Characterisation of Trabecular Bone Structure

Ian H. Parkinson and Nicola L. Fazzalari

Abstract The characterisation of trabecular bone structure has until recently relied on morphometric analysis of histological sections although there is now wide availability of bench top non-destructive X-ray-based imaging with the ability to resolve trabecular elements at resolution on the order of ~ 10 microns. The advent of non-destructive X-ray-based imaging, such as micro-computed tomography (micro-CT) has enabled measurements from image datasets, representing the three-dimensional structure of trabecular bone. Ex vivo studies into trabecular bone structure in osteoporosis have mainly focused on clinically relevant skeletal sites, such as the proximal femur, the distal radius and vertebral bodies. In vivo, the iliac crest and the sternum have been used to obtain material for the diagnosis of metabolic bone diseases including osteoporosis. Metaphyseal bone structure is determined early in development as secondary trabeculae emerge from the primary spongiosa in the epiphyseal plates during endochondral bone growth. After closure of the epiphyseal growth plates at skeletal maturity, bone remodelling becomes the predominant means by which bone is added or removed from the trabecular compartment. From the time of attainment of peak bone mass, studies show that there is a decrease in trabecular bone volume through to older age in both sexes, although not at all sites and not uniformly for males and females. Gender specific changes in trabecular bone are most evident at and after the menopause in females, which is associated with decreased estrogen and associated with reduced androgen production in males. The consequence of

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menopausal or age-related bone loss for females and males, respectively, is a marked increase in fracture incidence, although the changes to the trabecular bone architecture are different between sexes.

1 Introduction

Trabecular bone is found at the end of the long bones of the appendicular skeleton and in the vertebral bodies of the axial skeleton. The bone has a complex, porous spatial arrangement and the spatial complexity contributes to maximal strength for minimum mass for the skeleton as a whole [23]. The high mineral surface area associated with the arrangement of the trabecular bone elements provides a vast substrate on which cellular interaction with bone mineral material can occur.

The characterisation of trabecular bone structure has until recently relied on morphometric analysis of histological sections. While histological sections only provide a two-dimensional "snapshot" of the complex three-dimensional entity, the insights gained from these preparations have been validated and further developed by the data analysis from three-dimensional imaging modalities that have become the methods of choice for studying trabecular bone [12, 27, 75, 78]. The main difference between these methodologies is that of bias in estimating the dimensions of the trabecular bone structure from histological sections [78]. The bias originates from the use of Parfitt's idealized plate and rod model of trabecular bone structure [85]. The destructive nature of histological section preparation has limited the study of bone strength to parallel investigations in cross-sectional studies [2, 19, 20, 24, 28, 34, 37, 70, 78, 86, 89, 98, 104, 106].

There is now wide availability of bench top non-destructive X-ray-based imaging with the ability to resolve trabecular elements at resolution on the order of $\sim$10 microns. The advent of non-destructive X-ray-based imaging, such as micro-computed tomography (micro-CT) has enabled measurements from image datasets, representing the three-dimensional structure of trabecular bone [63, 79, 100, 101]. Subsequent mechanical testing of the same sample has enabled explanatory models of bone strength to be developed, which provide further understanding of the change in trabecular bone structure associated with ageing and disease [6, 64, 102]. These three-dimensional datasets have also been used as input for finite element analysis models to determine apparent mechanical properties of bone [62, 81]. To the present, trabecular bone has been studied at multiple skeletal sites, in all age groups from neonates to the elderly [4, 14, 42, 111]. Advantages given by non-destructive imaging of trabecular bone include the ability to subsequently perform mechanical testing on the bone samples, histological analysis and/or gene expression analysis [6, 105, 106].

Ex vivo studies into trabecular bone structure in osteoporosis have mainly focused on clinically relevant skeletal sites, such as the proximal femur, the distal radius and vertebral bodies [3, 35, 36]. In vivo, the iliac crest and the sternum have been used to obtain material for the diagnosis of metabolic bone diseases including

osteoporosis [69, 70, 89, 91], but ethical issues and the availability of clinical non-invasive imaging have greatly reduced this source of material. Societal sensitivities and government regulation have also restricted the availability of cadaveric material, which has tended to constrain these studies to an older age range thus limiting the clinical relevance of studies [34, 36].

Metaphyseal bone structure is determined early in development as secondary trabeculae emerge from the primary spongiosa in the epiphyseal plates during endochondral bone growth [14, 42]. Modelling of individual bones in childhood and adolescence occurs through periosteal apposition and endosteal resorption to change the size and shape of the cortical shell of the bone and the trabeculae in the trabecular bone compartment; these appeat to adapt in a coordinated manner to maintain the ability to withstand the extant loads [45, 82, 83, 109].

After closure of the epiphyseal growth plates at skeletal maturity, bone remodelling becomes the predominant means by which bone is added or removed from the trabecular compartment [45]. It is now well established that the architecture of trabecular bone is dependent on the forces acting upon it and the high surface area of the bone mineral in the bone tissue marrow facilitates the cellular events, which remove or deposit bone in a highly dynamic environment [45]. Coordination of the cellular events may be at the tissue level for events such as removal of damaged bone [82, 83], at the organ level for modelling due to changed usage pattern, or the whole body level for involutional events such as the menopause in females [90]. The consequences of these scenarios at all size scales can be to decrease the load needed to cause a fracture, where in-built safety margins in load carrying capacity are reduced [8].

From the time of attainment of peak bone mass, studies show that there is a decrease in trabecular bone volume with aging in both sexes [68], although not at all sites and not uniformly for males and females [9]. The specific cause of these age-related changes in individuals is unclear as most studies were cross-sectional in nature making it difficult to track the many factors that influence bone mass, such as the loading history of the individuals. While it is known that trabecular bone architecture changes according to the loading history of the individual, there are other factors, such as nutrition, co-morbidities, social activities and work activities that affect bone metabolism, independent of direct mechanical stimuli [54].

In females, changes in trabecular bone are most evident at and after the menopause, which is associated with decreased estrogen [22]. The greatly increased activation of osteoclasts associated with decreased oestrogen in menopausal females results in an imbalance between resorption and formation with a consequent net bone loss [90]. In males, the reduction in sex-hormone (androgen) production is typically more gradual but is associated with significant net bone loss over time as a consequence of increased resorption relative to formation [55, 103].

The consequence of menopausal or age-related bone loss for females and males, respectively, is a marked increase in fracture incidence, although the changes to the trabecular bone architecture are different between sexes [43, 109]. In general, menopausal females lose trabecular bone through perforation of trabeculae, which are then either completely removed or transformed from plate-like to rod-like

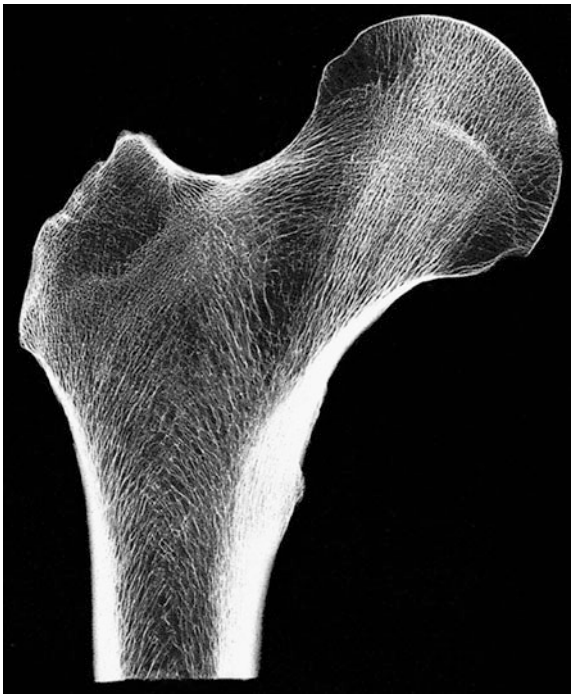

Fig. 1 X-ray image of a sagittal slice from the proximal femur, which shows the complex patterns formed by trabeculae

[1, 70]. In males, age-related bone loss occurs through gradual thinning of trabeculae, where the connectivity of the structure is largely intact but overall the structure has a reduced capacity to withstand load [1, 22, 101]. Similar findings have also been reported in micro-CT studies, which show that the bias in the estimation of the magnitude of trabecular dimensions using histological sections does not mask relative differences between groups [99].

2 Skeletal Distribution of Trabecular Bone: What and Where to Sample

Trabecular bone is found at the end of the medullary cavities of hollow long bones throughout the skeleton (Fig. 1). It forms a trabecular network, of interconnected rod-like and plate-like structures, which are found in greater or lesser proportion depending on the skeletal site [4, 85]. Interestingly, the skeletal sites with the greatest volume of trabecular bone are, in general, the sites where the majority of osteoporotic or fragility fractures occur [52, 53]. Hence, there has been and continues to be a strong focus on understanding the contribution of trabecular bone to mechanical strength of the bone as an organ and in how therapeutic intervention prevents loss of mechanical integrity [6, 10, 71, 94].

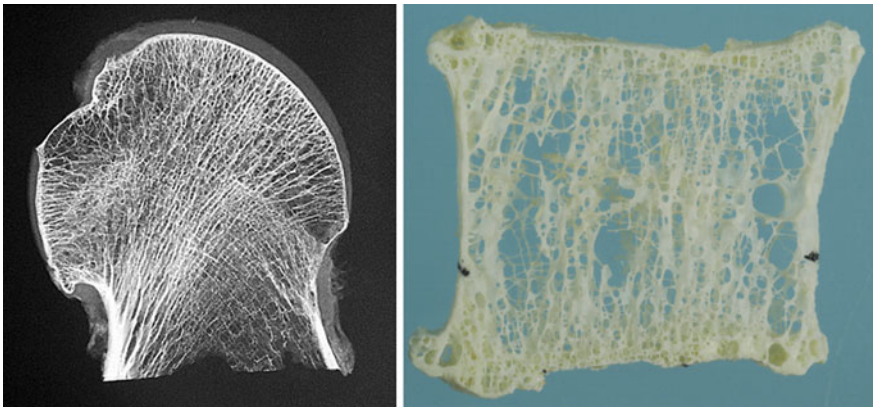

Fig. 2 X-ray image of sagittal femoral head slice (*left*) in contrast to a macerated sagittal slice from a vertebral body (*right*) showing marked differences in the amount of bone and the arrangement of trabeculae

Histological studies have shown, as expected, that there is considerable heterogeneity in the trabecular microstructure at clinically relevant sites [4, 46]. For example, bone volume fraction varies from less than 7% in the centrum of vertebral bodies to more than 25% in regions in the femoral head [3, 35] (Fig. 2). Consequent to this variability in bone volume fraction, the trabecular architecture is highly variable, not just in terms of how plate-like or rod-like it is but in the dimensions of the trabecular elements and how they are arranged in space [46–48]. Eckstein evaluated trabecular microstructure at seven sites (distal radius, L2 vertebra, femoral neck, femoral trochanter, iliac crest, calcaneus) in 79 female and 86 male cadavera, and reported that correlation coefficients between sites were weak to moderate (0.01–0.56). For example, BV/TV at one site explained only 10–32% of the variance of BV/TV at other sites.

The amount of trabecular bone adjacent to joints through which large forces are transmitted suggests that it plays an important role in maintaining the mechanical integrity of the joint complex and in the mechanical dynamics of the body a whole. Optimization of the micro-architecture of the trabecular network in response to extant loads imparts optimal strength with minimal mass [23, 41], with inbuilt safety margins [8]. The large mineral surface area afforded by the complex trabecular network provides a vast substrate on which directed cellular activity can interact with the bone mineral material [13]. This enables bone to be removed or laid down very rapidly in a coordinated manner in response to biomechanical or physiological signals [93].

As stated previously, there is considerable heterogeneity in the distribution of trabecular bone within skeletal sites [4, 98] and between skeletal sites [4]. Therefore, where a sample is taken is extremely important as to its relevance to the experimental questions that can be answered. In relation to studies of osteoporotic bone it is necessary that the samples have a structure that is equivalent to the structure where a fracture may have occurred or is at increased risk of occurring.

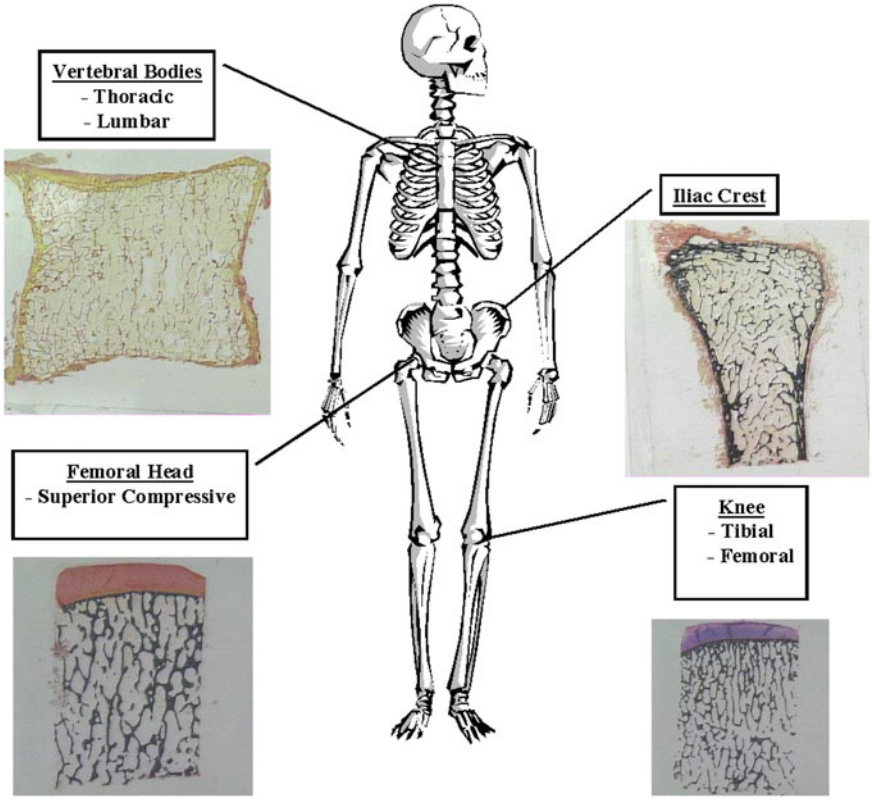

Fig. 3 Composite photomicrograph showing histological sections of trabecular bone from multiple skeletal sites

Availability of human trabecular bone samples has always and continues to be a problematical issue in experimental design. For ex vivo cadaveric studies, samples have been obtained from clinically-relevant skeletal sites, such as the proximal femur, the distal radius and vertebral bodies. These samples have enabled the morphology of the trabecular bone at sites where osteoporotic fractures occur to be characterised and for comparative studies (Fig. 3) [49–51]. Change in bone morphology over time, between sexes or between clinically-relevant groups can be determined [34, 92]. For in vivo studies, biopsies from the iliac crest and the sternum have been obtained because of ease of accessibility to these sites without the need for general anesthesia. These biopsies have enabled changes in bone morphology or cellular dynamics to be investigated from subsequent histomorphometric analysis.

It has become increasingly difficult to obtain human bone material due in part to changing societal attitudes, human ethics considerations for in vivo studies and clinical imperative. For cadaveric ex vivo studies, regulatory frameworks, in the western world, are at best highly restrictive but where access to human cadaveric material is allowed informed consent from next of kin is at the centre of all

requests for material [5]. These restrictions have had the consequence of generally limiting the age range of study cohorts to the older end of the human age-range, which while still clinically relevant it does not encompass all clinical groups. Surgically-sourced bone samples for ex vivo studies can be obtained through direct patient consenting protocols but rely upon motivated surgical, nursing and medical liaison staff. For in vivo studies, and where humans ethics approval for human experimentation is permitted, biopsies are most useful for monitoring changes in cellular dynamics and bone material properties in response to therapeutic interventions. In longitudinal studies, serial biopsies are obtained over an appropriate timescale [10]. In the past, iliac crest biopsies in particular, were useful in the diagnosis and characterisation of metabolic disorders affecting bone, including osteoporosis [21, 70]. However, advances in interpretation of bone serology and imaging have reduced the clinical imperative for in vivo bone biopsy [43, 103].

3 Histomorphometry of Trabecular Bone Structure

From the 1960s until the mid 1990s, histological sections were the most widely used tool for the study of trabecular bone structure. Pioneers in this field, such as Harold Frost and Michael Parfitt, developed histomorphometric techniques to enable the characterisation of trabecular bone micro-architecture as well as the cellular dynamics of bone [39, 85].

Protocols have been developed for resin embedding of undecalcified bone samples to enable thin sections (less than 10 micron thick when cut in the longitudinal direction) to be cut thus preserving the bone mineral phase. Such undecalcified sectioning allows bone components to be visualized by utilizing the physical chemistry of bone mineral, where a modified von Kossa technique [15] localizes the mineralized bone phase and haematoxylin and eosin (H&E) is used to stain the unmineralized or osteoid bone phase (Fig. 4). The H&E stain also enables visualization of bone cells, such as osteoclasts and osteoblasts at the bone surfaces in the same sections. Surfaces at which active mineralisation is occurring are localized by sequential administration of fluorescing compounds, which are incorporated, in vivo, at sites of mineralizing bone [39]. Together these techniques have provided a platform on which to conduct cross-sectional biopsy-based clinical studies or ex vivo laboratory-based studies.

In addition to observational descriptions of bone, quantitative protocols were developed through the adaptation of stereological techniques [40, 56, 86] specifically for the complex architecture of trabecular bone. Bone histomorphometry uses a suite of structural descriptors, which were developed based on idealized models of trabecular bone structure as plates, rods or mixed plates and rods [85]. Independent bone tissue measures such as bone mineral area, bone tissue area and bone mineral perimeter are applied to stylized models of trabecular bone structure and indices describing the average architectural properties are derived. These indices include independent measures of bone volume per tissue volume

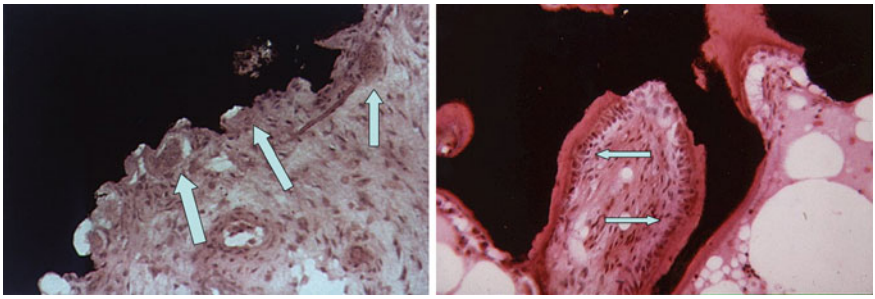

Fig. 4 Histological images of osteoclasts removing bone matrix (*left*) and osteoblasts laying down osteoid (*right*). Von Kossa's silver impregnation with Hematoxylin and Eosin counterstain. (*Black* is bone mineral, on the *left panel*, *arrows* indicate actively resorbing osteoclasts and on the *right panel arrows* indicate osteoblast actively laying down osteoid on bone surfaces)

(BV/TV, also called 'bone volume fraction'), bone surface per tissue volume (BS/TV) and bone surface per bone volume (BS/BV). From these independent measures, derived parameters include trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and trabecular number (Tb.N) [87]. These histomorphometric indices of bone structure have been universally adopted [84] and continue to be used.

Bone histomorphometry has also been extended to bone cell dynamics, including mineralization measured from fluorochrome labeling. By measuring the extent of bone surface with osteoid, resorption pits or active mineralization it is possible to derive indices of bone turnover [39, 106]. These techniques have provided insights into the temporal sequence of the components of the basic multicellular unit (BMU) [39], such that activation frequency for new BMUs can be calculated, the extent of resorption measured, the extent of osteoid measured and the rate of mineralization of the osteoid calculated [13]. More recently, the principles of bone histomorphometry have been adapted to quantify microdamage accumulation, where en bloc or bulk staining of whole bone samples preferentially stains areas of microdamage, which can be visualized after subsequent processing into resin and sectioning [30, 32, 72, 95].

The quantitative techniques available for study of trabecular bone structure and trabecular bone cellular dynamics encompass manual, semi-quantitative/interactive and automated techniques. Manual quantitation from histological sections of trabecular bone involves point counting, where a grid pattern overlaid on the section determines where sampling of the feature of interest occurs [56]. These point counts provide estimates of area or perimeter of the features of interest and by application of Parfitt's plate or rod model to the raw point counts, indices representing the trabecular bone structure are derived [84]. Point counting techniques have been applied to provide static indices of trabecular bone structure, such as bone volume fraction, trabecular thickness, trabecular separation and trabecular number as well as dynamic indices of bone structure, such as bone formation rate and bone apposition rate [40]. Semi-quantitative techniques typically utilize a camera-mounted microscope interfaced to a computer monitor

where customised or commercial (Bioquant, Bioquant Image Analysis Corporation, Nashville, TN; Osteomeasure, Osteometrics Inc., Decatur, GA) software enables features of interest to be delineated in spatial registration with the microscope image. Static and dynamic indices characterising the trabecular structure are calculated based on the same stereological principles employed for point counting techniques [18]. Automated quantitation of trabecular bone from histological sections has mainly been confined to a small range of static parameters derived from bone area, bone perimeter and tissue area measurements [86]. Essentially, only bone matrix can be reliably segmented by automated techniques in histological sections, hence only global static indices of bone structure are obtained; however, when performed in conjunction with manual or semi-quantitative techniques all descriptive bone parameters can be obtained.

The importance of these techniques developed over the past 50 years is underlined by their continued use today. While currently available imaging technology and techniques have extended the ability to visualize and quantify trabecular bone structure they have not replaced manual and interactive techniques but have become complementary to them.

4 Non-Destructive Imaging and Morphometry of Trabecular Bone

Notwithstanding the conceptual models developed to extrapolate measurements from histological sections to represent the three-dimensional micro-architecture of trabecular bone, the field has always strived to develop and adapt technology to enable true 3D analysis of this complex entity. The availability of benchtop high-resolution micro-computed tomography (micro-CT) in the mid 1990s was enthusiastically welcomed by the bone community [37, 46]. For ex vivo studies micro-CT scanners provide isotropic spatial resolution on the order of 10 microns albeit for samples less than 10 mm in diameter [76, 77]. However, for bone samples up to 50 mm in diameter 15 micron spatial resolution is achievable, which enables the thinnest trabecular elements (70 microns in diameter) to be resolved (Fig. 5) [77]. The non-destructive nature of micro-CT imaging means that complementary methodologies can be applied to the samples, subsequent to imaging. For example, conventional histology can be performed if cellular dynamics are of interest [78], or genetic analysis can be performed to identify genes associated with bone diseases [31, 58] or mechanical testing can be performed to enable predictive models of bone strength to be formulated [7, 38, 44, 62, 88, 102].

While laboratory-based micro-CT has become ubiquitous for quantitative bone studies there are other non-destructive imaging modalities available to researchers. Synchrotron facilities provide X-ray tomography but with a monochromatic X-ray source, which provides much better delineation between the mineral and non-mineral phases in bone than the polychromatic X-ray source used in laboratory-based micro-CT imaging [11, 17]. However, access to synchrotron facilities, while

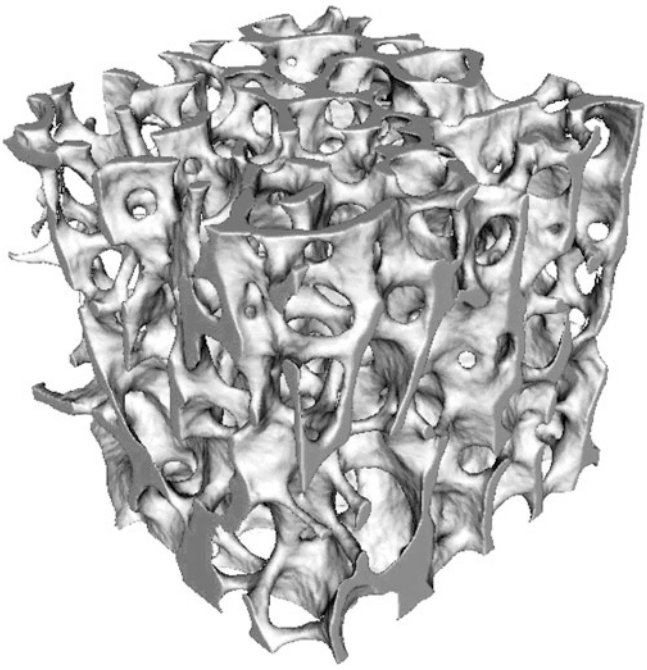

Fig. 5 3D rendering of a bone cube from the L4 vertebral body of 66 years old male with BV/TV = 9.6%

improving, is problematic and there are limited facilities in which large clinically-relevant bone samples can be imaged. In addition, synchrotron-derived datasets present challenges in data handling, where it is not unusual for a single sample to generate at least 100 GB of data, whereas a dataset from a laboratory-based micro-CT system will be less than 10 GB in size. These massive synchrotron datasets require computing resources not commonly available. For non-ionizing radiation imaging, magnetic resonance (MR) imaging is approaching the spatial resolution of micro-CT, where in-plane resolution is approaching 100 μm and apparent resolution below 100 μm with sub-voxel processing techniques [59]. However, compared to micro-CT imaging there are still limitations in the ability to accurately delineate the mineral and non-mineral phases, which have limited the adoption of this imaging modality for morphometric bone studies [57, 59, 61, 67].

One of the challenges of three-dimensional imaging of trabecular bone by whatever imaging modality is the ability to manipulate the wide range and large volume of data acquired from imaging, for 3D reconstruction and for morphometric analysis. However, consumer-level computers are able to process desktop micro-CT datasets reasonably efficiently, in terms of processing time, data generation and data storage needs.

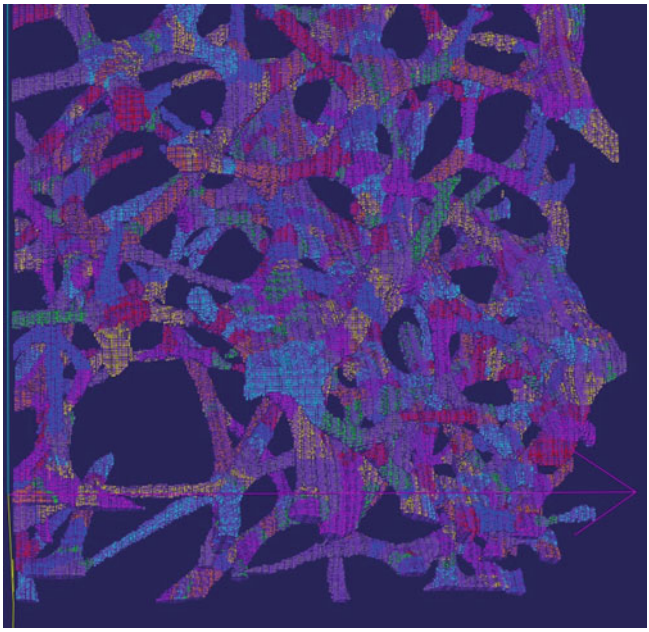

Fig. 6 Colour-coded 3D-rendered image of trabecular bone from the intertrochanteric region of the femur showing decomposition of the trabecular structure into individual trabeculae elements. (colour coding of individual trabeculae provides visual contrast between trabeculae and is not indicative of morphology)

The availability of 3D voxel-based datasets of trabecular bone has driven the development of quantitative tools to validate insights gained from histological studies and to extend morphometric capabilities to more realistic representations of the 3D structure of trabecular bone. Through implementation of an algorithm that fit spheres to 3D datasets, "real" measures of trabecular diameter and trabecular separation are possible [47, 48]. Together with algorithms that describe how plate-like or rod-like the structure is (Structure model index, SMI) [47, 48], whether there is preferential orientation of the structure (Degree of anisotropy, DA) [112] or how well connected the structure is (Connectivity Density. Conn. D) [80]. Suites of histomorphometric measures are available within commercially available micro-CT systems. More recently, in parallel, Stauber et al. [100, 101] and Liu et al. [63] have developed algorithms that volumetrically "decompose" the trabecular structure into individual elements, which are then classified as rods or plates (Fig. 6). These tools provide the size, shape and orientation of the individual trabeculae, which enables study into how individual trabecular morphology or orientation contributes to the mechanical properties of the structure as a whole [65, 102].

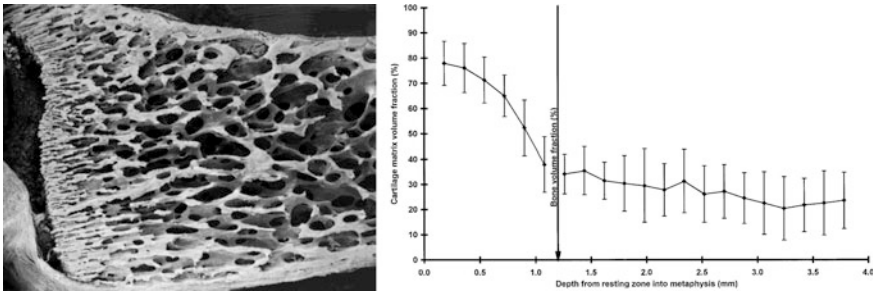

Fig. 7 Scanning electron microscope image of endochondral bone in human neonate rib (*left*), and graph showing change in cartilage volume fraction and bone volume fraction (*y*-axis) versus distance from the resting zone to the mid-metaphysis (*x*-axis) (*right*) [33] with permission

5 Trabecular Bone Structure in the Young

Although there have been relatively few studies describing trabecular bone structure in development and adolescence, the morphological events in bone growth are well described [14, 33, 42] (Fig. 7) and the rate of acquisition of bone mass has been shown to take place relatively slowly until puberty when the rate significantly increases [45]. The velocity of bone growth is different between boys and girls, reflecting differences in onset of puberty and in response to differences in musculature and while there is convergence in growth rates towards adulthood, clear sex-related size differences in the skeleton are maintained throughout life [45, 109].

There is less information as to the relative importance of the genetic template for bone structure versus the effects of environmental factors, i.e., nature versus nurture. In the neonate growth plate histological studies clearly show that the spatial arrangement of the columnar hypertrophic chondrocytes give rise to primary spongiosa and hence the secondary trabeculae [14, 42]. Whether the quality of adult trabecular bone structure is determined or influenced at this early stage has been hypothesized [33], although the ability of bone to adapt to the prevailing mechanical environment shows that genetic influence cannot fully determine an adult's bone microarchitecture.

There is a peak in fracture incidence in the young around the time of puberty, which in girls is approximately 11.5–12.5 years, and in boys is approximately 13.5–14.5 years [45]. These fractures are commonly associated with moderate trauma, with the majority occurring in the distal radius. While trabecular bone structure has not been directly implicated as contributing to susceptibility to fracture it has been shown that during adolescence there is a transient increase in longitudinal and circumferential growth of the cortex before there is a corresponding increase in bone mass through thickening of the cortex and consolidation of the trabecular bone structure within the medullary space [45, 60].

Notwithstanding the genetic or environmental effects on trabecular bone structure prior to adulthood [45], future susceptibility to osteoporotic fractures will be minimised, for an individual, if consideration is given to maximizing bone health during these formative years. It has been shown that the skeleton is capable of very high calcium absorption during growth [45], which allows for rapid modeling and optimization of the trabecular architecture, particularly at sites where the majority of osteoporotic fractures occur.

6 Trabecular Bone Structure in the Adult

Peak bone mass and thus, maximal bone strength is attained late in the 3rd decade of life [45]. Complete cessation of endochondral bone formation means that further change in trabecular bone structure is primarily through remodeling of existing trabeculae. Regulation of the bone basic multicellular unit (BMU) through the coordinated action of osteoclasts and osteoblasts has been well characterised at morphological, physiological and molecular levels, which has provided insights into how trabecular bone is maintained, repaired and remodeled in response to physiological or mechanical stimuli. Histomorphometric studies have shown that the sequence of cellular events that occur when bone is removed and subsequently replaced by unmineralized matrix (osteoid) can be quantified in time and space and in vivo fluorochrome labeling has enabled the rate of mineralization of the osteoid to be measured. Together these measured parameters provide a detailed snapshot of an individual's bone metabolism at the site of sampling. It has been suggested that remodeling can be targeted or untargeted [82, 83], where targeted remodelling is most usually a reparative process in response to damage accumulation [13, 82, 83], which is initiated by disturbance to the cannilicular network as microcracks progress within the bone matrix. The amount of bone turnover has been shown to be in excess of the that required to maintain mechanical competence therefore it is has been suggested that, while initially targeted, there is some remodeling that continues even when the initiating stimulus is no longer present [82, 83].

From the 4th to 6th decades studies have shown that trabecular bone volume fraction can decline by up to 40–50% for males and females [1, 45, 68, 73] although the rate of decline is sex-dependent, site-dependent and study cohort dependent. An exception to this general pattern is in lactation, where up to 10% of bone mass is lost in response to the nutritional imperative of milk production, where the bone loss is mediated by mammary gland-derived parathyroid hormone related-protein (PTHrP) in combination with low estrogen levels [16]. Fortunately, this insult to the skeleton is transient with rapid restoration of bone mass after weaning and it is not thought to infer greater susceptibility to fracture later in life.

In clinically relevant skeletal sites, there are significant differences in the bone volume fraction of trabecular bone between males and females [4], which are associated with differences in the trabecular microstructure [1]. There is also considerable variability in trabecular microstructure within skeletal sites

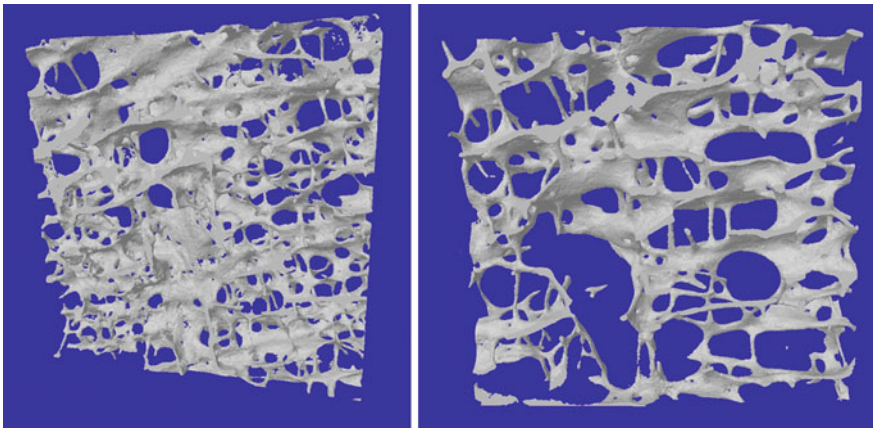

Fig. 8 3D rendering from micro-CT images of trabecular bone samples from human vertebral bodies of two individuals, which have similar bone volume fraction but differ markedly in architecture. (both samples were obtained from individuals over the age of 60, where the BV/TV was 9.3 and 8.6%, respectively)

[1, 29, 98, 110, 113], particularly at sites with large load-bearing. During the 4th to 6th decades and particularly before the menopause in women, the prevalence of low-impact or fragility fractures is low compared to older age groups. However, the incidence of fractures at sites such as the distal radius, the ribs and ankles rises significantly after the age of 35 [108].

7 Trabecular Bone Structure in Older Age

Large and clinically relevant changes in trabecular bone structure occur from the 6th decade of life onward (Fig. 8; Table 1). The Rotterdam study [96] shows that incidence of non-vertebral fractures in osteopenic and osteoporotic males and females (diagnosed based on BMD t-scores) more than doubles after the 7th decade. Fracture risk is site dependent and males and females have different distributions of prevalence in sites of fracture, for example the incidence rate per 1,000 person years for hip fractures in males is 3.0, whereas the incidence rate per 1,000 person years for females is 6.9 [96]. The age at which particular skeletal sites show increased incidence of fractures differs between the sexes, for example the incidence of distal radius fractures in females increases markedly from the age of 55, whereas in men the incidence of these fracture does not increase until after the age of 75 [96].

In females, there is accelerated loss of bone mass from the onset of menopause, which can be within the 5th decade but more usually in the 6th decade. Cessation of estrogen production removes an important control on osteoclast activation,

Table 1 Changes in trabecular bone structure or density with ageing from middle to old age

Study	Materials	Site (parameter)	Male versus female	Percent change per decade	
				Female	Male
QCT [25]	51 F, 50 M 19–96 years	L3 Vertebra (vBMD)	ns	−9%	−9%
Micro-CT [26, 66]	75 F, 75 M 52–99 years	L2 Vertebra (BV/TV)	ns	−9.1	ns
		Iliac crest (BV/TV)	ns	−15.5	ns
		Femoral neck (BV/TV)	F < M (−35%)	−13.4	ns
		Femoral troch. (BV/TV)	F < M (−19%)	ns	9.0
		Calcaneus (BV/TV)	ns	−8.3	ns
		Distal radius (BV/TV)	F < M (−30%)	ns	ns
HR-pQCT [74]	64 F, 66 M 60–99 years	Distal radius [region 1] (BV/TV)	F < M (−32%)	−11.4	−9.1

F female *M* male; *ns* not significant ($p > 0.05$)

which results in a large increase in osteoclastic resorption. This massive up-regulation in the removal of bone matrix is not matched by equivalent replacement of bone therefore there is a net loss of bone mass. The increased number and depth of resorption events results in perforation of individual trabeculae, particularly rod-like structures, which now unloaded are targeted for further resorption and completely removed. This process results in disconnectivity in the structure locally and as a whole and reduces the strength of the structure to a greater degree than accounted for by the loss of bone mass alone [97, 107]. In addition, plate-like trabeculae become perforated and over a number of cycles become more rod-like, which in turn are susceptible to complete perforation and removal. While the increased turnover in females returns to pre-menopausal levels after a number of years, the transformation of the trabecular bone structural can be dramatic with an associated increase in fracture risk.

In males, loss of bone mass from the 6th decade is more gradual than for females and is associated with decreased androgen production [68, 92]. There is increased bone activation of osteoclasts but not to the extent seen in menopausal females, which while resulting in decreased bone mass is not associated with resorption of sufficient depth to perforate trabeculae. Hence, there is generalized thinning of trabeculae but the overall structure remains intact. However, in both males and females there is an increased fracture incidence, which is not completely explained by the loss of bone mass, which again suggests that the mechanical integrity of the trabecular bone structure has been compromised [68].

The sex specific changes to trabecular bone structure in aging, described above, are generalizations, and for individuals will be a combination of trabecular

perforation and trabecular thinning, to a greater or lesser degree [9]. However, significant trabecular bone loss is likely to occur in all individuals over the age of 60 and the degree to which this bone loss contributes to an increase in fracture risk is dependent on multiple factors, including but not exclusively, peak bone mass, degree of sarcopenia, propensity to falls and sunlight exposure [45]. At the bone level alone, skeletal site, is an important risk factor, where the amount of bone, the trabecular bone architecture and the cortical bone morphological properties differs markedly at different sites [4].

8 Summary

A great deal of the knowledge of trabecular bone structure has been elucidated from quantitative methods developed for analysis of histological sections. From the pioneering work of Harold Frost in the 1960s to the present, the complex architecture of trabecular bone has been acknowledged as a three-dimensional entity, which has been optimized for its primary function of ensuring the habitual loads extant on the skeleton do not allow it to fracture. Despite the practical difficulties of describing a 3D structure from 2D images, workers in this field have developed and utilized powerful quantitative tools, collectively known as bone histomorphometry. These tools have provided quantitative characterization of the dimensions of trabeculae, the spatial arrangement between trabeculae the cellular dynamics at trabecular surfaces and the dynamics of bone mineralization.

While ex vivo investigations utilizing histomorphometry have provided comprehensive characterisation of trabecular bone structure and determined skeletal variation and morphological properties these observations have provided an understanding of why and how fractures can occur but not in whom they are going to occur. The X-ray-based imaging tools available today promise to enable in vivo study of individuals at equivalent spatial resolution to histology or ex vivo micro-CT imaging. The suite of tools available for the analysis of trabecular bone as a 3D structure has been significantly expanded with the development of tools that can isolate individual trabecular elements, enabling the morphology of these structures to be measured. Together with finite-element-based analysis, apparent mechanical properties can be obtained at the level of individual trabecular elements to fully characterise the ability of the structure to resist loads under varying conditions.

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