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The influence of age on adaptive bone formation and bone resorption

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ABSTRACT

Bone is a tissue with enormous adaptive capacity, balancing resorption and formation processes. It is known that mechanical loading shifts this balance towards an increased formation, leading to enhanced bone mass and mechanical performance. What is not known is how this adaptive response to mechanical loading changes with age. Using dynamic micro-tomography, we show that structural adaptive changes of trabecular bone within the tibia of living mice subjected to two weeks of in vivo cyclic loading are altered by aging. Comparisons of 10, 26 and 78 weeks old animals reveal that the adaptive capacity diminishes. Strikingly, adaptation was asymmetric in that loading increases formation more than it reduces resorption. This asymmetry further shifts the (re)modeling balance towards a net bone loss with age. Loading results in a major increase in the surface area of mineralizing bone. Interestingly, the resorption thickness is independent of loading in trabecular bone in all age groups. This data suggests that during youth, mechanical stimulation induces the recruitment of bone modeling cells whereas in old age, only bone forming cells are affected. These findings provide mechanistic insights into the processes that guide skeletal aging in mice as well as in other mammals.

1. Introduction

Adaptation is a key process by which the skeleton adjusts to changes in the loading environment via bone modeling and remodeling to modify bone mass and architecture. While modeling does not require coordination, an individual remodeling event is performed by the basic multicellular unit (BMU) and involves the coordinated action of osteoclasts that resorb bone followed in time by osteoblasts that form bone [1]. With aging a net negative modeling and remodeling balance occurs, whereby the volume of resorbed bone exceeds that of formed bone. Either the skeleton's ability to form new bone declines with increasing age or the appropriate stimulus required to form new bone in an aged skeleton is not perceived, thereby contributing to the pathogenesis of age-related bone mineral loss [2]. A number of human exercise trials demonstrate that physical stimuli that enhance osteogenesis in young people aren't as effective in older individuals [3–6].

Animal studies have shown varied results, reporting an increased [7,8], decreased [9–12], or no change in the response [13–16] of bone to loading with aging. However, most studies examined mechanoresponsiveness of cortical bone with aging, while few examined trabecular bone [16–19]. This is relevant because fractures often occur at skeletal sites containing trabecular bone. Additionally, many previous studies were exercise-based [8,10,11,13–16], which introduce systemic effects and do not allow for strict control of loading parameters. Only a few studies compared mechanoresponsiveness between different aged mice after in vivo loading: adolescent and adults [18–20] or adult and aged mice [16,21]. Brodt et al. [16] reported that loading enhanced bone formation indices in BALB/c mice, yet micro-computed tomography (micro-CT) measures showed bone loss in 7 month old mice and no gain in 22 month old mice. They suggested trabecular bone resorption was the reason for this apparent contradiction, although they were unable to detect an increase in osteoclast surface using TRAP (tartrate-resistant acid phosphatase) staining. Recently, we showed that trabecular bone in adult mice exhibited a reduced and delayed response to loading compared to the young mice, apparent in trabecular bone volume fraction and architecture [22]. A major limitation of our work and others studies is a lack of understanding how resorption contributes to the adaption process.

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Technology combining in vivo micro-CT with image registration overcomes these limitations to quantify the bone formation and resorption response to mechanical loading over time.

Bone (re)modeling (remodeling and modeling) in response to loading has been primarily investigated using static and dynamic histomorphometry or micro-CT methods. Dynamic bone histomorphometry is the standard method for evaluating alterations in bone formation (e.g. MAR (mineral apposition rate) and BFR (bone formation rate)), while measures of resorption are limited to identifying a scalloped or eroded surface, which may or may not have active resorption occurring. Resorption has also been detected using stereological methods [23], but these methods have remained relatively unpopular as they are quite labor-intensive. More commonly, TRAP staining of osteoclasts has been used to identify bone resorption. However, these methods do not allow investigation of temporal changes or the spatial, 3D distribution and volume of resorption. More recently serial block face imaging was introduced, allowing quantification of number and size of resorption cavities [24]. Unfortunately, this method has disadvantages: small sample volume, resorption cavities identified based on the presence of an eroded surface, allows examination of only a single time point within a particular specimen, and is not yet commercially available.

Micro-CT is widely used to measure 3D structural parameters of bone. Waarsing et al. [25] originally proposed using 3D data sets of the bone's structure acquired at consecutive time points from in vivo micro-CT to visualize remodeling after ovariectomy in rats. A similar method was reported by Müller's group to quantify (re) modeling in the mouse tail in response to loading and ovariectomy [26–28]. Using a similar method we recently showed that loading has a much stronger effect on formation than on resorption in cortical bone; specifically due to an increase in formation surface with mechanical stimulation that is conserved into old age [9]. Despite these studies, it remains unknown how aging influences the trabecular bone formation and resorption response to mechanical loading. This knowledge is of particular importance, since fractures occur primarily at trabecular bone sites.

In the current study we made use of this method to investigate age-related alterations in the formation and resorption response of trabecular bone to loading. We use a mouse tibial loading model, which unlike the mouse tail model, is non-invasive. We investigated the adaptive response of trabecular bone of young (10 week old), adult (26 week old), and elderly (78 week old) female C57Bl/6J mice over a two week period of controlled non-invasive tibial compressive loading. We hypothesized that trabecular bone adapts to mechanical loading by both increased formation and decreased resorption, and this mechanoresponsiveness would diminish with increasing age. To test this hypothesis, we established an image processing and analysis method based on longitudinal micro-CT imaging to create 3D data sets of bone (re)modeling and assess formation and resorption volume, surface area, thickness/depth and rate.

2. Material and methods

2.1. In vivo load-strain calibration

As we wanted to conduct a strain-matched study, earlier in vivo strain gauging measurements on 10 and 26 week old mice [22] and 78 week old mice [9], ($n = 7$/age) were used to determine the relationship between applied compression force and longitudinal bone tissue deformation at the level of the strain gauge site. This relationship was used to define the applied load that engendered +1200 με at the medial cortical midshaft of the tibia. Single element strain gauges (EA-06-015LA-120, Micromeasurements, USA) were attached to the medial surface of the tibial midshaft aligned with the bone's long axis [18,29]. While mice were anesthetized, a range of dynamic compressive loads (peak loads ranging from −2 to −12 N) were applied between the flexed knee and ankle and strain measurements recorded simultaneously using an in vivo loading device (Testbench ElectroForce LM1, Bose, USA).

2.2. In vivo mechanical loading

Twenty nine female C57Bl/6J mice (10 week old: $n = 6$, 26 week old: $n = 13$, 78 week old: $n = 10$) underwent in vivo cyclic compressive loading of the left tibia (Fig. 1A). The mouse's knee and ankle were positioned in the loading device into concave cups, through which a −1 N preload was applied (Testbench ElectroForce LM1, Bose, USA). The right tibia was not loaded and served as an internal control. The loading protocol consisted of 216 cycles applied at 4 Hz (mouse locomotory stride frequency) [30], delivering a max force of −11 N for 10 and 26 week and −9 N for 78 week old mice (engendering 1200 με in the tibia of all age groups). The strain level has been shown to be osteogenic [17,31], and corresponds to roughly two to three times the strains engendered on the medial tibia during normal walking in the mouse [18,32]. The waveform included 0.15 s symmetric ramp loading/unloading, 0.1 s rest insertion between load cycles and a 5 s pause between every 4 cycles. Loading was applied 5 days/week (M–F) for 2 weeks while mice were anesthetized. Calcein was given via intraperitoneal injection, 12 and 3 days before euthanasia to label bone apposition. Mice were sacrificed on day 15, three days after the last loading session. Animal experiments were performed according to procedures approved by the local legal representative (LAGeSo Berlin, G0333/09).

2.3. In vivo monitoring of bone (re)modeling

In vivo micro-CT at an isotropic voxel size of 10.5 μm (vivaCT40, Scanco Medical, Switzerland; 55 kVp, 145 μA, 600 ms integration time, no frame averaging) was performed at day 0, prior to the start of the loading experiment and on days 5, 10, and 15 to assess trabecular bone. The scan region began at the growth plate and extended 432 slices (4536 μm) in the distal direction (Fig. 1B). To prevent motion artifacts, mice were anesthetized and kept in a fixed position using a custom-made mouse bed during the scans. In the group of the 10 week old mice, one mouse died between day 10 and 15 and was therefore not imaged at the last time point. Additionally, from the 26 and 78 week old mice, one data set of day 5 and one of day 15 were excluded from the analysis due to motion artifacts. For validation of the image processing technique, additional scans were performed on a subset of mice at day 15 ex vivo (26 week old, $n = 3$; 78 week old, $n = 3$).

2.4. Three dimensional image registration to visualize bone (re)modeling kinetics

For each animal, micro-CT images of the same region acquired at different time points (day 0, 5, 10, and 15) were evaluated to assess adaptive changes due to mechanical loading. The problem which had to be solved was to geometrically align consecutive images in a common coordinate system. For all pre-processing steps ZIBAmira software (Zuse Institute, Germany) was used. Misalignment of raw data slices, an artifact caused by the scanner, was reduced by aligning slices using the least square approximation function implemented in AMIRA. Subsequently, the input for the registration algorithm was defined, therefore the later image (day 5, 10, 15) was rigidly translated in order to superimpose its center of gravity with the earlier “reference” image's center of gravity. The images of the later time point were registered onto the reference image using a 3D rigid registration. Normalized mutual information was used as optimization criterion [25]. To exclude background noise from the registration, the histogram range used for calculation of mutual information ranged from 1000 HU to the maximal grey value in the reference image. To reduce the risk of finding local minima a hierarchical strategy was applied, starting at a coarse resampling of the data sets, proceeding to finer resolutions. Interpolation has been shown to affect the outcome of morphometric analysis [33], therefore we use a Lanczos windowed sinc kernel as interpolator, which has been shown to produce interpolation results comparable to B-splines [34], which have been shown to lead to low interpolation errors [33]. Registered images were transformed into the coordinate system of the reference data set, so all images had a common coordinate system with the same voxel size. Images were cut to 10% of total tibial length, starting 50 μm below the growth plate. To facilitate segmentation, the fibula was manually labeled in the data set. All segmented data sets were checked visually for segmentation errors.

2.5. Automatic segmentation of bone volumes

Image post-processing was performed using software written in Matlab (2009b; The Mathworks, Inc. USA). The algorithm consisted of three parts: (A) Extracting the bone region: Images were Gaussian filtered (convolution kernel [3 3 3], standard deviation 0.65) and binarized into bone and background using a global threshold of 273/1000 (456 mg HA/cc). The threshold for segmenting data sets into bone and background was determined based on the grey value distribution of the data sets of the different groups [35]. For each scan a histogram of the grey values was calculated and analyzed and segmented data sets were checked visually after segmentation. (B) Segmentation to separate trabecular from cortical bone (Fig. 2): Voxels labeled as fibula were automatically removed from all data sets. Data sets were then slice-wise segmented into trabecular and cortical bone. First, a closing filter was applied to close holes, such as blood vessels in the cortical bone, and then the whole ring of the