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Bone adaptation to cyclic loading in murine caudal vertebrae is maintained with age and directly correlated to the local micromechanical environment

Floor M. Lambers, Gisela Kuhn, Claudia Weigt, Kathleen M. Koch, Friederike A. Schulte, Ralph Müller *

Institute for Biomechanics, ETH Zürich, Zürich, Switzerland

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ABSTRACT

The ability of the skeleton to adapt to mechanical stimuli (mechanosensitivity) has most often been investigated at the whole-bone level, but less is known about the local mechanoregulation of bone remodeling at the bone surface, especially in context of the aging skeleton. The aim of this study was to determine the local and global mechanosensitivity of the sixth caudal vertebra during cyclic loading (8 N, three times per week, for six weeks) in mice aged 15, 52, and 82 weeks at the start of loading. Bone adaptation was monitored with in vivo micro-computed tomography. Strain energy density (SED), assumed as the mechanical stimulus for bone adaptation, was determined with micro-finite element models. Mechanical loading had a beneficial effect on the bone microstructure and bone stiffness in all age groups. Mineralizing surface was on average 13% greater ($p < 0.05$) in loaded than control groups in 15- and 82-week-old mice, but not for 52-week-old mice. SED at the start of loading correlated to the change in bone volume fraction in the following 6 weeks for loaded groups ($r^2 = 0.69$–0.85) but not control groups. At the local level, SED was 14–20% greater ($p < 0.01$) at sites of bone formation, and 15–20% lower ($p < 0.01$) at sites of bone resorption compared to quiescent bone surfaces for all age groups, indicating SED was a stimulus for bone adaptation. Taken together, these results support that mechanosensitivity is maintained with age in caudal vertebrae of mice at a local and global level. Since age-related bone loss was not observed in caudal vertebrae, results from the current study might not be translatable to aged humans.

1. Introduction

Physical exercise or in vivo loading could provide a strategy to counteract bone loss with age (Langsetmo et al., 2012; Morseth et al., 2010; Srinivasan et al., 2012; Troy et al., 2013). In vivo loading models in animals have shown that bones adapt to mechanical stimuli (mechanosensitivity) (Chambers et al., 1993; Fritton et al., 2005; Gross et al., 2002; Hillam and Skerry, 1995; Lambers et al., 2011; Moustafa et al., 2009; Perry et al., 2009; Saxon and Lanyon, 2008; Sugiyama et al., 2012; van der Meulen et al., 2009). While load-induced changes in the bone microstructure, remodeling rates, and bone strength have been observed for cortical bone, less is known about how local mechanical strains affect bone remodeling at the tissue level in trabecular bone. It is assumed that high mechanical stimuli result in local bone formation and that the lack of mechanical stimuli leads to local bone resorption (mechanostat) (Burr, 2002; Skerry, 2006, 2008). Direct comparison of mechanical signals with bone remodeling has been difficult to assess in trabecular bone due to the complex microarchitecture and the lack of three-dimensional imaging methods for bone formation and bone resorption in vivo. Recently, a method for three-dimensional quantification of bone formation and resorption from serial in vivo micro-computed tomography (micro-CT) scans was developed (Schulte et al., 2011). Using such imaging combined with micro-finite element (micro-FE) modeling, it was shown that mechanical stimuli (strain energy density, SED) at the tissue level contribute to the regulation of bone adaptation of trabecular bone in 15-week-old mice (Schulte et al., 2013). If local mechanoregulation is disturbed with age, this would likely lead to reduced mechanosensitivity of the bone and contribute to a diminished response to mechanical loading with age. While the majority of studies show a beneficial effect of in vivo loading on bone mass in

* Correspondence to: Institute for Biomechanics, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland. Tel.: +41 44 632 4592.
E-mail addresses: floor.lambers@gmail.com (F.M. Lambers), gakuhn@ethz.ch (G. Kuhn), claudia.weigt@sl.ethz.ch (C. Weigt), kathleen.m.koch@gmail.com (K.M. Koch), fschulte@ethz.ch (F.A. Schulte), ram@ethz.ch (R. Müller).

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aged animals (Brodt and Silva, 2010; Lynch et al., 2011b; Saxon et al., 2005; Silva et al., 2012; Srinivasan et al., 2003; Turner et al., 1995; Wenger et al., 2010; Willie et al., 2013), several studies report no or minimal effects of physical exercise or vibration in aged animals (Christiansen et al., 2009; Lynch et al., 2010, 2011a; Rubin et al., 1992; Silbermann et al., 1990). Therefore, at the global level, mechanosensitivity seems to be maintained, but reduced in bone with increasing age. It is unclear how mechanical strains contribute to local bone remodeling at the tissue level in trabecular bone in elderly mice.

The aim of this study was to determine the global and local mechanosensitivity of caudal vertebrae during cyclic loading in mice aged 15, 52, and 82 weeks at the start of loading. Specifically, the goals were (1) to determine the global mechanosensitivity by longitudinally monitoring bone microstructure, bone remodeling and bone stiffness and (2) to determine the local mechanosensitivity by comparing initial SED at formed and resorbed bone surfaces to initial SED at quiescent surfaces. We hypothesized that SED measured on the tissue level is directly correlated to bone formation or bone resorption in the local bone matrix.

2. Materials and methods

2.1. Study design

All animal procedures were approved by the local authorities (Kantonales Veterinäramt Zürich, Zürich, Switzerland). To enable loading of the sixth caudal vertebra (CV6), stainless steel pins were surgically inserted in the fifth and seventh caudal vertebrae of female C57BL/6 mice (12-week-old mice: $N=20$, 48-week-old mice: $N=23$, 76-week-old mice: $N=16$, RCC Ltd, Füllinsdorf, Switzerland) after one week of settling. Due to swollen tissue around the pins, several mice had to be excluded from the experiment, eventually leading to the following group sizes: $N=5$ loaded and $N=6$ control for mice 15-week-old at the start of loading (W15); $N=6$ loaded and $N=11$ control for mice 52-week-old at the start of loading (W52); and $N=6$ loaded and $N=7$ control for mice 82-week-old mice at the start of loading (W82). Three to six weeks after insertion of the pins the scanning and loading regime was started. Mice were anesthetized with Isoflurane during loading and micro-CT scans (2–2.5%, Attane, Piramal Healthcare, Mumbai, Maharashtra, India).

2.2. In vivo cyclic loading

For mechanical loading, pins were fixed in a previously developed caudal vertebral axial compression device (Webster et al., 2008). The proximal pin was clamped tightly, while the distal pin was connected to a load cell which applied sinusoidal forces at a peak load of 8 N (plus a preload of 1 N) at 10 Hz for 3000 cycles (5 min) to CV6. This loading protocol was applied three times per week for six weeks for the loaded groups (8 N). For the control groups (0 N), the mice were placed in the loading device under the same anesthesia, but no loading was applied. Thus for the control group no additional cyclic loading was applied, but bones were also not unloaded.

2.3. In vivo micro-computed tomography

In vivo micro-CT scans of CV6 were obtained at the start of loading for each group, and subsequently after 4 and 6 weeks for 15-week-old mice, after 1, 2, 4, and 6 weeks for 52-week-old mice, and after 2, 4 and 6 weeks for 82-week-old mice. Time points differed between age groups, because data of 15-week-old mice were taken from another study (Lambers et al., 2013). Furthermore, because data from a scan 1 week after the start of loading did not contribute to calculation of dynamic remodeling rates or a better understanding of the mechanical loading response, the scan one week after the start of loading was omitted for 82-week-old mice. Vertebrae were scanned at a voxel size of 10.5 μm using a vivaCT 40 (Scanco Medical AG, Brüttisellen, Switzerland). Settings of the scanner were 55 kVp, 145 μA, 200 ms integration time, and 1000 projections per 180°. The radiation dose for each scan was estimated to be 640 mGy. Previous control experiments showed that five scans at this dose did not have an effect on the bone microstructure or bone remodeling rates in caudal vertebrae of 15-week-old C57BL/6 mice. Since older mice are less sensitive to radiation, no detrimental effects of radiation are expected

Table 1
Absolute bone microstructural parameters in trabecular bone at week 0 and 6 and percentage difference between week 0 and week 6 for the three age groups.

	Trabecular Tissue Volume (Tb.TV) [mm^3]	Trabecular Bone Volume (Tb.BV) [mm^3]	Trabecular Bone Volume Fraction (BV/TV) [%]	Specific Bone Surface (BS/BV) [mm^2/mm^3]	Trabecular Thickness (Tb.Th) [μm]	Trabecular Separation (Tb.Sp) [mm]	Trabecular Number (Tb.N) [1/mm]	Connectivity Density (Conn.D) [1/mm^3]
15-week-old								
8N Week 0	2.30 ± 0.09	0.38 ± 0.06[a,b]	16.7 ± 1.76[a,b]	38 ± 2.5[a,b]	71 ± 3.6[a,b]	0.32 ± 0.019	2.91 ± 0.18[b]	58 ± 5.7
8N Week 6	2.29 ± 0.12	0.50 ± 0.05[a,b]	21.6 ± 1.34[a,b]	30 ± 1.8[a,b]	86 ± 3.8[a,b]	0.31 ± 0.011	2.89 ± 0.11[b]	49 ± 4.5
Percent change	0%	30%[c]	30%[c]	−21%[c]	21%[c]	−3%[c]	−1%[c]	−16%[c]
0N Week 0	2.14 ± 0.23	0.36 ± 0.06	16.6 ± 2.23	36 ± 2.2	73 ± 3.6	0.32 ± 0.029	2.84 ± 0.23	54 ± 12
0N Week 6	2.16 ± 0.26	0.40 ± 0.08	18.3 ± 2.33	32 ± 2.6	79 ± 6.2	0.32 ± 0.022	2.79 ± 0.20[c]	41 ± 9.9
Percent change	1%	12%[c]	11%[c]	−10%[c]	8%[c]	0%[c]	−2%	−22%[c]
52-week-old								
8N Week 0	1.83 ± 0.14	0.50 ± 0.03	27.2 ± 2.95	27 ± 2.5	92 ± 5.5[b]	0.25 ± 0.017	3.44 ± 0.17	51 ± 7.7[a,b]
8N Week 6	1.93 ± 0.14	0.44 ± 0.02	23.1 ± 1.44	29 ± 1.7	90 ± 3.8[b]	0.28 ± 0.019	3.17 ± 0.15	43 ± 6.4[a,b]
Percent change	5%[c]	−10%[c]	−15%[c]	7%[c]	−1%	14%[c]	−8%[c]	−15%[c]
0N Week 0	1.81 ± 0.12	0.52 ± 0.05	28.8 ± 1.91	26 ± 1.2	92 ± 3.2	0.24 ± 0.014	3.52 ± 0.18	50 ± 7.6
0N Week 6	1.98 ± 0.15	0.46 ± 0.06	23.3 ± 2.45	30 ± 2.0	85 ± 4.3	0.27 ± 0.019	3.31 ± 0.18	53 ± 8.0
Percent change	9%[c]	−12%[c]	−19%[c]	15%[c]	−7%[c]	12%[c]	−6%[c]	9%[c]
82-week-old								
8N Week 0	2.01 ± 0.10	0.46 ± 0.09[a,b]	22.9 ± 4.88[a,b]	31 ± 4.2[a,b]	85 ± 7.4[a,b]	0.27 ± 0.032	3.34 ± 0.28[b]	56 ± 10
8N Week 6	1.97 ± 0.18	0.52 ± 0.07[a,b]	26.4 ± 4.60[a,b]	27 ± 3.0[a,b]	94 ± 6.8[a,b]	0.27 ± 0.031	3.30 ± 0.27[b]	45 ± 5.5
Percent change	−2%	14%[c]	17%[c]	−14%[c]	11%[c]	0%	−1%[c]	−18%[c]
0N Week 0	1.85 ± 0.20	0.50 ± 0.04	27.3 ± 3.34	27 ± 3.5	93 ± 8.7	0.27 ± 0.025	3.43 ± 0.26	60 ± 16
0N Week 6	1.90 ± 0.23	0.50 ± 0.04	26.6 ± 2.75	27 ± 2.9	93 ± 7.2	0.27 ± 0.024	3.28 ± 0.23	53 ± 8.7
Percent change	3%	1%	−2%	0%	0%	3%	−4% c	−10%

Values shown are mean ± standard deviation.

[a] Significant difference in percent change between the 8 N and 0 N group.

[b] Significant interaction between time and group.

[c] Significant change over time between week 0 and 6 for the group.