Journal of Biomechanics 66 (2018) 127–136

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Microstructural changes associated with osteoporosis negatively affect loading-induced fluid flow around osteocytes in cortical bone

Vittorio Gatti, Evan M. Azoulay, Susannah P. Fritton *

Department of Biomedical Engineering, The City College of New York, New York, NY 10031, USA

ARTICLE INFO

Article history:
Accepted 9 November 2017

Keywords:
Poroelasticity
Bone fluid flow
Lacunar-canalicular system
Osteoporosis
Osteocyte

ABSTRACT

Loading-induced interstitial fluid flow in the microporosities of bone is critical for osteocyte mechanotransduction and for the maintenance of tissue health, enhancing convective transport in the lacunar-canalicular system. In recent studies, our group has reported alterations of bone's vascular porosity and lacunar-canalicular system microarchitecture in a rat model of postmenopausal osteoporosis. In this work, poroelastic finite element analysis was used to investigate whether these microstructural changes can affect interstitial fluid flow around osteocytes. Animal-specific finite element models were developed combining micro-CT reconstructions of bone microstructure and measures of the poroelastic material properties. These models were used to quantify and compare loading-induced fluid flow in the lacunar-canalicular system of ovariectomized and sham-operated rats. A parametric analysis was also used to quantify the influence of the lacunar-canalicular permeability and vascular porosity on the fluid velocity magnitude. Results show that mechanically-induced interstitial fluid velocity can be significantly reduced in the lacunar-canalicular system of ovariectomized rats. Interestingly, the vascular porosity is shown to have a major influence on interstitial fluid flow, while the lacunar-canalicular permeability influence is limited when larger than 10^{-20} m^2. Altogether our results suggest that microstructural changes associated with the osteoporotic condition can negatively affect interstitial fluid flow around osteocytes in the lacunar-canalicular system of cortical bone. This fluid flow reduction could impair mechanosensation of the osteocytic network, possibly playing a role in the initiation and progression of age-related bone loss and postmenopausal osteoporosis.

1. Introduction

The movement of interstitial fluid within the bone microporosities is critical for bone physiology. Blood vessels and bone cells are embedded in the bone tissue inside interconnected porosities, where interstitial fluid permits the exchange of nutrients and signaling molecules. In cortical bone, blood vessels reside in the vascular porosity, and the osteocytes, the most abundant bone cells, reside in the lacunar-canalicular porosity. The two porosities are hierarchically interconnected and form a dual-porous structure where the extravascular fluid in the vascular porosity continually interchanges with the extracellular fluid in the lacunar-canalicular porosity (Cowin, 1999; Qin et al., 2002). Mechanically-induced deformation of bone tissue acts as a motive force for this fluid displacement, generating fluid pressure gradients that drive interstitial fluid into the lacunar-canalicular system towards the vascular porosity space and vice versa (Starkebaum et al., 1979; Zeng et al., 1994; Cowin et al., 1995). This loading-induced fluid flow has two important implications for bone physiology: (a) it enhances convective fluid transport in the lacunar-canalicular system, which is critical for osteocyte viability (Piekarski and Munro, 1977; Knothe-Tate et al., 2000; Wang et al., 2000); and (b) it generates forces on the osteocyte processes in the canalicular space, playing a critical role in bone mechanotransduction, coupling external mechanical loading with the osteocyte microenvironment (Weinbaum et al., 1994; You et al., 2001). Strong evidence suggests that osteocytes are able to translate these fluid-mediated physical stimuli to biochemical signals that regulate the processes of bone formation and bone resorption, maintaining the mechanical function of the bone structure (Klein-Nulend et al., 2013).

Alterations in the microstructure of cortical bone have been observed in several models of osteoporosis, including estrogen deficiency, aging, and disuse (Westerlind et al., 1997; Sharma et al., 2012; Tommasini et al., 2012; Britz et al., 2012). Both vascular porosity and lacunar-canalicular microarchitecture have been shown to change in response to a drop in estrogen level in a rat

* Corresponding author.
E-mail address: fritton@ccny.cuny.edu (S.P. Fritton).

https://doi.org/10.1016/j.jbiomech.2017.11.011
0021-9290/

model of postmenopausal osteoporosis (Sharma et al., 2012; Sharma et al., submitted for publication). These microstructural changes possibly affect the fluid flow in the lacunar-canalicular system, impairing osteocyte mechanosensation or affecting fluid-mediated molecular transport in the lacunar-canalicular system. Alteration in the loading-induced convection of small molecules within the lacunar-canalicular system of ovariectomized rats was reported by our group, supporting the hypothesis that estrogen deficiency changes the interstitial fluid flow microenvironment (Ciani et al., 2014).

Theoretical and experimental studies have demonstrated that interstitial fluid velocity in the lacunar-canalicular system depends on characteristics of mechanical loading, such as strain rate and strain magnitude, as well as characteristics of the vascular and lacunar-canalicular porosities, such as vascular pore morphology and lacunar-canalicular system permeability (Zhang et al., 1998; Steck et al., 2003; Swan et al., 2003; Fornells et al., 2007; Remond et al., 2008; Kumar et al., 2012; Pereira and Shefelbine, 2014). In particular, using poroelasticity theory to estimate fluid flow, researchers have shown that both vascular and lacunar-canalicular porosities influence interstitial fluid flow at the micro-scale (i.e., single osteons) and macroscale (i.e., cortical bone section) (Wang et al., 1999; Fornells et al., 2007; Cowin et al., 2009). In cortical bone, interstitial fluid flow is particularly influenced by the vascular porosity, which acts as a low-pressure fluid reservoir, where the loading-induced fluid pressure in the lacunar-canalicular system mainly relaxes (Cowin, 1999; Wang et al., 1999; Goulet et al., 2008). Furthermore, bone interstitial fluid flow depends on the morphology of the lacunar-canalicular system and on the permeability of the pericellular space in which the fluid moves (Weinbaum et al., 1994; Smit et al., 2002; Goulet et al., 2009; Wang et al., 2014).

The objective of this study was to investigate how changes in cortical bone microporosities affect loading-induced fluid flow around osteocytes. High-resolution animal-specific poroelastic finite element models were used to quantify and compare interstitial fluid flow within the osteocytic network of ovariectomized rats and sham-operated control rats. In addition, a parametric study using idealized bone geometries was performed to estimate the specific influence of vascular porosity and lacunar-canalicular permeability on the fluid velocity in the lacunar-canalicular system. The results of this work can offer a better understanding of how fluid flow in the lacunar-canalicular system is altered during conditions of bone loss, possibly affecting osteocyte viability and bone mechanotransduction.

2. Methods

2.1. Finite element analysis of interstitial fluid flow in the lacunar-canalicular system

To quantify fluid flow in cortical bone we used finite element analysis, assuming bone tissue to be an ideal fluid-saturated poroelastic material. The cortical bone tissue was modeled as a biphasic material with fully interconnected solid and fluid phases. Material parameters were assigned to model the bone matrix, where the solid phase represents the collagen-apatite mineralized structure, impermeable to fluids, and the porous space represents the fluid-saturated lacunar-canalicular system. The vascular porosity was included in all the models, but the fluid pressure and displacement in the vascular porosity were ignored because their influence on the fluid flow in the lacunar-canalicular system is negligible (Mak et al., 1997; Zhang et al., 1998). In addition, soft tissue within the vascular pores (i.e., blood vessels and nerves) and the lacunar-canalicular system (i.e., osteocyte body and processes) was not modeled because its influence on the poroelastic behavior of the tissue is negligible (Zeng et al., 1994; Weinbaum et al., 1994; Cowin, 1999).

Mechanically-induced deformation of bone tissue generates fluid pore pressure, which drives fluid displacement in the lacunar-canalicular porous space (Darcy's law):

$$\vec{v} = \frac{-k}{\phi_{lc}\mu}\nabla p \tag{1}$$

where $\vec{v}$ is the fluid velocity within the lacunar-canalicular porosity and $\bigtriangledown p$ is the load-induced fluid pressure gradient. The intrinsic permeability, k, defines the resistance opposed by the lacunar-canalicular porosity to the fluid displacement, while ϕ_{lc} is the lacunar-canalicular porosity and μ is the dynamic viscosity of the interstitial fluid. The poroelastic problem was solved using the coupled pore fluid diffusion and stress analysis in Abaqus (v6.13, Dassault Systemes).

2.2. Micro-CT derived finite element models

Animal-specific finite element models were generated from micro-CT scans collected as part of a previous study that investigated changes in the cortical microporosities in a rat model of postmenopausal osteoporosis (Sharma et al., submitted for publication). In that study, twelve 20-week-old female Sprague Dawley rats were divided in two groups: one underwent bilateral ovariectomy (OVX) while the other was sham-operated (SHAM). After 6 weeks animals were sacrificed and the right tibiae were scanned with a high-resolution micro-CT system (SkyScan 1172; Bruker) at a nominal isotropic voxel size of 1 μm (100 kV, 100 μA). Three-dimensional reconstructions were obtained with a back-projection algorithm (NRecon, v.1.6.5, SkyScan, Bruker), using beam hardening and ring-artifact correction algorithms, and a Gaussian low-pass filter to reduce noise. Density phantoms were scanned using the same parameters and were used to calibrate the grayscale images to obtain accurate measures of the mineral content. All procedures were conducted under Institutional Animal Care and Use Committee approval.

To create a finite element model for each animal, a volume of interest was selected from the anterior cortical region of the proximal tibial metaphysis, where we previously detected morphological changes in response to estrogen deficiency (Fig. 1). Three-dimensional datasets were segmented using the micro-CT analysis software (CTAn v1.14; Bruker). A global grayscale threshold value corresponding to a mineral content of 0.45 g/cm^3 was applied to binarize images, separating bone tissue from soft tissue (Palacio-Mancheno et al., 2014). Image noise was removed using three-dimensional de-speckle filters and basic morphological operations, assuming that the vascular pores are interconnected and that their minimum volume is 1000 μm^3 (Tommasini et al., 2012; Palacio-Mancheno et al., 2014). Similarly, the lacunar porosity was isolated from noise with three-dimensional filtering, assuming that a single lacuna volume falls in the range 100 μm^3 to 600 μm^3 (Tommasini et al., 2012). Vascular porosity morphology and lacunar density and porosity were quantified for each volume of interest (CTAn v1.14; Bruker). Vascular canal diameter and separation (Ca.Dm and Ca.Sp) were measured using the sphere-fitting algorithm, and vascular porosity (Ca.V/TV), lacunar density (N.Lc/TV), and lacunar porosity (ϕ_{lac}) were also calculated. In addition, the tissue mineral density (TMD) was estimated from the mean grayscale value of the voxels representing bone tissue, and converted to g/cm^3 with a calibration curve derived from the known density values of the scanned phantoms.

After segmentation and morphometric analysis, each volume of interest was converted to a three-dimensional voxel-based hexa-