Abstract

Osteoporosis is a skeletal disease with a strong aging-related prevalence. It is defined by low bone mass and compromised bone strength, leading to increased risk of fractures. Osteoporosis results from an imbalance in the bone remodeling process with aging in which more bone is being resorbed than being formed. The process of bone remodeling is thought to be controlled by osteocytes that steer the activity of osteoblast, the bone forming cells, and of osteoclasts, the bone resorbing cells. Osteocytes reside in cavities within the bone matrix called lacunae and interconnect through cell extensions that reside in small canals named canaliculi. Through this extensive communication network, the osteocytes sense mechanical loads upon bone. Osteocytes are able to modify their own microenvironment by removal and addition of bone at the surface of the lacunae. It has been shown that lacunae undergo morphological alterations with aging and bone pathologies. Whether these alterations affect the transmission of mechanical signals to osteocytes is not known, but if they would, than the disturbance in the regulation of bone remodeling such as seen with aging may reflect an impaired ability of the osteocytes to sense and/or respond to mechanical stimuli. In order to elucidate this potential pathway, the central hypothesis of this thesis was that changes in the osteocyte lacunar morphology would lead to changes in the mechanical conditions experienced by the osteocyte, which in turn would give rise to a modified response to mechanical loading. To test this hypothesis, four aims were defined: (i) to quantify the morphology of
individual osteocyte lacunae, (ii) to quantify age-related changes in the osteocyte lacunar morphology, (iii) to relate the local bone mechanical strain to the osteocyte lacunar morphology, and (iv) to relate the osteocyte lacunar morphology to bone mechano-biological response.

In order to reach those aims, we developed a method for three-dimensional (3D) imaging, segmentation and quantification of the osteocyte lacunar network in murine bone using high-resolution desktop micro-computed tomography (µCT). We further evaluated the accuracy and reproducibility of µCT-derived cortical bone microstructural parameters. We concluded that desktop µCT is a valuable tool to quantify the 3D characteristics of bone vascular canals as well as lacunae which can be applied to intact murine bones with high accuracy and reproducibility. Using this 3D imaging method, we investigated potential age-related variations in the 3D morphology of the osteocyte lacunar network in fibulae of young and old mice. We found that osteocyte lacunae are becoming smaller and more spherical with aging. Since variations in the size and shape of the lacunae may cause changes in the local mechanical environment of the osteocytes, this observation led us to hypothesize that the lacunae with a large volume and low sphericity, as found at young age, experience higher local strains than those at older age. To test this hypothesis, we quantified the bone strains in the proximity of the lacunae using µCT image-based micro-finite element modeling and related those to lacunar shape in young and old mice. Our results showed that the mechanical environment of the osteocytes alters by the morphology of osteocyte lacunae. We found that larger, thinner and less longitudinally oriented lacunae experience higher maximum effective strains. Since osteocytes can sense matrix strains directly via their cell bodies, these local variations may affect the osteocyte mechanoresponse. Thus, we hypothesized that larger lacunae will enhance the osteocyte response to mechanical loading because they would be experiencing higher strains. To test this hypothesis, we related osteocyte lacunar morphology to bone mechano-biological response. Specifically, we investigated the response of osteocytes to mechanical loading in fibulae of two groups of mice having different lacunar morphology. Namely lactating mice, which
have enlarged lacunae due to osteocytic osteolysis and age-matched virgin mice. The osteocyte mechanoresponse was measured by quantifying loading-related changes in sclerostin and β-catenin expression in osteocytes, as determined by immunohistochemistry. We found significantly less loading-induced sclerostin and more β-catenin expression by osteocytes residing in enlarged lacunae in the fibulae of lactating mice compared to those in virgin mice.

In conclusion, the data presented in this thesis provide evidence that osteocyte lacunar morphology affects bone mechanoresponse. Specifically, the data imply that osteocytes located in smaller lacunae will experience lower strains than those in larger lacunae and will respond less to identical mechanical loading. They also indicate that, at least in part, smaller lacunae such as found in aged bones are a potential causative factor for the reduced bone mechanoresponsiveness seen with aging, hence, are a potential causative factor for age-related bone loss that leads to osteoporosis. The results described in this thesis contribute to opening a new research direction in bone mechanobiology and provide more insight on the role of osteocyte lacunar morphology on functional bone adaptation and the disturbed bone remodeling process at old age. It is therefore promising for elucidating a pathway in the etiology of osteoporosis.