Current Osteoporosis Reports (2019) 17:105–115
https://doi.org/10.1007/s11914-019-00508-y

OSTEOCYTES (J KLEIN NULEND, SECTION EDITOR)

Inter-site Variability of the Human Osteocyte Lacunar Network: Implications for Bone Quality

Petar Milovanovic[1,2] · Björn Busse[1,3]

Abstract

Purpose of Review This article provides a review on the variability of the osteocyte lacunar network in the human skeleton. It highlights characteristics of the osteocyte lacunar network in relation to different skeletal sites and fracture susceptibility.

Recent Findings Application of 2D analyses (quantitative backscattered electron microscopy, histology, confocal laser scanning microscopy) and 3D reconstructions (microcomputed tomography and synchrotron radiation microcomputed tomography) provides extended high-resolution information on osteocyte lacunar properties in individuals of various age (fetal, children's growth, elderly), sex, and disease states with increased fracture risk.

Summary Recent findings on the distribution of osteocytes in the human skeleton are reviewed. Quantitative data highlighting the variability of the osteocyte lacunar network is presented with special emphasis on site specificity and maintenance of bone health. The causes and consequences of heterogeneous distribution of osteocyte lacunae both within specific regions of interest and on the skeletal level are reviewed and linked to differential bone quality factors and fracture susceptibility.

Keywords Osteocyte distribution · Inter-site differences · Osteocyte lacunar number · Mechanical loading · Bone strength · Aging · Bone development

Introduction

Bones have the exceptional characteristic to change in size, mass, and morphology through adaptation to present mechanical needs [1]. In the process of bone remodeling, osteoclasts remove the old bone matrix, while functionally coupled to osteoblasts that secrete collagenous matrix as basis for mineralization of newly formed bone [1–4].

Skeletal development, aging, and various diseases can cause alterations in the bone remodeling process. Changes in the bone remodeling dynamics may lead to imbalance between the degree of bone resorption and formation, which can increase the risk of fractures, such as in age-related bone loss [5].

With more than 25,000 osteocytes per mm^3 of mineralized bone matrix, osteocytes are the most abundant cell type in bone [6]. During the embedding of osteoblasts in osteoid, osteoblasts turn into pre-osteocytes and later on to osteocytes residing in newly formed mineralized matrix. Osteocytes develop multiple dendritic processes enabling them to form a global network throughout the bone tissue. This functional osteocyte network is a structural prerequisite for cellular connectivity and function, whereby dendritic connections are enabled by gap junctions formed by connexin 43 proteins [7]. The osteocyte network is encased within the fluid-filled lacunae and canaliculi, therefore serving the purpose of mechanosensation and mechanotransduction [8]. The lacuno-canalicular network provides a means of nutrient transport and waste removal, which are essential to osteocyte viability. These cells have been identified as central orchestrators of bone remodeling [9]. Osteocytes are the first cells to sense

This article is part of the Topical Collection on *Osteocytes*

✉ Björn Busse
b.busse@uke.uni-hamburg.de

[1] Department of Osteology and Biomechanics, University Medical Center Hamburg-Eppendorf, Lottestrasse 55A, 22529 Hamburg, Germany

[2] Laboratory for Anthropology and Skeletal Biology, Institute of Anatomy, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[3] Forum Medical Technology Health Hamburg (FMTHH), Heisenberg Research Group of Biomedical Sciences, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

mechanical alterations in bone and react to this by upregulating the expression of RANKL to attract osteoclasts [10], or by producing sclerostin to inhibit osteoblast function [11, 12]. In addition to osteocytes role in biomechanical signaling, osteocytes play an endocrine role through secretion of fibroblast growth factor 23 which acts on the kidney and regulates vitamin D and phosphate metabolism [13]. Osteocytes also express a high level of dentin matrix protein 1, regulating bone matrix mineralization [14].

Since bone remodeling is orchestrated by a global network of mechanosensitive osteocytes in a spatiotemporal manner [15], local osteocyte viability (Fig. 1a, b) and function play a critical role in maintaining bone's physiological repair processes, which is a prerequisite for preventing the accumulation of fatigue damage and microcracks that could subsequently develop into fractures [20, 21]. Therefore, local changes to the osteocyte network characteristics (Fig. 1a–k) need to be understood to shed more light on the origins of site-specific bone fragility in aging and disease.

Distribution of Osteocytes Among Different Skeletal Sites

The osteocyte lacuno-canalicular network spreads throughout the entire bone matrix. However, osteocytes' presence has been reported not to be homogenously distributed throughout the skeleton (Table 1), and even within an individual bone, there is a large variation in osteocyte density (Fig. 1d, e).

Although studies that have focused on inter-site differences of osteocyte density are rare, a few reports have indicated that different numbers of osteocyte lacunae per bone area are present in different skeletal sites. Hunter and Agnew [28••] analyzed undecalcified bone specimens from the femoral midshaft, diaphysis of the radius, and midshaft of ribs by optical microscopy in 30 individuals in the age group above 50 years. They found that the osteocyte lacunar number per bone area (Ot.Lc.N/B.Ar) was significantly higher in the radius in comparison to both femur and ribs (radius 1074.49 vs. femur 939.8 and rib 939.23/mm^2). While they did not find sex-related differences, there was a slight but significant age-related reduction in Ot.Lc.N/B.Ar exclusively occurring in the femur, while ribs were not subject to age-related changes. In future research, larger sample sizes and wider age ranges are needed to provide additional support for such site-specific peculiarities. Nevertheless, the available data shows that femurs, as "weight-bearing" long bones, are more affected by aging than "non-weight-bearing" bones such as the radius and ribs. More pronounced age-related loss of osteocyte lacunae in "weight-bearing" sites may stem from accumulating larger amounts of linear microdamage at these sites due to higher mechanical loading [28••].

Gauthier et al. performed synchrotron radiation (SR) microcomputed tomography on the midcortical region of the femoral diaphysis, femoral neck, and distal radius in eight women aged 50 to 91 years [29••]. They showed significantly higher numbers of lacunae per bone volume in the femoral neck than in the radius (26,123/mm^3 vs. 21,191/mm^3), while the femoral diaphysis showed slightly higher values than the radius (21,617/mm^3 vs. 21,191/mm^3). In addition to changes in lacunar number per bone volume, Gauthier et al. also showed inter-site differences in 3D lacunar shape, where more elongated and narrower lacunae were found in the radius than in the femoral neck. The authors also showed that osteocyte lacunar number per bone volume poorly correlated between the femoral neck and radius diaphysis ($r = 0.17$) or even between the femur's neck and diaphysis ($r = 0.62$) [29••]. Such findings suggest that extrapolations from one skeletal site to other skeletal sites are unreliable (even for bone regions that are in close proximity to each other, e.g., femurs' neck and diaphysis).

Additional information about the heterogeneity of the osteocytic network can be obtained from studies analyzing osteocyte lacunar density in a specific region of interest. Although comparisons between some studies are challenging due to methodological differences in quantitative imaging of different skeletal sites (2D methods: backscattered electron microscopy, histology, confocal laser scanning microscopy vs. 3D reconstructions: microcomputed tomography, synchrotron radiation microcomputed tomography; different sampling areas; different patient characteristics, etc.), the data provides valuable insights into the variability of the osteocytic network throughout the skeleton (Table 1).

The majority of reports that presented osteocyte lacunar data were focusing on femurs, as femurs are among the most common fracture sites. Frequently, researchers analyzed the subtrochanteric region of the femoral diaphysis [18, 22, 25••, 26••, 32–34], which has been a prominent site for atypical femoral fractures [35, 36]. Data on the osteocyte distribution in the midshaft of the femur is also available [37]. Although the femoral neck is the most frequently broken part of the femur, data about osteocyte characteristics associated with femoral neck bone quality have been rather scarce. Valuable information about the number and size of the femoral neck's osteocytes in young and aged women has been published recently [28••] (Table 1; also see the next section for more details).

Bach-Gansmo and coworkers investigated iliac crest cortical bone from 46 women and 42 men of a wide age range using SR-microCT and microCT [23]. They found unchanged lacunar volume with age and sex, but the number of lacunae per bone volume was higher in young (15,700/mm^3) than in aged individuals (14,600/mm^3) when women and men were pooled. They speculated that a decline in lacunar density with age arises from hypermineralization of some osteocyte lacunae, while a lack of changes in lacunar volume may reflect methodological limitations of microCT [17••]. Rolvien et al.

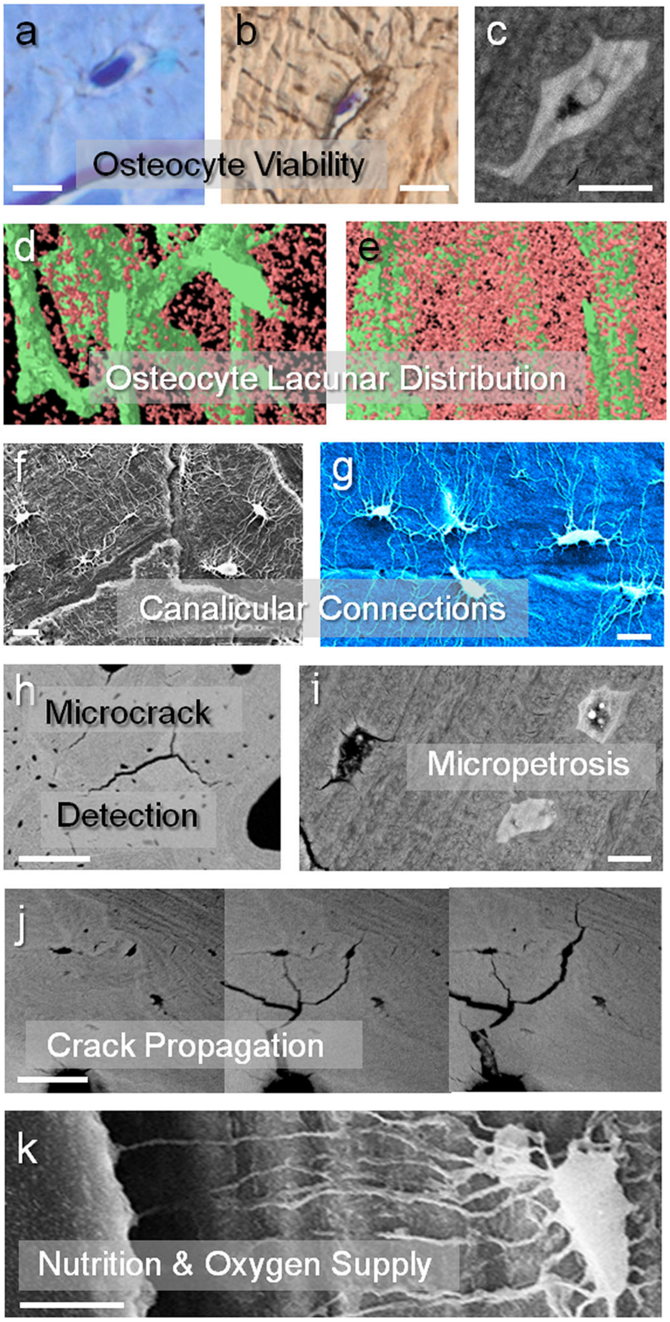

Fig. 1 Human osteocyte characteristics affecting bone quality. **a**, **b** Osteocyte viability is a prerequisite to orchestrate bone remodeling. Stained nuclei in histological sections signify viable osteocytes (left: toluidine blue stain, right: modified silver stain/thionin). **c** Osteocyte cell death/apoptosis often results in micropetrosis where the lacunar space is impregnated with calcified material (backscattered electron imaging, adapted from Milovanovic et al. [16•]). **d**, **e** The distribution of osteocyte lacunae is dependent on the skeletal site (e.g., left: superolateral vs. right: inferomedial femoral neck), mechanical loading, age, and/or disease status (microcomputed tomography, adapted from Rolvien et al. [17••]). **f**, **g** Canalicular connections between osteonal and interstitial osteocytes are important for cellular communication (scanning electron microscopy of acid-etched cortical bone specimens, adapted from Milovanovic et al. [18]). **h** Microcrack in interstitial bone. Systematic distribution of osteocytes in the bone matrix enables detection of microdamage. Preserved osteocyte lacuna-canalicular network is essential to initiate bone repair (backscattered electron microscopy). **i** Micropetrotic lacunae are permanent indicators of osteocytes' death and do not participate in microdamage detection but present themselves as highly mineralized spots in the bone matrix (backscattered electron imaging, adapted from Milovanovic et al. [16•]). **j** Osteocyte lacunae may affect the propagation of cracks (backscattered electron imaging, adapted from Busse et al. [19]). **k** Connections between osteocyte lacunae and Haversian canals via canaliculi ensure nutrition and oxygen supply to the cells (scanning electron microscopy of acid-etched cortical bone specimens, adapted from Milovanovic et al. [18]). Scale bars represent 10 μm in all panels except for (**h**) and (**j**) where scale bars display 100 μm

investigated transiliac crest bone biopsies in 2D in 30 adults with vitamin D deficiency and 30 control cases. The authors showed that vitamin D deficiency was associated with decreased numbers of viable osteocytes accompanied by impaired lacunar connectivity and increased osteocyte apoptosis [38]. While the number of osteocyte lacunae between 15 vitamin D-deficient and 15 vitamin D-sufficient older adults was similar in a 3D assessment of transiliac bone biopsies, spherical lacunae were 14% larger in volume in vitamin D-deficient subjects [19].

Specific Distribution of Osteocyte Lacunae Within a Single Bone Section

Not only does the osteocytic network differ between individual bones but even in a single cross section of bone various distributions of osteocytes can be found.

From 2D studies focusing on cortical bone histology [39–41] as well as from 3D microCT imaging of trabecular bone [42, 43], we can deduce that patterns of microarchitecture and porosity are very heterogeneous within the femoral neck. Rolvien et al. [17••] compared inferomedial and superolateral cortices of the femoral neck in young and aged women and demonstrated 19% higher osteocyte lacunar density (2D) and 72% higher lacunar number per bone volume in the inferomedial neck compared with the superolateral neck (Fig. 1d, e). Osteocyte lacunar density was lower in aged individuals, while there was a modest reduction in inter-site differences with aging (inferomedial neck had 22% higher Ot.Lc.N/B.Ar than superolateral neck in young cases, compared with 16% in aged cases). Of note, osteocyte lacunar size did not differ between the sites [17••].

In terms of osteocyte lacunar density, trabecular and cortical areas can be clearly distinguished. In iliac crest bone biopsies, it was shown that cortical bone had fewer osteocyte lacunae per bone area than trabecular bone [27].

In cross sections of long bones, subdivision into four anatomical quadrants (i.e. anterior, medial, posterior, lateral) often revealed substantially different densities of osteocyte lacunae highlighting heterogeneous osteocyte distribution within a single cross section. Our previous study showed a tendency of higher osteocyte numbers in the medial than in other quadrants of the femoral diaphyseal cross section in both sexes, especially in the periosteal region of young individuals [44]. Also, Carter et al. showed that the highest number of lacunae occurs in the medial zone of the femoral cortex in young healthy males [34] and suggested that differences in loading are likely responsible for differences in lacunar density between the quadrants. Subdivision of cortical bone into periosteal and endocortical compartments showed that osteocyte distribution varies already within the cortical shell of one individual. Specifically, we found in femoral diaphyseal cross sections that the periosteal compartment presented significantly higher numbers of osteocyte lacunae along with lower numbers of mineralized lacunae (i.e., micropetrotic lacunae) in comparison to the endosteal cortex [25••]. Together with a lower degree of mineralization in the periosteal compartment [25••], our findings reinforced the notion that the periosteal surface is associated with bone apposition (i.e., radial growth) continuously during life [45–47]. While Ashique and coworkers have not reported differences in the periosteal, midcortical, and endocortical zones when analyzing exclusively the anterior region in the proximal femoral diaphysis [22], our assessment of human femurs from young, aged, osteoporotic, and alendronate-treated elderly female donors [25••, 44] showed a significant elevation in osteocyte lacunar density towards the periosteal cortex when compared with the endocortical zone.

The Reasons and Origins for Inter-site Variability

There is still insufficient data to explain comprehensively the origins of inter-site variability of the osteocyte lacunar network. However, the prevalent opinion is that inter-site differences can be explained to a certain degree by different loading scenarios. Present osteocyte densities may be the result of former adaptation processes to the magnitude or type of mechanical loading through bone remodeling [34]. The femoral neck is indeed an interesting skeletal region to study such adaptation processes because the inferomedial and superolateral subregions are subject to different stress and strain rates [48, 49]. Under habitual mechanical loading of the skeleton, the inferomedial region of the femoral neck is subject to substantial compressive loads [49]. In sharp contrast, the superolateral region is only exposed to minor load [50]. The latter mechanical circumstance would provide fewer stimuli for osteocyte survival and likely result in lower osteocyte numbers in the superolateral neck due to relative unloading [17••]. This is further supported by the fact that the superolateral neck also exhibits a thinner cortex and thinner, more separated and frequently rod-shaped trabeculae [42, 43, 51]. Nevertheless, considering that the center of mass of the femoral neck is shifted towards the inferomedial border early after birth [52], it is likely that there is also certain genetic preprogramming of bone structure and cell distribution [52, 53]. Additional information about the significance of mechanical loading in relation to osteocyte distribution can also be obtained from studies on immobilization and disuse [54–57], while additional research is needed on the effects of prolonged bed rest or inactivity as it will become a common problem in the present society of ever-increasing ages [58].

Auditory ossicles are intriguing bones that are well-suited for studying the variations in osteocyte characteristics. Auditory ossicles are the smallest bones in the body, located

Table 1 Osteocyte lacunar number per bone area or volume in different skeletal sites of the human skeleton (listed studies were published from 2015 to 2019)

Authors	Skeletal site	Imaging method	Younger	Aged	Disease
Rolvien et al. 2018 [17••]	Femoral neck—cortex	BSE-SEM	< 50 years, 362.2/mm^2	> 70 years, 307.1/mm^2	–
Ashique et al. 2017 [22]	Femoral midshaft—cortex	CSLM	20–23 years, 695/mm^2	70–86 years, 546/mm^2	–
Bach-Gansmo et al. 2016 [23]	Iliac crest—cortex	SRμCT	< 57 years, 15,700/mm^3	> 57 years, 14,600/mm^3	–
Rolvien et al. 2018 [24•]	Auditory ossicles	BSE-SEM	< 25 years, 591.8/mm^2	> 60 years, 219.0/mm^2	–
Milovanovic et al. 2015 [25••]	Proximal femoral diaphysis—cortex	BSE-SEM	< 40 years, 344.6/mm^2	> 60 years, 270.6/mm^2	OPO, 296.3/mm^2 BP, 298.5/mm^2
Tong et al. 2017 [26]	Subtrochanteric femoral diaphysis—cortex	Histology	< 50 years, 241.1/mm^2	> 50 years, 197.4/mm^2	–
Akhter et al. 2017 [27]	Iliac crest—cortex	3DXRM	49.4 ± 1.9 years (women), 17,402/mm^3	54.6 ± 2.2 years (same persons), 17,583/mm^3	–
	Iliac crest—trabecular bone		49.4 ± 1.9 years (women), 22,428/mm^3	54.6 ± 2.2 years (same persons), 20,850/mm^3	–
Hunter and Agnew 2016 [28••]	Femoral midshaft	Optical microscopy	–	> 49 years, 939.8/mm^2	–
	Diaphysis of the radius		–	> 49 years, 1074.49/mm^2	–
	Midshaft of the rib		–	> 49 years, 939.23/mm^2	–
Gauthier et al. 2018 [29••]	Femoral neck	SRμCT	–	> 50 years, 26,123/mm^3	–
	Femoral diaphysis		–	> 50 years, 21,617/mm^3	–
	Radius		–	> 50 years, 21,191/mm^3	–
Bereshiem et al. 2019 [30]	Midshaft of the rib	BSE-SEM	< 40 years, 109.16/mm^2	> 70 years, 100.28/mm^2	–
Zimmermann et al. 2019 [31]	Femoral mid-diaphysis	BSE-SEM	Fetal and infant, 750.72/mm^2 Children 2–14 years, 386.9/mm^2	–	–

BSE-SEM, backscattered electron microscopy; *3DXRM*, 3D X-ray microscopy; *SRμCT*, synchrotron radiation microcomputed tomography; *OPO*, osteoporosis, *BP*, bisphosphonate-treated osteoporosis in the middle ear [59]. Specifically, the absence of bone remodeling in auditory ossicles makes them an interesting entity to study changes in osteocyte network viability across life. Recently, it has been shown that a large proportion of osteocytes in ossicles dies during the first year of life, which is associated with a striking decrease in viable osteocytes, lower osteocyte lacunar density, and an increase in mineralized lacunae (i.e., micropetrotic lacunae) [24•]. Considering that osteocytes need stimulation to survive [60], minor mechanical loading occurring during sound transmission is likely insufficient and rapidly leads to apoptosis of the majority of osteocytes [24•]. Obviously, viable osteocytes are not necessary for the function of auditory ossicles during sound transmission. Moreover, it seems that osteocyte death-driven initiation of a new bone remodeling cycle is suppressed to ensure ossicles' structural integrity for stable transmission of sound waves [24•]. Specifically, while bone remodeling would support the maintenance of the number of viable osteocytes, bone remodeling would create a temporary increase in porosity (i.e., resorption lacunae) which would likely damage the structural integrity and function of the small-sized ossicles. Therefore, a currently unknown mechanism of preventing osteocyte death–driven initiation of bone remodeling seems to be in play in auditory ossicles [24•].

Apart from the inter-site variations in osteocyte lacunar numbers, the key reason for the loss of the osteocyte network at a single site during life is osteocyte apoptosis [61–65]. Osteocyte apoptosis is a process that is frequently followed by mineralization of canaliculi and lacunae [16•, 24•, 25•]. There seems to be spatial selectivity in osteocyte death, given that most of the mineralized lacunae are found in old interstitial regions [25••, 44]; nevertheless, osteonal osteocytes may also die and leave micropetrosis as an imprint of previous osteocyte apoptosis [16•, 25••]. In midcortical bone of the femoral diaphysis in aged individuals, we observed in particular dramatic losses of canaliculi traversing cement lines [18]. Therefore, communication between multiple osteons and interstitial bone regions (Fig. 1f, g) is hampered [18] providing evidence for spatially heterogeneous disruption of the lacuno-canalicular network. In addition, Repp et al. identified intra-osteonal zones without signs of lacuno-canalicular network, possibly due to inaccessibility of these zones to fluorescent stain due to micropetrosis [37].

Inter-site differences in osteocyte distribution may also be related to local bone turnover changes. In Paget's disease of bone, a patchwork of lamellar and woven bone within a locally limited bone volume can be found due to a local bone remodeling defect, where osteocyte lacunae are substantially enlarged and more frequent [66]. Woven and lamellar bone show distinguishing characteristics in terms of the osteocyte lacunar morphology. In woven bone, osteocyte lacunae are larger and more densely packed in comparison to osteocyte lacunae in lamellar bone packets. The latter pattern was found in rats [67] but also in humans [31]. Hence, the proportion of woven bone per total bone would affect the overall osteocyte numbers. Zimmermann et al. showed microstructural properties of fetal/infant bone tissue with predominantly woven bone areas, whereas the bone from children between 2 and 14 years showed rather lamellar bone with smaller osteocyte lacunar sizes [31]. The occurrence of larger and densely packed osteocyte lacunae in the woven bone areas may originate from rapid bone formation leading to lower matrix deposition, and/or due to relative calcium deficiency, that causes enlarged osteocyte lacunae [4, 31, 54, 68, 69].

While the magnitude of experienced loads is considered crucial for osteocyte number and distribution, there are several studies also linking the magnitude and/or type of mechanical loading to lacunar size and shape [70]. The relationship between osteocyte characteristics and loading was further supported by specific mechanosensitivity responses by round-shaped and flat-shaped osteocytes [71, 72]. Namely, in vitro studies showed that the round-shaped osteocytes are more mechanosensitive than flat-shaped osteocytes [71], and Vatsa et al. reported that skeletal sites with a rather low loading (i.e., calvaria) are specifically equipped with round osteocytes [72]. It is our impression that this pattern of enlarged lacunae may be considered as a "safety factor" to ensure that osteocytes receive enough stimulation for survival, even in the skeletal sites that are neither strictly weight-bearing nor heavily loaded by muscle actions. Hemmatian et al. analyzed the fibula of lactating mice and showed that mechanical loading led to higher sclerostin expression in osteocytes, while osteocyte lacunae were larger compared to controls [73•]. The authors suggested that osteocytes residing in larger lacunae are more mechanoresponsive [73•]. Wu et al. [74•] conducted a study in patients with partly edentulous posterior maxilla who underwent maxillary sinus-floor elevation and analyzed osteocyte number, morphology, and orientation in the bone areas where a single tooth was missing (single gap) but also where several rear teeth were missing (free ends). While no significant differences in osteocyte numbers were found, bone tissue in single-gap regions showed 1.5 times higher osteocyte surface area and more cranially-caudally oriented osteocytes than in free ending regions in those cases with multiple teeth missing. The authors concluded that higher osteocyte area and anisotropic cranio-caudal orientation corresponded to higher and vertically oriented tensile strains observed in their finite element model [74•]. Additional studies providing three-dimensional datasets from bone undergoing different loading patterns would be helpful to further explore osteocyte lacunar shapes and sizes in the skeleton.

Importance of Osteocyte Characteristics for Bone Quality

The number of osteocyte lacunae and the viability of their inhabitant cells are important determinants of fracture susceptibility [25••, 44, 75]. In the superolateral femoral neck of elderly women, significantly lower osteocyte lacunar density was found in fracture cases in comparison to non-fracture cases (226 ± 27 vs. 247 ± 32/mm^2, $p \leq 0.05$) [75]. Further correlations between osteocyte lacunar density and fracture susceptibility were deduced from comparisons of superolateral and inferomedial regions of the femoral neck [17••]. Namely, the superolateral femoral neck is considered as a point of origin for hip fractures [76, 77]. The microstructure of the superolateral femoral neck typically reflects an adaptation to low habitual loads [48, 49], which becomes a problem during sideways falls where it suddenly experiences high impact stress and strain [75, 78, 79]. The superolateral femoral neck does not only reflect disadvantageous bone architecture since early childhood [52] but also reveals pronounced decay of microstructure during aging [42, 43, 51, 80, 81]. In contrast, the inferomedial femoral neck achieves better microarchitecture already in childhood [52], while also aging processes are less harmful to its microstructure [42].

Analysis of the proximal femoral diaphysis revealed that the number of osteocyte lacunae in alendronate-treated patients was higher than in treatment-naïve osteoporosis cases, while mineralized lacunae as the long-term witnesses of previous osteocyte death were less frequent following alendronate therapy [25••]. Indeed, there is both in vivo and in vitro data about bisphosphonates' mode of action, which includes the prevention of osteocyte apoptosis [82, 83]. The well-documented reduction of fracture risk following antiresorptive treatment therefore may be partially explained by BPs positive effects on the osteocyte network. Although in several pre-clinical animal studies significant accumulation of microdamage due to bisphosphonates suppressing bone turnover was of concern [84, 85], in humans, osteocyte viability and microdamage repair seem to be maintained during osteoporosis therapy with alendronate [25••, 86]. Osteocytes' viability is an important factor for maintaining bone quality and fracture resistance, while preservation of osteocytes' viability may be even more important in human bone in comparison to animal bone associated with shorter lifetimes. Of note, micropetrosis, i.e., lacunar occlusion (Fig. 1c) has so far only been reported in human bone but not in animal bone.

The human skeleton is exposed to a variety of loading scenarios. Depending on the load level, accumulation of microdamage represents a typical physiological phenomenon [87]. However, when the rate of microdamage formation exceeds the rate of tissue repair via appropriate remodeling, microcracks can develop more easily into macrocracks and bone fracture [88]. In physiological conditions, where the stress and strain is quite low, the microdamage accumulation process can be adjusted through osteocytes' capability to detect damages [89, 90]. Based on the so-called "scissor" model [91], it is assumed that the rupture of dendrites/canaliculi through microcracks spawns a new bone remodeling cycle starting with bone resorption [92, 93]. Because of osteocytes' ability to sense stress and strain [94], a decline of the widespread network of osteocytes represents a crucial risk for delay or absence of bone (re)modeling [18, 21, 25••].

Once cracking of the microstructure has taken place (Fig. 1h), osteocyte lacunae could affect crack propagation. Here, osteocyte lacunae represent small interfaces within the mineralized hard tissue consisting of mineralized lamellar bone, osteocyte lacunar walls with different mineralization characteristics (Fig. 1i) [16•] and soft cellular components within the lacunae. Such interfaces have the ability to either arrest or deflect propagating cracks (Fig. 1j) [95, 96]. Interestingly, microcracks can be often observed in interstitial bone regions (Fig. 1h) with high mineral content and low viable osteocyte density that are far away from nutrition and oxygen sources supplied by Haversian canals (Fig. 1k) [44]. However, bone fracture is essentially the result of low bone quality in combination with high-impact loads [75]. As bones are usually not subject to unusually high or traumatic impact forces, bone tissue with lower osteocyte density is not necessarily prone to fracture [97, 98]. The study of Zimmermann et al. [31] compared woven bone of infants with lamellar bones of children between 2 to 14 years; it was shown that lamellar bone has substantially higher stiffness and strength than woven bone despite osteocyte numbers being lower in lamellar bone. However, woven bone consists of enlarged osteocyte lacunae, undeveloped canalicular connections, disorganized collagen, and altered mineral-to-matrix ratios, which translates to inferior mechanical competence [31].

Conclusions

Recent research on osteocyte characteristics employing high-resolution techniques broadened our understanding on the distribution of osteocyte lacunae in various skeletal sites. While there is still a need for further research with special emphasis on osteocyte characteristics in various skeletal sites of the skeleton, currently available datasets reveal that the osteocyte lacunar network varies not only between different bones of the skeleton but also within individual bones. Here, differences were found between (i) anatomical quadrants of cross-sectioned bone, (ii) cortical and trabecular bone, (iii) periosteal and endocortical compartments, and (iv) osteonal and interstitial bone packets in human cortical bone. These differences might originate from specific distribution of mechanical loading and may be associated with a certain genetic preprogram pre-determining the osteocyte population. The origin of bone fragility is heavily influenced by alterations to the integrity of osteocyte network. When osteocytes' ability to sense/respond to mechanical signals diminishes and osteocytes are unable to orchestrate bone remodeling along repair of microdamage, the bone quality framework is endangered. Additional studies should provide further insights into the importance of osteocyte lacunar networks for maintaining bone quality while the effects of age, disease, mobility, lifestyle, and/or pharmacological interventions are of special concern to the integrity of the osteocytic network.

Acknowledgments The authors acknowledge the support from the German Research Foundation (DFG), the Alexander von Humboldt Foundation, and the Serbian Ministry of Education and Science (III45005).

Compliance with Ethical Standards

Conflict of Interest P. Milovanovic and B. Busse declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Currey JD. Bones: structure and mechanics. Princeton, N. J: Princeton University Press; 2002.
2. Busse B, Hahn M, Schinke T, Puschel K, Duda GN, Amling M. Reorganization of the femoral cortex due to age-, sex-, and endoprosthetic-related effects emphasized by osteonal dimensions and remodeling. J Biomed Mater Res A. 2010;92(4):1440–51. https://doi.org/10.1002/jbm.a.32432.
3. Jobke B, Milovanovic P, Amling M, Busse B. Bisphosphonate-osteoclasts: changes in osteoclast morphology and function induced by antiresorptive nitrogen-containing bisphosphonate treatment in osteoporosis patients. Bone. 2014;59:37–43. https://doi.org/10.1016/j.bone.2013.10.024.
4. Regelsberger J, Milovanovic P, Schmidt T, Hahn M, Zimmermann EA, Tsokos M, et al. Changes to the cell, tissue and architecture levels in cranial suture synostosis reveal a problem of timing in bone development. Eur Cell Mater. 2012;24:441–58.
5. Szulc P, Seeman E. Thinking inside and outside the envelopes of bone: dedicated to PDD. Osteoporos Int. 2009;20(8):1281–8. https://doi.org/10.1007/s00198-009-0994-y.
6. Buenzli PR, Sims NA. Quantifying the osteocyte network in the human skeleton. Bone. 2015;75:144–50. https://doi.org/10.1016/j.bone.2015.02.016.
7. Loiselle AE, Jiang JX, Donahue HJ. Gap junction and hemichannel functions in osteocytes. Bone. 2013;54(2):205–12. https://doi.org/10.1016/j.bone.2012.08.132.
8. Santos A, Bakker A, Klein-Nulend J. The role of osteocytes in bone mechanotransduction. Osteoporos Int. 2009;20(6):1027–31. https://doi.org/10.1007/s00198-009-0858-5.
9. Klein-Nulend J, Bonewald LF. The osteocyte. In: Bilezikian JP, Raisz LG, Martin TJ, editors. Principles of bone biology. 3rd ed. San Diego: Academic press; 2008. p. 153–74.
10. Bellido T. Osteocyte-driven bone remodeling. Calcif Tissue Int. 2014;94(1):25–34. https://doi.org/10.1007/s00223-013-9774-y.
11. Dallas SL, Prideaux M, Bonewald LF. The osteocyte: An endocrine cell ... and more. Endocr Rev. 2013;34(5):658–90. https://doi.org/10.1210/er.2012-1026.
12. Bonewald LF. The amazing osteocyte. J Bone Miner Res. 2011;26(2):229–38. https://doi.org/10.1002/jbmr.320.
13. Bonewald LF, Wacker MJ. FGF23 production by osteocytes. Pediatr Nephrol. 2013;28(4):563–8. https://doi.org/10.1007/s00467-012-2309-3.
14. Pereira RC, Juppner H, Azucena-Serrano CE, Yadin O, Salusky IB, Wesseling-Perry K. Patterns of FGF-23, DMP1, and MEPE expression in patients with chronic kidney disease. Bone. 2009;45(6):1161–8. https://doi.org/10.1016/j.bone.2009.08.008.
15. Prideaux M, Findlay DM, Atkins GJ. Osteocytes: the master cells in bone remodelling. Curr Opin Pharmacol. 2016;28:24–30. https://doi.org/10.1016/j.coph.2016.02.003.
16. • Milovanovic P, Zimmermann EA, vom Scheidt A, Hoffmann B, Sarau G, Yorgan T, et al. The formation of calcified nanospherites during micropetrosis represents a unique mineralization mechanism in aged human bone. Small. 2017;13(3):1602215. https://doi.org/10.1002/smll.201602215 **This study presents high-resolution data on the structure and composition of hypermineralized osteocyte lacunae as permanent traces of osteocyte apoptosis.**
17. •• Rolvien T, Vom Scheidt A, Stockhausen KE, Milovanovic P, Djonic D, Hubert J, et al. Inter-site variability of the osteocyte lacunar network in the cortical bone underpins fracture susceptibility of the superolateral femoral neck. Bone. 2018;112:187–93. https://doi.org/10.1016/j.bone.2018.04.018 **This study assessed cortical bone of the femoral neck—one of the most common fracture sites—from 12 female donors (age 34–86 years) with backscattered scanning electron microscopy and high-resolution microcomputed tomography (μ-CT). It highlights the lower osteocyte lacunar density in the superolateral subregion (lower habitual loading intensity) than in the inferomedial neck region (habitually highly loaded in compression), and suggests that reduced osteocyte numbers are linked to higher fragility of the superolateral neck.**
18. Milovanovic P, Zimmermann EA, Hahn M, Djonic D, Püschel K, Djuric M, et al. Osteocytic canalicular networks: morphological implications for altered mechanosensitivity. ACS Nano. 2013;7(9):7542–51. https://doi.org/10.1021/nn401360u.
19. Busse B, Bale HA, Zimmermann EA, Panganiban B, Barth HD, Carriero A, et al. Vitamin D deficiency induces early signs of aging in human bone, increasing the risk of fracture. Sci Transl Med. 2013;5(193):193ra88. https://doi.org/10.1126/scitranslmed.3006286.
20. Burr DB, Turner CH, Naick P, Forwood MR, Ambrosius W, Sayeed Hasan M, et al. Does microdamage accumulation affect the mechanical properties of bone? J Biomech. 1998;31(4):337–45. https://doi.org/10.1016/S0021-9290(98)00016-5.
21. Vashishth D, Verborgt O, Divine G, Schaffler MB, Fyhrie DP. Decline in osteocyte lacunar density in human cortical bone is associated with accumulation of microcracks with age. Bone. 2000;26(4):375–80.
22. Ashique AM, Hart LS, Thomas CDL, Clement JG, Pivonka P, Carter Y, et al. Lacunar-canalicular network in femoral cortical bone is reduced in aged women and is predominantly due to a loss of canalicular porosity. Bone Rep. 2017;7:9–16. https://doi.org/10.1016/j.bonr.2017.06.002.
23. Bach-Gansmo FL, Brüel A, Jensen MV, Ebbesen EN, Birkedal H, Thomsen JS. Osteocyte lacunar properties and cortical microstructure in human iliac crest as a function of age and sex. Bone. 2016;91:11–9. https://doi.org/10.1016/j.bone.2016.07.003.
24. • Rolvien T, Schmidt FN, Milovanovic P, Jähn K, Riedel C, Butscheidt S, et al. Early bone tissue aging in human auditory ossicles is accompanied by excessive hypermineralization, osteocyte death and micropetrosis. Sci Rep. 2018;8(1):1920. https://doi.

org/10.1038/s41598-018-19803-2 **The study shows that in auditory ossicles, the majority of osteocytes die within the first months and years of life. Despite abundant osteocyte apoptosis, bone remodeling is not initiated, which presents a safety factor to conserve the architecture of the auditory ossicles and ensure stable sound transmission throughout life.**

25.•• Milovanovic P, Zimmermann EA, Riedel C, Scheidt AV, Herzog L, Krause M, et al. Multi-level characterization of human femoral cortices and their underlying osteocyte network reveal trends in quality of young, aged, osteoporotic and antiresorptive-treated bone. Biomaterials. 2015;45:46–55. https://doi.org/10.1016/j.biomaterials.2014.12.024 **This study provides a detailed assessment of femoral cortex reorganization in young, aged, osteoporotic, and alendronate-treated female patients. Antiresorptive treatment showed favorable effects on osteocyte lacunar density and cell viability as reflected in a low occurrence of micropetrosis. Moreover, the study highlights differences in bone matrix mineralization between the femur, radius, iliac crest, and vertebral bone, and emphasizes differing trends in mineralization with bisphosphate treatment in relation to anatomical locations.**

26. Tong X, Malo MKH, Burton IS, Jurvelin JS, Isaksson H, Kröger H. Histomorphometric and osteocytic characteristics of cortical bone in male subtrochanteric femoral shaft. J Anat. 2017;231(5):708–17. https://doi.org/10.1111/joa.12670.

27. Akhter MP, Kimmel DB, Lappe JM, Recker RR. Effect of macroanatomic bone type and estrogen loss on osteocyte lacunar properties in healthy adult women. Calcif Tissue Int. 2017;100(6):619–30. https://doi.org/10.1007/s00223-017-0247-6.

28.•• Hunter RL, Agnew AM. Intraskeletal variation in human cortical osteocyte lacunar density: Implications for bone quality assessment. Bone Rep. 2016;5:252–61. https://doi.org/10.1016/j.bonr.2016.09.002 **The results from this study show differences in osteocyte lacunar number per bone area between the femur, radius, and rib. The authors suggest that although these skeletal sites systemically experience declines in osteocyte lacunar density, specific characteristics can be found at each anatomical site potentially due to age-related changes in mechanical loading.**

29.•• Gauthier R, Langer M, Follet H, Olivier C, Gouttenoire P-J, Helfen L, et al. 3D micro structural analysis of human cortical bone in paired femoral diaphysis, femoral neck and radial diaphysis. J Struct Biol. 2018;204:182–90. https://doi.org/10.1016/j.jsb.2018.08.006 **This SR-μCT study shows differences in osteocyte lacunar number per bone volume between paired femoral diaphysis, femoral neck, and radial diaphysis samples, suggesting that osteocyte characteristics at one skeletal site cannot be reliably transferred to other anatomical locations.**

30. Bereshiem AC, Pfeiffer SK, Grynpas MD, Alblas A. Use of backscattered scanning electron microscopy to quantify the bone tissues of midthoracic human ribs. Am J Phys Anthropol. 2019;168(2):262–78. https://doi.org/10.1002/ajpa.23716.

31. Zimmermann E, Riedel C, Stockhausen K, Chushkin Y, Schaible E, Schmidt F et al. Mechanical competence and bone quality develop during skeletal growth. J Bone Miner Res. 2019. https://doi.org/10.1002/jbmr.3730.

32. Bernhard A, Milovanovic P, Zimmermann EA, Hahn M, Djonic D, Krause M, et al. Micro-morphological properties of osteons reveal changes in cortical bone stability during aging, osteoporosis, and bisphosphonate treatment in women. Osteoporos Int. 2013;24(10):2671–80. https://doi.org/10.1007/s00198-013-2374-x.

33. Carter Y, Thomas CDL, Clement JG, Peele AG, Hannah K, Cooper DML. Variation in osteocyte lacunar morphology and density in the human femur — a synchrotron radiation micro-CT study. Bone. 2013;52(1):126–32. https://doi.org/10.1016/j.bone.2012.09.010.

34. Carter Y, Suchorab JL, Thomas CDL, Clement JG, Cooper DML. Normal variation in cortical osteocyte lacunar parameters in healthy young males. J Anat. 2014;225(3):328–36. https://doi.org/10.1111/joa.12213.

35. Shane E, Burr D, Abrahamsen B, Adler RA, Brown TD, Cheung AM, et al. Atypical subtrochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2014;29(1):1–23. https://doi.org/10.1002/jbmr.1998.

36. Donnelly E, Meredith DS, Nguyen JT, Gladnick BP, Rebolledo BJ, Shaffer AD, et al. Reduced cortical bone compositional heterogeneity with bisphosphonate treatment in postmenopausal women with intertrochanteric and subtrochanteric fractures. J Bone Miner Res. 2012;27(3):672–8. https://doi.org/10.1002/jbmr.560.

37. Repp F, Kollmannsberger P, Roschger A, Kerschnitzki M, Berzlanovich A, Gruber GM, et al. Spatial heterogeneity in the canalicular density of the osteocyte network in human osteons. Bone Rep. 2017;6:101–8. https://doi.org/10.1016/j.bonr.2017.03.001.

38. Rolvien T, Krause M, Jeschke A, Yorgan T, Puschel K, Schinke T, et al. Vitamin D regulates osteocyte survival and perilacunar remodeling in human and murine bone. Bone. 2017;103:78–87. https://doi.org/10.1016/j.bone.2017.06.022.

39. Bell KL, Loveridge N, Power J, Garrahan N, Meggitt BF, Reeve J. Regional differences in cortical porosity in the fractured femoral neck. Bone. 1999;24(1):57–64.

40. Bell KL, Loveridge N, Power J, Garrahan N, Stanton M, Lunt M, et al. Structure of the femoral neck in hip fracture: cortical bone loss in the inferoanterior to superoposterior axis. J Bone Miner Res. 1999;14(1):111–9. https://doi.org/10.1359/jbmr.1999.14.1.111.

41. Power J, Noble BS, Loveridge N, Bell KL, Rushton N, Reeve J. Osteocyte lacunar occupancy in the femoral neck cortex: an association with cortical remodeling in hip fracture cases and controls. Calcif Tissue Int. 2001;69(1):13–9. https://doi.org/10.1007/s00223-001-0013-6.

42. Djuric M, Djonic D, Milovanovic P, Nikolic S, Marshall R, Marinkovic J, et al. Region-specific sex-dependent pattern of age-related changes of proximal femoral cancellous bone and its implications on differential bone fragility. Calcif Tissue Int. 2010;86(3):192–201. https://doi.org/10.1007/s00223-009-9325-8.

43. Milovanovic P, Djonic D, Marshall RP, Hahn M, Nikolic S, Zivkovic V, et al. Micro-structural basis for particular vulnerability of the superolateral neck trabecular bone in the postmenopausal women with hip fractures. Bone. 2012;50(1):63–8. https://doi.org/10.1016/j.bone.2011.09.044.

44. Busse B, Djonic D, Milovanovic P, Hahn M, Püschel K, Ritchie RO, et al. Decrease in the osteocyte lacunar density accompanied by hypermineralized lacunar occlusion reveals failure and delay of remodeling in aged human bone. Aging Cell. 2010;9(6):1065–75. https://doi.org/10.1111/j.1474-9726.2010.00633.x.

45. Milovanovic P, Adamu U, Simon MJK, Rolvien T, Djuric M, Amling M, et al. Age- and sex-specific bone structure patterns portend bone fragility in radii and tibiae in relation to osteodensitometry: a high-resolution peripheral quantitative computed tomography study in 385 individuals. J Gerontol A Biol Sci Med Sci. 2015;70(10):1269–75. https://doi.org/10.1093/gerona/glv052.

46. Milovanovic P, Djuric M, Neskovic O, Djonic D, Potocnik J, Nikolic S, et al. Atomic force microscopy characterization of the external cortical bone surface in young and elderly women: potential nanostructural traces of periosteal bone apposition during aging. Microsc Microanal. 2013;19(5):1341–9. https://doi.org/10.1017/S1431927613001761.

47. Szulc P, Seeman E, Duboeuf F, Sornay-Rendu E, Delmas PD. Bone fragility: failure of oeriosteal apposition to compensate for increased endocortical resorption in postmenopausal women. J Bone Miner Res. 2006;21(12):1856–63. https://doi.org/10.1359/jbmr.060904.

48. Verhulp E, van Rietbergen B, Huiskes R. Load distribution in the healthy and osteoporotic human proximal femur during a fall to the side. Bone. 2008;42(1):30–5. https://doi.org/10.1016/j.bone.2007.08.039.

49. Rudman K, Aspden R, Meakin J. Compression or tension? The stress distribution in the proximal femur. Biomed Eng Online. 2006;5(1):12.

50. Mayhew PM, Thomas CD, Clement JG, Loveridge N, Beck TJ, Bonfield W, et al. Relation between age, femoral neck cortical stability, and hip fracture risk. Lancet. 2005;366(9480):129–35.

51. Djonic D, Milovanovic P, Nikolic S, Ivovic M, Marinkovic J, Beck T, et al. Inter-sex differences in structural properties of aging femora: implications on differential bone fragility: a cadaver study. J Bone Miner Metab. 2011;29(4):449–57. https://doi.org/10.1007/s00774-010-0240-x.

52. Milovanovic P, Djonic D, Hahn M, Amling M, Busse B, Djuric M. Region-dependent patterns of trabecular bone growth in the human proximal femur: a study of 3D bone microarchitecture from early postnatal to late childhood period. Am J Phys Anthropol. 2017;164(2):281–91. https://doi.org/10.1002/ajpa.23268.

53. Skedros JG, Baucom SL. Mathematical analysis of trabecular 'trajectories' in apparent trajectorial structures: the unfortunate historical emphasis on the human proximal femur. J Theor Biol. 2007;244(1):15–45.

54. Tsourdi E, Jähn K, Rauner M, Busse B, Bonewald LF. Physiological and pathological osteocytic osteolysis. J Musculoskelet Neuronal Interact. 2018;18(3):292–303.

55. Lin C, Jiang X, Dai Z, Guo X, Weng T, Wang J, et al. Sclerostin mediates bone response to mechanical unloading through antagonizing Wnt/β-catenin signaling. J Bone Miner Res. 2009;24(10):1651–61. https://doi.org/10.1359/jbmr.090411.

56. Moriishi T, Fukuyama R, Ito M, Miyazaki T, Maeno T, Kawai Y, et al. Osteocyte network; a negative regulatory system for bone mass augmented by the induction of Rankl in osteoblasts and Sost in osteocytes at unloading. PLoS One. 2012;7(6):e40143.

57. Gerbaix M, Gnyubkin V, Farlay D, Olivier C, Ammann P, Courbon G, et al. One-month spaceflight compromises the bone microstructure, tissue-level mechanical properties, osteocyte survival and lacunae volume in mature mice skeletons. Sci Rep. 2017;7(1):2659. https://doi.org/10.1038/s41598-017-03014-2.

58. Gill TM, Allore H, Guo Z. The deleterious effects of bed rest among community-living older persons. J Gerontol A Biol Sci Med Sci. 2004;59(7):755–61.

59. Pfaff C, Schultz JA, Schellhorn R. The vertebrate middle and inner ear: a short overview. J Morphol. 2018. https://doi.org/10.1002/jmor.20880.

60. Noble BS, Peet N, Stevens HY, Brabbs A, Mosley JR, Reilly GC, et al. Mechanical loading: biphasic osteocyte survival and targeting of osteoclasts for bone destruction in rat cortical bone. Am J Phys Cell Physiol. 2003;284(4):C934–43. https://doi.org/10.1152/ajpcell.00234.2002.

61. Tomkinson A, Reeve J, Shaw RW, Noble BS. The death of osteocytes via apoptosis accompanies estrogen withdrawal in human bone. J Clin Endocrinol Metab. 1997;82(9):3128–35. https://doi.org/10.1210/jc.82.9.3128.

62. Noble BS, Stevens H, Loveridge N, Reeve J. Identification of apoptotic changes in osteocytes in normal and pathological human bone. Bone. 1997;20(3):273–82.

63. Tomkinson A, Gevers EF, Wit JM, Reeve J, Noble BS. The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res. 1998;13(8):1243–50.

64. Weinstein RS, Nicholas RW, Manolagas SC. Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. J Clin Endocrinol Metab. 2000;85(8):2907–12. https://doi.org/10.1210/jc.85.8.2907.

65. Kogianni G, Mann V, Noble BS. Apoptotic bodies convey activity capable of initiating osteoclastogenesis and localized bone destruction. J Bone Miner Res. 2008;23(6):915–27. https://doi.org/10.1359/jbmr.080207.

66. Zimmermann EA, Kohne T, Bale HA, Panganiban B, Gludovatz B, Zustin J, et al. Modifications to nano- and microstructural quality and the effects on mechanical integrity in Paget's disease of bone. J Bone Miner Res. 2015;30(2):264–73. https://doi.org/10.1002/Jbmr.2340.

67. Hernandez CJ, Majeska RJ, Schaffler MB. Osteocyte density in woven bone. Bone. 2004;35(5):1095–9. https://doi.org/10.1016/j.bone.2004.07.002.

68. Remaggi F, Canè V, Palumbo C, Ferretti M. Histomorphometric study on the osteocyte lacunocanalicular network in animals of different species. I. Woven-fibered and parallel-fibered bones. Ital J Anat Embryol. 1998;103:145–55.

69. Mulder L, Koolstra JH, den Toonder JMJ, van Eijden TMGJ. Relationship between tissue stiffness and degree of mineralization of developing trabecular bone. J Biomed Mater Res A. 2008;84A(2):508–15. https://doi.org/10.1002/jbm.a.31474.

70. van Oers RFM, Wang H, Bacabac RG. Osteocyte shape and mechanical loading. Curr Osteoporos Rep. 2015;13(2):61–6. https://doi.org/10.1007/s11914-015-0256-1.

71. Bacabac RG, Mizuno D, Schmidt CF, MacKintosh FC, Loon JJWAV, Klein-Nulend J, et al. Round versus flat: bone cell morphology, elasticity, and mechanosensing. J Biomech. 2008;41(7):1590–8.

72. Vatsa A, Breuls RG, Semeins CM, Salmon PL, Smit TH, Klein-Nulend J. Osteocyte morphology in fibula and calvaria — is there a role for mechanosensing? Bone. 2008;43(3):452–8.

73.• Hemmatian H, Jalali R, Semeins CM, JMA H, van Lenthe GH, Klein-Nulend J, et al. Mechanical loading differentially affects osteocytes in fibulae from lactating mice compared to osteocytes in virgin mice: possible role for lacuna size. Calcif Tissue Int. 2018;103(6):675–85. https://doi.org/10.1007/s00223-018-0463-8 **These results suggest that osteocytes in fibulae from lactating mice with large lacunae respond more distinct to mechanical loading than those from virgin mice, as evidenced via sclerostin expression. The authors conclude that osteocytes residing in large lacunae show an effective response to mechanical loading.**

74.• Wu V, van Oers RFM, Schulten EAJM, Helder MN, Bacabac RG, Klein-Nulend J. Osteocyte morphology and orientation in relation to strain in the jaw bone. Int J Oral Sci. 2018;10(1):2. https://doi.org/10.1038/s41368-017-0007-5 **This study reports osteocyte data in relation to jaws in areas where a single tooth was missing or where several rear teeth were missing. The authors linked osteocyte characteristics to strain distribution as shown in a finite element model.**

75. Milovanovic P, Rakocevic Z, Djonic D, Zivkovic V, Hahn M, Nikolic S, et al. Nano-structural, compositional and micro-architectural signs of cortical bone fragility at the superolateral femoral neck in elderly hip fracture patients vs. healthy aged controls. Exp Gerontol. 2014;55:19–28.

76. PMd B, Manske SL, Ebacher V, Oxland TR, Cripton PA, Guy P. During sideways falls proximal femur fractures initiate in the superolateral cortex: evidence from high-speed video of simulated fractures. J Biomech. 2009;42(12):1917–25.

77. Tang T, Cripton PA, Guy P, McKay HA, Wang R. Clinical hip fracture is accompanied by compression induced failure in the superior cortex of the femoral neck. Bone. 2018;108:121–31. https://doi.org/10.1016/j.bone.2017.12.020.

78. Zani L, Erani P, Grassi L, Taddei F, Cristofolini L. Strain distribution in the proximal Human femur during in vitro simulated sideways fall. J Biomech. 2015;48(10):2130–43. https://doi.org/10.1016/j.jbiomech.2015.02.022.

79. Kheirollahi H, Luo Y. Identification of high stress and strain regions in proximal femur during single-leg stance and sideways fall using QCT-based finite element model. Int J Med Health Biomedi Bioeng Pharm Eng. 2015;9(8):633–40.
80. Milovanovic P, vom Scheidt A, Mletzko K, Sarau G, Püschel K, Djuric M, et al. Bone tissue aging affects mineralization of cement lines. Bone. 2018;110:187–93. https://doi.org/10.1016/j.bone.2018.02.004.
81. Thomas CDL, Mayhew PM, Power J, Poole KES, Loveridge N, Clement JG, et al. Femoral neck trabecular bone: loss with aging and role in preventing fracture. J Bone Miner Res. 2009;24(11): 1808–18. https://doi.org/10.1359/jbmr.090504.
82. Ye T, Cao P, Qi J, Zhou Q, Rao DS, Qiu S. Protective effect of low-dose risedronate against osteocyte apoptosis and bone loss in ovariectomized rats. PLoS One. 2017;12(10):e0186012.
83. Bellido T, Plotkin LI. Novel actions of bisphosphonates in bone: preservation of osteoblast and osteocyte viability. Bone. 2011;49(1):50–5. https://doi.org/10.1016/j.bone.2010.08.008.
84. Mashiba T, Turner CH, Hirano T, Forwood MR, Johnston CC, Burr DB. Effects of suppressed bone turnover by bisphosphonates on microdamage accumulation and biomechanical properties in clinically relevant skeletal sites in beagles. Bone. 2001;28(5):524–31. https://doi.org/10.1016/S8756-3282(01)00414-8.
85. Mashiba T, Hirano T, Turner CH, Forwood MR, Johnston CC, Burr DB. Suppressed bone turnover by bisphosphonates increases microdamage accumulation and reduces some biomechanical properties in dog rib. J Bone Miner Res. 2000;15(4):613–20. https://doi.org/10.1359/jbmr.2000.15.4.613.
86. Krause M, Soltau M, Zimmermann EA, Hahn M, Kornet J, Hapfelmeier A, et al. Effects of long-term alendronate treatment on bone mineralisation, resorption parameters and biomechanics of single human vertebral trabeculae. Eur Cell Mater. 2014;28: 152–63 discussion 63-5.
87. Ritchie RO. The conflicts between strength and toughness. Nat Mater. 2011;10(11):817–22. https://doi.org/10.1038/nmat3115.
88. Ma S, Goh EL, Jin A, Bhattacharya R, Boughton OR, Patel B, et al. Long-term effects of bisphosphonate therapy: perforations, microcracks and mechanical properties. Sci Rep. 2017;7:43399. https://doi.org/10.1038/srep43399.
89. Jan GH, Michael F, Eilis F, Thomas CL, David T. Microdamage: a cell transducing mechanism based on ruptured osteocyte processes. J Biomech. 2006;39(11):2096–103.
90. Noble B. Bone microdamage and cell apoptosis. Eur Cell Mater. 2003;6:46–55.
91. Taylor D, Mulcahy L, Presbitero G, Tisbo P, Dooley C, Duffy G, Lee TC The scissors model of microcrack detection in bone: work in progress. MRS Online Proc Lib 2010;1274. https://doi.org/10.1557/PROC-1274-QQ08-01.
92. Dooley C, Tisbo P, Lee T, Taylor D. Rupture of osteocyte processes across microcracks: the effect of crack length and stress. Biomech Model Mechanobiol. 2012;11(6):759–66. https://doi.org/10.1007/s10237-011-0349-4.
93. Colopy SA, Benz-Dean J, Barrett JG, Sample SJ, Lu Y, Danova NA, et al. Response of the osteocyte syncytium adjacent to and distant from linear microcracks during adaptation to cyclic fatigue loading. Bone. 2004;35(4):881–91. https://doi.org/10.1016/j.bone.2004.05.024.
94. Klein-Nulend J, Bakker A. Osteocytes: mechanosensors of bone and orchestrators of mechanical adaptation. Clin Rev Bone Miner Metab. 2007;5(4):195–209. https://doi.org/10.1007/s12018-008-9014-6.
95. Brock GR, Kim G, Ingraffea AR, Andrews JC, Pianetta P, van der Meulen MCH. Nanoscale examination of microdamage in sheep cortical bone using synchrotron radiation transmission x-ray microscopy. PLoS One. 2013;8(3):e57942. https://doi.org/10.1371/journal.pone.0057942.
96. Reilly GC. Observations of microdamage around osteocyte lacunae in bone. J Biomech. 2000;33(9):1131–4. https://doi.org/10.1016/s0021-9290(00)00090-7.
97. Cummings SR, Nevitt MC. Non-skeletal determinants of fractures: the potential importance of the mechanics of falls. Osteoporos Int. 1994;4(1):S67–70. https://doi.org/10.1007/bf01623439.
98. Greenspan SL, Myers ER, Kiel DP, Parker RA, Hayes WC, Resnick NM. Fall direction, bone mineral density, and function: risk factors for hip fracture in frail nursing home elderly. Am J Med. 1998;104(6):539–45.