Microarchitecture, the key to bone quality

Maria Luisa Brandi1

Bone has the ability to adapt its shape and size in response to mechanical loads via a process known as modelling in which bones are shaped or reshaped by the independent action of osteoblasts and osteoclasts. Remodelling is a process that maintains mechanical integrity of the skeleton, allowing it to selectively repair and replace damaged bone. During adulthood, bone remodelling is the dominant process; after the age of 40 years, the age-related decline in bone mass increases the risk of fracture, especially in women. Osteoporosis is defined as a reduction in bone mass and an impairment of bone architecture resulting in thinning and increased cortical porosity, bone fragility and fracture risk. As new products and methods have been developed, focusing on bone fragility, effective and sensitive non-invasive means able to detect early changes in bone fragility process have also been developed. Due to limitations in assessing fracture risk and response to therapy, the evaluation of bone mineral contents by bone densitometry is progressively replaced by new non-invasive and/or non-destructive techniques able to estimate bone strength, providing structural information about the pathophysiology of bone fragility by quantitative assessments of macro- and microstructural bone features. Dxa and volumetric QCT quantify bone macrostructure, whereas high-resolution CT, microCT, high-resolution MR and microMR assess bone microstructure. Knowledge of bone microarchitecture is a clue for understanding osteoporosis pathophysiology and improving its diagnosis and treatment; the response of microarchitecture parameters to treatment should allow assessment of the real efficacy of the osteoporosis therapy.

KEY WORDS: Bone modelling, Bone remodelling, Osteoporosis, Microarchitecture.

Introduction

The framework of the human body is provided by the 206 separate bones of the skeleton. This anatomic entity contains 99% of the total body calcium, and plays a major role in its preservation; it also protects vital organs, contains bone marrow and is the site of attachment of muscles and tendons.

The shape of bones results from a process known as ‘modelling’, whereas the process that continuously renews the bones is known as ‘remodelling’. Bone tissue is a composite of both flexible and rigid components: a flexible and tough extracellular matrix is made up of type 1 collagen, proteoglycans and a number of non-collagenous proteins; within this matrix, the rigid one, bone mineral—predominantly hydroxyapatite—, is deposited [1]. It also contains high amounts of growth factors and bone morphogenetic proteins. Bone cells are the osteoclasts, which are bone-resorbing cells. The osteoblasts, the main function of which is to synthesize and subsequently mineralize the osteoid, produce many factors that regulate osteoclast development and function. Osteocytes are terminally differentiated osteoclasts, which become embedded in bone matrix. Osteocytes are connected to one another and to osteoclastic cells on the bone surface by an extensive network of canaliculi, which contain the bone extracellular fluid; they act as mechanosensors in the bone, sensing physical strains and initiating the appropriate modelling or remodelling response [1, 2].

Macroscopically, there are two types of bones: (i) the cortical bones (compact), which constitute ~80% of the skeleton and are found in the shafts of long bones such as the femur, tibia and radius and outer surfaces of the flat bones (skull, mandible and scapula); and (ii) the trabecular bone (cancellous) found mainly at the end of long bones and at the inner parts of flat bones [1]. The relative proportions of the two types of bone vary considerably among different skeletal sites: the cancellous: cortical bone ratio is about 75:25 in the vertebra, 50:50 in the femoral head and 95:5 in the shaft or diaphysis of the radius [1]. In the cortical bone, the periosteum is the outer fibrous structure of all bones, which contains the blood vessels that nourish the bone, nerve endings, osteoblasts and osteoclasts, and which is anchored to the bone by Sharpey’s fibres that penetrate into the bone tissue. The endosteum is a membranous sheath that constitutes the inner surface which is in direct contact with the marrow, and that also contains blood vessels, osteoblasts and osteoclasts.

Mechanisms of bone modelling and remodelling at healthy and postmenopausal stages

Bone modelling

A particular feature of the bone is its ability to adapt its shape and size in response to mechanical loads. This mechanical adaptation is generated by a process known as modelling, in which bones are shaped or reshaped by the independent action of osteoblasts and osteoclasts. Modelling occurs vigorously not only during growth, but also, in the adult, in response to a mechanical load such as in tennis players in whom the radius of the playing arm has a thicker cortex and a larger external diameter than the contralateral radius. Conversely, rapid bone loss may be induced by the unloading of the skeleton during bed rest or space flight [3].

Bone modelling differs from bone remodelling, because in this process bone formation is not coupled with prior bone resorption. The modelling process is less frequent than the remodelling one, but it does occur in normal subjects [4] and may be increased by some pathological states [5, 6].

Bone remodelling

Another feature of the human bone is the process of remodelling, a surface-based phenomenon that involves the removal of a quantum of bone by osteoclasts followed by the deposition of new bone by osteoblasts in the cavity formed [2]. The remodelling process by which the bone is renewed constantly occurs throughout life: at any one time, when ~10% of the bone surfaces in the adult skeleton are undergoing active remodelling, the remaining 90% are found to be quiescent. The duration of the remodelling cycle is ~6 months, most of this time being occupied by formation; ~10% of the skeleton is renewed by remodelling each year [2].
The remodelling process is accomplished at the level of the ‘bone remodelling unit’, which groups the different cell types [7–10] in four distinct phases, which are now clearly identified: quiescence/activation, resorption, reversal and formation (Fig. 1).

The quiescence/activation phase refers to the event that transforms a previously quiescent bone surface into a remodelling one, involving recruitment of circulating mononucleated osteoclast precursors [11], penetration of the bone lining cell layer and fusion of the mononuclear cells to form multi-nucleated pre-osteoclasts [1].

The resorption phase refers to the osteoclastic resorption that is regulated by local cytokines and systemic hormones [11–14]. During this phase, specific types of proton pumps and other ion channels in the osteoclast membrane transfer hydrogen ions to the resorbing compartment, and this acidic solution dissolves the mineral component of the matrix while a number of lysosomal enzymes are secreted and digest the organic phase of the matrix. Resulting from this process, saucer-shaped resorption cavities are created on the surface of the cancellous bone (Howship’s lacunae), and cylindrical tunnels form within the cortex [1]. Resorption is first accomplished by multinucleated osteoclasts and later by mononucleated cells [1, 15, 16]. This phase concludes with osteoclast apoptosis and is followed by reversal [1, 2, 17].

During the reversal phase, the resorption lacuna is inhabited by mononuclear cells (monocytes, osteocytes liberated from the bone by osteoclasts, and pre-osteoblasts recruited to initiate the formation phase of the cycle). It is during this phase that coupling mechanisms (resorption always followed by formation) must work in an efficient and balanced manner. In the absence of efficient coupling and bone balance, each remodelling transaction would result in a net loss of bone. Bone remodelling units on the periosteal surface of cortical bone produce a slightly positive bone balance so that with ageing, the periosteal circumference increases. On the other hand, remodelling units on the endosteal surface of cortical bone are in negative balance so that the marrow cavity enlarges with age. In addition, the balance is more negative on the endosteal surface than on the periosteal surface, which results in age-related cortical thickness decline.

Normal and osteoporotic bone structures

At the macroscopic level, the normal cortical bone appears dense and solid, whereas cancellous bone is a lace-like structure of interconnected trabecular plates and bars surrounding marrow-filled cavities. At the light microscope level, both cortical and cancellous bone is composed of BSUs or osteons [1]. The normal trabecular bone is composed of internal rods or plates that form a 3D branching lattice oriented along the lines of stress. The trabecular interstices of the axial skeleton are the primary repository of red bone marrow, therefore trabecular bone lies in close proximity with the marrow-derived cells that participate in bone turnover. Bone loss initially starts at the bone surfaces; therefore, changes in bone mass occur earlier and to a greater extent in trabecular bone than in skeleton regions that are primarily cortical.

Osteoporosis is a systemic disease defined as a reduction in bone mass associated with an impaired bone architecture: disruption of trabecular continuity by trabecular perforation,
resulting in reduced connectivity of the trabecular bone structure, increased bone fragility and increased fracture risk; and thinning and increased porosity of the cortices occur, with the conversion of the normal plate-like trabeculae into thinner rod-like structures (Fig. 3) [20]. These changes result from the combination of the increased osteoclastic activity and the reduced osteoblast function that characterizes postmenopausal osteoporosis.

Evaluation of bone structure: new imaging techniques, CT and MR

Traditional techniques

Besides conventional radiographs, bone densitometry has long been the standard technique to assess bone mineral content despite the fact that this technique provides important information about osteoporotic fracture risk. Recent clinical investigations indicate that BMD only partly explains bone strength and show limitations of BMD measurements in assessing fracture risk and monitoring the response to therapy [21–27].

New techniques

As new products and methods have been developed by molecular and cellular research focusing on bone fragility, it became essential to develop effective and sensitive non-invasive means by which early changes in the fracture repair process can be detected [20]. New specialized non-invasive and/or non-destructive techniques that are able to provide structural information about local and systemic skeletal health, the propensity to fracture and the pathophysiology of bone fragility have been developed, enabling quantitative assessments of macro- and microstructural bone features, and improving our ability to estimate bone strength.

Quantitative assessment of bone macrostructure can be provided by DXA and CT (Table 1), particularly volumetric QCT (vQCT), whereas assessment of the trabecular bone microstructure may be obtained by high-resolution CT (hrCT), microCT, high-resolution MR (hrMR) and microMR. vQCT, hrCT and hrMR are generally applicable in vivo, whereas microCT and microMR are principally used in vitro [21]. These currently available advanced imaging modalities help to investigate bone fragility and to define the skeletal response to innovative therapies and assess the biomechanical relationships [20]. QCT allows separate analysis of the trabecular and cortical compartments. The analysis of cortical bone, in particular at the hip, is important to estimate fracture risk, and this technique has been utilized in several clinical trials [28, 29]. MicroCT is a technique particularly adapted to 3D analysis of human iliac crest bone biopsies, investigating evolution of trabecular structure under treatment (Fig. 4).

Despite the progress made with these techniques, certain issues remain, such as the important balances between spatial resolution and sampling size, or between signal-to-noise and radiation dose
or acquisition time, which need to be considered further, as do the complexity and expense of the methods vs their availability and accessibility. The relative merits of these sophisticated imaging techniques must be weighed with respect to their applications as diagnostic procedures, requiring high accuracy or reliability, compared with their monitoring applications, requiring high precision or reproducibility [21].

Evaluations of bone microarchitecture and strength

Fundamentally, a fracture occurs when the external force (or load) applied to a bone exceeds its strength. Whether or not a bone will be able to resist the fracture [22] depends on the amount of bone present, the spatial distribution of the bone mass, the cortical and trabecular microarchitecture and the intrinsic properties of each of the bone components. Imaging techniques that can measure one or more of the determinants of bone strength may enhance clinical management of osteoporosis and enhance new drug development [22]. Several novel non-invasive techniques for the assessment of bone quality and strength are currently being investigated in clinical studies. They aim to quantify various determinants of bone strength such as 3D bone geometry, volumetric bone density, microarchitecture and properties of the bone matrix [22]. Finite element analysis, by combining bone geometry with material characteristics to predict bone strength, holds promise as a biomechanically based technique for fracture assessment, and imaging modalities capable of assessing trabecular architecture may be particularly useful in assessing subtle treatment-based changes in bone strength [22]. Finite element methods are becoming increasingly popular for quantifying the elastic and failure properties of trabecular bone [30].

Few methods are currently clinically validated to assess and monitor the evolution of microarchitecture in bone diseases. The most developed studies relate to microarchitectural measurements obtained by bone histomorphometry with the use of new algorithms, which can assess 2D various characteristics of the trabeculae, such as thickness and connectivity [31]. Histomorphometry or quantitative histology is the analysis of histological sections of bone resorption parameters, formation and structure. This modality is the gold standard for assessing bone because it is the only method available for the direct in situ analysis of bone cells and their activities [32] (Table 2). It has been shown that microarchitecture parameters should be obtained by using several independent techniques; microCT, micro-MRI and synchrotron also allow the measurement of 3D trabecular microarchitecture in a non-destructive way on bone specimens [30]. The methods of MR and microCT image analysis are reliable, but preferably should be used in combination as to obtain valid conclusions [33].

The ability to assess the risk of fracture, evaluate new therapies, predict implant success and assess the influence of bone remodelling disorders requires specific measurement of local bone micromechanical properties. To assess these properties, two new methods are used: (i) nano-indentation is a reliable method for...
assessing the intrinsic micromechanical properties of single BSUs such as the hardness and modulus of dry and wet bone tissue, with a high spatial resolution [34–36]. This technique contributes to the current understanding of structure-function relationships throughout the trabecular bone structural hierarchy [37, 38]. (ii) The three-point bending test is also used to evaluate the mechanical properties of bone. The bone is placed in the material-testing machine on two supports and load is applied on the middle of the bone shaft [39, 40].

Implications

Fractures that result from osteoporosis are a major and growing concern for public health systems. As the population ages, the number of fractures worldwide will double or triple in the next 50 years [22]. The ability of a bone to resist fracture (or ‘whole bone strength’) depends not only on the bone mass but also on its spatial distribution (macro- and microarchitecture), and the intrinsic properties of the materials that constitute the bone [41]. Quantitative assessment of macro- and microstructural bone features improve our ability to estimate bone strength. Thus, knowledge of bone microarchitecture appears to be clue for understanding the pathophysiology of osteoporosis and improving both its diagnosis and its treatment. The response of microarchitecture parameters to treatment should allow assessment of the real efficacy of a treatment for osteoporosis. Another clue is a computer-driven fracture risk assessment (FRAX) tool that has been developed by WHO to evaluate fracture risk of patients. It is based on individual patient models that incorporate the risks associated with clinical risk factors as well as BMD at femoral neck. The FRAX algorithms give the 10-year probability of fractures [42].

Elderly women have the highest prevalence of osteoporosis and the highest risk of falling, making them likely to experience osteoporotic fractures. However, early treatment of osteoporotic women, as early as at menopause, should maximize the efficacy of the long-term osteoporosis therapy and prevent the devastating consequences known to occur at an older age. Since anti-resorptive treatments, such as bisphosphonates, also strongly decrease bone formation by a coupling effect besides fixing osteoporosis-induced bone loss, and since anabolic treatments, such as PTH, increase bone resorption by the same effect, a new generation agent, strontium ranelate, seems to be characterized by a dual mechanism of action which increases bone formation and decreases bone resorption with a net improvement in the two main components of bone strength, BMD and bone quality (bone microarchitecture, its intrinsic properties and its geometry, i.e. its shape and dimensions).

### Table 2. Standardized nomenclature of histomorphometric parameters, from the ASBMR (the American Society of Bone and Mineral Research)

<table>
<thead>
<tr>
<th>Parameters of bone structure</th>
<th>Abreviation, unit</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters expressing the amount of bone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancellous bone volume</td>
<td>Cn-BV/TV, %</td>
<td>The percentage of spongy bone tissue including mineralized bone and osteoid</td>
</tr>
<tr>
<td>Total bone volume</td>
<td>BV/TV, %</td>
<td>This is an estimate of bone mass</td>
</tr>
<tr>
<td>Cortical width</td>
<td>Ct.Wi, mm</td>
<td>The thickness of the cortices</td>
</tr>
<tr>
<td>Wall thickness</td>
<td>W.Th, mm</td>
<td>The width of complete trabecular bone</td>
</tr>
<tr>
<td><strong>Parameters reflecting trabecular bone microarchitecture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular thickness</td>
<td>Tb.Th, mm</td>
<td>The width of the trabeculeae</td>
</tr>
<tr>
<td>Trabecular separation</td>
<td>Tb.Sp, mm</td>
<td>The distance between trabeculeae</td>
</tr>
<tr>
<td>Trabecular number</td>
<td>Tb.N, mm</td>
<td>The density of trabeculeae</td>
</tr>
<tr>
<td><strong>Parameters of bone formation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoid volume</td>
<td>OV/BV, %</td>
<td>The fraction of trabecular tissue that is not calcified</td>
</tr>
<tr>
<td>Osteoid thickness</td>
<td>O.Th, μm</td>
<td>The average width of osteoid seams</td>
</tr>
<tr>
<td><strong>Dynamic parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral apposition rate</td>
<td>MAR, μm/day</td>
<td>It expresses the rate of progression of the mineralization front</td>
</tr>
<tr>
<td>Mineralizing surfaces</td>
<td>dLS/BS, %</td>
<td>The extent of double, single or total tetracycline labelled surfaces expressed as a percentage of total trabecular bone surfaces</td>
</tr>
<tr>
<td>Osteoid surface</td>
<td>sLS/BS, %</td>
<td></td>
</tr>
<tr>
<td>Osteoid volume</td>
<td>LS/BS, %</td>
<td></td>
</tr>
<tr>
<td><strong>Derived parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone formation rate</td>
<td>Cn-BFR/BS, μm²/μm²/day</td>
<td>The amount of mineralized bone made per unit of trabecular bone per day</td>
</tr>
<tr>
<td>Adjusted apposition rate</td>
<td>Aj.AR, μm²/μm²/day</td>
<td>The amount of mineralized bone made per day per unit of osteoid-covered surface</td>
</tr>
<tr>
<td>Formation period</td>
<td>Fp, days</td>
<td>The mean time needed to build a new bone structural unit</td>
</tr>
<tr>
<td>Mineralization lag time</td>
<td>Mt, days</td>
<td>The mean interval between the deposition of osteoid and the mineralization</td>
</tr>
<tr>
<td>Activation frequency</td>
<td>Cn-BFR/W.Th, per year</td>
<td>Number of bone multi-cellular units born per year represents the probability that a new cycle of bone modelling will start on the bone surface</td>
</tr>
<tr>
<td><strong>Bone resorption parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eroded surface</td>
<td>ES/BS, %</td>
<td>The percentage of trabecular bone surface eroded</td>
</tr>
<tr>
<td>Osteoclast number</td>
<td>N.Oc/BS</td>
<td>Number per millimetre of trabecular bone</td>
</tr>
<tr>
<td>Erosion depth</td>
<td>E.De, μm</td>
<td>Derived from the number of eroded lamellae in each resorption cavity and the lamellar thickness</td>
</tr>
</tbody>
</table>

This table has been reproduced with permission from Current Medicine Group 2009© from: Rizzoli R, The atlas of postmenopausal osteoporosis, 3rd edition. London: Current Medicine Group. The table from ‘The atlas of postmenopausal osteoporosis’ was adapted from Parfitt MA, Drezner MK, Glorieux FH et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Bone Miner Res 1987;2:595–610. *Calculated from the trabecular bone area, volume and the length of the bone marrow interface. **Calculated from the mean apposition rate and the mineralizing surfaces.

### Rheumatology key messages

- Osteoporosis is defined as a reduction in bone mass associated with impaired bone microarchitecture.
- Ability of bone to resist fracture depends on its spatial distribution and intrinsic material properties.
- Response of microarchitecture parameters to treatment allows assessment of the real efficacy of an osteoporosis drug.

### Acknowledgements

Technical editing assistance for the preparation of this manuscript was provided by Mediscript. This assistance was funded by Servier.
Supplement: This paper forms part of the supplement entitled ‘Improvement of bone microarchitecture: the foundation for a better protection against osteoporotic fractures’. This supplement was supported by an unrestricted grant from Servier.

Disclosure statement: M.L.B. is a consultant for and has received honoraria and grant/research support from MSD, Procter & Gamble, Servier, Nycomed, Glaxo, NPS and Amgen.

References


42 Kanis J. FRAX WHO Fracture risk assessment tool. www.shef.ac.uk/FRAX/. (26 August 2009, date last accessed)