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Longitudinal effects of Parathyroid Hormone treatment on morphological, densitometric and mechanical properties of mouse tibia

Yongtao Lu[a,b,c,1], Maya Boudiffa[d,1], Enrico Dall'Ara[c,d,*], Yue Liu[a], Ilaria Bellantuono[c,d], Marco Viceconti[b,c]

[a] Department of Engineering Mechanics, Dalian University of Technology, Dalian, China
[b] Department of Mechanical Engineering, The University of Sheffield, Sheffield, UK
[c] Insigneo Institute for in Silico Medicine, The University of Sheffield, Sheffield, UK
[d] MRC Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA), Department of Oncology and Metabolism, The University of Sheffield, Sheffield, UK

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ABSTRACT

The use of Parathyroid Hormone (PTH) as bone anabolic is limited due to cost-benefit assessments. Preclinical studies evaluating the effects of PTH on bone have reported variable and often contradictory results. Here, we have applied a new approach using a combination of *in-vivo* longitudinal μCT, image processing techniques and finite element models to monitor early local changes in the whole tibia (divided in 40 compartments) and mechanical properties of female C57BL/6J mice treated with PTH 1-34, compared to controls. Compared with standard 3D bone morphometric analysis, our new approach allowed detection of much smaller and localised changes in bone mineral content (BMC) at very early time points (1 week vs 3 weeks with standard methods) and showed that changes do not occur uniformly over time and across the anatomical space. Indeed, in the PTH treated mice, significant changes in BMC were observed in the medial and posterior sectors of the proximal tibia, a week after treatment, and in the medial sector of the tibia midshaft region a week later ($p < 0.05$). By the third week, two thirds of the regions showed significantly higher values of BMC ($p < 0.05$). The effect of PTH on bone regional volume is similar to that on BMC, but there is almost no effect of PTH on bone tissue mineral density. The differences in estimated mechanical properties became significant after three weeks of treatment ($p < 0.05$). These results provide the first evidence of an early and localised PTH effect on murine bone, and show that our novel partitioning approach, compared to the standard evaluation protocol, allows a more precise quantification of bone changes following treatment, which would facilitate preclinical testing of novel mono- and/or combination therapies throughout the bone.

1. Introduction

Osteoporotic fractures are a major clinical problem that increases the mortality and morbidity of our ageing society (Johnell and Kanis, 2005; Kanis and Johnell, 2005). Despite being the only FDA approved anabolic drug for the treatment of osteoporosis (Neer et al., 2001), teriparatide (PTH 1-34) is recommended for use only as secondary line of intervention due to cost-benefit considerations. Therefore, finding ways to reduce teriparatide dosage while still retaining its positive effects is the focus of intense investigation (Li et al., 2015).

Preclinical studies are useful tools to understand how this may be achieved. However, testing for the effect of parathyroid hormone (PTH) on bone in mono- or combination therapy has reported variable results in mice (Bouxsein et al., 2005; Ferrari et al., 2005; Pierroz et al., 2010; Wu et al., 2010). This is thought to be due to the cross-sectional design of the studies, which limits the power to detect meaningful differences, and to the way the 3D bone micro-architecture is analysed, by using sample regions (e.g. the proximal/distal region and/or the midshaft region of long bones), thus underrepresenting the response of the whole bone (Altman et al., 2014; Bouxsein et al., 2010; de Bakker et al., 2015). PTH has been shown to increase trabecular bone density and femoral strength measured by three-points bending mechanical testing in juvenile (Bartlow et al., 2016), young (Vrahnas et al., 2016) and adult (Johnston et al., 2007) mice. This *ex vivo* approach allows only for comparison of mechanical properties between groups of different animals in standard cross-sectional designs, including the intrinsic inter-

* Correspondence to: Department of Oncology and Metabolism and INSIGNEO Institute for in Silico Medicine, University of Sheffield, Sheffield, UK.
E-mail address: e.dallara@sheffield.ac.uk (E. Dall'Ara).
[1] These authors contributed equally to this work.

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subject variability and limiting the power of detecting small longitudinal changes in the same animals. Furthermore, three-points bending tests are affected by experimental artefacts (Wallace et al., 2014) and are scarcely informative of the changes in bone strength due to changes in the cortical and trabecular bone properties in different regions of the bone.

In vivo imaging techniques can be used to non-invasively analyse the same tissues of the same mice longitudinally, reducing the measurement variability due to inter-subject differences (Dall'Ara et al., 2016). In particular, the combination of *in vivo* micro computed tomography (μCT) and image registration improve the measurement of bone properties by reducing measurements uncertainties (Campbell et al., 2014). Recently this approach has been used to study in mice models the effect of interventions and/or mechanical loading on bone remodeling in the trabecular centrum of the caudal vertebrae (Lambers et al., 2012; Levchuk et al., 2014; Schulte et al., 2011), in the proximal tibia (Ausk et al., 2013; Buie et al., 2008; Stadelmann et al., 2011) or in the tibia midshaft (Birkhold et al., 2015, 2014a). However, little is known about early spatiotemporal effects of PTH on the morphological and densitometric properties of the whole mice bones. The three-dimensional μCT images of the mouse tibia can be converted into finite element (FE) models for non-invasive estimation of its mechanical properties (Pereira et al., 2015; Razi et al., 2015a). *In vivo* μCT-based FE models of bone portions have been used to investigate the relationship between bone remodeling and mechanical stimuli in para-physiological loading conditions (Levchuk et al., 2014; Razi et al., 2015b) and to study bone healing (Casanova et al., 2016). To the authors' knowledge no studies have used the *in vivo* μCT-based FE models to reveal longitudinal changes in the whole tibia mechanical properties and their relations with the spatiotemporal changes of bone morphological and densitometric properties. This would be fundamental to understand if the preclinically tested intervention would be effective in improving the bone competence of resisting fractures, which is the final clinical goal of anti-osteoporosis treatments.

The aim of this study is to quantify longitudinal effects of PTH on the properties on the tibia of female C57BL/6J mice by using a combination of *in vivo* μCT imaging, image processing and finite element analysis.

2. Material and methods

2.1. Animals and treatment

Ten 13-weeks-old female C57BL/6J mice were purchased from Harlan Laboratories (Bicester, UK). They were housed in the University of Sheffield's Biological Services Unit with a twelve-hour light/dark cycle at 22 °C and free access to food and water. All the procedures complied with the UK Animals (Scientific Procedures) Act 1986 and were reviewed and approved by the local Research Ethics Committee of the University of Sheffield (Sheffield, UK). At 18 weeks of age, mice (n = 5/group) received a daily i.p. injection of either PTH (hPTH 1-34, Bachem, Bubendorf, Switzerland) at 100 ng/g/day 7 days a week ("WT + PTH" group) or vehicle ("WT" group). PTH was prepared in 1% acetic acid and 2% heat inactivated mouse serum in HBSS (Hank's Balanced Salt Solution, Gibco®). The treatment was given until the age of 22 weeks (end of the experiment).

2.2. In-vivo μCT imaging

The whole right tibia of the mice was imaged using the Scanco *in-vivo* μCT (vivaCT 80, Scanco Medical, Bruettisellen, Switzerland). A baseline scan was performed at 14 weeks of age, then weekly follow up scans starting the second week after the initial scan, for seven weeks (Fig. 1). The scanner was operated at 55 keV, 145 μA, 32 mm FOV, 1500/750 samples/projections, 200 ms integration time and a nominal isotropic image voxel size of 10.4 μm. A third-order polynomial beam hardening correction algorithm provided by the manufacturer, determined using the 1200 mg HA/cm^3 wedge phantom, was applied to all the scans. The image grey values were then converted into HA-equivalent BMD values by means of the calibration law suggested by the manufacturer and based on weekly quality check performed with a five-rod densitometric calibration phantom.

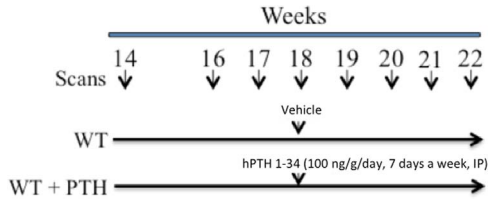

Fig. 1. Overview of the experimental design. Scans were performed at week 14 and then weekly from week 16 to week 22. Starting from week 18, mice received a daily i.p. injection of PTH ("WT + PTH" group, 100 ng/g/day) or vehicle ("WT" group) 7 days a week.

As *in vivo* μCT imaging is based on ionising radiation, there is an effect on the biological response of the imaged animal, which may affect the outcomes of the study. Taking into account this effect, we used the same scanning procedure for both groups at each time step and we reported data normalized for the baseline scans. The chosen scanning protocol induced 513 mGy dose to the mouse. Previous studies on C57BL/6J mice reported that repeated scans inducing 776 mGy affect significantly only trabecular bone and scans inducing 434 mGy were found not to affect cortical nor trabecular parameters (Laperre et al., 2011). Therefore, in order to evaluate the radiation effect with our scanning protocol, at the end of the longitudinal study we compared the bone mineral content in the scanned and the non-irradiated contralateral tibiae.

2.3. Standard 3D morphometric analysis

For trabecular bone analysis, a region of 1.0 mm height was chosen 0.2 mm below the growth plate as previously described (Lu et al., 2016). For cortical bone analysis, a region of 1.0 mm height was chosen in the tibia midshaft (Lu et al., 2016). Regions of interest for each compartment were manually marked and 3D bone parameters were generated, namely: trabecular bone volume fraction (BV/TV), number (Tb.N), separation (Tb.Sp) and thickness (Tb.Th) and average cortical thickness (Ct.Th). The analyses were performed with the software provided by the manufacturer of the μCT (V6.5-3, Scanco Medical, Bruettisellen, Switzerland).

2.4. Image processing and 4D analyses analysis

Image processing was performed as previously described (Lu et al., 2016). Briefly, rigid registration was applied to align tibiae along the longitudinal axis and to achieve correct rotation around this axis. For the registration, the tibia from the baseline scan was taken as the reference (Razi et al., 2015a). The tibiae from the following scans and from other mice were rigidly registered to the tibia of the baseline using a Quasi-Newton optimizer and the Euclidean distance as the similarity measure (Amira 5.4.3, FEI Visualization Sciences Group, France). Afterwards, the tibiae were divided in different regions and the bone properties in each region were tracked in time. In particular, after the image transformation, the tibial length (L) was measured from tibial most proximal voxel to the most distal voxel and a region of 80% of L was cropped out starting from the area below the proximal growth plate (Matlab, 2015a, The Mathworks, Inc. USA). Then, the obtained volume of interest (VOI) was partitioned into compartments (Fig. 2) using an in-house developed Matlab code. In the tibial longitudinal (proximal-distal) direction, the VOI was divided into ten regions with the same length to minimise the effect of tibial growth on the measured data.