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Review

Osteocytes and the bone lacunar-canalicular system: Insights into bone biology and skeletal function using bone tissue microstructure

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Determining the impact of diet, reproductive status, biomechanics, and disease on bone structure is key to understanding skeletal biology, pathology, and for making predictions regarding skeletal function in fossil species. Skeletal growth and remodeling are regulated, in part, by bone matrix-embedded osteocytes (Bonewald, 2011). As an osteoblast differentiates into an osteocyte, the cell extends dendritic processes toward neighboring osteocytes and surface lining cells (Fig. 1). These processes become surrounded by bone, where they and the cell body reside in fluid-filled channels called canaliculi and lacunae, respectively. Osteocyte density and the dimensions of the lacunae and canaliculi can be affected by age, reproductive and hormone status, and taxonomy (Mullender et al., 1996; Sharma et al., 2012; Qing et al., 2012; Stein and Werner, 2013; Lai et al., 2015), which could influence one of the primary functions proposed for the osteocyte lacunar-canalicular system (LCS): bone mechanobiology. The goal of this review is to highlight recent studies that have shown how measurable features of the LCS could be diagnostic of age, disease, reproductive status, taxonomy, and life history and to discuss how variation in LCS architecture could affect skeletal functional adaptation to physical challenge.

Bone mechanobiology is the process by which bone cells sense and respond to changes in the functional demands placed upon the skeleton, resulting in increased bone formation and larger bone dimensions with increased mechanical loading or bone resorption with skeletal disuse. This mechanism is initiated at the cellular level when physical loading of the skeleton creates bone tissue stress or strain gradients that produce pressure differentials in the LCS, driving the extracellular fluid from regions of high pressure to areas of lower pressure (Fritton and Weinbaum, 2009). As bones are loaded under greater loads, the fluid flow rate through the LCS increases, generating greater shear and drag forces on the osteocytes. The greater the mechanical stimulus at the osteocyte, the greater the bone anabolic signal that is conveyed to nearby bone-forming osteoblasts (Robling et al., 2008; Moustafa et al., 2012). With disuse, fluid flow through the LCS decreases below habitual levels and osteocyte cell death and bone resorption ensue (Aguirre et al., 2006).

The mechanobiological mechanism is hampered by age. Recent *in vivo* bone loading studies in rodents have shown that as animals age the amount of bone formed in response to applied mechanical loads decreases (Holguin et al., 2014; Main et al., 2014; Razi et al., 2015), which matches observations in humans (Kannus et al., 1995). In mice, it appears that the adaptive mechanism becomes *desensitized* rather than fully defunct, since a robust anabolic response can be rescued in older animals by increasing the tissue strain stimulus (Lynch et al., 2011).

Increasing age and/or changes in circulating hormone concentrations can be linked to structural changes in the LCS. In humans, osteocyte density decreases sharply between 20 and 40 years of age, which increases LCS discontinuity (Qiu et al., 2002). While gonadectomy studies have shown that estradiol, itself, is not necessary for generating the skeletal response to mechanical load (Hagino et al., 1993; Fritton et al., 2008; Sinnesael et al., 2015), a loss of circulating estradiol alters the dimensions of the LCS (Sharma et al., 2012). Six weeks following ovariectomy, porosity in the metaphyseal cortical and cancellous bone tissue increased (+56% and +16%, respectively), as

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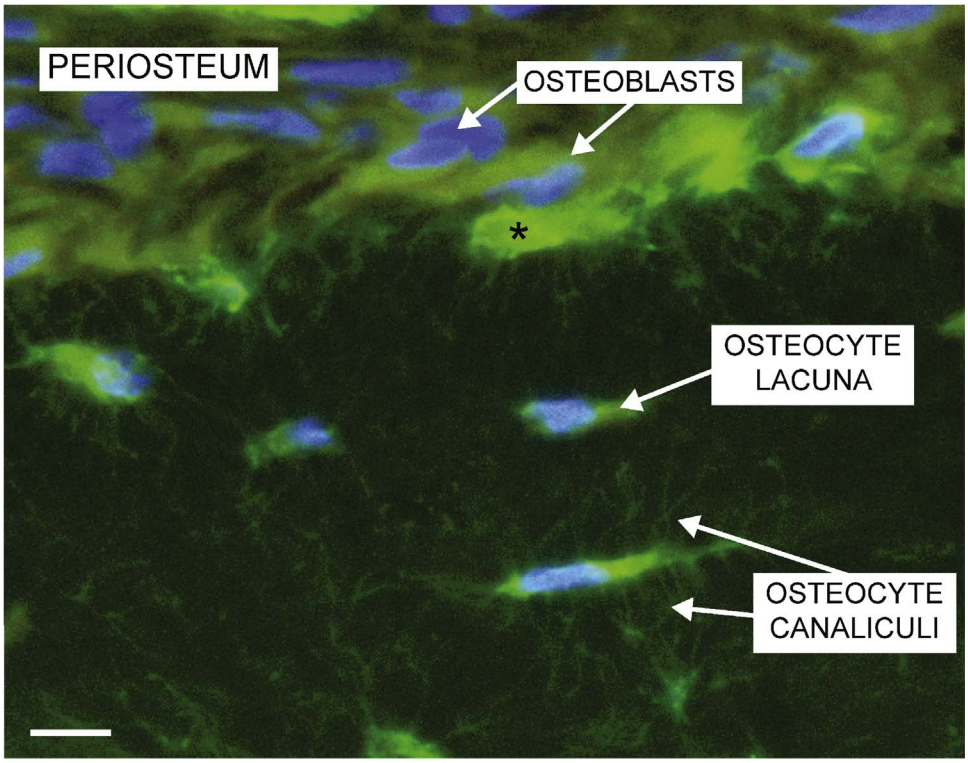

Fig. 1. Cortical bone from a mouse lumber vertebra. Osteocytes and their dendritic cytoplasmic processes lie within lacunae and canaliculi. Osteocytes differentiate from osteoblasts lying on the bone surface. The cell labeled with the (*) is an early differentiating osteocyte (indicated by the intense green fluorescence) that is extending/receiving dendritic processes toward/from osteocytes deeper in the bone matrix and is overlain by the next generation of bone-forming osteoblasts. Green and red (faint) fluorescence is derived genetically and results from a cross between mT/mG mice and DMP1-8kb-Cre mice (Muzumdar et al., 2007; Bivi et al., 2012). Blue fluorescence is DAPI which indicates DNA in the cell nucleus. Scale bar = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

did cancellous and cortical canalicular diameter (+48% and +26%, respectively). For a given load-induced strain gradient, the greater the diameters of the canaliculi, the lower the fluid velocity and resulting surface shear strains and drag forces on the dendritic processes. Osteocytes would perceive this as a reduced stimulus, similar to disuse conditions.

Lactation also increases canalicular and lacunar dimensions (Qing et al., 2012). In mice, lactation was associated with a 50% increase in canalicular diameter. Unlike sex hormone withdrawal, the osteolysis that occurs during lactation is reversible following weaning. The resorbed bone is restored 3–4 weeks following weaning in rodents, while in humans the lost bone is recovered in 6–12 months (Wysolmerski, 2013). It is unknown if increased physical loading of the skeleton during and following lactation might mitigate the effects of this osteolysis or aid in the re-accrual of bone following weaning.

Most of what we know of the LCS and osteocyte regulation of bone biology is derived from *in vitro*, rodent, and human studies. The pitfalls of basing our knowledge of osteocytes and LCS architecture on one bone or one taxon was recently highlighted by a comparative study that broadly sampled birds and non-avian dinosaurs (D'Emic and Benson, 2013). This study found a more than five-fold difference in osteocyte lacunar volume across different skeletal elements in the emu (*Dromaius novaehollandiae*). Contrary to this inter-element variation, lacunar volumes in the femoral mid-diaphysis of six avian genera only varied by about two-fold. Additionally, inter-individual variation within the same bone (femur) for a given species was generally less than 15%. If norms for osteocyte and LCS measures could be established for given bones within a given species at a given age, it may be possible to use osteocyte measures to gain information about life history or pathology. Given the large variation in osteocyte volumes between the bones of the skeleton, it is intriguing to question how lacunar volume may be related to relative growth rates in different bones, functional usage, and mechanosensitivity.

In addition to osteocyte volume, osteocyte density also likely affects bone biology. Using a comparative sample including amphibians, reptiles, birds, and non-avian dinosaurs, Stein and Werner (2013) found that osteocyte density generally decreases as body mass increases. Although there were limited amphibians and extant sauropsids in the sample, amphibians generally had lower osteocyte lacunar densities (OLDs) than the fully terrestrial placental taxa examined. Of the lizard species sampled, the active predatory species (*Tupinambis*, *Varanus*) had higher OLDs than the herbivorous *Iguana*. Both results suggest that OLD may be linked to the relative magnitude or frequency of mechanical loads experienced by the limb skeleton, though phylogenetic history could factor into this trait as well. Future studies examining the plasticity of OLD within a species, in individuals experiencing different skeletal loading regimes during growth or adulthood, could provide information regarding biomechanical or life history signals in the bones of living and extinct taxa.

Just as disease- or injury-induced skeletal pathology can be preserved in the fossil record (Wolff et al., 2009), functional skeletal adaptation can be identified on an individual level or within a population of animals experiencing differential functional usage of the skeleton. A recent example described two *Maiasaura* tibiae, found in a larger assemblage, which showed little to no external pathology (Cubo et al., 2015). However, histological sections showed fibrolamellar bone deposition under the periosteal surface similar to the response observed on the caudal-lateral surface of the radius in ulnar osteotomy experiments in ungulates (Lanyon et al., 1982). The pathology in the tibiae may have resulted from functional overloading of the tibia due to a hypothesized fibular fracture. Recent advances in the isolation of fossil osteocytes and identification of their proteinaceous contents could permit analysis of the cellular changes that occurred with the organ-level changes in bone structure in fossil taxa (Schweitzer et al., 2013).

In conclusion, the dimensions of the LCS and osteocyte density

could potentially provide diagnostic information regarding taxonomy, disease, reproductive status, or age. Future lab-based bone adaptation and growth studies and neontological and paleontological anatomical studies are critical for establishing baseline information regarding the possible factors influencing LCS architecture. Through characterization of the LCS in non-pathological and pathological conditions in living taxa, we will be able to use LCS characteristics for interpreting fossil bones.

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