

Osteocyte size, shape, orientation, and population density

Scaling relationships, interpretation of load history, and mechanical consequences

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Keywords

Osteocyte, osteocyte lacuna, canaliculi, osteons, osteon morphotypes, bone

Summary

Despite being encased in lacunae, osteocytes are extensively interconnected and have several mechanisms that enable them to physically and chemically appraise their environment and adjust to it. In the perspective that cell-cell and cell-matrix interactions mediate these functions and are critically important during the formation of a mechanically competent bone organ, we focus on several considerations: (1) osteocyte lacunae are not always occupied by living cells and the percent lacuna vacancy can increase with aging, some diseases, and experimental perturbations, (2) the potential for the population density and/or sizes and shapes of osteocytes (or of their lacunae) and of their cell pro-

cesses (typically seen as the canaliculi in which they reside) in helping investigators interpret the load history of a bone or bone region, and (3) scaling relationships between osteocyte density and various parameters, including animal mass and metabolism. We also point out that all of these considerations are being impacted by high-resolution three-dimensional imaging technologies that allow increased accuracy when quantifying details of lacunar-canalicular geometries.

Schlüsselwörter

Osteozyt, Osteozytenlakunen, Kanalikuli, Osteone, Osteon-Morphotypen, Knochen

Zusammenfassung

Auch wenn sie in Lakunen eingebettet sind, sind Osteozyten umfangreich verschaltet und

verfügen über eine Vielzahl von Mechanismen, die es ihnen ermöglicht, ihre Umgebung in physikalischer und chemischer Hinsicht zu erkunden und sich den Gegebenheiten anzupassen. Unter dem Blickwinkel betrachtet, dass diese Funktionen über Zell-Zell- und Zell-Matrix-Interaktionen vermittelt werden und von besonderer Bedeutung für die Bildung eines mechanisch kompetenten Knochens sind, konzentrieren wir uns auf verschiedene Betrachtungen: (1) Nicht alle Osteozytenlakunen sind von Osteozyten besiedelt und der Anteil leerer Lakunen kann im Alter sowie durch Krankheit und experimentelle Perturbationen zunehmen, (2) das Potenzial für die Zelldichte und/oder Größe und Form der Osteozyten (oder ihrer Lakunen) und ihrer Zellprozesse (typischerweise als Kanalikuli betrachtet), die den Forschern dabei helfen, die Belastungsart eines Knochens und einer Knochenregion zu interpretieren, und (3) die Skalierungsbeziehung zwischen der Osteozytendichte und verschiedenen Parametern, einschließlich Tiergewicht und Stoffwechsel. Wir zeigen außerdem auf, dass alle diese Betrachtungen durch hochauflösende 3D-Bildgebungsverfahren beeinflusst werden, da diese eine höhere Genauigkeit bei der Quantifizierung von Einzelheiten der lakuno-kanalikulären Geometrie erlauben.

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Größe, Form, Orientierung und Zelldichte von Osteozyten

Skalierungsbeziehung, Interpretation der Belastungsart und mechanische Konsequenzen

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Osteocyte cell bodies reside within the mineralized matrix in spaces called lacunae. Despite being encased in a lacuna, an osteocyte is extensively interconnected with its neighboring osteocytes via cell processes that extend from the cell body into dozens (usually) of canaliculi that surround its lacuna (1, 2). Therefore, osteocytes have been described as forming a neural-like

network that is inter-connected with gap junctions (3, 4). In turn, they likely employ several mechanisms that enable them to physically and chemically appraise their environment and adjust to it. For example, they sense matrix strains, detect microdamage, and activate repair mechanisms, while also being able to participate in local and systemic calcium balance.

Variations in osteocyte viability and lacuna population densities (Ot.Lc.Den)

Osteocyte viability can vary, and sometimes dramatically, even in cases where the individuals being analyzed are advancing into middle age (but are not per se

“elderly”), have diseases that affect bone health, or have been subjected to experimental perturbation (5–9). However, in studies of the normal functions of embedded osteocytes, it is common that the percentage of osteocytes that are alive cannot be determined. ► Table 1 (see online supplementary material to this article on www.osteologie-journal.de) lists selected studies that have used 2D imaging to examine osteocyte lacuna population densities (Ot.Lc.Den)¹ and/or osteocyte viability in various bones.

In our studies of regional variations in Ot.Lc.Den in limb bones from horses, deer, elk, and sheep, we were not able to determine percent osteocyte viability because the tissues were obtained many days after the animal had died (10, 11). But even if the tissues had been obtained immediately at the time of death, the only way to determine osteocyte viability is with immediate tissue processing using special fixation procedures and staining. In view of this deficiency, we argued that osteocyte viability was likely high because we used relatively young adults and because lacunae were not plugged with hypermineralized tissue, which has been associated with aging, ischemia, or necrosis. These observations and the estimated younger ages of our animals led to our suggestion that the percentage of dead osteocytes is small; this is consistent with observations of ~90% lacuna occupancy in the dorsal cortex of third metacarpals (MC3s) from horses similar in age to those used that we examined (12).

However, the theoretical idea that lacuna vacancy can be largely ignored in normal younger individuals leads to the problem of when (at what age) does this issue become important? Investigators might take comfort in knowing that studies have reported that the percentage of vacant lacunae at age ~80 years in normal human males and females is about 10% in cancellous bone from iliac crest biopsies (7, 8) and about 14% in cortical bone of ribs (13). However, age and glucocorticoid treatment were found to be associated with signifi-

Key Points

- Osteocyte lacunae are not always occupied by living cells, even in younger bones.
- Osteocyte population densities and sizes/shapes do not correlate with load history.
- Morphologies of osteocyte processes (canaliculi) do not correlate with load history.
- Osteocyte density does not scale simply/straightforward with metabolism or body mass.
- The “stressed volume effect” should be considered in studies of osteocyte physiology.

Kernbotschaft

- Bereits in jungem Knochen sind nicht alle Osteozytenlakunen von Osteozyten besiedelt.
- Die Zelldichte sowie die Größe beziehungsweise Form der Osteozyten korrelieren nicht mit der vorherrschenden Belastungsart.
- Die Morphologie der Osteozytenfortsätze (Kanalikuli) korreliert nicht mit der vorherrschenden Belastungsart.
- Die Zelldichte der Osteozyten skaliert nicht eindeutig mit Stoffwechselfparametern oder dem Körpergewicht.
- Bei der Bewertung der Osteozytenphysiologie sollte der „stressed volume effect“ berücksichtigt werden.

cantly increased osteocyte lacuna vacancy (7, 8). In a study of cancellous bone from trans-cortical iliac crest biopsies from humans with and without a hip fracture, Mullender et al. (14) reported that controls had ~18% empty lacunae (including a subgroup of individuals ≤55 years old) and osteoporotic patients had ~22% empty lacunae. Although the difference between these two groups was not statistically significant, these percentages seem high. There are cases where the percent of empty lacunae is even higher. For example, using tibiae of normal and immobilized (by sciatic neurectomy) rats, Iwamoto et al. (9) found a significant increase in empty lacunae when compared to age- and sex-matched controls (40% versus 34% empty lacunae in

immobilized and control bones, respectively). Power et al. (5) reported ~48–55% empty lacunae in cortical bone from femoral neck cortices of elderly human females. These data are generally consistent with earlier studies showing that the percentage of empty lacunae increases, and often markedly, with age in humans (14–19). Consequently, as emphasized by Vashishth et al. (20), the typical age-related decline in osteocyte density is probably more pronounced than the age-related decline in lacuna density.

It is important to note that sex can influence age-related changes in Ot.Lc.Den and/or lacunar shape-related morphologies. Using the femoral shafts from 30 women aged 20–86 years that were imaged using synchrotron-radiation micro-CT, Carter et al. (21) found no significant relationship between Ot.Lc.Den and age. However, a significant reduction in lacunar volume with age ($p < 0.001$) was observed, in addition to changes in lacunar morphology. When divided into two groups (<50 and >50 years) the younger group’s lacunae were ~30% larger, flatter ($p < 0.001$) and less spherical ($p < 0.001$). In contrast to these data in human females, and also using synchrotron-radiation micro-CT, Carter et al. (personal communication, submitted study) found that Ot.Lc.Den declines over the lifespan in the femoral shafts of males (36 men aged 18–92 years). They also showed that lacunar morphology demonstrated a tendency towards being more spherical and less elongate. These data suggest that sex plays a role in age-related changes in lacunar parameters. However, Busse et al. (22) did not detect sex-related differences in their study of age-related changes in Ot.Lc.Den in cortical bone of human femora from males and females across a broad age range. For additional data and discussion of age-related variations in human Ot.Lc.Den see their detailed study (22).

Previous studies investigating Ot.Lc.Den have also reported conflicting results in additional contexts. Jast and Jasiuk (23) used high-resolution micro-CT to examine tibial cortical bone in Sprague-Dawley rats (ages 3, 12, 32, 42, 60 and 72 weeks). Despite the marked increase in animals’ size across this range, no statistically significant differences

¹ In this review, osteocyte lacuna population density (Ot.Lc.Den) usually refers to the number of osteocyte lacunae per bone area, which is abbreviated as N.Ot.Lc/B.Ar (as shown in Table 1).

in Ot.Lc.Den or lacunar volume were found. Studies of human vertebral trabecular bone and femoral cortical bone (20, 24) have demonstrated that the relationship between Ot.Lc.Den and age is dependent on sex and is tissue specific. This could, in part, account for the discrepancy between results obtained by Mullender et al. (19) (a positive relationship with animal size and osteocyte density) and those found in Jast and Jasiuk (23) (no relationship with animal size and osteocyte density). Notably, osteocyte density in rats was reported to be $\sim 93,000 \text{ mm}^3$ by (19), which markedly differs from the $\sim 42,000 \text{ mm}^3$ reported by (23). This might reflect differences in the bone (femur vs. tibia) and/or bone “type” (cancellous vs. cortical); Mullender et al. analyzed femoral cancellous bone compared with the tibial cortical bone in Jast and Jasiuk (23). This discrepancy might also be influenced by differences in strain magnitudes between the rats used (Wistar rats vs. Sprague-Dawley rats). Perhaps this also is influenced by reduced accuracy of measurements made using 2D (19) vs. 3D (23) imaging (discussed below).

Interpreting load history and adaptation: density, size, and shape of osteocytes

In an analytical study estimating matrix strains in bone surrounding osteocytes, McCreadie and Hollister (25) demonstrated that lacunar shape is an important determinant of the strains experienced by the osteocytes. In loaded animal models, lacunar volume has been reported to increase (26). Conversely, lacunar volume decreases with decreased loads (27, 28). When subjected to increased load, osteocytes also become flatter and less spherical (28) and, similarly, in bones habitually subjected to relatively low strains (e.g., some skull bones) the lacunae are smaller and more spherical than in bones that habitually experience high strains (e.g., limb bones) (29). These studies support the hypothesis that osteocytes can adjust their lacunar morphology with respect to their localized loading environment, possibly re-

Biomechanically important structural and material characteristics in diaphyseal bone hierarchical organization¹

Structural Characteristics

- Bone length
- Diaphyseal curvature
- Cross-sectional shape and robusticity (e.g., moments and axes of area [inertial])
- Average and regional cortical thickness variations

Material Characteristics

- Mineral content (% ash)
- Microstructure
 - Secondary osteon population density and fractional area (On.N/T.Ar, On.Ar/T.Ar)
 - Secondary osteon cross-sectional area, shape, and orientation
 - Secondary osteon morphotypes (e.g. bright, alternating, parallel-fibered, hooped)
 - Mineral heterogeneity (e.g., relatively highly mineralized interstitial bone, young osteons, etc.)
 - Collagen fiber heterogeneity
 - Porosity (e.g., Haversian canals, primary vascular canals)
 - Lamellar organization of various osteon morphotypes
 - Variations in primary histologic organization (e.g., laminar vs. reticular vascular patterns in fibrolamellar/plexiform bone)
 - Osteocyte and lacunar population density, osteocyte lacuna-canalicular geometries
- Nanostructure
 - Predominant collagen fiber orientation (CFO), collagen density
 - Types and densities of collagen molecular cross-links
 - Mineral crystallite orientation, size, and heterogeneity
 - Spatial distribution of non-collagenous proteins (e.g., osteopontin and osteocalcin)

¹ Cancellous (trabecular) bone is not considered here

flecting their suggested role in mechanosensation and other mechanobiological and nutrient delivery functions. van Oers et al. (30) also speculated that differences in osteocyte and lacunar shape could stem from differences in mechanical loading between bone regions and with aging.

Processing strain-related information across an entire bone cross-section requires the presence of a cellular network that communicates across broad regions of a bone (e.g., across an entire diaphyseal cross-section). For this to work, the most likely resident cells with this function are osteocytes because they:

1. are the most numerous bone cells,
2. exhibit modified inter-cellular gap junctions and cell-cell molecular and/or electrical-like transmission, and
3. form gap junctions with bone lining cells that reside on the porous surfaces where osteoclast migration or activation commences for modeling/remodeling processes.

In these perspectives it is reasonable to speculate that differences in the distributions of osteocyte densities (based on Ot.Lc.Den) and/or their shape might correlate with load history. With keen interest in this possibility, we first focused on determining if regional variations in Ot.Lc.Den correlate with load history. More specifically, we sought to determine if the regional non-uniform strain mode and magnitude distribution that is typically produced by habitual unidirectional bending in a bone or bone region could be deduced from variations in the distributions of osteocyte lacunae. This idea is based on the fact that compression-loaded regions usually receive higher strains than tension-loaded regions, and neutral axis (prevalent/predominant shear) regions have the lowest strains. This type of analysis, where load history is retrospectively interpreted from variations in Ot.Lc.Den, lacunar shape and volume, or other histomorphological characteristics (see box “Biomechanically important structural and material characteristics in diaphyseal bone hierarchical organization” on this page), reflects our research that often involves examination of cadaveric material. The impetus for seeking correlates of regional variations in cell density

and/or histomorphological characteristics with regional variations in load history is the pervasive idea that bone tissue perceives regional non-uniformities in the strain environment, and adjusts its matrix organization accordingly (even at the osteocyte lacunar-canalicular level).

We conjectured that regional differences in osteocyte densities would be present and would reflect differences in the sensitivity of the cellular network for monitoring and ensuring a normal range of strains and detecting microdamage (10, 31). However, we did not find correlations of Ot.Lc.Den with strain history (modes and magnitudes) in relatively simply loaded bones where these relationships should be obvious (including no relationship across a bone's cortex from the periosteal region to the medullary canal) (10, 11). This result then led to this question: what cellular or histomorphological characteristic, or characteristics, are helpful for interpreting load history of a bone or bone region?

Some readers might think that regional variations in secondary osteon (Haversian system) population density can be very helpful for interpreting load history. In fact, secondary osteon population densities and other osteon morphologic characteristics (e.g., size, shape, etc.) receive great attention in anthropological studies that are aimed at distinguishing species or deciphering the load history within regions of limb bones and ribs. But our review of studies that have considered this possibility reveals that osteon population density and size do not correlate strongly with load history (32). Although there is evidence that osteon size tends to increase toward the endosteum (33), inconsistency in this relationship suggests that, when present, it might be an effect of proximity to the marrow and not a consequence of low strain (34, 35).

We have shown that these gross morphological parameters of osteons are a distant second to the regional patterns in the distribution of predominant collagen fiber orientation and/or secondary osteon morphotypes in reflecting a habitual bending versus torsional load history. These collagen fiber-orientation-based characteristics are stronger correlates than regional variations in mineral content, osteon population densities, Ot.Lc.Den, and likely also osteocyte lacunar shape and size (32, 36).

Interpreting load history and adaptation: number of canaliculi per osteocyte and other canalicular parameters

We re-examined the literature to see if the number of osteocyte canaliculi per cell, or some other canalicular morphological parameter (e.g., length, branching, and modifications in their number), might change with strain characteristics (e.g., magnitude and rate). Some findings related to this issue are reported by Bozal et al. (26) who used orthodontic appliances to alter the

loading on rat alveolar (jaw) bone. They measured several osteocyte and lacunar parameters including the number of osteocyte cell processes and the number of canaliculi surrounding each lacuna. No significant changes in the number of osteocyte cell processes and canaliculi were found between the compression-loaded and tension-loaded sides of the bone at baseline and as early as one hour after loading. However, cytoplasmic cell processes increased in length by 29% in compression and 38% in tension without changes in cell process width after compression loading and an 18% decrease in process width after tension loading. Canaliculi increased in width by 10% in compression and 14% in tension; however, canalicular length did not change significantly. One hour after applying compression loading, Bozal et al. (26) also reported a 44% increase in lacunar volume and only minor changes occurred in osteocyte volume with a 24% decrease in cell body length:width ratio. One hour after applying tension loading, they also found a 29% increase in lacunar volume and a 54% decrease in osteocyte volume, in addition to a 19% decrease in the ratio of cell body length:width ratio.

These results are intriguing because they show phenotypic plasticity of the lacunar-canalicular network as a result of modifications of the strain environment. The challenge is to determine what mechanisms are even more proximate than the matrix strains in causing these changes and whether or not they could be used to distinguish bones habitually loaded in bending (i.e., tension- vs. compression-loaded regions) vs. bones subjected to mixed loading (e.g., substantial torsion/shear). Future studies should consider the possibility that some aspects of the osteocytic network might be more closely associated with fluid-flow dynamics. For example, even though longitudinal strains are low in neutral axis regions, circumferential strain gradients and fluid flux are high (37). As previously suggested (10), variations in circumferential strain gradients might be correlated with variations in Ot.Lc.Den or other lacunar-canalicular characteristics.

Some parameters that might influence osteocyte densities in cortices of limb bones

- Histologic type (e.g., woven; fibrolamellar; secondary osteons)
- Percentage of secondary bone
- Remodeling rate
- Diaphyseal location: mid diaphysis vs. metaphysis
- "Envelope" (periosteal, middle, endocortical)
- Local rate of osteogenesis (mineral apposition rate) that produced the tissue
- Mean tissue age (viable osteocytes and Ot.Lc.Den decrease with age)
- Prevalent/predominant strain mode and other local strain- or strain-related characteristics (e.g., fluid-flow dynamics; strain gradients)
- Size/resolution and number density of "sensed" entity (i.e., microdamage)
- Metabolic requirements/Mineral homeostasis
- Nutrient delivery¹

¹ Means/efficiency of nutrient delivery differ significantly between cancellous and cortical bone. For example, in cortical bone the osteocytes can be in closer proximity to the vascular supply and there is greater importance of fluid-flow-mediated delivery driven by functional loading. In contrast, nutrient delivery to trabecular bone is more strongly dependent upon transport/diffusion from the marrow (42).

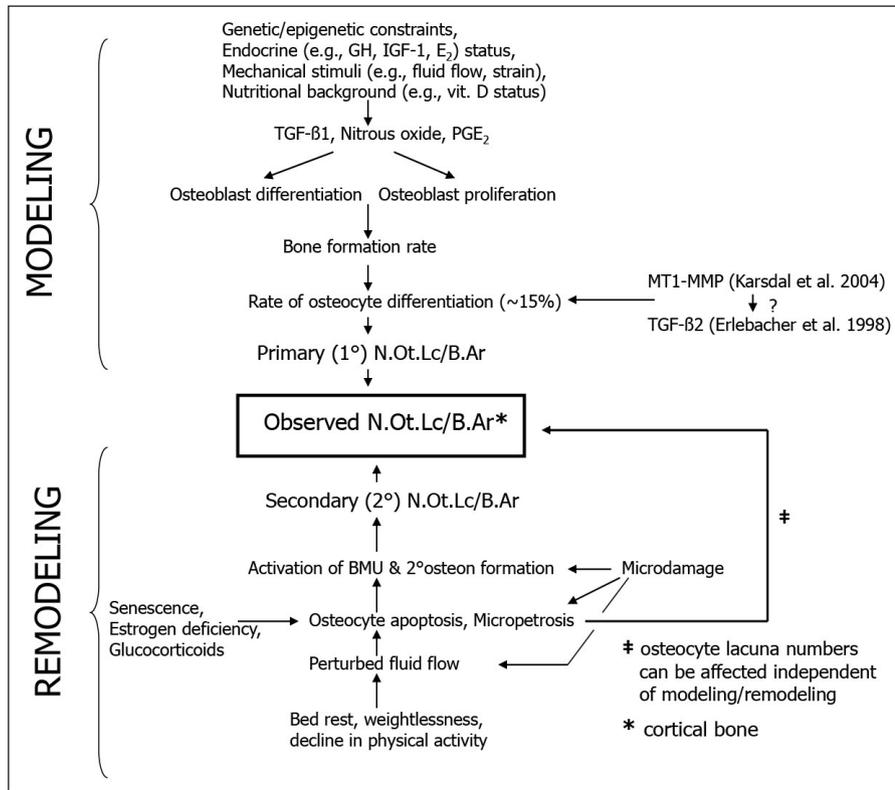


Fig. 1 Modeling and remodeling and related influences on osteocyte densities in cortical bone (81, 82). The roles played by sclerostin, WNT signaling pathways, and other factors and pathways are shown in ► figure 2.

Scaling relationships with osteocytes and related parameters

We warn readers that what follows becomes very confusing because of the myr-

riad and often confounding issues that should be considered in studies of potential scaling relationships between Ot.Lc.Den and body mass, metabolism, or other physiological parameters (see box “Some parameters that might influence osteocyte

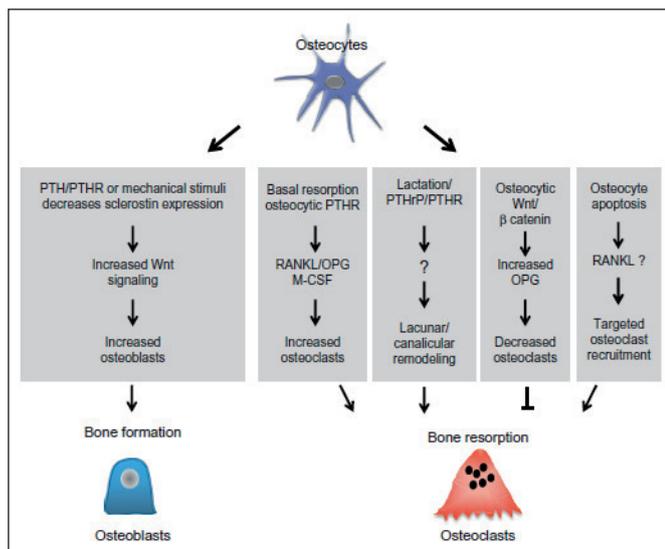


Fig. 2 Functions of osteocytes involved in the regulation of osteoblast and osteoclast production; after (83) with permission of Elsevier

densities in cortices of limb bones” on page 95; ► Fig. 1, ► Fig. 2).

Mullender et al. (19) investigated relationships between trabecular morphology, animal size, and osteocyte density in the femoral heads of 30 adult animals of five mammalian species (rat, rabbit, Rhesus monkey, pig, and cow). They reported that osteocyte density was inversely related to the size of the animals in each species. In turn they speculated that because basal metabolic output is related to body mass, osteocyte density may be related to metabolic rates. However, as described further below, the findings of Mullender et al. contradict results reported previously by Marotti et al. (38), Hobdell and Howe (39), and also those subsequently conducted by Remaggi et al. (40) and Ferretti et al. (41). This contradiction may reflect Mullender and co-workers’ examination of cancellous (trabecular) bone compared to (40, 41) who examined cancellous and cortical (compact) bone with various histological types (woven, parallel-fibered, and lamellar). The means and efficiency of nutrient delivery also clearly differ between cancellous and cortical bone (42).

When analyzing woven bone (lacking secondary osteons) and parallel-fibered bone (secondary osteons are common) in long bones (mainly tibiae and femora) of ten mature animal species (including human), Marotti et al. (38) found that neither Ot.Lc.Den nor lacunar volume correlate with animal body size. Instead, parallel-fibered bone has lower Ot.Lc.Den than woven bone. To explain this finding they emphasized that the main purpose of woven bone is to deposit relatively irregular and unorganized bone for animals that are growing rapidly. Hence rapid bone deposition would require the recruitment of additional osteocytes, as also recognized later by others (43–45).

Hobdell and Howe (39) examined cortical bone from the mandibles of five mammalian species and from long bone (unspecified) diaphyses from 15 reptilian species wherein they focused on primary bone in their Ot.Lc.Den analysis. Among the mammalian species, they reported only small differences in Ot.Lc.Den, most notably, the similar results in their mouse and elephant specimens.

Using woven and parallel-fibered bone from the mid-diaphyses of long bones of seven animal species, Remaggi et al. (40) measured osteocyte canalicular density, which they expressed as the number of canaliculi per 10 μm length of bone rather than the number of cell processes or canaliculi per individual osteocyte lacuna. Their data from frogs, rabbits, horses, dogs, and humans show increased canalicular densities in woven bone compared to parallel-fibered bone ($p < 0.001$). Their data from chicks and cows were similar ($p = 0.5$). But, unlike Mullender et al. (19), they did not find any correlation between osteocyte density and body size of the species. However, they emphasized that Mullender et al. (19) analyzed cancellous bone without making any distinction for potential histological variations. Consequently, Remaggi and co-workers noted that some species (e.g. rat and rabbit), whose skeleton undergoes little or no remodeling, the bone tissues making up the bony trabeculae of cancellous bone may differ considerably from remodeled trabeculae of other species (monkey, pig, cow). Using the same techniques and specimens in Remaggi et al. (40), Ferretti et al. (41) analyzed parallel-fibered bone and lamellar bone (woven bone was not examined). Consequently, these two studies show:

1. increased osteocyte canalicular density in parallel-fibered when compared to lamellar bone ($p < 0.001$), and
2. woven bone contains the highest osteocyte canalicular density followed by parallel-fibered and lamellar bone.

The findings of these two studies led to the general conclusion that osteocyte shape, size, and density, and canalicular density mainly depend on the microarchitecture of the bone tissues rather than species. But they could not explain the bigger lacunar area in their frog specimens compared with the other animals.

Bromage et al. (46) found an inverse relationship between Ot.Lc.Den in lamellar bone and body mass of 9 mammalian species ($r = -0.85$; $p < 0.01$). They suggested that Ot.Lc.Den reflects the rate of osteoblast proliferation, transformation, and incorporation into bone during growth. Cubo et al. (44) also found cellular density, among a number of other histomorpho-

metric parameters, to be significantly correlated to femoral growth rate. Osteocyte lacunar densities should therefore be higher in mammals with rapid growth, small body mass, and whose osteoblast proliferation rates would lead to higher incorporation. Ot.Lc.Den can also vary depending on the developmental history of the bone tissue, as has been shown in woven bone in the rat when formed via intramembranous osteogenesis compared to endochondrally derived woven bone (45).

In view of the studies discussed above, we suggest that there are five most important mechanobiological influences on Ot.Lc.Den (see box "Relative priorities of general mechanobiological factors or requirements on osteocyte density" on this page). However, as discussed below, their order of importance might require substantial adjustment in view of additional considerations.

Additional considerations for studies of scaling relationships

Variations in osteocyte-related parameters between regions of the same bone can confound attempts to detect scaling relationships. An example of this can be found in the data from Canè, Marotti et al. (47). They analyzed cortical and cancellous bone from cross-sections from femora, tibiae,

and humeri of three mature dogs. They found that Ot.Lc.Den is not uniform throughout the skeleton of these animals. They found that Ot.Lc.Den changes significantly between different locations of the bones: highest in cancellous bone of the metaphyses and lowest in cortical bone of the diaphyses.

D'Emic and Benson (48) examined osteocyte lacuna volume from primary parallel-fibered cortical bone from 69 femora and humeri specimens from 35 extant bird species. The body masses ranged from 10–120,000 g. They used polarized light microscopy and 100 μm transverse sections to obtain these data, and then ran correlations with bone mass and metabolic rate. They found a scaling relationship in which increasing avian body mass ($r^2 = 0.57$; $p < 0.001$) and mass-specific basal metabolic rate ($r^2 = 0.12$; $p < 0.001$) correlate with increased osteocyte volume. Though statistically significant, the relationships were admittedly weak.

In our study of domesticated turkey ulnae (49), we found high Ot.Lc.Den compared to mammals of a similar size. High Ot.Lc.Den in the turkey ulna is consistent with high Ot.Lc.Den in domesticated chickens (38, 40). Although these three studies used 2D measurements, the two bird species (*Buteo buteo*, *Struthio camelus*) examined by Stein and Werner (43) using quasi 3D transmitted light microscopy also revealed high Ot.Lc.Den when compared to mammals (► Tab. 2 [see online supplementary material to this article on www.osteologie-journal.de]). As previously speculated (49), it may be that substantially greater Ot.Lc.Den in avian species compared to mammals is a function of their relatively higher specific metabolic rate (metabolic rate per kilogram body mass).

Christen et al. (50) provide a novel explanation for the typically moderate-to-strong negative correlation that has been shown between body mass and Ot.Lc.Den in mammals. Using a computational model of bone modeling and remodeling, they found an inverse relationship between bone turnover rates and animal size, which might indicate that cancellous bone scaling could be linked to metabolic rather than mechanical adaptations. In this context it is likely that:

Relative priorities of general mechanobiological factors or requirements on osteocyte density¹

Listed in order of putative importance: top = most important, bottom = least important

1. Histology/Tissue Constraints
2. Microdamage Detection
3. Social/Communication
4. Nutrition
5. Metabolism

¹ The order of importance might need to be arranged differently for cancellous vs. cortical bone. The "volume effect" is also an important consideration in this context. See text for discussion.

1. metabolism should be moved farther up the list shown in box “Relative priorities of general mechanobiological factors or requirements on osteocyte density” on page 97, and
2. the order of importance of the listed factors/requirements differs for cancellous vs. cortical bone.

Contradictions between intra-species and inter-species comparisons

Here we examine two studies that used intra-species comparisons and did not find the inverse relationships between Ot.Lc.Den and body or bone mass shown above in inter-species comparisons.

Vashishth et al. (51) found that for both male and female humans of all ages (range: 23–91 years) extracellular matrix volume (which is an expression for bone mass) for cortical and cancellous bone can be predicted ($r^2=0.98$) by Ot.Lc.Den. In other words, increased Ot.Lc.Den values correlate with increased bone mass of the specimens that they analyzed. They concluded that bone mass is determined by the control of osteocyte number. This finding deemphasizes the role of mechanical adaptation with regards to body mass regulation, claiming instead that loading indirectly influences bone mass by affecting “osteocyte recruitment and death.”

Bromage et al. (52) examined Ot.Lc.Den in 11 femur specimens from adult humans of Bantu African origin (from 2D backscattered electron images) and ran correlations with age, height, and body mass. They reported a significant correlation with body height ($r=0.63$, $p=0.04$). Though based on a small sample size, this finding contradicts the findings of the aforementioned studies suggesting that animal size is inversely related to Ot.Lc.Den. Bromage et al. reconcile this discrepancy by arguing that metabolic differences between species necessitate fewer osteocytes in larger animals because they are growing over longer periods of time compared to smaller species. However, because metabolism should not differ widely within a specific species, size differences result from a greater number of osteocytes, which allow bones to grow more during the same amount of time.

Does the “stressed volume effect” play a role in scaling relationships?

The “stressed volume effect” (or “volume effect”) is a concept that is based on the idea (proven experimentally) that larger structures are more prone to failure than smaller structures (32, 53–55). This is because failures are initiated at flaws in the material of a structure, and larger volumes are more likely to contain a significant flaw (56). In applying this concept to a large-volume vs. a small-volume bone made of the same material, Taylor (57) argues that the larger bone is more prone to fatigue failure than the smaller bone. In this situation, natural selection would favour a mechanism for reducing and repairing the higher probability of flaws in large-volume bones. The volume effect also implies that bones in larger animals cannot withstand as much stress in daily use as bones in smaller animals. Hence, a bone from a large animal would be comparatively weaker, with the stress concentrator effect of osteocyte lacunae also contributing disproportionately. Taylor (57) suggests that this paradox is resolved in larger animals by enhancing the bone material through histological modifications. The potentially deleterious consequences (e.g., the accumulation of microfractures leading to fracture) of the volume effect in larger bones can be minimized using additional methods, including,

1. adjusting the animal’s ambulatory activities (e.g., elephants canter, and never gallop), and
2. adjusting joint and bone alignment (i.e., “posture”) and the bone’s cross-sectional geometric properties in order to reduce deleterious levels of strain and bending moments (58, 59).

We find it notable that these important issues are not considered in the aforementioned studies that have considered the possibility of scaling relationships involving osteocyte densities, Ot.Lc.Den, and other lacunar parameters.

Modify the order of the mechanobiological factors/requirements listed in the box?

In view of contradictions described above regarding potential scaling relationships and accommodations that minimize the volume effect, readers should consider re-ordering the list shown in box “Relative priorities of general mechanobiological factors or requirements on osteocyte density” on page 97. For example, we placed “microdamage detection” relatively high on the list because of its obvious importance in helping avoid fractures by repairing microdamage. But the population densities of the “microdamage detectors” (i.e., the osteocytes) do not increase as might be expected in larger bones (in larger animals). This is because it is likely that adjustments at the tissue level and modifications of animal ambulation/behavior and/or bone-joint mechanics effectively nullify the volume effect. Consequently, microdamage detection might play a role that has lesser importance than what is shown in the box.

Emerging 3D imaging technologies

► Table 2 lists selected studies that have used 3D imaging to examine Ot.Lc.Den in various bones. Recent studies using high-resolution imaging in 3D support the general idea that 2D data can be highly errant (2, 60). Cardoso et al. (60) state: “While LM and CLSM [light microscopy and confocal laser scanning microscopy] can provide detailed assessment of bone microarchitecture, the histological processing required for these imaging modalities, the low penetration depth, and the errors arising from 2D mechanical sectioning are among the inconveniences of these approaches. New interference approaches, non-linear imaging, and super-resolution microscopes may achieve imaging below the optical limit, 20 nm.”

Hannah et al. (61) assert that the best way to study the lacunar-canalicular network and Ot.Lc.Den is with 3D imaging techniques. They argue that studies reporting 3D data derived from 2D images (62) and unaltered 2D data (5) are insufficient

when it comes to studying the complex 3D structures of bone histology. Dong et al. (63) also echo this idea. Limitations of even the highest resolution 3D imaging technologies currently available include the relatively reduced ability to discern fine details such as cement lines and patterns of collagen lamellar organization. However, improvements are expected as technological advances continue to be made (64).

Conclusions

Important considerations in studies of osteocyte mechanobiology include cell viability and potential scaling relationships between metabolism or body/bone mass and osteocyte lacunar population density, size and lacunar-canalicular geometries. Osteocyte and osteocyte lacunar distribution and related morphological parameters can also depend on species, sex, age, bone type (cortical vs. cancellous), local histology and cell recruitment rates, bone location (e.g., diaphyseal vs. metaphyseal), as well as developmental history. The stressed volume effect, typical ambulatory activities, and joint and bone functional mechanics and postures can be important in these contexts.

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Conflict of interest

The authors report no conflict of interest.

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