PTH signaling mediates perilacunar remodeling during exercise

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Abstract

Mechanical loading and release of endogenous parathyroid hormone (PTH) during exercise facilitate the adaptation of bone. However, it remains unclear how exercise and PTH influence the composition of bone and how exercise and PTH-mediated compositional changes influence the mechanical properties of bone. Thus, the primary purpose of this study was to establish compositional changes within osteocytes' perilacunar region of cortical bone following exercise, and evaluate the influence of endogenous PTH signaling on this perilacunar adaptation. Raman spectroscopy, scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS) were used to evaluate tissue composition surrounding individual lacuna within the tibia of 19 week old male mice exposed to treadmill running for 3 weeks. As a result of exercise, tissue within the perilacunar region (within 0–5 μm of the lacuna wall) had a lower mineral-to-matrix ratio (MMR) compared to sedentary controls. In addition, exercise also increased the carbonate-to-phosphate ratio (CPR) across both perilacunar and non-perilacunar regions (5–10 μm and 10–15 μm from the lacuna walls). Tibial post-yield work had a significant negative correlation with perilacunar MMR. Inhibition of PTH activity with PTH(7-34) demonstrated that perilacunar remodeling during exercise was dependent on the cellular response to endogenous PTH. The osteocytes' response to endogenous PTH during exercise was characterized by a significant reduction in SOST expression and significant increase in FGF-23 expression. The potential reduction in phosphate levels due to FGF-23 expression may explain the increase in carbonate substitution. Overall, this is the first study to demonstrate that adaptation in tissue composition is localized around individual osteocytes, may contribute to the changes in whole bone mechanics during exercise, and that PTH signaling during exercise contributes to these adaptations.

Introduction

Bone is a complex living tissue with the unique ability to model and remodel its structure and mechanical properties to support sustained loads. As a composite structure, the mechanical properties of bone are influenced by its composition and spatial distribution [6,9]. Modeling and remodeling of bone tightly regulate its composition and distribution to optimize mechanical properties [36]. Although extensive work has identified cellular mechanisms that promote bone formation and remodeling, the impact of many of these signaling pathways on the mechanical properties of bone remains unclear. Understanding how mechanisms at the cellular level influence tissue composition and distribution of bone at the matrix-level, and also the mechanical properties of bone at the tissue and whole bone-levels, is vital for developing treatments that improve bone quality.

Bone quality can improve in response to various forms of exercise. There is an increase in bone metabolism in humans at the onset of exercise [27,38] that is followed by an increase in bone mineral content [42]. However, there is limited understanding of how exercise influences the mechanical properties of bone, given that increased mineral content or bone quantity does not always translate into improved mechanical function [20]. To this end, animal studies have identified structural and tissue-level mechanical properties that increase during various forms of exercise, such as swimming, jumping, voluntary running in a cage wheel or running on a treadmill [15,18,19,21,22,26]. For example, young adult mice running on a treadmill consistently exhibit increased structural and tissue-level properties, such as post-yield properties of the tibia [15,18,29,43,44]. Both structural and tissue-level properties can increase following exercise despite no significant changes in size, geometry, or mineral content [15,26]. However, investigation of tissue composition reveals increased carbonate-to-phosphate ratio, mineral-to-matrix ratio, and ratio of mature to immature collagen cross-links that correspond with strength and post-yield displacement gained during exercise [26,43]. Given that these findings represent a global average taken across the entire intracortical region, it remains unclear where these compositional changes are localized or where they may originate from, both of which are vital towards understanding how bone optimizes its strength without altering its shape and size.

The mechanical properties, mineral composition, and collagen cross-links that increase with exercise are considered a function of the dynamic loading and changes in calcitropic hormones that invoke specific cellular responses. In particular, we have demonstrated that the cellular response to endogenous parathyroid hormone (PTH) released during exercise facilitates an increase in stiffness and post-yield displacement, and reduction in yield displacement compared to sedentary controls [15]. Although PTH and mechanical loading are primarily associated with increased bone formation, both forms of stimuli also cause structural changes in the lacuna-canaliculi system of cortical bone [3,7,33]. In response to PTH, or in the absence of mechanical loads during spaceflight, lacunae volume can increase significantly [3,32]. Lactation similarly increases lacuna size, mediated by the calcitonin receptor [11], as well as the osteocytes' response to PTH [32]. This enlargement of the lacuna is often referred to as ‘osteocytic osteolysis’ [33], and can be counteracted by the addition of new tissue [37]. This local remodeling is indicative of the osteocytes' ability to not only alter the lacuna structure, but also modify the mass density and chemical composition of the perilacunar tissue [16].

To facilitate perilacunar remodeling, osteocytes can dissolve the surrounding mineral to enlarge their lacuna space by increasing acidity and degrading the mineral and matrix through tartrate-resistant acid phosphatase (TRAP), cathepsin-k (Ctsk), carbonic anhydrase 2 (CaII), and matrix metalloproteinase (MMP) activity [17,25,31,32,40]. In osteocyte-specific PTH/PTH-related protein receptor knockout mice, inhibition of lacuna enlargement and TRAP expression was observed during lactation [11,32]. With regard to the matrix, osteocyte expression of MMPs allows the lacuna and canaliculi structure to be maintained, while the loss of MMP expression leads to a decrease in mechanical properties of bone, specifically whole bone stiffness, strength, and modulus [31,40]. Replacing the mineral or matrix in the perilacunar zone is less understood. Given the similarities to osteoblasts, osteocytes have the capacity to generate alkaline phosphate (ALP) and type-1 collagen (COL1), along with other non-collagenous proteins [4]. As a result, osteocytes have the capacity to alter the mineral or matrix composition, such that the structural and/or tissue-level mechanical properties of bone are affected without requiring global changes to bone size or geometry.

We therefore hypothesized that endogenous PTH signaling during exercise mediates changes in the perilacunar tissue composition, and that this perilacunar adaptation contributes to the mechanical property changes that result from exercise. The purpose of this study was to: (1) establish that changes in perilacunar composition occur following short-term exercise, and (2) demonstrate the influence endogenous PTH signaling has on perilacunar adaptation during exercise.

Results

Perilacunar tissue composition is altered during exercise

Compared to sedentary controls, the exercise group exhibited a significantly smaller mineral-to-matrix ratio (MMR) within the perilacunar region (0 to 5 μm from the lacuna wall), but not in either non-perilacunar region (5 to 10, and 10 to 15 μm from the lacuna wall) (Fig. 1). The exercise group also exhibited a significantly greater carbonate-to-phosphate ratio (CPR) compared to sedentary controls across both perilacunar and non-perilacunar regions. This increase in CPR indicates a more carbonated apatite throughout the bone of mice subjected to exercise.

Inhibition of the cellular response to endogenous PTH was achieved by treating mice with PTH(7-34) during both exercise and sedentary conditions. Perilacunar MMR was not significantly different between exercise and sedentary groups treated with PTH(7-34). The CPR in the perilacunar and