0%)	276 (60.1%)
Higher	161 (32.9%)	82 (9.1%)	82 (11.2%)	34 (7.4%)
Current smoker	22 (4.5%)	59 (6.5%)	41 (5.6%)	27 (5.9%)
Alcohol consumption				
None	131 (26.8%)	212 (23.5%)	322 (44.1%)	84 (18.3%)
1-6	197 (40.3%)	410 (45.4%)	285 (39.0%)	195 (42.5%)
7-13	129 (26.4%)	205 (22.7%)	96 (13.2%)	131 (28.5%)
14-20	30 (6.1%)	66 (7.3%)	23 (3.2%)	41 (8.9%)
>20	2 (0.4%)	11 (1.2%)	4 (0.5%)	8 (1.7%)
Physically active compared to others				
Not at all	19 (3.9%)	12 (1.3%)	31 (4.2%)	9 (2.0%)
A little	75 (15.3%)	113 (12.5%)	173 (23.7%)	68 (14.8%)
Somewhat	257 (52.6%)	495 (54.8%)	322 (44.1%)	233 (50.8%)
Very	138 (28.2%)	284 (31.4%)	204 (27.9%)	149 (32.5%)
Educational attainment				
Below GCSE	156 (31.9%)	222 (24.6%)	427 (58.5%)	84 (18.3%)
GCSE	158 (32.3%)	356 (39.4%)	164 (22.5%)	169 (36.8%)
A-level	80 (16.4%)	142 (15.7%)	81 (11.1%)	72 (15.7%)
Degree	95 (19.4%)	184 (20.4%)	58 (7.9%)	134 (29.2%)
Current use of AOM	173 (35.4%)	16 (1.8%)	33 (4.5%)	4 (0.9%)
Ever used oestrogen/HRT	185 (37.8%)	472 (52.2%)	111 (15.2%)	176 (38.3%)
Currently taking calcium	476 (97.3%)	0 (0.0%)	8 (1.1%)	0 (0.0%)
Currently taking Vit D	252 (51.5%)	118 (13.1%)	71 (9.7%)	57 (12.4%)
Years since menopause				
Less than 10 years	71 (14.5%)	0 (0.0%)	0 (0.0%)	459 (100.0%)
10-19 years	162 (33.1%)	685 (75.8%)	12 (1.6%)	0 (0.0%)
20-29 years	141 (28.8%)	217 (24.0%)	326 (44.7%)	0 (0.0%)
30 or more years	115 (23.5%)	2 (0.2%)	392 (53.7%)	0 (0.0%)
Falls in previous 12 months				
None	288 (58.9%)	608 (67.3%)	435 (59.6%)	321 (69.9%)
Once	125 (25.6%)	206 (22.8%)	185 (25.3%)	78 (17.0%)
2 times or more	76 (15.5%)	90 (10.0%)	110 (15.1%)	60 (13.1%)
Fracture since 45 years	165 (33.7%)	90 (10.0%)	201 (27.5%)	31 (6.8%)
Family history of hip fracture	84 (17.2%)	130 (14.4%)	83 (11.4%)	74 (16.1%)
Number of comorbidities				
0	124 (25.4%)	271 (30.0%)	100 (13.7%)	167 (36.4%)
1	144 (29.4%)	319 (35.3%)	171 (23.4%)	152 (33.1%)
2	110 (22.5%)	207 (22.9%)	229 (31.4%)	93 (20.3%)
3	72 (14.7%)	88 (9.7%)	127 (17.4%)	32 (7.0%)
4+	39 (8.0%)	19 (2.1%)	103 (14.1%)	15 (3.3%)

*Mean (SD)

The cluster analysis was restricted to participants with complete data for all variables that were used in the cluster analysis algorithm (n=2582)

BMI was derived from self-reported height and weight

Associations between SPR and DXA aBMD parameters

The relationships between SPR and DXA aBMD parameters are presented in Table 5. Higher SPR was associated (p<0.02) with subsequent lower aBMD of the total hip, femoral neck and total lumbar spine in unadjusted analysis; the association regarding femoral neck aBMD was robust to adjustment (p=0.003), whereas for total hip it was reduced by almost 40%. The total hip encompasses the whole of the proximal femur region. In these women, it may be that the adjustment for body size and weight would have a much greater effect on this region of interest than on the femoral neck, which is a defined ROI-size not determined by the size of the bone. Also, whilst the total hip was reduced by 40%, the difference remains, albeit of borderline significance using the arbitrary p<0.05 as the cut-off (p=0.058).

Table 5 Standard deviation difference in mean DXA aBMD parameters (95%CI) per higher band of self-perceived fracture risk at baseline.

Parameter	Unadjusted Estimate (95% CI)	P	Adjusted* Estimate (95%CI)	P
Whole body total	-0.09 (-0.21,0.03)	0.143	-0.09 (-0.22,0.04)	0.172
Total hip	**-0.16 (-0.26,-0.05)**	**0.005**	-0.11 (-0.22,0.00)	0.058
Femoral neck	**-0.18 (-0.29,-0.08)**	**0.001**	**-0.18 (-0.29,-0.06)**	**0.003**
Total lumbar spine	**-0.13 (-0.24,-0.02)**	**0.018**	-0.12 (-0.24,0.00)	0.053

DXA: Dual-energy X-ray absorptiometry; P:P-value; aBMD: Areal bone mineral density
Self-perceived fracture risk was used as an ordinal variable with the following bands: 'much lower'; 'a little lower'; 'about the same'; 'a little higher'; and 'much higher'
*Adjusted for age at time of DXA scan, follow-up time, height, weight-for-height residual, physical activity, smoking status, alcohol consumption, education, time since last menstrual cycle, use of anti-osteoporosis medication, calcium and vitamin D supplements, and oestrogen/hormone replacement therapy (pill/skin patch)
Significant associations (p<0.05) are highlighted in bold

Associations between SPR and radial HR-pQCT parameters

The associations between SPR and radial HR-pQCT parameters are presented in Table 6. Higher SPR was associated with lower trabecular volumetric density and number, and higher trabecular separation in unadjusted and adjusted analysis (p<0.03).

Table 6 Standard deviation difference in mean HR-pQCT parameters (95%CI) per higher band of self-perceived fracture risk at baseline

| HR-pQCT parameter | Unadjusted | | Adjusted* | |
	Estimate (95%CI)	P	Estimate (95%CI)	P
Radius				
Total area	-0.03 (-0.16,0.09)	0.609	0.02 (-0.11,0.15)	0.783
Trabecular area	-0.01 (-0.13,0.12)	0.933	0.04 (-0.09,0.17)	0.526
Cortical area	-0.12 (-0.24,0.01)	0.068	**-0.14 (-0.27,-0.00)**	**0.047**
Cortical thickness	-0.09 (-0.21,0.04)	0.159	-0.12 (-0.26,0.02)	0.090
Cortical volumetric density	-0.02 (-0.14,0.11)	0.767	-0.09 (-0.23,0.04)	0.172
Cortical porosity	-0.09 (-0.21,0.04)	0.176	0.00 (-0.14,0.14)	0.997
Cortical pores diameter	-0.03 (-0.15,0.10)	0.682	-0.01 (-0.16,0.14)	0.906
Trabecular volumetric density	**-0.16 (-0.28,-0.04)**	**0.010**	**-0.16 (-0.31,-0.02)**	**0.027**
Trabecular number	**-0.18 (-0.31,-0.06)**	**0.004**	**-0.19 (-0.33,-0.04)**	**0.010**
Trabecular thickness	-0.04 (-0.17,0.08)	0.499	-0.05 (-0.20,0.10)	0.522
Trabecular separation	**0.18 (0.06,0.30)**	**0.004**	**0.18 (0.04,0.33)**	**0.011**
Tibia				
Total area	-0.01 (-0.12,0.10)	0.852	0.00 (-0.10,0.11)	0.953
Trabecular area	0.02 (-0.09,0.13)	0.745	0.03 (-0.08,0.13)	0.636
Cortical area	**-0.15 (-0.25,-0.04)**	**0.008**	**-0.12 (-0.23,-0.01)**	**0.038**
Cortical thickness	**-0.13 (-0.24,-0.03)**	**0.015**	-0.10 (-0.21,0.02)	0.093
Cortical volumetric density	-0.06 (-0.17,0.05)	0.287	-0.07 (-0.18,0.05)	0.240
Cortical porosity	0.00 (-0.11,0.11)	0.952	0.03 (-0.10,0.15)	0.682
Cortical pores diameter	-0.01 (-0.12,0.10)	0.832	0.02 (-0.11,0.14)	0.791
Trabecular volumetric density	**-0.16 (-0.27,-0.06)**	**0.003**	**-0.14 (-0.26,-0.01)**	**0.036**
Trabecular number	-0.09 (-0.20,0.02)	0.109	**-0.13 (-0.26,-0.01)**	**0.035**
Trabecular thickness	**-0.11 (-0.22,-0.01)**	**0.040**	-0.03 (-0.15,0.10)	0.688
Trabecular separation	0.11 (-0.00,0.22)	0.055	**0.14 (0.02,0.26)**	**0.027**

P: P-value; HR-pQCT: High resolution peripheral quantitative computed tomography
Self-perceived fracture risk was used as an ordinal variable with the following bands: 'much lower'; 'a little lower'; 'about the same'; 'a little higher'; and 'much higher'
*Adjusted for age at time of HR-pQCT scan, follow-up time, height, weight-for-height residual, physical activity, smoking status, alcohol consumption, education, time since last menstrual cycle, use of anti-osteoporosis medication, calcium and vitamin D supplements, and oestrogen/hormone replacement therapy (pill/skin patch)

Significant associations (p<0.05) are highlighted in bold

Associations between SPR and tibial HR-pQCT parameters

The associations between SPR and tibial HR-pQCT parameters are also presented in Table 6. Higher SPR was associated with lower cortical area and thickness as well as lower trabecular volumetric density and thickness in unadjusted analysis (p<0.05); relationships for cortical area and trabecular volumetric density were robust in adjusted analysis (p<0.04). Higher SPR was related to higher trabecular separation in adjusted analysis (p=0.027) and associations before adjustment were borderline significant

(p=0.055). When additionally adjusted for total hip aBMD, no associations regarding radial or tibial HR-pQCT parameters were robust.

Sensitivity analysis

In this subgroup, 69 women had a fracture since age 45 years, 31 were using AOM, 63 had a family history of hip fracture, and 141 women had at least one of these characteristics. These groups have been identified as higher risk and this prior knowledge/experience is likely to increase their SPR score, and may have led to previous BMD testing. We were therefore interested to investigate the associations between higher SPR and aBMD and HR-pQCT parameters in groups where participants with prior fracture, AOM use, family history of hip fracture and any of these three characteristics were excluded (data not shown). When each of these four sets of exclusions were applied, higher SPR was associated with lower femoral neck aBMD in unadjusted and adjusted analysis. When women on AOM at baseline were excluded, higher SPR remained associated with lower radial trabecular number and higher trabecular separation both before and after adjustments. When women with previous fractures were excluded, higher SPR remained associated with lower radial trabecular number and higher trabecular separation in adjusted analyses; relationships were borderline significant when those with a family history of hip fracture were excluded. Higher SPR was related to lower tibial trabecular volumetric density when women with family history of hip fracture were excluded; in the other sets of sensitivity analyses, no other associations regarding tibial parameters were robust in both unadjusted and adjusted analysis. When all three exclusions were applied, no tibial or radial associations were robust.

Discussion

In this study, we have identified personal characteristics associated with self-perception of risk of fracture. A cluster analysis of baseline participant characteristics identified one cluster, in which higher SPR, prior history of fracture since age of 45, current use of AOM, vitamin D and calcium supplementation clustered together. Hence this seems to identify women who, through prior fracture experience, have initiated and remained on therapy and acknowledge their higher fracture risk. However, despite greater use of anti-osteoporosis medications, a higher SPR was still related to impaired bone density

and microarchitecture measured a median of 7.5 years later. Associations were similar even when separately excluding the following groups of participants: previously experienced a fracture since age 45; reported a family history of hip fracture; and taking AOM. Although associations regarding tibial and radial HR-pQCT parameters were attenuated when participants with any of these three characteristics were excluded, this could have been due to the reduction in sample size and robust associations between higher SPR and lower femoral neck aBMD remained after these exclusions.

To our knowledge this is the first time that associations between SPR and DXA aBMD and HR-pQCT parameters among postmenopausal women have been examined, and suggests that women can correctly identify personal factors associated with heightened osteoporosis risk, but despite uptake of AOM, that risk remains elevated at around 7.5 years later. Findings from this study demonstrated that higher SPR bands are related to decrease in areal BMD at femoral neck and lower tibial trabecular volumetric density. There is evidence to suggest that 1SD decrease in BMD is associated with a 1.5-3-fold times higher fracture risk [113]. Our data suggests that it is likely that there will be increased fracture risk in women with higher SPR as they continue to lose bone and age.

There are limitations to our study. These are observational data that demonstrate associations, but not causality, and need to be tested in other populations. Secondly, the SPR questionnaire has not been validated. Finally, there is no information available if the participants had a DXA scan performed prior. Women who have had a prior fracture had or took bone-specific treatment may well have had a DXA scan. It would not be unexpected that those participants rated their SPR as higher compared to other women of the same age. Those women were likely to integrate the bone protective behaviour and measures into their daily life resulting in a 'self-fulfilling prophesy'. However, even if it is taken into account, we still observed lower aBMD and less favourable HR-pQCT parameters around 7 years later in this group. In many ways, this group represent the 'best case' scenario of osteoporosis care in that women have been identified as osteoporotic, recognise this diagnosis and remain on therapy to counteract this risk. The situation in many clinical cases may be much worse. Longer follow up of this group could be highly beneficial.

In the current study, the higher SPR was associated with higher FRAX scores for MOF and hip fracture. However, SPR of osteoporosis and osteoporotic fractures has been reported to be underestimated in postmenopausal women worldwide. Rothmann et al observed that women participating in the Risk-Stratified Osteoporosis Strategy Evaluation (ROSE) study underestimated their fracture risk compared to the risk estimated by FRAX [208]. Similarly, findings from GLOW showed that women at increased fracture risk generally perceive their risk to be lower or about the same as women of the same age [207,232]. Furthermore, it was previously demonstrated in GLOW that SPR of fracture does capture some aspect of fracture risk not currently measured using the conventional fracture prediction tool FRAX [221] The perception of personal risk has been shown to modify an individual's behaviour related to their bone health [189,221]. Heightened self-perceived risks of osteoporosis and fracture significantly increases the likelihood of seeking medical advice hence, increasing the chances, in appropriate individuals, of being given a diagnosis of osteoporosis – a well-known predictor of treatment initiation [189]. Moreover, heightened self-perceived risks of fracture is known to be associated with BMD testing.

Although the positive effect of risk perception on BMD testing has been previously described, the analysis of the relationship between the results of bone microarchitecture parameters and fracture risk perception is novel. There is evidence that other factors independent of aBMD, including skeletal properties of trabecular microstructure examined by HR-pQCT, contribute to fracture risk [138,143,143]. This study suggests that there is association between SPR and bone microarchitecture. Taking osteoporosis medications was strongly associated with a higher self-perceived fracture risk in this study. This concurs with findings from a cross-sectional analysis of GLOW where women with higher SPR were more likely to report AOM use than women with lower SPR [221]. Barcenilla-Wong et al. also reported that elevated self-perceived risk of fracture increases the likelihood of taking AOM prospectively [189].

In conclusion, we have identified individual characteristics correlated with higher SPR, considered how they cluster together and studied relationships between SPR and subsequent objectively assessed bone health. This is particularly notable as previous research has suggested that while women often underestimate fracture risk, a higher SPR is associated with health seeking behaviour and better compliance with OP

medication, as we observed in this study. An exploration of SPR through further studies, including qualitative work, may allow development of novel fracture prediction methods, and strategies to reduce fracture risk.

5. Discussion

5.1 Main findings

The research underpinning this thesis is sited in the GLOW study and has three key elements: an investigation of how bone microarchitecture (and certain aspects of that microarchitecture that might cluster together), as assessed by HR-pQCT, relate to fracture risk; the relationship between adiposity and SPR to bone microarchitecture, and finally an investigation of whether SPR relates to uptake and persistence of anti-osteoporosis medication not only in the UK cohort but the study population as a whole.

We have attempted to replicate previous reports that certain bone microarchitecture clusters associate with heightened fracture risk. We demonstrated that microstructural parameters of the bone evaluated by HR-pQCT are different between healthy participants and fracture participants at skeletal regions containing predominantly trabecular bone. In a subsequent cluster analysis, higher fracture risk and lower trabecular density and number, and consequently higher trabecular separation clustered together to predict fracture

We have examined the relationships of bone microarchitecture with adiposity. We found that in postmenopausal women there were significant trends in HR-pQCT parameters suggesting favourable bone microarchitecture at both radius and tibia with an increase of BMI category. However, there appeared to be a less favourable tibial profile among women with morbid obesity. There were different FMI patterns at the radius and tibia. At the radius FMI residuals were associated with parameters of bone size and trabecular architecture, whereas at the tibia, FMI residuals were associated with the cortical compartment parameters, bone size and trabecular architecture.

We investigated the determinants of SPR, and relationships between SPR and subsequent bone density and microarchitecture. We have identified the following personal characteristics associated with self-perception of risk of fracture: lower physical activity and educational attainment; use of AOM and calcium supplements; greater number of falls in the previous year; history of fracture since aged 45; family history of hip fracture; and increased comorbidity. We have shown that women can correctly identify personal factors associated with heightened osteoporosis risk, but

despite uptake of AOM, that risk remains elevated at around 7.5 years later. Findings from this study demonstrated that higher SPR bands are related to a decrease in areal BMD at the femoral neck and lower tibial trabecular volumetric density, despite higher use of AOM.

Finally, we investigated whether SPR is associated with incident fracture independent of traditional fracture risk prediction tools and assessed if SPR is related to uptake and persistence of AOM in the cohort as a whole. Within each SPR category, risk of fracture increased as the FRAX categorisation of risk increased. We demonstrated that SPR of fracture does capture some aspect of fracture risk not currently measured using the conventional fracture prediction tool FRAX. In GLOW only 11% of women with a lower baseline SPR were taking AOM at baseline, compared with 46% of women with a higher SPR. AOM use tended to increase in the years after a reported fracture. However, women with lower SPR who fractured still reported lower AOM rates than women with or without a fracture but a higher SPR.

Each of the main findings will be discussed separately below.

5.2 Relationships between bone microarchitecture and fracture

This study demonstrated that microstructural parameters of the bone evaluated by HR-pQCT are different between healthy participants and fracture participants at skeletal regions containing predominantly trabecular bone. We showed that various indices of bone microarchitecture of the radius, most notably cortical porosity, trabecular density, trabecular number and trabecular separation, appeared to be compromised among postmenopausal UK women with a previous history of fracture. Our findings are in agreement with previous studies that have investigated the differences in bone microarchitecture by fracture status in postmenopausal women. Differences have been described in many HR-pQCT–derived trabecular parameters between fractured individuals and controls with lower trabecular density, number and thickness in those with a prevalent fracture [126,128,129,133]. In terms of cortical parameters, reduced cortical area, thickness and vBMD were reported in those with a fracture at radius and tibia [126,128–130]. Cortical porosity generally did not differ by fracture status; however, Sundh and colleagues, in studies of women with prevalent hip fracture [131] and in older men with any fracture [132] reported cortical porosity to be higher when compared to controls.

However, in our study, unexpectedly, history of fracture was associated with lower cortical porosity. Fracture cases had higher cortical area, consistent with findings from other cohorts, however, they also had higher cortical vBMD which is probably due to the lower porosity. This observation has now been made in both the Hertfordshire and GLOW cohorts, and warrants further investigation [143].

Similarly prospective studies showed that postmenopausal women with fracture had total vBMD and trabecular vBMD significantly lower at both sites radius and tibia compared those without fracture in all studies [134–138]. Women with incident fractures had significantly lower cortical parameters (area and thickness at the radius [135,136] and tibia [136,137], vBMD at the radius[135] and tibia[136] and trabecular parameters (lower trabecular number at the radius[135,137] and tibia[136,137] and higher trabecular separation at the radius[135] and tibia[136]) compared with control women. A recent meta-analysis [139] reported that fracture associated differences increased with age and were consistently larger in the radius than in the tibia, especially for trabecular measures: Tb. vBMD, trabecular thickness and trabecular number.

5.3   Bone phenotype clustering and fracture risk

HR-pQCT has been utilized in research settings to examine skeletal properties of cortical bone and trabecular microarchitecture, that may contribute to fracture risk. We used statistical cluster analysis, based upon mathematical assumptions to define bone phenotypes taking into account all parameters measured by HR-pQCT [143]. We found one cluster with a significantly higher fracture risk. Individuals in this cluster had lower trabecular density and number, and consequently higher trabecular separation compared to the wider sample. In this cluster, hip aBMD was significantly lower. Our findings are in agreement with the findings from the Hertfordshire Cohort Study (HCS). Edwards and colleagues [143] identified high risk bone phenotype characterised by low trabecular density and number, low hip aBMD, suggesting a consistency of phenotype.

There was one more similar phenotype identified in the cluster analysis in Hertfordshire and GLOW cohorts; this was characterised by higher trabecular area and lower cortical area, thickness and density. In contrast to our findings, Edwards and colleagues reported association with higher fracture risk. As described in the discussion section of our published manuscript participants of the HCS were older, and there were also

differences in phenotype between genders. This observation warrants further investigation in different cohort and confirmation of increased risk of fracture in individuals with high risk bone phenotypes prospectively.

5.4 **Relationship between adiposity and bone microarchitecture**

In this study, we found that in postmenopausal women there were significant trends in HR-pQCT parameters suggesting favourable bone microarchitecture at both radius and tibia with an increase of BMI category. We observed different FMI patterns at the radius and tibia; at the radius FMI residuals were associated with parameters of bone size and trabecular architecture, whereas at the tibia, FMI residuals were associated with the cortical compartment parameters, bone size and trabecular architecture. However, there appeared to be a less favourable tibial profile among women with morbid obesity.

Extensive epidemiological studies have reported that elevated body weight or body mass index are positively associated with aBMD of the lumbar spine, femoral neck, distal radius, proximal femur and leg [127,154–156] and that a decrease in body weight leads to bone loss [157]. Similarly, in our study, an increase of aBMD assessed by DXA was observed with increased BMI. The generally accepted explanation of this relationship is that a larger body mass induces greater mechanical loading on bone, with a consequent increase in aBMD [77,156]. However, the higher aBMD in an obese individual may not be due to similar bone microarchitecture and may not confer greater strength than a lower aBMD in a lighter person.

More recently studies have begun to use HR-pQCT to assess bone microarchitecture in obesity. We found significant increase in cortical area, thickness, cortical volumetric density and trabecular number and decrease in trabecular separation parameters at the radius as BMI category increased. At the tibia there was significant increase in cortical area, thickness, volumetric density and trabecular microarchitecture: trabecular number, trabecular volumetric density and decrease in trabecular separation and thickness parameters as BMI category increased. However, at tibia this pattern was reversed in morbid obesity with a fall in some tibial parameters (compared to participants without hypertension or diabetes who were class 2/3 obese) mainly of the trabecular compartment i.e. trabecular volumetric density (due to lower trabecular number and higher trabecular separation). Sornay-Rendu and colleagues also investigated

associations between obesity and measures of bone micro-architecture in elderly women, but same between different classes of obesity. They reported that higher total vBMD was observed in obesity; due to greater cortical thickness, area and vBMD and greater trabecular vBMD due to greater trabecular number with a lower trabecular separation. At the tibia, positive trend was observed in cortical thickness in obesity, but it was not significant [127]. In the obese group cortical porosity was 21% lower at the tibia, whilst no differences in cortical porosity were observed at the radius. Sornay-Rendu et al. reported greater percentage differences in microarchitectural parameters at the distal tibia compared to the distal radius in the obese group versus the non-obese group. However, the increase of all parameters in obese women was lower relative to the excess of weigh for BMI [127]. The differences in relationships at the radius and tibia may reflect differences due to the weight bearing/non-weight bearing nature of the two sites.

One of possible explanations is that the protective effects of obesity on BMD are due to mechanical loading effects of high body weight on bone. Bone modelling occurs in response to changes in mechanical loading to maintain skeletal competence. The mechanostat hypothesis proposes that strain magnitudes could stimulate bone modelling following tissue deformation in response to mechanical loading thresholds, hence in obesity, bone mass at weight bearing sites would be greater than in non-obese individuals [261].

Apart from contributing to the loading effect of high body weight on the skeleton, adipose tissue is a highly active endocrine organ known to be involved in the production and release of cytokines and other related molecules. Leptin is produced by adipocytes, its level is proportional to the individual's total body fat mass and is therefore elevated in obesity [262]. Osteoblasts and chondrocytes have leptin receptors. Associations between leptin and BMD are complex; leptin may increase osteoblast differentiation and proliferation, and through the RANKL-OPG axis, inhibit osteoclastogenesis [263,264]. In contrast, leptin appears to reduce bone formation and increase resorption when it acts via the central nervous system[265]. Overall, it appears that the peripheral effects predominate, as most studies report positive associations between leptin and aBMD [266–268]. This relationship is particularly marked in postmenopausal women [268]. Adiponectin, like leptin, is produced in fat cells, but levels are inversely related to BMI [269]. It has been shown that there is a negative association between adiponectin levels and

aBMD [268,270]. Furthermore, adiponectin is involved in glucose synthesis in the liver; increasing insulin sensitivity and reducing serum insulin [271]. Adipocytes also produce oestrogen and in postmenopausal women this is the main source. Oestrogen is established to have beneficial effects on bone. Insulin is also found at higher levels in obesity, in some cases leading to insulin resistance. Moreover, 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 [272]. Blood samples were not available in our study to test cytokines levels, though such research would be valuable and could increase our understanding of these complex relationships.

5.5   Relationship between SPR and actual fracture risk.

Several studies suggest under-appreciation of osteoporosis-related fracture risk [197,208,273,274]. In a study sited in a South Australian community the knowledge of osteoporosis risk factors was very limited. Women with one or more risk factors had no increased perception of future osteoporosis risk compared with women having no such risk factor. Moreover, risk was wrongly self-perceived to be higher among younger (age 45 to 54 years) than older (>55) women [275]. In other work, in a community-based study from the Southwestern United States only 16% of women perceived themselves to be at higher risk of osteoporosis compared with 63% who thought their risk was low. Women also held misconceptions about osteoporosis risk and protective factors [197]. Among older (mean age 67.5years) patients with recent fragility fractures an osteoporosis diagnosis was reported in 56 (44%) participants, but only 17% thought their fracture was related to osteoporosis. Fewer than 50% believed they were at increased risk of future fractures [276]. Similarly, Rothmann et al, observed that women participating in the Risk-Stratified Osteoporosis Strategy Evaluation (ROSE) study underestimated their fracture risk compared to the risk estimated by FRAX. Although it demonstrated that women did have some understanding of the importance of some risk factors such as prior fracture, parental history and falls [208].

In GLOW, it has also previously been reported that that people with an increased fracture risk commonly underestimate their actual risk [207]. Among women whose actual risk was increased based on the presence of any one of seven risk factors for fracture, the proportion who recognized their increased risk ranged from 19% for smokers to

39% for current users of glucocorticoid medication. Only approximately 30 % of women with a previous fracture after age 45, the most potent risk factor for future fractures, viewed themselves to be at higher risk for subsequent fractures than their peers, while 21% who had a prior fracture saw themselves as having lower risk [207]. Furthermore, in GLOW cohort disease-specific perception of fracture risk and incident fracture rate was assessed [232]. The findings were in agreement with previous studies showing that postmenopausal women with morbidities tend to under-appreciate their risk; for all morbidities women who perceived their fracture risk to be "much or a little lower" than average actually had increased rates of incident fracture compared with morbidity free women.

We have extended those findings and considered self-perception of fracture risk further, by assessing how well a person's fracture risk perception aligned with fracture probability as assessed by FRAX [221]. Our findings showed that within each SPR category, risk of incident fracture increased as FRAX categorisation of risk increased. In a statistical model both variables FRAX risk and SPR were highly significant (p<0.0001), suggesting that a woman's own perception of fracture risk is an additional predictor of fracture beyond that calculated by FRAX. We have explored increased number of falls, as a potential partial explanation. However, even in our model including falls, SPR remained a significant independent predictor of fracture.

Risk communication is a key element in fracture prevention and greater focus should be on education interventions that may help; a woman's perception of her own risk of fragility fracture should be considered when counselling her regarding management of osteoporosis.

5.6 Relationship between SPR and anti-osteoporosis medications (AOM) use

It is well documented that AOM use among women with osteoporosis or following osteoporotic fracture are low and adherence to medication is low. The problem of low levels of recognition and treatment post fragility fracture has been documented in a systematic review by Elliot-Gibson et al. [273]. Among the 29 reports reviewed on treatment following fracture, rates were variable but generally low; with use of

bisphosphonates treatment range from 0.5% to 38%. It was consistent with findings from a cross-sectional analysis that examined NHANES data from 1999/2000 and 2001/2002 to identify risk factors for fractures and use of anti-resorptive prescription medication in women aged 65 and older [274]. Only 17% of the women in that study with prior fracture were taking specific antiresorptives with an additional 15% on HRT. Similarly, a prospective cohort study of 2,075 women from Quebec contacted 6 to 8 months after their fragility fracture reported that only 15.4% had started pharmacological therapy [277]. Finally, the data from the multinational study involving postmenopausal women from 10 countries demonstrated that only 17% of untreated women report using AOMs after a fracture [255]. Not only initiation of AOM is poor, but also adherence is a major problem, with less than 50% of those starting oral BPs continuing them for more than one year [278].

The consequences of problems with AOM adherence are large numbers of patients with osteoporosis who, without effective treatment and adherence to treatment, continue to be at high risk for fracture and the associated morbidity and mortality [279]. Hence, numerous studies have examined the reasons why patients fail to comply with treatment; the reasons include real or perceived adverse events, complicated dosing regimens and a lack of knowledge surrounding osteoporosis and the importance of fracture prevention [280].

Barcenilla-Wong and colleagues described the association between concern and risk perception to self-reported AOM use [189]. In that study patients were more likely to take AOM at the follow-up assessment with higher self-ratings of fracture risk ("a little higher": OR: 1.99; 95% CI 0.80–4.92; "much higher": OR 5.21; 95% CI 1.77–15.3). In a previous report from GLOW, Gelbach et al found that FRAX variables were generally less robust predictors of medication behaviour than self-perceived fracture risk [257].

The current study showed that SPR was associated with AOM uptake [221]. Women with higher SPR were more likely to report AOM use than women with lower SPR. AOM use was higher in the year after an incident fracture for women in both groups with lower or higher SPR. However, among women with lower SPR who reported an incident fracture, use of AOM was consistently lower than that of women with higher SPR regardless of their fracture status. Findings from this study add to growing body of

evidence that women, who initiate and use AOM appear to be motivated partially by SPR.

5.7   Relationship between correlates of SPR and bone microarchitecture

In this study we reported that higher SPR was associated with lower physical activity and educational attainment; use of anti–osteoporosis medications (AOM) and calcium supplements; greater number of falls in the previous year; history of fracture since aged 45; family history of hip fracture; and increased comorbidity. Women with higher baseline SPR had poorer subsequent bone health. Even after adjustments that included AOM use, higher SPR was associated with lower radial trabecular volumetric density and number, and higher trabecular separation; lower tibial cortical area and trabecular volumetric density. It is the first time that associations between SPR and DXA aBMD and HR-pQCT parameters among postmenopausal women have been examined.

5.7.1.1   Relationship between bone microarchitecture and physical activity and educational attainment

Most of the studies that examined the relationship between education and BMD identified a protective effect of greater education [281,282]. For example Gur et al. [283] identified a protective relationship between education and BMD in comparisons between Turkish women across the four levels of no formal education, elementary education, high school education, and university education. Similarly Ho et al. [284] identified a tertiary level of education was more protective against lower BMD compared with no formal education, a primary school education, or a secondary school education in Chinese women. Little is known of the definitive reasons why educational attainment may influence BMD; the relationship between education and BMD may be related to non-occupational factors that impact health (due to sedentary occupations) such as the greater level of physical activity and nutritional intake of more educated individuals. In our study, we showed the association between educational attainment and SPR, which may influence osteoprotective behaviours in that group.

Exercise is one of the modifiable factors associated with improved bone health outcomes, such as high bone mineral density and strength. Bone mineral density, strength and architecture, change and adapt to help the skeleton to cope with the loading

environment and prevent injuries. High-impact loading exercises are thought to provide the greatest benefit with examples in athletes: Olympic fencers had greater cortical thickness and area and higher trabecular density than matched controls [285], tennis players [286,287] or baseball players [288], are observed to have a greater bone mass in the more active limb. Some studies have also shown an association between weight-bearing physical activity and bone mineral density in middle-aged women and men [289–292]. These studies have reported either a higher BMD in physically active pre- and postmenopausal women compared to inactive women in this same age group or less bone loss in physically active women compared to inactive women [289–291]. A meta-analysis of randomised controlled trials that examined effect of exercise on parameters of bone structure and strength in postmenopausal women found exercise in postmenopausal women decreases bone loss by maintaining cortical and trabecular volumetric BMD [293]. However, more research is still needed to further explore the effects of exercise on bone geometry in that group. In our study higher SPR was associated with lower physical activity. This appears contradictory to what one may expect based on evidence of beneficial effect of exercise on bone health discussed above. Women's' behaviour may be driven by lack of information or other factors (fear of fracture for example); it would be interesting to explore this in more detail through focus groups sessions.

5.7.1.2 Relationship between bone microarchitecture and use of anti–osteoporosis medications (AOM) and calcium supplements

Maintaining a calcium intake of at least 1000-1200 mg/day has long been recommended for older individuals to treat and prevent osteoporosis [1]. The mechanism by which calcium intake affects bone health is by increasing bone mineral density. A systematic review and meta-analysis of randomised controlled trials in older adults (aged >50) found that increasing calcium intake from dietary sources or by taking calcium supplements produces small non-progressive increases in BMD, which are unlikely to lead to a clinically significant reduction in risk of fracture [294]. In contrast AOM, have been repeatedly shown to significantly improve bone density and microstructure [295].

Hence, it is not unexpected that in our study both anti–osteoporosis medications and calcium supplements were found to be associated with higher SPR [221]. However, despite greater use of anti-osteoporosis medications, a higher SPR was still related to impaired

bone density and microarchitecture measured a median of 7.5 years later. Findings from this study demonstrated that higher SPR bands are related to decrease in areal BMD at femoral neck and lower tibial trabecular volumetric density. Our data suggests that it is likely that there will be increased fracture risk in women with higher SPR as they continue to lose bone and age. The relationship between AOM utilisation and poorer trabecular density is likely to be due to reverse causality. In order to be started on a AOM, 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. Clearly, if the AOM 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.

5.8   Limitations and strengths

5.8.1   Study Cohort

An assessment was made to determine whether individuals that took part in the current study differed significantly from those initially recruited to the UK arm of the GLOW study. This was based on demographic, anthropometric and lifestyle data collected at baseline. It was found that individuals that were recruited to the UK arm of the GLOW study at baseline but did not take part in the current study were older and had a higher BMI . 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 Osteoporosis Centre to undergo the required tests. Secondly, those 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 [296]. 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. Indeed, women who took part in the current study had higher educational attainment and lower comorbidity.

This project investigates internal associations and it is not thought that these are likely to be significantly altered by the small differences shown. Similarly physicians at each site who agreed to participate may not be representative of all physicians in an area with respect to osteoporosis recognition and management. However, it must still be taken into consideration when interpreting the study results, in particular with respect to generalisability.

5.8.2 Data from questionnaires

The data collected from questionnaires in the GLOW study are self-reported and conducted by postal questionnaire rather than objectively measured. While this approach is subject to limitations of recall and recall bias, it has the advantages of efficiency and methodological consistency.

Baseline data included self-reported weight, smoking status, alcohol consumption, physical activity, history of fracture, AOM use and comorbidities, but also patient concern of osteoporosis and fracture risk. Both smoking status and alcohol consumption were measured in 2012, are subject to change. As with physical activity, 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. The spectrum of diseases was limited, but did include cardiovascular, respiratory, endocrine, gastroenterology and neurological conditions. The lack of a broader range of diseases is a potential limitation.

The fracture status since age of 45 was ascertained at baseline, and then updated fracture status at each stage of study follow up. As with the demographic and lifestyle factors above, it was self-reported. The accuracy of self-report for fracture is site dependent, with false positive rate at approximately 11% [297]. Although the accuracy of the assessment in the current study is not certain, it is likely to be similar.

AOM use was recorded at each stage of follow up and included a range of available pharmacological treatments for osteoporosis at that time. Consequently, analysis of the treated population was possible with inclusion of those women who start or stop

medications, as well as those who have a high degree of persistence. with the range of information available it was possible to assess for fracture risk factors and risk scores.

5.8.3 Dual energy X-ray absorptiometry

Femoral neck, total hip and total lumbar spine aBMD is used as the current standard of care for the assessment of aBMD using radiological imaging in NHS. In this project, relationships between fracture status and bone microarchitecture were adjusted for total hip aBMD to ascertain whether HR-pQCT might offer additional information above and beyond DXA. As the technique used to scan the areas 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.

The main limitations of body composition assessment by DXA are technical issues. It also relies on several assumptions including that fat has a constant attenuation and that lean mass estimates are not affected by hydration. The scanning bed does have a maximum value for weight, although it did not result in the exclusion of any individuals from this study. However, it can be difficult to position obese participants on the table, with the limited size of the scanning area. It may lead to soft tissue being excluded from the scan. In that situation half body scanning can be used. There are limitations of this method including imperfect positioning of the central line by operator or anatomical differences between the two sides of the body [298].

DXA is as an appropriate technique for the measurement of body composition, as it is simple, widely available, and only associated with a low radiation exposure; and currently remains the gold standard for the assessment of osteopenia and osteoporosis.

5.8.4 High-resolution peripheral quantitative computed tomography

Unlike DXA, HR-pQCT enables the distinction between cortical and trabecular bone compartments, allowing the study of bone microstructure. There is less soft tissue present at distal sites and therefore HR-pQCT is likely to be less affected by soft tissue confounding than DXA, as HR-pQCT enables high resolution imaging of the non-weight bearing distal radius which is a common fracture site and of the weight bearing tibia [64].

However, there are some limitations to this technique. Whilst trabecular number and density are directly measured, the majority of trabecular outcomes are derived, including trabecular thickness and separation [299]. The scanner produces a value for each voxel based on the average density contained within it. Although it is a high – resolution tomography, the partial volume effect may influence the results of image analyses. Defining the cortical and trabecular compartments remain a challenge [300]. The 82μm resolution achieved with the HR-pQCT scanner is close to the thickness of a trabecula. Assessments of cortical porosity are also limited by the resolution of HR-pQCT, with cortical porosity assessments confined to detection of Haversian canals and larger resorption cavities resolvable by HR-pQCT [64]. Accurate segmentation of the cortex from the trabeculae at the transitional zone can be difficult as both operator (delineating the periosteal surface) and software (during the automated analysis) levels ,particularly when the cortex is porous or when cortical thickness is low [64].

Furthermore, movement artefact is a common problem, in particular, measures of microarchitecture are more sensitive to such effects when compared to geometric and density assessments [64]. In this study, in the event of movement on the first image, the scan was repeated. To minimise this issue, we used a cast and aimed to maintain free from disturbance environment. Despite that, there were still some images with significant artefacts that needed to be excluded from the statistical analyses. In general, as in this study, motion artefact tends to be a greater problem at the radius than the tibia.

Despite these limitations, bone microarchitecture as assessed by HR-pQCT correlates well with transiliac bone biopsies [71]. Among imaging modalities, HR-pQCT gives the most detailed *in vivo* assessment of bone microarchitecture and allows to identify the specific structures on which factors, such as body 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 and the distal tibia, as a weight-bearing bone also provides additional value.

5.8.5   Study design

The timeline of variable acquisition is important when attempting to attribute causality in any association. In this study, information on demographic information, medical history, risk factors for osteoporosis-related fracture, perception about fracture risk and

osteoporosis, medication use, health care utilization, physical activity and physical function and p of life were obtained from baseline questionnaire, approximately 7.5 years prior to the collection of DXA and HR-pQCT data. Body composition was collected contemporaneously with bone microarchitecture. Interferences are made based on known biological pathways and a general understanding of human physiology. Sometimes, it is not possible to delineate the direction of causality in this study and this has been taken into consideration when interpreting the findings.

5.9 Implications for clinical practice and future work

In the management of osteoporosis, fracture prediction is of great importance to allow targeting of therapy to those at highest risk. The research suggests that HR-pQCT may provide additional discrimination independent of aBMD for this purpose [140,143]. The current study has demonstrated the pattern of differences in bone microarchitecture by fracture status in women, suggesting that alterations of trabecular architecture are likely to play an important role in skeletal fragility associated with osteoporosis. This approach may have clinical utility in patients where such scans are available, as it allows the incorporation of a large number of variables acquired during a scan to be combined into a bone phenotype that may be more useful for a clinician and patient alike. However, HR-pQCT is an expensive technology, it's not widely available and it is important to consider whether it is feasible that it would be incorporated into clinical practice. Clearly there are implications regarding cost, lack of clinical certification and this may well preclude wide spread use in a general clinical setting at present. However, our findings suggest that it still has utility is specialised centres or in a research setting. Having demonstrated cross-sectional relationships between bone microarchitecture and fracture, the next step would be to assess whether HR-pQCT has the potential to predict fracture prospectively. However, a great deal of further work is required to determine whether it provides a clinically meaningful benefit in fracture prediction in longitudinal studies given the cost and potential barriers to using this technology in a clinical setting.

Furthermore, in this study we have observed a significant trend suggesting favourable bone microarchitecture with increased BMI category in postmenopausal women. However improvements in parameters indicative of better bone health, were not proportional to the increase in body weight; and there also appeared to be a less favourable tibial profile among women with morbid obesity. Understanding better the

relationships between obesity, fat mass and bone microarchitecture, and the impact of comorbidity, may give insights into targeted interventions for prevention of osteoporotic fractures later in life. Further research is required to confirm the mechanisms by which obesity is protective against some types of fracture, and increases the risk of other types of fracture. Whether weight history, duration of obesity or different classes of obesity affect associations between obesity and bone density and structure is unclear and warrants further research.

Moreover, this project provides evidence that not only HR-pQCT, but also SPR in women may add to fracture prediction. We have demonstrated that SPR of fracture does capture some aspect of fracture risk not currently measured using the conventional fracture prediction tool FRAX. It provides an educational challenge, as women at increased fracture risk generally perceive their risk to be lower or about the same as women of the same age [259]. Patient and healthcare provider awareness of individual fracture risk is essential for accurate planning and successful implementation of prevention strategies. We have identified personal characteristics associated with self-perception of risk of fracture. However, further research is required to increase our understanding of what determines the risk in focus groups or a qualitative studies, and develop subsequently educational interventions that may increase the awareness of womens' perception of their own risk of fragility fracture.

Finally, therapeutic options can significantly reduce the risk of osteoporosis-related fractures, but suboptimal use of AOM and low adherence among women who have started AOM are recognised problems This study provides evidence that SPR is associated with self-reported AOM uptake, and women with higher SPR are more likely to report AOM use than women with lower SPR. Reporting findings from a real-life cohort, increases awareness, and ultimately may educate and empower patients. These observations suggest that education interventions may help to improve medication uptake and adherence, and that SPR should be considered when counselling women regarding management of osteoporosis

6. Conclusions

In conclusion, microstructural parameters of the bone evaluated by HR-pQCT appear to be different at skeletal regions containing predominantly trabecular bone between healthy postmenopausal women and those with fracture; with lower trabecular density and number, and higher trabecular separation among those with higher fracture risk. Microstructural parameters of cortical and trabecular microarchitecture change with increased BMI category, at both radius and tibia, the trend suggest favourable results. However at the tibia this pattern is reversed in morbid obesity with less favourable tibial parameters mainly of the trabecular compartment.

Our data suggest that self-reported risk of fracture capture an aspect of fracture risk not currently measured using FRAX, and that greater self-reported risk also translates to improved osteoporosis medication uptake. Furthermore we have identified individual characteristics that correlated with higher SPR, and showed that higher SPR, prior history of fracture since age of 45, current use of AOM, vitamin D and calcium supplementation cluster together. Examination of the relationships between SPR and subsequent objectively assessed bone health by DXA and HR-pQCT revealed that higher SPR bands are related to a decrease in areal BMD at the femoral neck and lower tibial trabecular volumetric density. It may suggest that women who, through prior fracture experience, have initiated and remained on therapy and acknowledge their higher fracture risk. However, despite greater use of AOM, a higher SPR was still related to impaired bone density and microarchitecture.

The main importance of these relationships is the potential to improve fracture prediction and therefore the targeting of therapy, including the possibility of educational interventions to improve AOM uptake and adherence. These findings require further investigation in prospective longitudinal studies of mixed methodology.

7. Appendices

7.1 Appendix 1 – Invitation letter

Re: Global Longitudinal Registry of Osteoporosis in Women

Date as postmark

Dear

Over the last 5 years you have made a valuable contribution to this study by completing study questionnaires regarding your bone health.

We are now inviting participants to attend Southampton General Hospital to have two bone density scans performed; a DXA scan (which you may have had before) and a HRpQCT scan (which is a research scan). If you choose to have these bone scans performed, you will be providing further valuable information that will be used to improve patient care for all women. The two scans together will take about 30 minutes to perform, and do not require you to undress or be in a confined space.
If you do decide to take part we will be able to provide transport to and from the scanner.

We are enclosing an information leaflet for you to read about these scans in more detail. If you choose to participate, simply complete the enclosed reply slip, and return it in the stamped, self-addressed envelope provided. You will then be contacted to arrange an appointment at a time convenient to you.

If you would rather not be contacted again concerning this study, you can return the enclosed form using the prepaid envelope and we will remove your name from the list of potential study participants.

Any information you provide will remain strictly confidential and no reports or publications will ever identify study participants by name. Whether you choose to participate or not, it will have no effect on the medical care you receive at your GP practice. We will forward a copy of your DXA scan result to your own doctor.

Thank you for your help. Please direct any questions about this study to Mrs Janet Cushnaghan, at the MRC Lifecourse Epidemiology Unit, Southampton on 02380777624, or by email at jc@mrc.soton.ac.uk.

Yours sincerely

Dr Michael Moore   Professor Cyrus Cooper

Invitation letter v2 27/8/2013

7.2 Appendix 2 - Participant information sheet

Introduction
You are being invited to take part in an extension to the GLOW 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.

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. It is currently diagnosed by DXA. Recently, a new more detailed scanning technique has been developed called HRpQCT which is high-resolution peripheral quantitative computed tomography. This study will allow us to study how drug treatments and other lifestyle factors impact upon bone health in a 'real life' setting.

Why have I been chosen?
You have been chosen because you have previously helped us with another research study (GLOW). The answers you have given previously would be linked to your scan results.

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 this information sheet to keep and asked to sign a consent

Version 3.0, 09/01/14.

form. You are still free to withdraw at any time without giving a reason.

What will happen to me if I take part?
A researcher will contact you to arrange a mutually convenient time to attend the Osteoporosis Centre at Southampton. We can arrange for a taxi to collect you and bring you to the appointment if necessary. When you attend for your scans, you will be met by one of the research staff. We will then ask you to sign a consent form. Your DXA scan will then be carried out. This involves lying on a firm table whilst an x-ray arm passes over you.

The second test is the high-resolution peripheral quantitative computed tomography (HRpQCT) scan. This scan will involve us carefully positioning your forearm and then your lower leg 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 90 seconds for each limb and will produce three dimensional images of the bones. None of the scans performed would require your head or body to be enclosed within a tunnel.

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 as described above.

Your GP will be informed of your participation following your completion of the study.

What are the possible benefits of taking part?
There are no specific benefits to you in taking part in the study. We hope that the results will forward our understanding of bone health which may be of benefit to patients in the future.

What are the possible disadvantages and risks of taking part?
Along with the DXA which you are having, the HRpQCT will also involve a small dose of radiation (approximately 5μSv). 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 6μSv per day) is all around us in the soil and air. Radiation from the HRpQCT scan used will expose you to the equivalent of approximately one days natural background radiation in Southampton.

What will happen if anything goes wrong?
In the unlikely event that you are harmed by taking part or you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal University complaints mechanisms are open to you.

Please contact Dr Martina Prude - Head of Research Governance, Research Governance Office, Corporate Services, Building 37, Room 4055, University of Southampton, University Road, Highfield, Southampton, SO17 1BJ. Telephone: 02380 595058. Alternatively you could contact Patient support services: 023 8077 7222 ext. 6325 or email PatientSupportServices@uhs.nhs.uk or the Independent Complaints and Advocacy Service (ICAS) on 0300 456 2370.

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.

What will happen to the study results?
The results of your DXA scan will be sent out to your doctor, but not your HRpQCT, this is because researchers are still deciding how best to use results of HRpQCT to treat patients. The overall results of the study 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 Lifecourse Epidemiology Unit website. We will be happy to discuss the results with you when the study is completed, and will be able to let you know where you can obtain a copy of the published results.

Will I be reimbursed for my time?
There will be no reimbursement for time.

Version 3.0, 09/01/14.

Contact for further information
If you have any further questions then please contact Mrs Janet Cushnaghan at the MRC Lifecourse Epidemiology Unit by telephone 02380777624 or email jc@mrc.soton.ac.uk

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.

University Hospital Southampton **NHS**
NHS Foundation Trust

Study Title:

Global **L**ongitudinal Registry of **O**steoporosis in **W**omen

Participant Information Sheet

Study Site:
University Hospital Southampton
Tremona Road
Southampton
SO16 6YD

Coordinating Site:
MRC Lifecourse Epidemiology Unit

Telephone: 02380 777624
Fax: 02380 704021
http://www.mrc.soton.ac.uk

7.3 Appendix 3 - Informed consent

Global Longitudinal Registry of Osteoporosis in Women

CONSENT FORM

Name of Lead Investigator: Dr Michael Moore

Please initial boxes.

1. I confirm that I have read and understand the information sheet dated 09/01/2014 (version 3.0) 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 receiving only my DXA scan results.

4. I agree to take part in the above study.

5. 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   Date   Signature
(Please print)

_____ _____ _____
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 notes.

7.4 Appendix 4 - Baseline questionnaire

Bone Health Survey
United Kingdom Version 1.0

Required
SLUGID (7)

No selection = blank
Single choice = checked = 1
Multichoice = unchecked = 0
Field name = unchecked = blank

(s) = single choice
(m) = multi choice

Study ID
Required

STUDYID (13)

Survey Instructions

- Your participation in this study is voluntary. If you come across a question you would rather not answer, go on to the next question.

- Answer all the questions by filling in the circle to the left of your answer.

- Please fill in circles completely: Correct: ●   Incorrect: ⊠ ⌀

- Please print neatly within the boxes: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 |

- You are sometimes asked to skip over some questions in this survey. When this happens you will see an arrow with a note that tells you what question to answer next, like this:

  ○ Yes → **If Yes, go to question 1**
  ○ No

Confidential: No information shall be presented or published in any way that would permit identification of any individual.

---

1. In thinking about your health, how concerned are you about osteoporosis?

1 ○ Very concerned   OSTEOCONCERN(s)

2 ○ Somewhat concerned

3 ○ Not at all concerned

2. In the last 12 months, have you talked to a doctor or other health provider about osteoporosis, including things like testing, treatment, or prevention?

1 ○ Yes   OSTEOTALK(s)

0 ○ No

3. Has a doctor or other health provider ever told you that you had osteoporosis?

1 ○ Yes → **If Yes, go to question 5 on page 2**

0 ○ No   OSTEOTOLD(s)

4. Has a doctor or other health provider ever told you that you did not have osteoporosis, but had osteopenia, low bone density or were at risk for osteoporosis?

1 ○ Yes   OSTEORISK(s)

0 ○ No

5. How would you rate your own risk of getting osteoporosis compared to other women your age?

1 ○ Much lower   RATEOSTEO(s)

2 ○ A little lower

3 ○ About the same

4 ○ A little higher

5 ○ Much higher

6. How would you rate your own risk of fracturing or breaking a bone compared to other women your age?

1 ○ Much lower   RATEFRACTURE(s)

2 ○ A little lower

3 ○ About the same

4 ○ A little higher

5 ○ Much higher

7. Since you turned 45 years old, have you broken any of the following bones?

	1	0	BROKEN_(s)
Collar bone or clavicle	○ Yes	○ No	CLAVICLE
Upper arm	○ Yes	○ No	ARM
Wrist	○ Yes	○ No	WRIST
Spine	○ Yes	○ No	SPINE
Rib	○ Yes	○ No	RIB
Hip	○ Yes	○ No	HIP
Pelvis	○ Yes	○ No	PELVIS
Ankle	○ Yes	○ No	ANKLE
Upper leg	○ Yes	○ No	UPPERLEG
Lower leg	○ Yes	○ No	LOWERLEG

8. Have you ever had a test to check the strength of your bones, usually called a bone density test?

1 ○ Yes   BONETEST(s)

0 ○ No

3 ○ Don't know

9. In the last 12 months, how many times have you fallen?

0 ○ No times (zero) FALLEN(s)

1 ○ Once

2 ○ 2 times or more

10. Have you ever talked with a doctor or other health provider about things you can do to prevent falls at home, such as adding railings or removing loose rugs?

1 ○ Yes PREVENTFALLS(s)

0 ○ No

11. For each of these drugs, please mark whether you are <u>currently</u> taking, if you have <u>ever</u> taken, or if you have <u>never</u> taken each drug? (The generic name for each drug is listed in parentheses.)

Drug Name	DRUG_(s)	<u>Currently</u> taking	Not taking now but taken in the past	<u>Never</u> taken
		1	2	3
Actonel (risedronate)	ACTONEL	○	○	○
Aredia (pamidronate)	AREDIA	○	○	○
Boniva (ibandronate)	BONIVA	○	○	○
Didronel (etidronate)	DIDRONEL	○	○	○
Evista (raloxifene)	EVISTA	○	○	○
Forteo (teriparatide)	FORTEO	○	○	○
Fosamax (alendronate)	FOSAMAX	○	○	○
Livial (tibolone)	LIVIAL	○	○	○
Miacalcin (calcitonin)	MIACALCIN	○	○	○
Protelos (strontium ranelate)	PROTELOS	○	○	○
Zometa (zoledronic acid)	ZOMETA	○	○	○
Calcium supplements	CALCIUM	○	○	○
Rocaltrol (calcitriol)	ROCALTROL	○	○	○
Vitamin D or multi-vitamin with Vitamin D	VITAMIND	○	○	○
Cortisone or Prednisone	CORTISONE	○	○	○
Depo Provera	DEPO	○	○	○
Estrogen or hormone replacement (pill or skin patch)	ESTROGEN	○	○	○
Arimidex (anastrozole)	ARIMIDEX	○	○	○
Aromasin (exemestane)	AROMASIN	○	○	○
Femara (letrozole)	FEMARA	○	○	○
Tamoxifen	TAMOXIFEN	○	○	○

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12. Did your mother have osteoporosis?

1 ○ Yes   MOMOSTEO(s)

0 ○ No

3 ○ Don't know

13. Did your mother ever break or fracture her hip?

1 ○ Yes   MOMFRACTURE(s)

0 ○ No

3 ○ Don't know

14. Did your father ever break or fracture his hip?

1 ○ Yes   DADFRACTURE(s)

0 ○ No

3 ○ Don't know

Now we would like to ask you some questions about your general health.

15. In the last 12 months, how many times did you visit a doctor or other health care provider to get medical care for yourself?

0 ○ No times (none)   VISIT(s)

1 ○ 1 - 2 times

2 ○ 3 - 5 times

3 ○ 6 or more times

16. In the last 12 months, how many times did you stay at least one night in the hospital?

0 ○ No times (none)   STAY(s)

1 ○ 1 time

2 ○ 2 times

3 ○ 3 or more times

17. In thinking about your health, how concerned are you about high blood pressure or hypertension?

1 ○ Very concerned   HYPERCONCERN(s)

2 ○ Somewhat concerned

3 ○ Not at all concerned
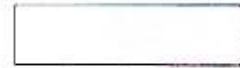
18. In the last 12 months, have you talked to a doctor or other health provider about high blood pressure or hypertension, including things like testing, treatment, or prevention?

1 ○ Yes   HYPERTALK(s)

0 ○ No

19. Has a doctor or other health provider ever told you that you had high blood pressure or hypertension?

1 ○ Yes   HYPERTOLD(s)

0 ○ No

20. In thinking about your health, how concerned are you about heart disease?

1 ○ Very concerned   HEARTCONCERN(s)

2 ○ Somewhat concerned

3 ○ Not at all concerned

21. In the last 12 months, have you talked to a doctor or other health provider about heart disease, including things like testing, treatment, or prevention?

1 ○ Yes   HEARTTALK(s)

0 ○ No

22. Has a doctor or other health provider ever told you that you had heart disease?

1 ○ Yes   HEARTTOLD(s)

0 ○ No

23. In thinking about your health, how concerned are you about high cholesterol?

1 ○ Very concerned   CHOLESCONCERN(s)

2 ○ Somewhat concerned

3 ○ Not at all concerned

24. In the last 12 months, have you talked to a doctor or other health provider about high cholesterol, including things like testing, treatment, or prevention?

1 ○ Yes   CHOLESRISK(s)

0 ○ No

25. Has a doctor or other health provider ever told you that you had high cholesterol?

1 ○ Yes CHOLESTOLD(s)

0 ○ No

26. How long ago did you have your last menstrual period?

1 ○ Less than 10 years ago LASTMENSTRUAL(s)

2 ○ 10 - 19 years ago

3 ○ 20 - 29 years ago

4 ○ 30 or more years ago

27. Has a doctor or other health provider ever told you that you had any of the following health conditions or problems?

HP_ (s)		1	0
Asthma	ASTHMA	○ Yes	○ No
Chronic bronchitis or emphysema	EMPHYSEMA	○ Yes	○ No
Osteoarthritis or degenerative joint disease	JOINT	○ Yes	○ No
Rheumatoid arthritis	ARTHRITIS	○ Yes	○ No
Stroke	STROKE	○ Yes	○ No
Ulcerative colitis or Crohn's disease	COLITIS	○ Yes	○ No
Celiac disease	CELIAC	○ Yes	○ No
Parkinson's disease	PARKINSON	○ Yes	○ No
Multiple sclerosis	SCLEROSIS	○ Yes	○ No
Cancer	CANCER	○ Yes	○ No
Diabetes (Type-1)	DIABETES	○ Yes	○ No

28. In general, would you say your health is

1 ○ Excellent   GH01(s)
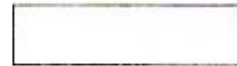
2 ○ Very Good

3 ○ Good

4 ○ Fair

5 ○ Poor

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29. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
	1	2	3
Vigorous activities, such as running, lifting PF01 heavy objects, participating in strenuous sports	○	○	○
Moderate activities, such as moving a table, PF02 pushing a vacuum cleaner, bowling, or playing golf	○	○	○
Lifting or carrying groceries PF03	○	○	○
Climbing **several** flights of stairs PF04	○	○	○
Climbing **one** flight of stairs PF05	○	○	○
Bending, kneeling, or stooping PF06	○	○	○
Walking **more than a kilometer** PF07	○	○	○
Walking **several hundred meters** PF08	○	○	○
Walking **one hundred meters** PF09	○	○	○
Bathing or dressing yourself PF10	○	○	○

30. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time **during the past 4 weeks** . . .

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
	1	2	3	4	5
Did you feel full of life? VT01	○	○	○	○	○
Did you have a lot of energy? VT02	○	○	○	○	○
Did you feel worn out? VT03	○	○	○	○	○
Did you feel tired? VT04	○	○	○	○	○

31. Do you usually need your arms to assist yourself in standing up from a chair?

1 ○ Yes ARMASSIST(s)

0 ○ No

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32. In the past 30 days, not including walking around inside your home, how many days did you walk for <u>at least 20 minutes</u> at a time?

0 ○ 0 days (none)   WALKDAYS(s)
1 ○ 1 - 2 days
2 ○ 3 - 5 days
3 ○ 6 - 9 days
4 ○ 10 - 19 days
5 ○ 20 or more days

33. In general, how physically active are you compared to other women your age?

1 ○ Very active   ACTIVE(s)
2 ○ Somewhat active
3 ○ A little active
0 ○ Not at all

Please mark the box that indicates the statement that best describes <u>your health today</u>.

34. Thinking about mobility, which of the following best describes you?

1 ○ I have no problems in walking about   EQ5D_M(s)
2 ○ I have some problems in walking about
3 ○ I am confined to a bed

35. Thinking about self-care, which of the following best describes you?

1 ○ I have no problems with self-care   EQ5D_S(s)
2 ○ I have some problems washing or dressing myself
3 ○ I am unable to wash or dress myself

36. Thinking about your usual activities (for example: work, study, housework, family or leisure activities), which of the following best describes you?

1 ○ I have no problems performing my usual activities   EQ5D_U(s)
2 ○ I have some problems with performing my usual activities
3 ○ I am unable to perform my usual activities

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37. Thinking about pain/discomfort, which of the following best describes you?

1 ○ I have no pain or discomfort　　　EQ5D_P(s)
2 ○ I have moderate pain or discomfort
3 ○ I have extreme pain or discomfort

38. Thinking about anxiety/depression, which of the following best describes you?

1 ○ I am not anxious or depressed　　　EQ5D_A(s)
2 ○ I am moderately anxious or depressed
3 ○ I am extremely anxious or depressed

39. Do you currently smoke cigarettes?

2 ○ Yes, I smoke every day　　　SMOKE(s)
1 ○ Yes, I smoke some days
0 ○ No, I do not smoke

40. On average, how many drinks do you have each week? (A drink is one bottle or half pint of beer, one glass of wine, or one mixed alcohol drink or measure of spirit)

0 ○ No drinks (none)　　　DRINK(s)
1 ○ Fewer than 7 drinks per week
2 ○ 7 - 13 drinks per week
3 ○ 14 - 20 drinks per week
4 ○ More than 20 drinks per week

41. Do you currently have any private health insurance?

1 ○ Yes　　HEALTHINSURANCE(s)
0 ○ No

169

42. In what month were you born?

1-12

BIRTH_M
Write in numeric format

43. In what year were you born?

1 9 ☐ ☐ BIRTH_Y
Write in year

44. How tall are you now?

TALL_CM
Write in centimeters

or

TALL_FEET
Write in feet

0-11

TALL_INCHES
Write in inches

45. How tall were you at age 25?

TALL25_CM
Write in centimeters

or

TALL25_FEET
Write in feet

0-11

TALL25_INCHES
Write in inches

46. How much do you weigh now?

WEIGH_KG
Write in kilograms

or

WEIGH_ST
Write in stones

WEIGH_LB
Write in pounds

47. In the last 12 months, have you lost 5 or more kilograms (12 lb) without trying?

1 ○ Yes   LOST(s)

0 ○ No

48. What is the highest grade or level of school that you have completed?

1 ○ CSE O level GCSE   EDUCATION(s)

2 ○ A level

3 ○ Degree

49. Today's date is:

1-31   1-12

☐ ☐ ☐ ☐ 2 0 0 ☐   Write in numeric format

day   month   year
TODAY_D TODAY_M TODAY_Y   Not required
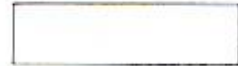
50. Please write here the name of your usual doctor:

_____

**Your assistance in completing this survey is greatly appreciated.
Please return the survey in the enclosed envelope.**

Thank you!

Questions 28-30 are from the SF-36 Health Survey V2.0 Copyright 1994. The Health Institute, New England Medical Center. All rights reserved. Reproduced with permission of the Medical Outcomes Trust.
Questions 34-38 are from the EQ-5D   © Euro QoL 1990

7.5   Appendix 5 - Ethical approval document

NHS
Health Research Authority

NRES Committee London - South East

Bristol Research Ethics Committee Centre
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Whitefriars,
Lewins Mead,
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Tel: (0117) 3421382
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02 January 2014

Dr Michael Moore
Senior Lecturer and Deputy Director of PCRNSW
University of Southampton
Aldermoor Health Centre,Aldermoor Close
Southampton SO16 5ST

Dear Dr Moore

Study title:	**Global Longitudinal Registry of Osteoporosis in Women (GLOW)**
REC reference:	**07/MRE01/30**
Amendment number:	**Substantial Amendment 7; 27/08/2013**
Amendment date:	**18 October 2013**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The sub committee's understanding is that participants in the Southampton arm of the main study will be recruited into another study assessing the diagnostic value of the HRpQCT scan.

The sub-committee understand that Dexa scan remains the gold standard for osteoporosis diagnosis. Both these diagnostic tests will be carried out on participants at 5 years follow up.

The GP will only receive the results of the Dexa scan only since HRpQCT scan is still developmental. The sub-committee is of the view that this is not made clear in the PIS that the HRpQCT scan is still being researched to assess it's diagnostic usefulness and only the results of the Dexa scan will be sent to the participants GP.

The committee requests that the above points are clarified in the PIS and consent forms.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

A Research Ethics Committee established by the Health Research Authority

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMPs): Substantial Amendment 7; 27/08/2013		18 October 2013
Letter of invitation to participant	2	27 August 2013
Participant Information Sheet	2.0	27 August 2013
Participant Consent Form	2.0	27 August 2013
Protocol: GLOW Protocol UK v4		27 August 2013
GP/Consultant Information Sheets	1.0	27 August 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

07/MRE01/30:	Please quote this number on all correspondence

Yours sincerely

Mr Wai Yeung - REC Assistant

pp Professor David Caplin
Chair

E-mail: nrescommittee.london-southeast@nhs.net

Enclosures: *List of names and professions of members who took part in the review*
Copy to: *Dr Martina A Dorward, University of Southampton*

A Research Ethics Committee established by the Health Research Authority

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