Acta Biomaterialia 21 (2015) 154–164

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Skeletal maturation substantially affects elastic tissue properties in the endosteal and periosteal regions of loaded mice tibiae

Sara Checa [a,*], Bernhard Hesse [a,b,c], Paul Roschger [d], Marta Aido [a,b], Georg N. Duda [a,b], Kay Raum [a,b], Bettina M. Willie [a]

[a] Julius Wolff Institute, Charité – Universitätsmedizin Berlin, Germany
[b] Berlin-Brandenburg School for Regenerative Therapies (BSRT), Berlin, Germany
[c] European Synchrotron Radiation Facility, Grenoble, France
[d] Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Center Meidling, 1st Med. Dept., Hanusch Hospital, Vienna, Austria

ARTICLE INFO

Article history:
Received 20 January 2015
Received in revised form 9 March 2015
Accepted 14 April 2015
Available online 18 April 2015

Keywords:
Bone adaptation
Skeletal growth and maturation
Elastic material properties
Scanning acoustic microscopy
Quantitative backscattered electron imaging

ABSTRACT

Although it is well known that the bone adapts to changes in the mechanical environment by forming and resorbing the bone matrix, little is known about the influence of mechanical loading on tissue material properties of the pre-existing and newly formed bone. In this study, we analyzed the newly formed and pre-existing tissue after two weeks of controlled in vivo axial compressive loading in tibia of young (10 week-old) and adult (26 week-old) female mice and compared to the control contralateral limb, by means of scanning acoustic microscopy. Additionally, we used quantitative backscattered electron imaging to determine the bone mineral density distribution within the newly formed and pre-existing bone of young mice. No significant differences were found in tissue stiffness or mineral density in the pre-existing bone tissue as a result of external loading. In the endosteal region, 10 and 26 week loaded animals showed a 9% reduction in bone tissue stiffness compared to control animals. An increase of 200% in the mineral apposition rate in this region was observed in both age groups. In the periosteal region, the reduction in bone tissue stiffness and the increase in bone mineral apposition rate as a result of loading were two times higher in the 10 compared to the 26 week old animals. These data suggest that, during growth and skeletal maturation, the response of bone to mechanical loading is a deposition of new bone matrix, where the tissue amount but not its mineral or elastic properties are influenced by animal age.

1. Introduction

Physical activity is thought to be a promising noninvasive treatment to reduce the risk of bone fracture. It is clear from human exercise [1–6] and preclinical studies [7–9] that loading can have a beneficial effect by increasing the bone size and altering the bone shape (i.e. increases in cortical thickness, bone cross sectional area, and/or cross sectional moment of inertia) [10–12] leading to increased bone strength [13,14]. We and others [7,8,15] have shown that the formation and resorption response to mechanical loading diminishes with increasing age and there is already a dramatic reduction in the bone formation response to in vivo mouse tibia loading at skeletal maturation. Using 3D dynamic in vivo morphometry we reported a 78% difference in mineralizing surface normalized to bone surface (MS/BS) and 86% difference in eroded surface normalized to bone surface (ES/BS) in loaded tibia from young compared to adult mice. In contrast, there was only an 18% difference in MS/BS and a 50% difference in ES/BS in loaded tibia from adult compared to elderly (78 week old) mice [7].

Bone strength and therefore, the risk of fracture, is not only determined by the amount of bone and/or its distribution (i.e. bone geometry), but also by tissue quality. Although several studies have examined the effect of loading on whole bone strength [14,16] and how macroscopic changes in bone mechanical properties due to loading are affected by age [17,18], it is unclear how loading affects tissue level material properties [19,20]. Loading may alter the bone quality by affecting the basic constituents of bone tissue: mineral and/or collagen. This knowledge is of clinical relevance, since changes in bone mass alone are not able to explain alterations in fracture risk [21–24].

Unloading of the bone during spaceflight has been shown to alter bone matrix ultrastructure and mineralization, suggesting

* Corresponding author at: Julius Wolff Institute, Charité – Universitätsmedizin Berlin, Campus Virchow-Klinkum, Institutsgebäude Süd/Südstraße 2, Augustenburger Platz 1, 13353 Berlin, Germany. Tel.: +49 (0)30 450 559789; fax: +49 (0)30 450 559938.
E-mail address: sara.checa@charite.de (S. Checa).

http://dx.doi.org/10.1016/j.actbio.2015.04.020
1742-7061/

degradation of the bone material properties [25–28]. Exercise studies led to alterations in the organic matrix by increased bone collagen synthesis [29] and/or altered tensional properties of the collagen network [30,31]. Unfortunately, most previous studies on the topic have focused on young animals or how exercise early in life affects bone quality later in life [32,33]. Isaksson et al. [30] showed that voluntary exercise (continuous access to a running wheel beginning at the age of 1 month in C57Bl6 male mice) led to improved tensional properties of the collagen network when the mice were still young and growing (2 and 4 month old), but not when the mice were adults (6 month old). The authors attributed the exercise-related improved mechanical properties in the young mice to increased remodeling of the bone, along with reorientation of the collagen fibrils. They reported no difference in the collagen content or crosslinks between running mice and sedentary controls of any age.

Interestingly, in tibiae from 16-week old male C57Bl6 mice, Kohn et al. [34] reported that 21 days of daily treadmill running led to increased ultimate strength, without changes in bone size or shape. Since there was no increase in bone formation rate or intracortical remodeling, they suggested that changes in the pre-existing bone quality, via enhanced mineralization (increased mineral-to-matrix ratio), mineral composition (decreased carbonate:phosphate ratio) and the organic matrix (increased collagen maturity), were responsible for the increased structural and tissue level mechanical properties. The addition of a latency period of two weeks after exercise in a subsequent study, further increased the predicted tissue-level stiffness and strength in tibiae from 8 week old C57Bl6/129 male mice, that they attributed to enhanced bone extracellular matrix quality [35]. These data by Kohn et al. [34] were quite unique in that they were able to show that in the absence of intracortical remodeling they observed changes in bone composition taking place in pre-existing tissue, since there was no new tissue formed by their loading model. In addition to a number of possible cellular mechanisms, Kohn et al. proposed several physically–chemically mediated mechanisms to explain the mechanically mediated alterations in minerals including: (1) pressure-induced phase changes, substitutions [36], or transformations [37], (2) changes in mineral chemistry via substitutions or crystal size, which could be due to increased mineral ion concentrations along with increased fluid flow near crystallites having a large surface/volume ratio [38,39], and (3) changes in the interface between mineral and collagen [40,41], for example the structural water between the mineral and collagen interface [42]. Another possible mechanism for the changes they observed in bone composition of pre-existing tissue could be perilacunar/canalicular remodeling [43,44]. Studies have shown that disuse or microgravity led to perilacunar modification in the form of enlarged lacunae [45,46], suggesting that loading may also influence this process.

Studies using controlled noninvasive loading models such as the mouse tibia or rat ulnae have suggested that changes in pre-existing tissue only occur during so-called fatigue loading, when loads are applied that engender strains that lead to microdamage and subsequent intracortical remodeling. In studies where the mouse tibial loading model elicits an anabolic response in the absence of intracortical remodeling and microdamage, load levels that engender +1200 με on the medial surface of the tibial midshaft [47,48] are commonly used. The +1200 με corresponds to roughly two to three times the strains engendered during normal walking in the mouse [47,49]. Although at this strain level there appears to be an absence of intracortical remodeling, it remains unclear if there are still changes in the quality of the pre-existing tissue via other mechanisms such as those proposed by Kohn et al. [34].

While changes in bone mass and architecture with two weeks of strain-matched (1200 με at tibial midshaft) controlled in vivo mouse tibial loading were confirmed in earlier studies on 10 and 26 week old female C57Bl6 mice [7,50], it is unclear whether changed loading conditions altered the bone tissue elastic properties. In this study, we hypothesized that (1) mechanical loading of the bone increases the elastic properties of the pre-existing bone tissue to accommodate the imposed mechanical loads and that (2) newly formed bone is less mineralized and less stiff in young compared with adult animals. To test these hypotheses, we used non-destructive spherically focused ultrasound at 200 MHz to investigate the adaptation of bone tissue elastic properties in response to controlled in vivo mechanical loading. In addition, we used quantitative backscattered electron imaging (qBEI) to determine if loading altered the bone mineralization density distribution (BMDD) at the microscopic level. We assessed the cortical bone response of postpubescent, young (10 week old) and adult (26 week old) female C57Bl/6J mice after two weeks of non-invasive tibial compression loading.

2. Materials and methods

Histomorphometric and FTIR analyses were previously performed and reported for the same samples [50,51]. For the sake of clarity, the preparation of the samples is briefly described here.

2.1. Sample preparation

The left tibiae of 10-week old and 26-week old female C57Bl/6J mice underwent in vivo cyclic compressive loading ($n = 7$/age). Loading parameters included: 216 cycles applied daily at 4 Hz, 5 days/week (M-F), for 2 weeks, delivering −11 N loads. The right tibia served as an internal control. Calcein was administered via intraperitoneal injection, 12 and 3 days before euthanasia, so that newly mineralized tissue could be identified between the double fluorochrome labels. At day 15, the right and left tibiae were dissected, embedded in polymethylmethacrylate and sectioned in 20-μm thick sections (1 per bone) in the transverse plane at the mid-shaft.

In this study, these samples were used to perform scanning acoustic microscopy and quantitative backscattered electron imaging as described below.

2.2. Scanning acoustic microscopy (SAM)

Bone elastic properties of the loaded and control limbs of the 10 and 26 week old mice were measured using scanning acoustic microscopy. Samples were measured using a custom-made scanning acoustic microscope (SAM200Ex, Q-BAM, Halle, Germany) equipped with a spherically-focused transducer with a nominal center frequency of 200 MHz, which provides a spatial resolution of approximately 8 μm in the focal plane. All measurements were performed in distilled and degassed water at 25 °C. The sample surfaces were aligned in the focal plane of the transducer. The scan field included the tibia and the fibula, which allowed easy recognition of the anatomical orientation. The scan increment was set to 4 μm in x and y directions (parallel to the sample surface). For each (x, y) position the entire pulse echo signal $V_{x,y}(t)$ was stored. After the x, y scan, another x-scan with variable transducer-sample distance (z-scan) was performed for each sample within a bone area in order to collect reference data for a defocus correction.

The following steps were applied to convert the measured pulse echo signals $V_{x,y}(t)$ into calibrated maps of the stiffness coefficient c_{33}. Each signal was upsampled and bandpass filtered using an FFT-based interpolation method and Type II Chebychev filters [52]. The Hilbert transform was computed for each pulse echo signal. Each signal consisted of a part related to the actual pulse echo (signal I) of the measured specimen plus some part originating from the