Bone Reports 5 (2016) 252–261

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

# Intraskeletal variation in human cortical osteocyte lacunar density: Implications for bone quality assessment

Randee L. Hunter [a,b,*], Amanda M. Agnew [a,c]

[a] Skeletal Biology Research Laboratory, School of Health and Rehabilitation Sciences, The Ohio State University, Columbus, OH, United States
[b] Division of Radiologic Sciences and Therapy, School of Health and Rehabilitation Sciences, The Ohio State University, Columbus, OH, United States
[c] Department of Anthropology, The Ohio State University, Columbus, OH, United States

ARTICLE INFO

*Article history:*
Received 2 August 2016
Received in revised form 7 September 2016
Accepted 11 September 2016
Available online 12 September 2016

*Keywords:*
Osteocyte
Osteocyte lacunar density
Cortical bone
Intracortical porosity
Bone quality

ABSTRACT

Osteocytes and their lacunocanalicular network have been identified as the regulator of bone quality and function by exerting extensive influence over metabolic processes, mechanical adaptation, and mineral homeostasis. Recent research has shown that osteocyte apoptosis leads to a decrease in bone quality and increase in bone fragility mediated through its effects on remodeling. The purpose of this study is to investigate variation in cortical bone osteocyte lacunar density with respect to major factors including sex, age, and intracortical porosity to establish both regional and systemic trends. Samples from the midshaft femur, midshaft rib and distal one-third diaphysis of the radius were recovered from 30 modern cadaveric individuals (15 males and 15 females) ranging from 49 to 100 years old. Thick ground undecalcified histological (80 μm) cross-sections were made and imaged under bright field microscopy. Osteocyte lacunar density (Ot.Lc.N/B.Ar) and intracortical porosity (%Po.Ar) were quantified. No significant sex differences in Ot.Lc.N/B.Ar or %Po.Ar were found in any element. Linear regressions demonstrated a significant decrease in osteocyte lacunar density (Ot.Lc.N/B.Ar) and increase in intracortical porosity (%Po.Ar) with age for the sex-pooled sample in the femur ($R^2 = 0.208$, 0.297 respectively) and radius ($R^2 = 0.108$, 0.545 respectively). Age was unable to significantly predict osteocyte lacunar density or intracortical porosity in the rib ($R^2 = 0.058$, 0.114 respectively). Comparisons of regression coefficients demonstrated a systemic trend in the decrease in osteocyte lacunar density (Ot.Lc.N/B.Ar) and increase in intracortical porosity (%Po.Ar) with age. In each element, intracortical porosity was significantly negatively correlated with lacunar density for which the radius demonstrated the strongest relationship ($r = -0.746$). Using pore number (Po.N) as a proxy for available vascularity to support the osteocyte population, Po.N was able to predict 61.8% of variation in osteocyte lacunar number (Ot.Lc.N) in the rib. The femur and radius also demonstrated significant relationships between these variables ($R^2 = 0.560$ and 0.397 respectively). The results from this study indicate that although the femur, radius and rib may be experiencing systemically influenced declines in osteocyte lacunar density, there may be differential effects at each anatomical site potentially due to age related changes in mechanical loading. With decreasing osteocyte lacunar density in each element, intracortical porosity increased with likely direct impacts on gross bone strength. This study provides a foundation upon which to build interpretations of osteocyte lacunar density values and their effect on differential fracture risk for aging individuals.



## 1. Introduction

Bone strength, or the ability to resist fractures and successfully incur regular insults while functioning properly, is determined by its composition and structure (Brandi, 2009; Currey, 2003; Seeman and Delmas, 2006) and better conceptualized as "bone quality." Moving beyond solely considering measures of bone mass, research into the multi-faceted and hierarchical components of bone quality have increasingly included factors affecting the cellular machinery of bone. In fact, increasing age has been shown to increase fracture risk independently from measured bone mass or mineral density (Nicks et al., 2012; Seeman, 2007) supporting a paradigm shift in the way in which bone quality is quantified. Determining factors of bone quality have been expanded to include not only mass, but also microarchitecture, material properties of the extracellular matrix, microdamage accumulation, osteocyte density, and remodeling rate (Burr, 2014; Burr and Akkus, 2014). Recently, another paradigmatic shift towards the importance of cortical microstructure in fracture resistance has been introduced (Agnew and Bolte, 2011; Nicks et al., 2012; Seeman, 2015). In order to better elucidate bone quality and quantify fracture risk for individuals at any age, the field must move beyond a "trabeculo-centric" view (Seeman, 2015). This manuscript investigates variation in one of the multi-factorial and hierarchical aspects of cortical bone maintenance and fracture resistance: the osteocyte.

* Corresponding author at: Skeletal Biology Research Laboratory, 2063 Graves Hall, 333 W. 10th Avenue, Columbus, OH 43210–2205, United States.
*E-mail address:* Randee.Hunter@osumc.edu (R.L. Hunter).

http://dx.doi.org/10.1016/j.bonr.2016.09.002

The mechanism by which the physiological (both mechanical and systemic influences) environment affects change (i.e., functional adaptation) in cortical bone is orchestrated by the osteocyte lacunocanalicular network conducting a cellular team towards maintaining bone quality and preventing gross failure. Osteocytes are ideally situated to integrate both metabolic (systemic) stimuli (Bellido and Hill, 2014; Bellido et al., 2013) and mechanical stimuli as the mechanotransducers of the skeletal system (Han et al., 2004; Klein-Nulend et al., 2013; Nicolella et al., 2006; Schaffler et al., 2014) maintaining their local microenvironment and in turn the integrity of the bone as a whole (Bonewald, 2007, 2011; Seeman, 2006). Recently, Jilka and O'Brien (2016) provided an extensive review concerning the pivotal role of osteocyte control on age related bone loss. In order to govern bone functional adaptation, there must be adequate osteocyte cell numbers in any given bone (Ma et al., 2008; Vashishth et al., 2002). Osteocyte apoptosis, classified as a form of tissue damage itself (Seeman, 2006), can result from a multitude of endogenous and exogenous factors and can lead to increased fracture risk by an overall decrease in bone quality (Ma et al., 2008; Noble and Reeve, 2000; Noble et al., 1997). Systemic factors including increasing endogenous oxidative stress and glucocorticoids, and decreasing levels of sex hormones with increasing age have all been shown to have a pro-apoptotic effect on osteocytes (Almeida, 2010; Bellido and Hill, 2014; Bonewald, 2011; Jilka et al., 2013; Piemontese et al., 2015; Tomkinson et al., 1997). In addition to the systemic factors presumably affecting the global osteocyte population, maintaining site specific physiological or optimal mechanical loading, is essential to osteocyte viability (Aguirre et al., 2006; Hughes and Petit, 2010). Optimal levels of mechanical strains are necessary for movement of interstitial fluid throughout the lacunocanalicular network responsible for delivery of nutrients to the entombed cell population (Hughes and Petit, 2010; Jilka et al., 2013) and has demonstrated a protective mechanism to mitigate adverse systemic factors (Bonewald and Johnson, 2008). Disuse and/or linear microcracks interrupting fluid flow can result in hypoxia and apoptosis (Burr, 2014; Frost, 1960a; Jilka et al., 2013; Kennedy et al., 2014; Verborgt et al., 2000). The lacunar space occupied by viable osteocytes is maintained until apoptosis, upon which time either targeted removal and repair by a basic multicellular unit (BMU) is initiated (Burr, 2014; Cardoso et al., 2009) or the empty lacuna is filled with mineralized tissue (Frost, 1960b). Thus, the existence of the lacunae is dependent on the viability of its occupant (Knothe Tate et al., 2004). Apoptotic osteocytes signal surviving neighboring osteocytes to release RANKL to signal initiation and direct a targeted intracortical remodeling event (Kennedy et al., 2012). These remodeling events result in new intracortical porosity as well as supplying a new complement of osteocytes embedded in the recently laid down matrix. Thus, as the bones' mechanosensing cell, osteocytes conduct the adaptive response to its loading environment by translating mechanical signals into chemical signals affecting bone metabolism, but are themselves dependent on a customary strain level of mechanical loading to properly function.

Changes in bone strength and resulting differential fracture risk are likely due to site-specific changes in the cortex of susceptible bones rather than global changes affecting all skeletal envelopes equally (Thomas et al., 2006). To investigate the effects of such influences as chronological age and sex on changes in osteocyte lacunar density and ultimately how this contributes to differential fracture risk across the skeleton, it is imperative to establish intra-individual and inter-individual variation from all skeletal envelopes of comparable anatomical locations. This study is the first to attempt to establish a baseline understanding of intra-skeletal variation in osteocyte lacunar density in human cortical bone. Osteocyte lacunar density has often been used as a proxy for osteocyte cell density in both cortical and trabecular bone (Bach-Gansmo et al., 2015, 2016; Miszkiewicz, 2016; Qing et al., 2012; Teti and Zallone, 2009). Before reported patterns of decreasing density with age or variation between sexes can be translated to tangible effects on fracture risk, a systematic investigation into basic factors affecting variation in osteocyte density must be conducted. Thus, the goals of this study were two-fold: First, we investigated systemic variation in cortical osteocyte lacunar density between multiple anatomical sites as it relates to influential factors, specifically chronological age and sex. This study is the first to analyze systemic human cortical bone variation in osteocyte lacunar density represented by three anatomical sites experiencing varying mechanical stimuli to establish age and sex related trends. Second, by initiating and controlling the components of the basic multicellular unit (BMU), osteocyte density has a direct impact on intracortical porosity; a relationship we are the first to explore across the entirety of the cortex at multiple skeletal sites with implications for bone quality and fracture resistance.

## 2. Materials and methods

### 2.1. Samples

Skeletal samples were obtained from modern embalmed postmortem human subjects (PMHS) received through The Ohio State University's Whole Body Donor program. A total of 30 individuals of known demographics were chosen as representative of the population for which fracture risk assessments are routinely performed. The total sample ranged in age from 49 to 100 years old including 15 males (mean age of 77.8 ± 13.45 years) and 15 females (mean age of 75.87 ± 11.89 years). Cause of death was not a determining factor as these individuals were experiencing a myriad of conditions which may influence osteocyte viability systemically and/or mechanically as a consequence of lifestyle variations. The only exclusion criterion was evidence of macroscopic changes to the skeletal elements of interest which could have included healed or active infections, prosthetics, or gross evidence of bony metastases.

Each PMHS was represented by three anatomical locations: midshaft femur, distal one-third of the diaphysis of the radius, and midshaft of the 6th rib; samples were obtained from each location in 2 cm blocks. These sites were chosen due to their varying loading environments and clinical significance. The femur represents a weight bearing bone which may experience age related changes associated with activity decline (Robling and Stout, 2003). The rib experiences a consistent loading environment from pulmonary ventilation independent of age and is often used as an indicator of systemic effects on skeletal metabolism within an individual (Agnew and Stout, 2012; Eleazer and Jankauskas, 2016). Lastly, the cortex of the distal one third of the diaphysis of the radius undergoes an intermediate amount of variation in mechanical loading with advancing age and is an important clinical site for age-associated fragility fractures (Court-Brown and Caesar, 2006). The total sample consisted of 30 femoral and radius sections and 29 rib sections (ribs were unavailable for one PMHS).

Each skeletal block was macerated, cleaned of remaining soft tissue and marrow, and de-greased. Ribs were embedded in epoxy resin to prevent damage to the fragile cortex during sectioning, neither femoral nor radius sections were embedded. Undecalcified histological sections were prepared using standard techniques (Maat et al., 2001). Thick sections were cut using an Isomet saw (Buehler, IL) and ground to a uniform thickness of 80 μm to reduce any lacunar density quantification errors attributable to variability in section thickness. The resulting 89 sections were mounted to glass slides using Permount. Imaging of slides was performed with CellSens dimension software on an Olympus VS120 slide scanner at 40× magnification under bright field light for visualization of osteocyte lacunae.

### 2.2. Data collection

Composite images were used to quantify basic histomorphometric parameters as well as osteocyte lacunar data across each cross-section (Table 1). Due to their size, femoral total subperiosteal area (Tt.Ar) and endosteal area (Es.Ar) were measured using ArcGIS version 10.1