Different Sclerostin Response to Cycling and Running at the Same Exercise Intensity

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Abstract

Recreational cycling is a popular activity which stimulates and improves cardiovascular fitness. The corresponding benefits for bone are unclear. This study examined the effect of running (high-impact) vs. cycling (low-impact), at the same moderate-to-vigorous exercise intensity, on markers of bone formation (N-terminal propeptide of type I collagen, PINP) and bone resorption (C-telopeptide of type I collagen, CTX-1), a non-collagenous bone remodeling marker (osteocalcin), as well as bone-modulating factors, including parathyroid hormone (PTH), irisin (myokine) and sclerostin (osteokine). Thirteen healthy men (23.7 ± 1.0 y) performed two progressive exercise tests to exhaustion (peak VO_2) on a cycle ergometer (CE) and on a treadmill (TM). On subsequent separate days, in randomized order, participants performed 30-min continuous running or cycling at 70% heart rate reserve (HRR). Blood was drawn before, immediately post- and 1h into recovery. PTH transiently increased (CE, 51.7%; TM, 50.6%) immediately after exercise in both exercise modes. Sclerostin levels increased following running only (27.7%). Irisin increased following both running and cycling. In both exercise-modes, CTX-1 decreased immediately after exercise, with no significant change in PINP and osteocalcin. At the same moderate-to-vigorous exercise intensity, running appears to result in a greater transient sclerostin response compared with cycling, while the responses of bone markers, PTH and irisin are similar. The longer-term implications of this differential bone response need to be further examined.

Introduction

Bone is a living dynamic tissue that adapts to mechanical stresses, such as exercise [1]. While the effects of weight-bearing activities on bone adaptation have been well documented [2, 3], there are few studies on non-weight-bearing activities, such as cycling, reporting conflicting findings [4, 5]. For this reason, current guidelines for bone health recommend weight-bearing activities, such as running or jumping [6, 7]. On the other hand, recreational cycling is very popular and beneficial for cardiovascular fitness. Recent findings suggest that high-intensity cycling may induce bone turnover changes to a similar degree as high-impact exercise [8], but the acute effects at moderate-to-vigorous intensity are still not clear.

The action of osteoblasts for bone formation and osteoclasts for bone resorption is a tightly coupled and regulated process for bone remodeling and the repair of micro fractures [9]. As such, this process of bone remodeling plays a paramount role in the adaptation of bone to mechanical forces and stress. Bone turnover markers (BTM) that are released during this process reflect the dynamic molecular changes within the bone and offer an opportunity to examine the bone response to different stimuli, including exercise [3, 10]. The specific BTM that are widely used include the C-telopeptide of type-1 collagen (CTX-1), a collagen degradation fragment, N-terminal propeptide of type 1 collagen (PINP), a residue from collagen formation, and osteocalcin, a bone non-collagenous protein, [11, 12].

In addition, changes in circulating hormone levels (e.g., parathyroid hormone, PTH), or osteokines that are secreted from bone cells to regulate the bone remodeling process may offer insight into the acute effects on the bone preceding the changes in BTMs. An important candidate for mediating the exercise impact

on the bone is sclerostin, an osteocyte-secreted osteokine that inhibits the Wnt-pathway, thus preventing osteoblast differentiation and function, and subsequently, decreasing bone formation [13]. In animal studies, sclerostin has been shown to increase in response to prolonged mechanical unloading and to decrease with mechanical loading [14]. In contrast, recent studies in humans have consistently demonstrated unintuitive transient increase in sclerostin following intense intermittent cycling and running exercise [15–17]. However, the BTM and sclerostin response to an acute bout of continuous moderate-to-vigorous cycling and running has not been examined.

The bone remodeling process can also be affected by the adjacent contracting muscles, directly by mechanical tension forces or indirectly through secreted myokines [18]. Irisin, a myokine that was originally highlighted for its potential role in browning white adipose tissue and thermogenic effects [19], has recently been shown in animals to affect bone remodeling [20]. Furthermore, in athletes, irisin levels are correlated with bone mineral density [21]. Irisin receptor was identified as integrin receptor on the osteocyte, leading to sclerostin expression [22]. Although irisin targets bone resorption, intermittent treatment with irisin has been shown to improve bone mass in mice [23]. Thus, the effect of exercise on irisin and its role in bone metabolism and remodeling is still unclear.

Despite advancements in our understanding of the mechanisms and regulating pathways of bone remodeling in response to exercise, further investigation is needed to determine the potential beneficial effects of different modes of exercise, especially those of low impact, on bone health. The aim of this study was to examine the effect of a 30-min submaximal continuous running (high-impact) and cycling (low-impact), at the same moderate-to-vigorous exercise intensity (70% heart rate reserve), on bone turnover markers and bone regulatory factors.

Methods

Participants

Thirteen healthy adult males (23.7 ± 1.0 years old) participated in the study. Table 1 presents participants' physical characteristics and response to the progressive peak VO_2 test. Highly-trained athletes and individuals with a history of any chronic medical conditions or medication use were excluded from the study. The Institutional Review Board at the University of California Irvine cleared the study and written informed consent was obtained from all participants upon enrollment.

Table 1. Anthropometric and Physiological Characteristics (n=13)

Age (years)	23.7 ± 1.0
Height (m)	1.75 ± 0.02
Body mass (kg)	78.3 ± 2.5
BMI (kg/m ²)	25.5 ± 0.7
CE VO _{2 peak} (ml/kg/min)	44.2 ± 2.3
CE Peak HR (bpm)	187 ± 2.9
TM VO _{2 peak} (ml/kg/min)	52.0 ± 2.2
TM Peak HR (bpm)	199 ± 2.9

Values are means ± SE.

BMI - body mass index. VO_{2 peak} - peak oxygen consumption. CE-cycle ergometer. TM-treadmill

Study design

All participants completed four exercise sessions in the Pediatric Exercise and Genomics Research Center Human Performance Laboratory. Two of the sessions were performed on a cycle ergometer (CE) (Lode Excalibur Sport Ergometer, Netherlands) and two on a motorized treadmill (TM) (Full Vision Trackmaster TMX428CP, Newton, Kansas, USA). In each exercise mode (cycling and running), participants performed a test of maximal aerobic capacity (sessions 1 and 2, in random order), and subsequently, a 30 min bout of submaximal exercise at 70% heart rate reserve (sessions 3 and 4, in counterbalanced order), as described below. This intensity was chosen because it has been shown to result in cardiovascular and metabolic benefits and is recommended by the American Heart Association [24] and the Physical Activity Guidelines for Americans [25]. All 4 sessions were completed within 30 days.

Sessions 1 & 2: Determination of peak HR and peak VO₂

Each participant performed a progressive ramp exercise test protocol on both the CE and TM, in random order. Those visits were performed at least 48 hours apart and no more than 9 days apart. Gas exchange was measured using the Sensor Medics metabolic system (Ergoline 800 S, Yorba Linda). Prior to the onset of exercise, participants rested in a recliner chair for 15 minutes, as resting heart rate (HR) was recorded using Polar HR monitor. For the CE ramp test, participants first sat comfortably without pedaling (“resting”) on the CE for 3 min and then began unloaded pedaling for 1 min prior to the commencement of the ramp test. The CE test protocol consisted of incremental increase in the work rate of 20-30 Watts/min, until the participant could no longer maintain a steady cadence and cadence fell below 60 RPM for >20s, despite encouragement by the investigators. For TM testing, a comfortable running pace was first determined for each participant. The TM test protocol consisted of 1-minute warmup at 3.5 mph, 1.5% grade, followed by 0.5 mph/min speed increase until reaching the comfort speed. From this point, the incline was incrementally increased at 0.5%/min to the limit of the participant’s tolerance. During both

tests, participants were encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath and peak VO_2 ($\text{VO}_{2\text{ peak}}$) was calculated as the highest value of a rolling average of 20 seconds in the last 2 minutes of the test.

Sessions 3 & 4 (Exercise Challenge)

At least 48 hours and no more than 14 days following the last $\text{VO}_{2\text{ peak}}$ evaluation, participants performed a 30-min constant exercise challenge at the same 70% HR reserve on either the CE or TM and came back to the lab within 5-15 days to perform the same exercise challenge in the other exercise mode. Target HR was calculated using the formula: $\text{HR}_{\text{rest}} + 0.7(\text{HR}_{\text{peak}} - \text{HR}_{\text{rest}})$. HR was monitored continuously using a Polar heart rate monitor.

Blood Sampling and Analysis

On the day of the exercise challenge, participants arrived at the laboratory between 7:30-8:30 AM, after an overnight fast of at least 10 hours. An indwelling catheter was inserted into the antecubital vein. After 30 min rest in a semi-reclined position, baseline blood samples were collected into EDTA vacutainers. Additional blood was collected immediately after the exercise (while the participants were still on the CE or TM), and 1-hour post-exercise (while the participants were again in a semi-reclined position). Thus, all samples were collected in the morning to control for circadian effects. EDTA vacutainers were immediately centrifuged at $1500 \times g$ for 15 minutes and plasma was separated and stored in aliquots at -80°C for subsequent analysis.

Plasma PTH was determined using a commercial ELISA assay (ALPCO, Cat # 21-IPTHU-E01), with a detection range from 11 to 971 pg/ml and a sensitivity of 1.57 pg/ml. This assay determines intact PTH 1–84, which is the biologically active form of the hormone. PTH intra-assay coefficient of variation (CV) average of 5% whereas, inter-assay CV average of 2%. Plasma sclerostin was determined using a commercial ELISA Assay (R&D, Cat # DSST00) with a detection range from 31.3 to 2000 pg/ml and a sensitivity of 3.8 pg/ml. Sclerostin intra-assay CV average of 0.5%; whereas inter-assay CV average 0.2%. Plasma N-terminal propeptide of type I collagen (PINP) was determined using a commercial ELISA Assay (Abbexa, Cat # abx250337) with a detection range from 15.6 to 1000 pg/ml and a sensitivity of 9.38 pg/ml. PINP intra-assay CV average of 12.6%; whereas inter-assay CV average 5%. Plasma C telopeptide of type I collagen (CTX-1) was determined using a commercial ELISA Assay (Immunodiagnostic, Cat # AC-02) with a detection range from 0.17 to 2.28 ng/ml and a sensitivity of 0.020 ng/ml. CTX-1 intra-assay CV average of 6%; whereas inter-assay CV average 5%. Plasma osteocalcin was determined using a commercial ELISA Assay (Invitrogen, Cat # KAQ1381) with a detection range from 2.3 to 89.3 ng/mL and a sensitivity of 0.08 ng/ml. Osteocalcin intra-assay CV average of 3%; whereas inter-assay CV average 1%. Plasma irisin was determined using a commercial ELISA Assay (Phoenix Pharmaceuticals, Cat # EK-067-29) with a detection range from 0.1 to 1000 ng/mL and a sensitivity of 1.4 ng/ml. Irisin intra-assay CV average of 9%; whereas inter-assay CV was 9%.

Corrections for Plasma Volume Changes

Hemoglobin (Hb) and hematocrit (Hct) were analyzed at the clinical pathology laboratory at University California Irvine Medical Center. Percent plasma volume change (% Δ PV) from pre- to post-exercise was calculated for each participant using the Dill and Costill equation [26]: % Δ PV = 100X((Hb pre/Hb post) X (100-Hct post)/(100-Hct pre)-1). The average plasma volume change immediately after exercise was not significantly different between exercise modes (-14.80 $\pm$ 1.91% in CE and -11.85 $\pm$ 4.27% in TM). Plasma sclerostin, CTX-1, osteocalcin and PINP levels immediately after exercise were corrected for plasma volume changes using the formula: (parameter) uncorrected *(1+ % Δ PV/100). Plasma volume returned to baseline levels by 1-hour post exercise, and was no significant differences from baseline. Since actual concentration of hormone determines its biological effect, PTH and irisin [27], were not corrected for plasma hemoconcentration in order to account and reflect their biological activity.

Statistical analysis

There were no missing samples from a total of 78 blood samples (13 participants X 6 sampling times). Data were assessed for normality by Shapiro-Wilk test. For each variable, change over time and differences between exercise modes were assessed using a 2-Way ANOVA for repeated measures. The main effects were 'Exercise mode' (TM, CE) and 'Time' (Pre, Post, and Rec). Paired t-tests (with Bonferroni multiple comparison correction) were used to decompose significant effects. Friedman's Two-way Nonparametric ANOVA was used in cases of osteocalcin and CTX-1 which had a non-normal distribution (based on the Shapiro-Wilk evaluation), with non-parametric paired comparisons to assess potential interaction. The level of significance was set to $P \leq 0.05$.

Results

During the exercise challenge on the CE and TM, HR remained stable at 70% HR reserve (CE 156.7 $\pm$ 0.4 bpm; TM 159.3 $\pm$ 0.7 bpm). A significant main effect for time was observed for PTH ($P < 0.001$), with neither a significant exercise mode effect nor a mode-by-time interaction (Fig. 1). PTH transiently increased immediately after exercise in both CE and TM (CE, 51.7%; TM, 50.6%), and returned to near baseline levels 1-hour post-exercise. There was a significant main effect for time also found in irisin levels ($p = 0.047$), increasing by 6.5% following TM and 2.75% following CE, with no significant exercise mode effect or interaction (Fig. 1). A significant mode-by-time interaction was observed in sclerostin ($F(2, 48) = 4.59$, $P = 0.015$), reflecting an increase after TM running (27.7%), which was not evident after CE cycling (Fig. 1).

In terms of bone turnover markers, a significant main effect for time ($p = 0.004$) was found for CTX-1 (Fig. 2), with no significant exercise mode effect and no significant time-by-mode interaction. CTX-1 decreased immediately following both CE (19.4%) and TM (19.9%) and returned to near baseline levels 1 hour into recovery. There were no significant effects for PINP or osteocalcin (Fig. 2).

Discussion

This study provides evidence that 30-min of cycling (CE, low-impact) and running (TM, high-impact) at moderate-to-vigorous intensity results in similar PTH, irisin and BTMs responses, but a different sclerostin response. These results suggest that mechanical impact on the bone is mediated through sclerostin activity.

Sclerostin, an inhibitor of bone formation has been shown to play a major role in mechano-transduction in bone. It decreases following prolonged mechanical loading and increases during prolonged mechanical unloading in animal studies [14]. Similarly, sclerostin increases following bed rest in healthy men [28, 29]. Thus, it would be anticipated that sclerostin would decrease following high impact exercise, but interestingly, it was found to increase following exercise such as plyometric jumps in young men (but not in boys) [30] and TM running at low [31] and high intensity [32]. The increase in sclerostin levels following high impact exercise would suggest a catabolic effect on bone, as seen following prolonged bed rest. However, this discrepancy might be explained by a counterintuitive sclerostin secretion pattern, as already known for PTH [33]. According to this pattern, while persistently high levels of sclerostin (e.g., during prolonged bed rest), like PTH will lead to a bone catabolic effect, a transient or intermittent increase of its secretion (e.g., following high impact exercise) will activate the remodelling process and lead to an anabolic response. It also seems that the sclerostin response to exercise may be intensity-dependent, with high-intensity intermittent cycling and running leading to a similar increase in sclerostin [17], but a dissimilar response following moderate-to-vigorous continuous cycling and running, as in the present study. Therefore, further studies are needed to understand the mechanisms for these inconsistencies in the exercise-induced behavior of sclerostin.

Similar to previous studies [30, 34], PTH transiently increased immediately post-exercise in both modes, a response potentially linked to calcium homeostasis. Indeed, although we did not measure electrolytes in the present study it has been previously shown that PTH responds abruptly to changes in circulating calcium and phosphorus post-exercise [35], while calcium infusion during exercise attenuate this response [36]. Changes in PTH levels also depend on exercise intensity, with higher PTH response to higher exercise intensity [37]. However, it is still not clear if the transient exercise-induced increase in PTH has a direct anabolic effect on bone, similar to that seen with PTH injection, or following exercise in rodents [38].

Irisin, a myokine that has endocrine effect on adipose tissue [19], is also a candidate for mediating the osteocytes' response to mechanical loading [20]. Recently, it has been shown to bind directly to integrin receptor, a molecule that attaches the osteocyte to the extracellular matrix, and leads to sclerostin expression [22]. Similar to other exercise studies [39–41], we found a slight increase in plasma irisin following both exercise modes. Further studies are needed to clarify if this post-exercise irisin change has any effect on the bone, especially since the increase in irisin levels were accompanied by an increase in sclerostin levels following TM running.

There was no acute change in BTMs levels except the transient decrease in CTX-1 following both exercise modes. CTX-1 response to exercise has been shown to depend on exercise intensity: decreasing following

60 minutes of low intensity running [42] but increasing following 60 minutes of high intensity cycling [43] and high-intensity interval running and cycling [16, 44]. Since the exercise intensity in this study was moderate-to-vigorous it was anticipated that CTX-1 levels would increase. Thus, the decrease that we observed probably reflects an effect of the continuous versus intermittent exercise protocol. Alternatively, the unexpected decrease in CTX-1 may be related to its known diurnal changes. That is, CTX-1 peak in the morning, i.e., at the time we took the baseline measure, and decreases to a minimum at noon [45], at about the time exercise ended. Since we did not have a non-exercising control group we couldn't control for CTX-1 diurnal variation. The rapid and transient decrease in CTX-1 levels without change in PINP or osteocalcin does not support an immediate bone remodeling effect. However, bone formation follows bone resorption, so it is possible that the bone formation markers increase later than the 1-hour post-exercise.

In conclusion, bone turnover markers response to exercise is an intertwined interaction of local responses mediated through locally-secreted cytokines, as well as through systemic signals such as hormones. The complexity of the bone response to exercise may be a reflection of the dual role of the bone as a structural support and as a mineral reservoir. Moreover, exercise can have different effects (metabolic and mechanical) on bone, depending on impact, intensity, and/or duration of the exercise, so it is not surprising to find inconsistent bone markers responses to various exercise challenges. In this study we controlled for both exercise intensity and duration, in two popular exercise modalities, running (high mechanical impact) and cycling (low mechanical impact), using target HR, which is a practical and common tool to prescribe exercise in the field, using an intensity that is recommended by physical activity guidelines. Our results demonstrate the complexity of the bone response to exercise. While PTH and sclerostin, both of which have a resorption effect on bone, increased (sclerostin only following running) there was decrease in CTX-1, a bone resorption marker, immediately post-exercise. The different sclerostin response between the exercise modes might reflect the different mechanical loading. Thus, more studies are needed to examine the underlying mechanisms in the acute bone response to exercise of different modes and intensities.

Declarations

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Ethics declarations

This study was funded by NIH U01-TR002004 and PERC Systems Biology Fund.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: This study was approved by Institutional Review Board at the University of California Irvine and carried out in accordance with the principles set out in the Declaration of Helsinki.

Informed consent: Written informed consent was obtained from all participants upon enrollment.

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Figures

Figure 1

Circulating levels of parathyroid hormone (PTH), irisin and sclerostin. Data are means $\pm$ SEM, sclerostin values are corrected for changes in plasma volume * denotes significant time effect $p < 0.05$ # denotes significant mode-by-time interaction.

Figure 2

Circulating levels of bone turnover markers. Data are means $\pm$ SEM, values are corrected for changes in plasma volume * denotes significant time effect $p < 0.05$ CTX-1- C-telopeptide of type I collagen; PINP- N-

terminal propeptide of type I collagen