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REVIEW ARTICLE

Bone Quality: The Determinants of Bone Strength and Fragility

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Abstract Bone fragility is a major health concern, as the increased risk of bone fractures has devastating outcomes in terms of mortality, decreased autonomy, and healthcare costs. Efforts made to address this problem have considerably increased our knowledge about the mechanisms that regulate bone formation and resorption. In particular, we now have a much better understanding of the cellular events that are triggered when bones are mechanically stimulated and how these events can lead to improvements in bone mass. Despite these findings at the molecular level, most exercise intervention studies reveal either no effects or only minor benefits of exercise programs in improving bone mineral density (BMD) in osteoporotic patients. Nevertheless, and despite that BMD is the gold standard for diagnosing osteoporosis, this measure is only able to provide insights regarding the quantity of bone tissue. In this article, we review the complex structure of bone tissue and highlight the concept that its mechanical strength stems from the interaction of several different features. We revisited the available data showing that bone mineralization degree, hydroxyapatite crystal size and heterogeneity, collagen properties, osteocyte density, trabecular and cortical microarchitecture, as well as whole bone geometry, are determinants of bone strength and that each one of these properties may independently contribute to the increased or decreased risk of fracture, even without meaningful changes in aBMD. Based on these findings, we emphasize that while osteoporosis (almost) always causes bone fragility, bone fragility is not always caused just by osteoporosis, as other important variables also play a major role in this etiology. Furthermore, the results of several studies showing compelling data that physical exercise has the potential to improve bone quality and to decrease fracture risk by influencing each one of these determinants are also reviewed. These findings have meaningful clinical repercussions as they emphasize the fact that, even without leading to improvements in BMD, exercise interventions in patients with osteoporosis may be beneficial by improving other determinants of bone strength.

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1 Introduction

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fractures, but the clinical criterion for making a diagnosis of osteoporosis is based only on the bone mineral density (BMD) score, which has to be −2.5 standard deviations or lower than the average bone mass of healthy young adults to enable a diagnosis [1]. BMD is then the chief parameter for diagnosing osteoporosis and a major surrogate for assessing how bone tissue responds to interventions for the improvement of bone health. Nevertheless, the majority of fragility fractures occur in individuals who do not have osteoporosis according to these standards [2–6], stressing the notion that BMD is just one among several indicators of bone health and that assessment of fracture risk should also rely on other bone properties. Our aim is to identify what these other properties are, their importance to whole bone strength, and what experimental findings exist showing that they can be modified by physical exercise.

BMD variance only explains a portion of the bone mechanical strength variance since age-related declines in bone strength are disproportionately steeper than decreases in BMD [7]. Reductions in fracture risk following bisphosphonate treatment are also frequently disproportionate to changes in BMD [8–12], suggesting that improvements may be obtained without BMD increases, while increases of up to 35 % in BMD following sodium fluoride treatment have been associated with increases in fractures [13, 14]. Newer imaging methods, such as quantitative computerized tomography (QCT), can complement dual-energy X-ray absorptiometry (DXA) information due to its ability to assess volumetric BMD and bone geometry and to differentiate between cortical and trabecular bone compartments [15]; it has also shown moderate to high correlations with DXA-derived areal BMD (aBMD) measurements [16, 17]. Some studies argue that QCT provides a significantly better prediction of vertebral [18, 19] and femoral neck [20] strength than DXA, while others have shown that bone strength prediction by QCT in the femoral neck is only marginally superior [21, 22] or even inferior [23] to DXA. It is therefore apparent that, despite newer imaging techniques looking promising, a significant portion of fracture risk variation is still not captured by these methods.

It is well established that the skeleton benefits from regular physical activity. Bone mass is generally higher in athletes than in sedentary individuals [24–30], and prospective studies show that exercise increases bone mass in humans [31–34] and experimental animals [35–38]. While substantial at young ages [39–42], exercise-induced increases in BMD are minor in adults [43–49]. In spite of this apparently minute effect, sedentary behavior is a known risk factor for hip fracture [50], with physically active men and women having up to half the risk of hip fracture than sedentary counterparts [51, 52]. Then, if only minor increases in BMD are attained with exercise, this reduction in fracture risk in physically active adults must be achieved by modifying other meaningful properties that contribute to bone strength, independently of BMD, as well as other non-skeletal variables, such as falling risk, that significantly influence fracture occurrence [53, 54]. It is thus remarkable that BMD changes are still the main surrogate for assessing exercise-induced bone health improvements despite the findings showing that improvements in mechanical bone properties are independent of changes in BMD [37, 55–57], and may be, for instance, due to changes in the bone shape [55, 57] or matrix composition [58, 59] and may even be obscured by extraosseous [60] and bone marrow adipose tissue distribution changes associated with exercise [61], as they significantly increase the inaccuracies of the DXA measurements. It should then be argued that a decrease in BMD is one of the possible manifestations of osteoporosis and that bone strength or fragility is multifactorial. The aim of this review is therefore to discuss a variety of points concerning skeletal health, which make up this complex array of determinants of bone strength and fragility. Moreover, the modifications of bone quality by exercise training or regular physical activity will be considered to draft a picture about exercise-dependent bone health benefits.

2 What are the Modifiable Properties in Bone that Influence Its Strength?

Bone strength is the maximal amount of load tolerated before structural failure occurs [62]. Failure gradually builds within the material as micro-cracks develop when strains reach an unbearable critical limit [63]. Bone strength and toughness are therefore highly dependent on the ability of the bones to dissipate the stresses that lead to increases in strain, as well as by the micro-structural properties that prevent crack propagation.

The higher resolution of newer imaging and mechanical testing procedures [64, 65] has highlighted the significance of several bone properties other than BMD for bone strength. It is now well established that several factors, organized in a hierarchical fashion [66], contribute to bone strength [67–69] and are therefore regarded as determinants of bone quality [70]: (i) whole bone morphology, defined by the amount and distribution of bone tissue; (ii) the overall composition of bone tissue, depending on the proportion of hydroxyapatite, water, type I collagen, and other non-collagenous proteins; and (iii) the biophysical properties of these components, such as the degree and type of collagen cross-linking and the mineral crystal size and their crystallinity [70] (Fig. 1). Therefore, impaired bone strength might result from decreases in the amount of bone mass, changes in bone micro-architecture or geometry, in the biophysical properties of the bone tissue, or even from a combination of all the previous. To complicate things further, many of these properties have a U-shaped relationship with bone strength (i.e., bones may become fragile because they have a too low [71] or a too high [72, 73] degree of mineralization), and properties that might increase resistance to one kind of mechanical demand (i.e., static loading) may be deleterious in other kinds (fatigue loading), all depending on the type of stress to which the bone is being subjected [74]. We can take, for example, bisphosphonates, which by inducing osteoclast death decrease bone turnover [75, 76], consequently increasing the degree of bone mineralization [77] and thereby decreasing fracture risk [78]. Teriparatide, by increasing bone turnover [79, 80], instead leads to an average decrease in bone tissue mineralization [81], and yet is also shown to decrease fracture risk [9, 82]. These examples emphasize

that, as other determinants of bone strength besides mineralization degree are also affected by these drugs [71, 83], looking to just one of the determinants can be extremely deceiving. This is why it is important to recognize the several determinants of bone strength and fragility and how they are affected by disease and by the interventions designed to improve bone health, namely by exercise.

2.1 Bone Material Properties

Bones are composite materials made predominantly of type I collagen and, to a minor extent, of other non-collagenous proteins and proteoglycans [84] in which hydroxyapatite crystals are laid down to grow during biomineralization [85]. This two-phase composite nature of the bone fabric enables it to absorb stresses by elastic deformation and to endure high loads before fracturing. The mineral phase is mainly responsible for the ability to resist deformation (stiffness), while collagen fibers allow energy absorption (toughness) [68, 84]. Therefore, variations in either fraction may affect the bone mechanical properties and thereby the fracture risk.

2.1.1 Organic Matrix

Type I collagen is laid down by osteoblasts during bone formation. It is first synthesized as the precursor procollagen, formed by three polypeptide chains in a triple helix, stabilized by post-translational modifications and disulfide bonds. Following secretion into the extracellular matrix, procollagen is cleaved of the N- and C-terminals, enabling spontaneous self-assembly into collagen fibrils that are further stabilized by post-translational modifications that allow the formation of intermolecular and interfibrillar cross-links [86].

The importance of collagen for bone strength becomes obvious in pathologies such as osteogenesis imperfecta [87] and scurvy [88–90], in which a deficient collagen structure substantially increases fracture risk. Inter- and intra-chain cross-links are a key feature for the mechanical properties of collagen, since they maintain polypeptide chains in a closely organized fibrillar structure. Cross-links can be formed by enzymatic and non-enzymatic processes, namely by the formation of advanced glycation end-products (AGEs) [86]. The most abundant enzymatically derived collagen cross-links are pyridinoline and deoxypyridinoline, while pentosidine is the most common AGE-derived cross-link [86].

Abnormalities in collagen cross-links have been associated with increased fracture risk [91–93]. Low pyridinium cross-link content was shown to reduce bending strength by 26 % and the elastic modulus by 30 % in bone from experimental animals despite unchanged bone mass [91], and bone strength in humans was reported to be associated to the cross-links profile [92]. For instance, in vertebral trabecular bone, pyridinoline cross-link content was correlated with ultimate strain, while the pyridinoline/deoxypyridinoline ratio was correlated with ultimate stress

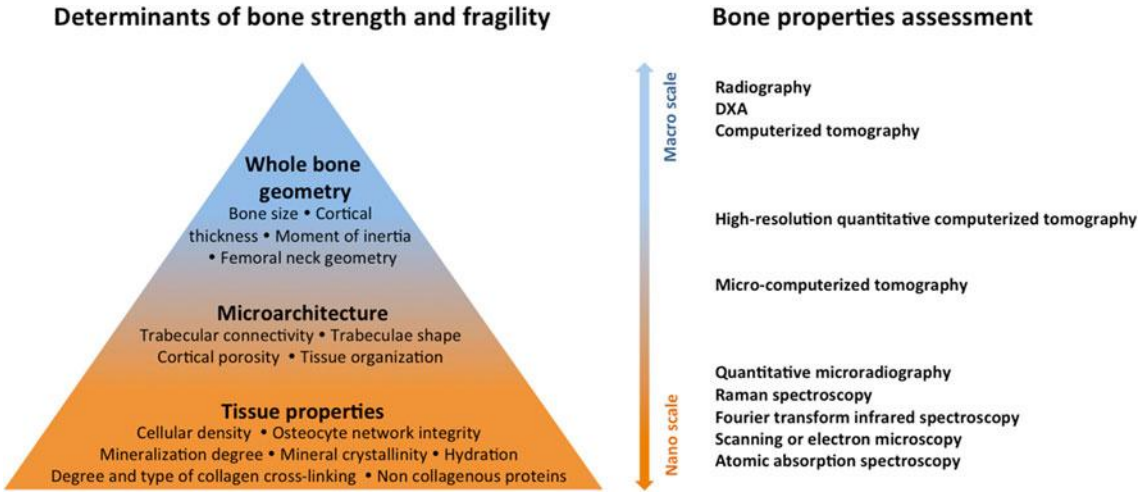

Fig. 1 The several traits influencing bone strength are schematically depicted in the *left* of the figure. The *base* of the pyramid is represented by the properties of bone tissue, which comprise the relative amount and biophysical properties of either the organic or the inorganic components. The *centre* of the pyramid shows the micro-architectural properties that are defined by the way the bone tissue is spatially organized inside the trabecular and cortical components. Finally, at the *top* of the pyramid, supported by the remaining determinants, the gross morphological traits that define whole bone geometry are represented, which are key determinants for the way the bones dissipate the stresses generated during loading periods. The *right half* of the figure lists some of the numerous laboratory methods, ranging from a nano-scale to the macro-scale, that can be used to investigate these bone properties. *DXA* dual-energy X-ray absorptiometry

and elastic modulus [93]. Collagen assembly and cross-link formation [94] are also influenced by non-collagenous proteins present in the bone matrix; hence, these are also suggested to influence the collagen mechanical properties [95]. Collagen fiber orientation was also identified as an important predictor of bone tensile strength [96]. SAMP6 mice, for instance, which have weak and brittle bones, have disorganized collagen fibrils and a low overall collagen content [97].

Glycation is a common protein post-translational modification mediated by reactive aldose or ketose sugars and other metabolic intermediates that can react with free amino groups in lysine, hydroxylysine, or arginine residues, forming adducts to proteins or promoting protein cross-linking [98]. Non-enzymatic cross-links due to glycation are associated with deterioration of bone mechanical properties (Fig. 2). Several in vitro studies show that an increase in bone AGEs increases tissue brittleness, assisting with the accumulation of micro-damage that decreases bone strength [99–101]. Non-enzymatic glycation tends to increase with aging in human bone and is associated with decreases in bone toughness [102].

As previously mentioned, the amount of strain that bone tissue can withstand before fracturing is largely dependent on the ability of the bone tissue to dissipate forces applied to it, preventing in this way the formation of micro-cracks [63]. This illustrates the importance of limiting non-enzymatic glycation for the maintenance of optimal bone mechanical properties. Studies performed on human cadaveric vertebral bodies demonstrate that the ability of individual trabeculae to deform correlates with the amount

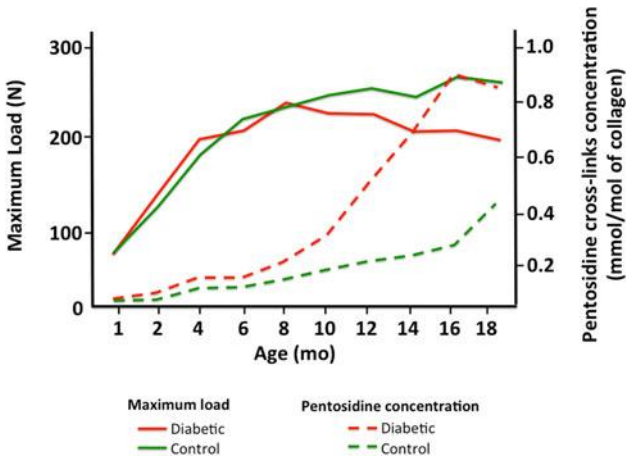

Fig. 2 The relationship between femur maximal load (unadjusted for the animal's weight) assayed by three-point bending and the degree of pentosidine cross-links in the bone matrix in spontaneously diabetic rats. It appears that the degree of collagen glycation is considerably elevated in diabetic rats, and that above a certain degree of pentosidine concentration, the femur maximal load is negatively influenced [284]

of pentosidine cross-links; this was assumed to contribute to about 9 % of the deformation variance [103]. AGE-associated cross-links are also major contributors to the increased bone fragility in patients with diabetes, especially type II diabetes, who commonly have increased BMD [104, 105]. Women with higher levels of urinary excretion of pentosidine are also at higher risk of having vertebral fractures [106], and it was recently recognized that FRAX® (fracture risk assessment tool) underestimates fracture risk in patients with diabetes as it disregards bone matrix changes [107], among other abnormalities [108].

It appears that not only is the proportion of bone constituents abnormal in fragile bones, but also the matrix composition. Bones from osteoporotic patients were shown to have an altered expression of type III and IV collagen when compared with healthy individuals [109]. However, the major differences reported between osteoporotic and normal bones are in terms of collagen hydroxylation and cross-link formation. For instance collagen from the femoral head of osteoporotic women has a higher degree of hydroxylated lysine residues than that from non-osteoporotic women [110], and collagen from bone of otherwise healthy premenopausal women with spontaneous low-trauma fractures displays a higher ratio of non-reducible/reducible collagen cross-links than normal [111].

Despite the limited number of studies investigating alterations of the organic bone matrix induced by exercise, there are still some findings suggesting that exercise may improve its biomaterial properties [55, 112–114] and that lack of mechanical stimulation also leads to abnormalities in bone collagen structure [115]. Experiments on mice, for instance, show that exercise increases the tensile strength of de-mineralized bones despite no changes in the total amount of collagen or collagen cross-links [112], suggesting that changes in mechanical properties may be attributed to improvements in collagen network organization. Improvements in bone strength, stiffness, and ductility in exercised mice have also been reported despite no changes in bone size or shape [113]. Tissue-level mechanical testing of bones from exercised rats also showed a significantly higher post-yield deformation in the tibiae [114] and a higher ultimate toughness and post-yield toughness in the femur than that from sedentary controls, despite no differences in bone mass or geometry [55]. Biomechanical improvements of the bone matrix in exercise-trained experimental animals might be associated with enzymatically mediated collagen post-translational modifications [116] or even with improvements in the collagen network organization [117]. However, this particular area clearly warrants further research. It has also been suggested that exercise, by influencing bone turnover, may promote the renewal of bone matrix collagen and thereby limit the formation of non-enzymatic cross-links [118]. However,

there are no experimental findings supporting this hypothesis.

2.1.2 *Inorganic Matrix*

The bone tissue inorganic fraction is of crucial importance for bone strength and stiffness [68, 72, 84]. The degree of mineralization, for instance, correlates significantly with bone tissue elastic modulus and maximal strength, even after adjusting for bone tissue volume and micro-architecture [72] (Fig. 3). Treatment with bisphosphonates was also shown to increase bone mineralization, thereby decreasing fracture risk without changes in bone tissue volume or micro-architecture [119]. The degree of bone tissue mineralization is mostly determined by the rate of bone turnover [75]. During the formation of new bone, osteoblasts secrete the organic matrix that initially serves as a scaffold for the formation of the primordial mineral templates (primary mineralization). New bone then progressively undergoes further mineralization (secondary mineralization), due to a gradual increase in mineral crystal number and size [85]. If bone turnover is too high, secondary mineralization does not occur efficiently, as this slow process does not have sufficient time to finish before a new remodeling unit reabsorbs the new bone again, leading to a decrease in the overall bone mineralization and consequentially to a decrease in bone stiffness [77, 120, 121]. Adequate balance between bone formation and resorption is therefore crucial for bone quality, and several studies have highlighted its contribution to skeletal fragility. For instance, fracture incidence in post-menopausal women was shown to be associated with the rate of bone resorption, independent of other fracture risk predictors, such as BMD or previous fractures [122]. Changes in bone turnover markers, but not in BMD, have also been associated with vertebral fracture reduction in women undergoing treatment with raloxifene [123]. Interestingly, it was estimated that BMD changes only explain about 4 % of the fracture risk reduction in patients undergoing raloxifene therapy, while the remaining 96 % is attributable to improvements in other BMD-independent properties [124], making raloxifene therapy studies a very good example of the extent to which bone mechanical properties can vary independently of BMD changes.

Excessively increased bone mineralization may also compromise bone strength and increase the risk of atypical fractures that, although an infrequent outcome in patients enrolled in long-term treatment with bisphosphonates, are a growing clinical concern [125, 126]. Bone tissue at atypical fracture sites, for instance, has been shown to be heavily mineralized [127]. As excessively reduced bone turnover decreases the renewal of bone tissue, it consequently increases the accumulation of older and more extensively

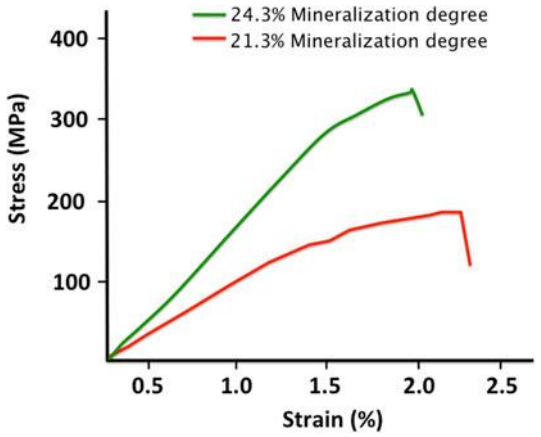

Fig. 3 The results of a 3-point bending biomechanical test performed on the femur of two different experimental animals of the same age but with different degrees of mineralization. It shows that the more mineralized bone has a higher Young modulus (slope of the stress–strain curve within the elastic region during the bending test) and achieves a higher maximal stress than does the less mineralized bone (unpublished observations)

mineralized bone [77, 128], which has two main biomechanical disadvantages. First, it makes bone more brittle [129], and therefore unable to absorb energy by elastic deformation. Consequently, loads applied during everyday movements will be dissipated through structural failure, initially by developing micro-cracks that progress until eventually reaching complete fracture. Second, one of the main purposes of bone turnover is to selectively remove damaged old bone and replace it with new, mechanically more competent bone tissue. An excessively low bone turnover rate, therefore, leads to the accumulation of damaged bone [130, 131], with reduced elastic properties [132], facilitating micro-crack proliferation and fracture occurrence. Therefore, it is not surprising that too much suppression of bone turnover during long-term use of anti-resorptive therapies has been associated with the occurrence of spontaneous fractures and with fractures failing to heal [133]. An adequate rate of bone turnover is therefore necessary for achieving a properly mineralized bone structure that best combines stiffness and brittleness, since hypomineralized bone tends to be very weak, and hypermineralized bone too brittle.

Still, what matters is not only the degree of mineralization but also the individual characteristics of the hydroxyapatite crystals, namely, their size and shape. As bones from younger experimental animals with increased bone strength have a wider variety of crystal sizes than older animals, who have mostly large mineral crystals, bone strength seems to be favored by greater mineral crystal size heterogeneity [134]. An overall reduction in crystal heterogeneity with aging is also observed in humans [135] and in long-term bisphosphonate-treated patients

with fragility fractures [127]. Conversely, bones from patients treated with raloxifene, which have a significantly reduced fracture risk [136], display greater mineralization heterogeneity [137]. As mineral crystals grow between collagen fibrils [138], it is possible that excessive crystal growth damages collagen fibers, thereby affecting the tissue mechanical properties. Interestingly, parathyroid hormone (PTH) administration increases bone turnover as well as bone tissue mineral crystal size heterogeneity and is associated with improved bone mechanical strength [139]. As PTH increases bone turnover [140], it also promotes the removal of older bone with larger mineral crystals, which is then replaced by new bone tissue with smaller mineral crystals, thereby increasing crystal size heterogeneity. A higher degree of crystallinity (orderliness of the crystal minerals) is also associated with higher bone strength and stiffness and was shown to explain between 6.7 and 63.5 % of the variation in the bone mechanical properties in humans [141].

Mineral crystal growth in bone is regulated by many factors that can either contribute to or inhibit mineral crystal formation [142–144]. By influencing crystal growth and maturation, the presence of some proteins seems critical in determining the inorganic properties of the bone tissue and consequently its mechanical competence [145–147]. Hence, changes with age in the secretion of proteins that might be involved in the biomineralization process [148] could have repercussions in the mineral crystal growth pattern.

There are some data showing that exercise can improve bone inorganic matrix quality. QCT techniques, by analyzing voxels of bone volume, are able to quantify the amount of mineral within each voxel and provide a true measure of the mineral density. Experimental animals subjected to exercise training and analyzed by QCT were shown to have a higher degree of bone matrix mineralization than untrained controls [112, 114]. Whole body vibration has been successfully used as a model to study the effects of exercise on bone quality [149] and has shown promising effects by increasing bone mass in athletes [150] as well as in patients confined to bed rest [151]. Notably, whole body vibration also increased bone tissue mineralization in animal models [152]. A recent systematic review [153] on the effects of exercise in postmenopausal women also reports an improved degree of mineralization in exercised women even though BMD measurements remained unremarkable in most cases. However, exercise-induced improvements in the inorganic bone component seem to be not restricted to mineralization degree. There are also findings showing that bone matrix from exercised animals has a higher water content [55, 154], which is assumed to confer additional biomechanical advantages [155] as the tissue become less brittle and more able to accommodate the loads applied to the bone before developing micro-damage.

2.2 Bone Cellular Activity

Bone cells comprise osteoblasts, osteoclasts, and osteocytes. Among these, osteocytes are the more prevalent and have a fundamental role in regulating bone remodeling [156]. Osteocyte apoptosis [157, 158] locally activates osteoclasts and increases bone resorption. Decreases in transforming growth factor (TGF)-β secretion [159], increases in receptor activator of nuclear factor kappa-B ligand (RANK-L) [160, 161] and macrophage colony-stimulating factor (M-CSF) secretion [157], as well as formation of apoptotic bodies [162], are involved in osteoclast activation. Further, bone matrix micro-damage, by triggering osteocyte apoptosis, leads to the recruitment of osteoclasts, thereby increasing intra-cortical bone remodeling [163]. Osteoclast activation therefore derives from the loss of the constitutive inhibition of osteocytes over osteoclasts [164] and from the release of stimulatory factors following osteocyte death [157, 162]. Although this mechanism promotes the renewal of damaged bone with worse mechanical properties [163], large increases in osteocyte death can lead to excessive bone resorption and increased intra-cortical porosity, ultimately compromising bone strength [165]. Osteocytes are therefore essential for targeted bone remodeling, and several findings suggest that damages to the osteocyte network may affect bone quality by hindering the repair of damaged bone [166] or by promoting excessive bone resorption.

In addition to recruiting osteoclasts, osteocytes also orchestrate the formation of new bone in response to mechanical loading by recruiting osteoblasts. This is achieved by detecting mechanical signals, namely interstitial fluid flow or direct cell strain [167, 168], and by releasing signaling molecules, such as nitric oxide (NO) [169], prostaglandins [170, 171], sclerostin [172], and many others [173, 174] that modulate osteoblast activity. NO, for instance, enhances osteoblast activity following mechanical stimulation, and blockage of NO synthesis inhibits mechanically induced bone formation [175, 176]. Prostaglandins are also released by osteocytes following mechanical stimulation [177] and stimulate osteoblast activity and bone formation [178, 179]. Osteocytes from mechanically stimulated bones also secrete lower levels of sclerostin [180], a negative regulator of the anabolic Wnt/β-catenin signaling pathway [181]. Osteocytes also express a group of proteins, known as the SIBLING (small integrin-binding ligand N-linked glycoprotein) family, that directly influence bone mineralization [182]. Matrix extracellular phosphoglycoprotein (MEPE) expression, for instance, a negative regulator of bone formation [183], is

enhanced by mechanical loading [184], while dentin matrix protein (DMP)-1 [185], a protein involved in bone mineralization, [186] is up-regulated by mechanical loading [187]. Together, these findings highlight the importance of osteocytes in the regulation of several key aspects of bone formation and resorption. It is therefore not surprising that there is a strong association between osteocyte density and fracture occurrence and that ablation of osteocytes leads to decreases in bone strength [165]. For instance, [188] a 34 % lower osteocyte density was identified in women who would later experience spontaneous vertebral compression fractures. Aging [189], lack of estrogen [190], use of glucocorticoids [191–193], and alcohol abuse [194–197] are all associated with loss of osteocyte viability and increases in bone fragility. The number of empty osteocyte lacunae, an indicator of osteocyte death, is also inversely correlated with femur diaphysis ultimate stress in experimental animals [198].

The association between decreases in osteocyte density and bone strength also seems to be linked to bone hydration status. Water is responsible for some hydraulic properties in hard tissues, and dehydrated structures have increased brittleness [199]. Much of the bone water is estimated to reside in the canalicular system surrounding the osteocyte body and its dendritic projections [200, 201]. Death of osteocytes and disruption of the peri-cellular matrix may therefore affect hydration status, compromising bone strength [202]. Both bone hydration [203] and osteocyte density [189] tend to decrease with age, and mechanical testing shows that dehydration increases bone tissue brittleness and decreases its strength [155], while hydration increases cortical bone strength 2.5-fold [204]. Age-dependent decreases in osteocyte density are also associated with the accumulation of heavily mineralized bone matrix within the empty lacunae [205], an event known as micropetrosis [206]. This excessive mineralization, which, as previously mentioned, greatly diminishes the ability of bones to tolerate loading without developing micro-damage, together with the decrease in osteocyte density, may contribute to an increased susceptibility to damage formation and failure of successful bone repair in the elderly [189]. Collectively, these findings support the crucial role of osteocytes as gatekeepers in the defense of bone matrix quality and show that the maintenance of a healthy osteocyte network is an absolute requirement for the safeguard of healthy bone tissue turnover and integrity. Nevertheless, osteocyte apoptosis is also required, to some extent, for targeted bone remodeling and damage repair to occur, and certainly excessive blocking of osteocyte apoptosis should also be detrimental for bone tissue mechanical properties.

Osteocytes are highly sensitive to mechanical stimulation, and their own viability relies on such stimuli, as osteocyte death is enhanced by the lack of skeletal muscle tension and weightlessness [207], which may be related to decreases in oxygen diffusion and consequent osteocyte hypoxia [208]. In turn, loading reduces the number of apoptotic osteocytes in experimental animals by 40 %, which was associated with an 80 % reduction in bone resorption surface [209]. Osteocytes exposed to fluid shear stress also show an increased expression of anti-apoptotic genes [210]. This expression appears to rely on several signaling pathways within the osteocyte, namely, on the activation of integrins and subsequent signaling through Src kinases [211] as well as by the activation of prostaglandin receptors and subsequent signaling through protein kinase A or β-catenin [212]. The existence of these overlapping pathways highlights the importance of the relationship between mechanical loading and osteocyte viability for skeletal homeostasis. Recent studies have also shown that physically active ovariectomized (OVX) rats had a significantly higher osteocyte density than sedentary controls [198] and that physically active mice do not display the same decreases in osteocyte density with age as sedentary counterparts [213]. Ex vivo studies with human bone also support the hypothesis that mechanical loading significantly reduces osteocyte apoptosis [214]. Mechanical stimulation brought by exercise training seems therefore a favorable strategy to counteract the increase in osteocyte apoptosis associated with ageing, estrogen loss, inflammation, or glucocorticoids [212, 215], an outcome that is not captured by changes in BMD and that might influence bone resistance to fracture.

2.3 Bone Micro-Architecture

Bone biomechanical competence depends not only on the amount of bone tissue and on its biophysical properties, but also on its micro-architecture—the way the bone tissue is spatially organized. With a larger surface-to-volume ratio, trabecular bone is rapidly affected by increases in bone resorption. Individual trabeculae become progressively thinner, shifting from a plate-like shape to a rod-like shape while trabecular separation increases. Progressive perforation of individual rods leads to the loss of trabecular connectivity and reduces the number of trabeculae, resulting in trabecular micro-architecture deterioration [216–218]. These changes in trabecular micro-architecture rapidly compromise bone strength in regions where trabecular bone predominates, such as long bone extremities and vertebral bodies [219, 220], independently of changes in the cortical bone shell [221]. Studies on human volunteers reveal average decreases of about 27 % in trabecular bone volume (TBV) in the distal radius of women and men aged from 20 to 90 years [222]. However, women mostly display a decrease in trabecular number and a consequent increase in trabecular separation, while men mostly show a

decrease in trabecular thickness [222]. Further, decreases in TBV do not occur at equal rates within the same bone region [223]. Changes in trabecular micro-architecture with aging have a major influence on bone biomechanical properties. A study estimating the contribution of TBV and micro-architecture to murine vertebra compressive strength show that they explain 91 % of compressive strength variability [224]. In another study with postmenopausal women [225], TBV and micro-architecture of the distal radius and tibia explained 96 %, while TBV alone explained between 37 and 67 % of the mechanical properties [226]. Moreover, trabecular bone micro-architecture significantly influences bone strength, independently of BMD [227]. Bone biopsies from patients with osteopenia who had or had not experienced previous fractures showed that, despite no differences in TBV or trabecular thickness, patients with previous fractures had a higher trabecular separation and a lower interconnectivity index than those without fractures [227]. Trabecular micro-architecture variations within the same vertebra are also shown to correlate more strongly than BMD with the site of fracture and the load to failure during compression testing [228, 229].

Weakening of the cortical bone compartment is also of major importance for fragility fractures. Cortical bone represents a substantial amount of the total bone mass, especially in the appendicular skeleton [230], therefore cortical micro-architecture deterioration may significantly compromise bone strength [231]. Additionally, in elderly individuals, the contribution of cortical bone to the femoral neck strength is higher due to their inferior TBV [232]. In a recent study, it was estimated that cortical porosity was responsible for a 6 % decrease in the tibia stiffness and for a significant transfer of the load from the cortical compartment to the already weakened trabecular compartment [233]. Imbalances in bone remodeling in osteoporotic patients is also associated with changes in the cortical bone architecture, namely, with a progressive increase in intracortical porosity. The cortical bone Haversian channel network provides a surface for the action of osteoclasts, which may enlarge the diameter of the channels. This increases bone porosity and leads to cortical bone trabecularization, which consequentially compromises bone strength [234, 235]. Studies in humans have shown a consistent relationship between increasing age and cortical bone porosity, which, notably, was largely undetected by aBMD measurements [234]. A comparison of cortical porosity in young and elderly volunteers also revealed that cortical porosity was higher in older than in young individuals despite identical BMD values [236]. These findings show that individuals with identical BMD values can have different cortical bone micro-architectural deterioration, and possibly different fracture risks. Biomechanical studies also show that human bone stiffness correlates inversely with cortical bone porosity [237], while cortical bone porosity in experimental animals correlates inversely with ultimate stress [198]. These results therefore demonstrate that micro-architectural changes in either cortical or trabecular bone are responsible for significant variations in the bone mechanical properties and, importantly, that many of these changes may remain undetected by BMD measurements.

Several findings suggest that exercise improves trabecular and cortical bone micro-architecture, thereby enhancing bone strength in a way that may be overlooked by BMD findings. Studies on the influence of life-long physical activity on bone quality show that physically active men have higher vertebral and femoral neck TBV than physically inactive men [238]. Physically active women also have a 6.9 % higher trabecular bone density in the distal tibia than less active women [239]. Studies on experimental animals also confirm these observations. For instance, mice subjected to cyclic compression loading showed increases in TBV, trabecular number, and trabecular thickness when compared with non-loaded controls [240]. Similar findings were also observed in young orchidectomized mice [241], suggesting that exercise improved trabecular bone micro-architecture even in the absence of physiologic levels of sex steroids. These adaptations have been observed in both male and female experimental animals, which suggest they are sex independent [242]. Various protocols of exercise also seem able to improve trabecular bone micro-architecture. Treadmill running (10 weeks) increased TBV, number, thickness, and connectivity and decreased trabecular separation in young male rats [243], while 8 weeks of treadmill running was sufficient to increase TBV in young female rats [244]. Resistance exercise (4–8 weeks) also increased TBV and trabecular thickness in male rats, despite that no differences in BMD, assayed by DXA, were detected between sedentary and exercise-trained animals [245]. Resistance training was also shown to prevent decreases in TBV in the lumbar vertebra of orchidectomized rats despite no detectable changes in bone mineral content (BMC) or BMD [246]. These benefits have been observed not only in healthy but also in osteopenic experimental animals. For instance, OVX rats submitted to whole body vibration showed increased vertebral TBV, number, thickness, and connectivity, improving overall biomechanical properties [152]. Exercise is also of paramount importance for cortical bone integrity. For instance, turkey radii deprived of mechanical loading suffer a substantial increase in intracortical porosity [247], and increases in cortical porosity with age in both OVX and intact female rats can be prevented by running-wheel exercise [198]. Exercise therefore seems to be able to prevent age-related increases in intra-

cortical porosity, which might be overlooked by DXA-derived aBMD measures [236].

2.4 Bone Geometry

Bone geometry parameters such as size, shape, cortical thickness, and cross-sectional area (CSA) are closely related with bone strength [248] and fracture occurrence [249]. A previous study found that fractures were more frequent in men with lower cortical thickness but found no association between fracture and cortical BMD [250]. Moreover, changes in cortical bone geometry, such as increases in cortical thickness and perimeter are associated with higher bone strength and lower fracture risk [251–253]. Bone strength differences between African and Caucasian postmenopausal women [254, 255], and between older men and women [256, 257], have also been attributed to differences in femoral shaft CSA. Cortical perimeter is a crucial geometric parameter of bone strength, because increasing a hollow cylinder diameter provides exponential increases in resistance to bending and torsion without necessary increases in bone mass [62]. Interestingly, some have argued that increases in bone diameter seen with aging or menopause are a compensatory mechanism for the decreases in bone mass and trabecular architecture, thus enabling the bone to maintain its strength [257–259]. Therefore, it is possible that the major adaptations in bone geometry seen in bones that have substantial deterioration in other parameters of bone quality may in fact be a sign of weakened bones.

Femoral neck geometry is also relevant for bone quality, even though there are conflicting results on how several parameters can predict fracture risk independently of BMD. For example, despite having lower bone mass than Caucasian women, Japanese women have fewer femur fractures; which has been attributed to their more favorable femoral neck geometry [260]. This observation has led to the development of software for DXA scanners that can provide additional information about bone geometry (hip–axis length, femoral neck–shaft angle, femoral neck CSA, and cross-sectional moment of inertia [CSMI]), and to the computation of equations that, based on these parameters, can more accurately predict the risk of fracture [261–263]. Combination of BMD and upper femur geometry information improves the fracture risk estimation provided by BMD alone [264], highlighting the importance of geometry for femoral strength. Previous studies also show that hip fractures are more frequent in women with higher femoral neck width, femoral shaft width, and longer femoral neck axis [265–267]. African women also have smaller bone widths and hip axis lengths than Caucasian women, and these geometrical differences are also thought to be associated with hip fracture occurrence differences between these two populations, independent of differences in BMD [268]. Nevertheless, others were unable to identify a relationship between femoral neck axis length and fracture risk [269]. Neck–shaft angle and femoral neck width were also found to be increased in those with greater fracture risk [270]. Increases of one standard deviation in the neck–shaft angle have been associated with 2.45 and 3.48 % increases in fracture risk in men and women, respectively, while increases in femoral neck width were associated with 2.15 and 2.40 % increases in fracture risk in men and women, respectively [270]. However, neck–shaft angle and femoral neck width were not found to be significantly associated with hip fracture risk in another study, while an increase in each standard deviation in hip axis length doubled the risk of hip fracture, independent of age and BMD [271]. Forces acting on the femoral neck after a sideways fall are also determined by the proximal femur geometry [272], and those with a longer femoral neck moment arm have a greater risk of hip fracture after a sideways fall, due to a greater concentration of forces in the femoral neck [273]. Interestingly, a recent study has identified an association between genetic polymorphisms involved in the bone mineralization pathway and several femoral neck geometrical parameters, suggesting that both traits are interconnected [274]. Anti-resorptive and anabolic drugs for osteoporosis have also been shown to modify proximal femur geometry, thereby increasing bone strength [275, 276].

Femoral neck and diaphysis geometry can be influenced by exercise, improving bone resistance without necessarily increasing its mass. For instance, sprinters have a higher tibial midshaft CSA than healthy non-athletes [277], and young female athletes have a thicker cortical bone in the femoral neck than do sedentary controls [278]. Moreover, differences in bone structure between athletes and non-athletes do not necessarily reflect differences in bone mass, as female runners were shown to have a higher cortical bone CSA, CSMI, and bone strength index (BSI) than age-matched controls, despite no differences in BMD [279]. Retrospective studies also suggest that higher levels of habitual physical activity in young adult men [39] and pre-pubertal girls [280] are associated with the development of a larger femoral diaphysis cross-section. Older physically active women also tend to have a higher radius and tibia BSI than sedentary age-matched women [239]. Exercise intervention trials also support these observational findings. For instance, early pubertal exercise-trained girls showed significantly higher increases in femoral neck CSA and cortical thickness than age-matched controls [281]. Exercise-trained postmenopausal women also revealed a significant expansion of the bone CSA than sedentary controls, without any relevant increases in

aBMD [57], while the decrease of tibial-shaft strength index in older women participating in an exercise training program was significantly lower than in sedentary controls, despite there being no difference in femoral neck BMC between the groups [282]. Results from experimental studies with exercise trained and sedentary female rats also show that exercised animals have a higher cortical thickness and higher breaking load than sedentary animals, despite no differences whatsoever in BMC [37]. These results clearly suggest that, although exercise training may have reduced effects on BMD, it may still have substantial effects on other meaningful determinants of bone resistance to fracture, namely, on bone geometry.

3 Conclusions

Despite that the diagnosis of osteoporosis is based on the assessment of BMD, this measure is only able to provide insight regarding the quantity of bone tissue, which is manifestly insufficient as a measure of bone quality, given that bone strength is dependent on a large variety of interconnected factors. Skeletal fragility can therefore result from problems arising from each single factor, or from several of the individual determinants of bone strength. Therefore, while osteoporosis (almost) always causes increases in bone fragility, bone fragility is not always caused just by osteoporosis (normally diagnosed by a BMD that is −2.5 or more standard deviations lower than the average bone mass of healthy young adults). In other words, osteoporosis (low bone mass) should be more accurately considered as just a feature of the disease (skeletal fragility) and not as its synonym, as has also been proposed previously [283]. This consideration has major implications for all those enrolled in the treatment of individuals who have had, or who are at risk of having, a fragility fracture, since assessment of treatment success should not be based merely on changes in BMD, but preferably on the highest number of features that can provide a more complete picture of bone strength adaptations. From the findings reviewed in this paper, it is clear that bone strength depends on several determinants. The pharmaceutical industry has achieved much progress, showing that some drugs are able to improve bone strength and reduce fracture risk by improving these determinants without necessarily increasing BMD, raloxifene probably being the best example. Despite some attractive studies showing that exercise might also improve several of the determinants for bone strength, further investigation is warranted by those focused on the benefits of exercise training on bone health in order to complete the complicated mosaic by including new pieces of knowledge.

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