

High force eccentric exercise enhances serum tartrate-resistant acid phosphatase-5b and osteocalcin

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Abstract

We investigated the effects of eccentric contractions (ECs) on bone metabolism markers and the relationship between bone metabolism and skeletal muscle related protein. Seventeen young untrained men were divided into two groups and performed either 60 or 30 maximal ECs. We measured serum levels of osteocalcin (OC), bone alkaline phosphatase, cross-linked N-telopeptide of type I collagen (NTx), and tartrate-resistant acid phosphatase 5b (TRACP-5b), growth hormone (GH), and insulin-like growth factor-1 (IGF-1). Blood samples were collected for up to five days after ECs. OC with 60 ECs were significantly higher than with 30 ECs (2 hours; $p < 0.05$, day 1 and day 5; $p < 0.01$). TRACP-5b with 60 ECs were significantly higher than with 30 ECs (day 3 and day 5; $p < 0.001$). IGF-1 and OC were significantly positively correlated with 60 ECs (2 hours, day 1, and day 5; $p < 0.05$). There were also significant positive correlations between IGF-1 and NTx with 60 ECs (2 hours, $p < 0.01$; day 1, $p < 0.05$). We found that one bout of severe ECs caused increases in OC and TRACP-5b, which promote increased bone metabolism. Our results suggest that contraction-induced IGF-1 may activate OC and NTx in acute response.

Keywords: Bone Metabolism, Lengthening Contractions, IGF-1, Muscle Injury, Myokine

Introduction

It is well known that bone mass and strength are regulated by endocrine and nutritional factors as well as exercise¹⁻³. In particular, dynamic and high-impact exercise effectively increases bone strength^{2,4}. However, it has also been reported that long-distance runners have lower bone mass than sedentary subjects⁵. Thus, while appropriate mechanical stimulation is effective for increasing bone strength, it can have a negative effect on bone mass when the exercise load is excessive.

The effect of transient mechanical stimulation on serum bone metabolism markers is controversial⁶⁻⁸. Previous study on bone metabolism responses to resistance exercise has

shown that both bone formation and resorption markers decrease significantly 24 hours after exercise⁷; however, Whipple et al. reported that bone formation markers increase significantly⁸. Other studies on aerobic exercises such as running or pedaling have revealed that bone formation and resorption markers increase⁹ or decrease¹⁰ in a manner similar to that in resistance training⁶. There is no consensus among these studies because of the full-body workout such as running, pedaling, and resistance exercise. Thus, the bone metabolism response to quantitative exercise volume remains unclear.

Exercise not only exerts mechanical stimulation on bone but also results in changes in endocrine responses¹¹⁻¹³. In particular, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) enhance bone formation and resorption and are essential for maintaining and improving bone strength^{11,12}. Previous studies have shown that GH and IGF-1 knockout mice have decreased osteoblast activity^{14,15}. In contrast, in mice overexpressing GH, both bone formation and resorption increase, bone resorption surpasses bone formation, and the quality of the bone structure subsequently deteriorates¹⁶, suggesting that GH and IGF-1 do not always positively influence bone metabolism. The associations between GH/IGF-1 and bone metabolism markers after acute exercise have not been determined.

The authors have no conflict of interest.

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Edited by: J. Rittweger

Accepted 4 November 2013

Table 1. Physical characteristics and total work out put during eccentric contractions.

	60ECs	30ECs
Age (years)	20.1±0.5	20.0±0.4
Height (cm)	171.8±1.9	168.4±1.9
Weight (kg)	65.0±2.8	64.9±1.9
Body fat (%)	15.1±1.5	15.9±1.0
Lean body mass (kg)	55.0±1.8	54.5±1.7
Total work (Nm/BW)	50.5±1.8	32.9±1.4 ***

Means ± S.E.
*** p<0.001
ECs: eccentric contractions

Table 2. Comparisons of the time course of changes in blood lactate, creatine kinase, and myoglobin.

		pre	post	2 hours	day 1	day 3	day 5
Blood lactate (mmol/l)	60ECs (n=9)	1.0±0.1	3.8±0.3	0.9±0.1	0.7±0.06	0.9±0.1	0.7±0.07
	30ECs (n=8)	1.3±0.2	2.3±0.2	1.1±0.1	1.0±0.1	0.8±0.06	0.9±0.08
Creatine kinase (IU/l)	60ECs (n=9)	301.4±87.0	380.9±96.3	360.9±84.0	390.3±48.5	2417.3±678.9	4792.7±649.2
	30ECs (n=8)	237.6±48.9	251.3±50.2	256.8±45.6	415.4±74.9	837.1±394.5	1197.0±436.8
Myoglobin (ng/ml)	60ECs (n=9)	25.3±4.1	31.2±4.7	40.6±5.0	40.0±10.2	156.3±47.9	137.2±36.0
	30ECs (n=8)	20.1±2.5	25.2±3.8	49.0±16.4	37.4±10.9	58.1±21.7	88.1±30.3

Means ± S.E.

* p<0.05, ** p<0.01, *** p<0.001

ECs: eccentric contractions

Recent studies have shown that exercise increases bone resorption and decreases pH and the oxidative environment¹⁷. Brandao-Burch et al. found that oxidation not only activates osteoclasts but also inhibits bone formation and reduces calcification¹⁸. However, contradicting studies found no association between lactic acid concentrations and bone metabolism markers after exercise^{19,20}.

The aim of the present study was to investigate the acute responses of bone metabolism markers as a result of simple eccentric movements of 2 intensities. Previous studies mainly used resistance training, jumping (plyometric), running, or pedaling as the mode of exercise^{7,19,20}. However, these movements are complex and their work output is unclear. Isokinetic dynamometers such as Biodex and Cybex are used for quantitative force analyses²¹⁻²³ and for activating GH/IGF-1 in the local biceps muscle during the acute phase²⁴. Furthermore, the associations among GH and IGF-1, blood lactate, and bone metabolism markers were investigated. We hypothesized that (1) eccentric contractions (ECs) with a larger work output would have a negative effect on bone metabolism, (2) increased blood lactate concentrations due to ECs would activate bone resorption markers, and (3) ECs-induced GH and IGF-1 would increase bone metabolism.

Materials and methods

Subjects

The subjects were 17 healthy untrained men (age, 20.1±0.4 years) who had not been involved in any regular resistance training for at least 2 years before this study were recruited. All subjects had no chronic disease, who were not receiving supplements, and who were non-smokers. The risks involved were explained to all study participants before the start of the study, and written informed consent was obtained. This study was conducted after receiving approval from the Ethics Committee of Juntendo University (ID; 24-118). After body composition measurements, the participants were randomly divided into 2 groups: the 60 ECs group (60 ECs) and the 30 ECs group (30 ECs). Dietary energy requirement was calculated for each subjects and macronutrient content was 15% protein, 55% carbohydrate, and 30% fat. Average daily energy intake was 2228±158 kcal. The number of subjects was determined by a sample size estimation using the data from previous studies²¹ in which maximal eccentric contractions. The estimation was based on the effect size of 1, alpha level of 0.05, a power (1-β) of 0.80, and an expected difference of 10% for the difference in muscle recovery at 5 days post-exercise, and it was shown that at least 12 subjects were necessary.

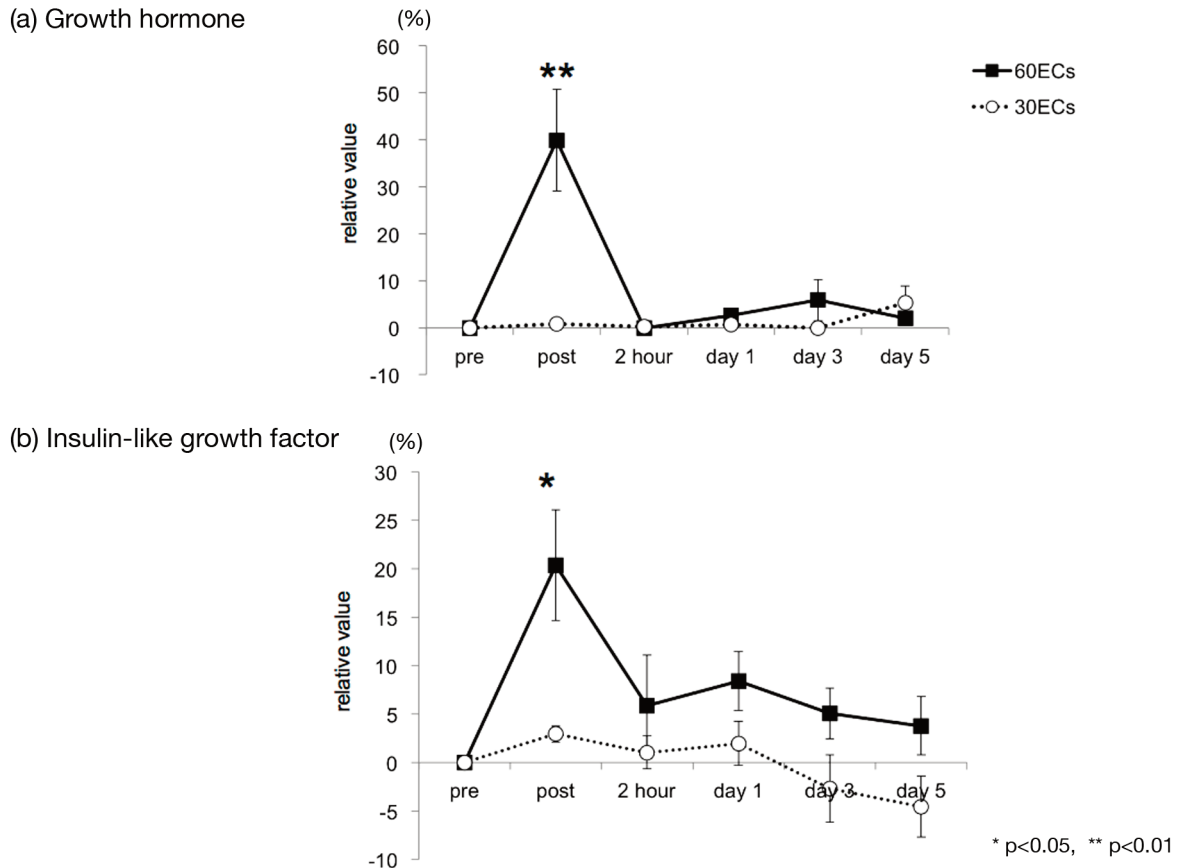


Figure 1. The time course changes in growth hormone (GH; a) and insulin-like growth factor-1 (IGF-1; b). Both GH (a) and IGF-1 (b) was significantly higher with 60 ECs than 30 ECs at pre (GH: $p<0.01$, IGF-1: $p<0.05$). Values are means \pm S.E.

Study design

The experiments were performed over a 6-day period. All subjects fasted for 8 hours until completion of blood collection 2 hours after ECs. Also, excessive exercise from 48 hours before ECs until 5 days after ECs was not allowed. Blood samples were collected at rest (pre-exercise) and immediately (post-exercise), 2 hours, 1 day, 3 days, and 5 days after ECs. The samples were collected at the same time each day to minimize intra-day variations.

Body composition

Body composition (weight, body fat, and lean body mass) was measured using a Tanita MC-190 body composition analyzer (Tanita Corp, Tokyo, Japan). The measurement procedure requires that each participant stands barefoot on the analyzer and holds a pair of hand grips. The device uses multiple-frequency (5, 50, 250, and 500 kHz) bioelectrical impedance analysis technology and has 8 tactile electrodes: 2 in contact with the palm and thumb of each hand and 2 in contact with the anterior and posterior aspects of the sole of each foot.

Procedure of eccentric contractions

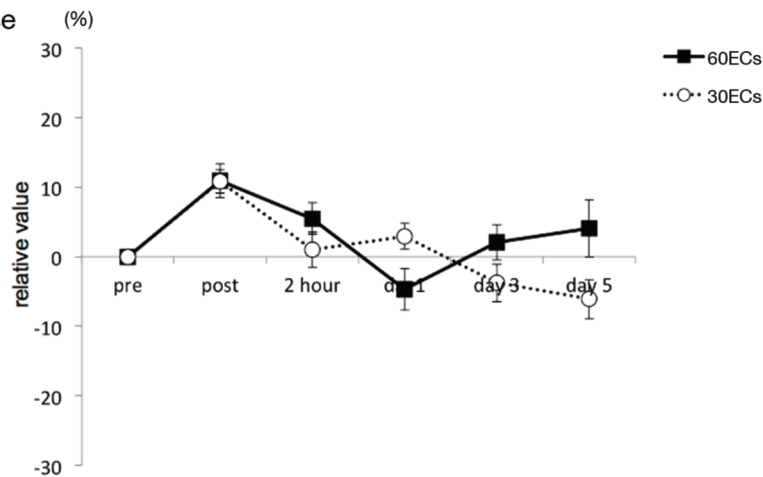
All participants performed maximal ECs of the elbow flexors

using their nondominant arm and a Biodex System 3 (Biodex Medical Systems, Inc., Shirley, NY, USA) based on exercise protocols used in previous studies^{21,25}. The position of the shoulder was rotated externally to 45° with the trunk and waist secured with a strap. The center of the elbow that was being measured was aligned with the axis of the dynamometer, and the upper arm was secured on a pad. 60 ECs were performed in 10 sets of 6 maximum contractions and 30 ECs were performed in 5 sets of 6 maximum contractions. A 2-min resting period was scheduled between the sets in both groups. The range of motion of the ECs was 120° and velocity was 30 deg/sec in two groups. Total work output was calculated from the sum of the amount of work for each set per body weight using the software of the Biodex Medical Systems (Systems 3 Application Software for Window XP, Biodex Medical Systems, Inc., Shirley, NY, USA).

Blood analysis

The blood samples were allowed to clot at room temperature (25°C) and then centrifuged at 3000 rpm for 10 min at 4°C. They were stored at -20°C until analysis. Bone alkaline phosphatase (BAP) and osteocalcin (OC) were measured as indicators of bone formation. BAP was measured using a chemiluminescent enzyme

(a) Bone-alkaline phosphatase



(b) Osteocalcin

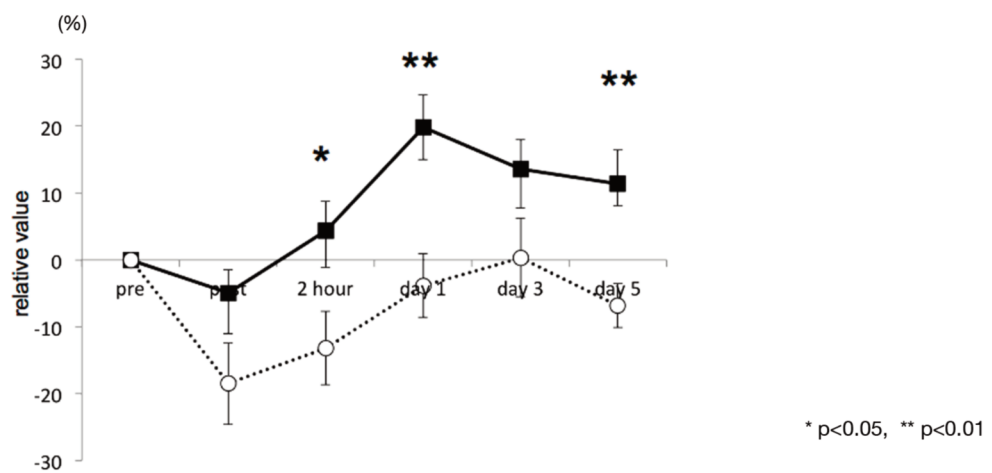


Figure 2. The time course changes in bone-alkaline phosphatase (BAP; a) and osteocalcin (OC; b) OC (b) was significantly higher with 60 ECs than 30 ECs at 2 hours, day 1, day 3, and day 5 (2 hours, day 1: $p < 0.05$; day 3, day 5: $p < 0.01$). Values are means \pm S.E.

immunoassay (EIA). The intra- and inter-assay coefficients of variation (CVs) were 6.8% and 5.0%, respectively. OC was measured by radioimmunoassay (RIA), and the intra- and inter-assay CVs were 4.1% and 6.3%, respectively. Cross-linked N-telopeptide of type I collagen (NTx) and tartrate-resistant acid phosphatase-5b (TRACP-5b) were measured as indicators of bone resorption. NTx was measured by enzyme-linked immunosorbent assay, and the intra- and inter-assay CVs were 5.9% and 6.3%, respectively. TRACP-5b was measured by RIA, and the intra- and inter-assay CVs were 2.2% and 2.9%, respectively. We also measured creatine kinase (CK), myoglobin (Mb), blood lactate, GH, and IGF-1. A sample for blood lactate was collected in a refrigerated capillary tube (20 μ l) and immediately added to a sample solution. These samples were analyzed using Biosen C-line (EKF-Diagnostics, Tokyo, Japan). Serum CK levels were measured using a Japan Society of Clinical Chemistry Standardized Method, and the intra- and inter-assay CVs were 4.0% and 4.1%, respectively. Serum Mb levels were measured by RIA, and the intra- and inter-assay CVs were 6.0% and 3.9%, respectively. IGF-1, and GH levels were also meas-

ured by RIA. These intra- and inter-assay CVs, IGF-1 were 2.4% and 3.3%, respectively, and GH were 3.0% and 2.0%, respectively.

Statistical analysis

All results are presented as means \pm standard errors. We used Student's *t*-tests to compare experimental groups for age, height, body weight, body fat, lean body mass, and total work. Two-way analysis of variance followed by Bonferroni tests was used to compare the blood analysis results. Pearson's product-moment correlation coefficient was used to assess associations between variables. $P < 0.05$ was considered significant. SPSS software for Windows was used for statistical analysis (SPSS Japan Inc., Tokyo, Japan).

Results

Physical characteristics of the subjects and the total work output from ECs are shown in Table 1. No significant differ-

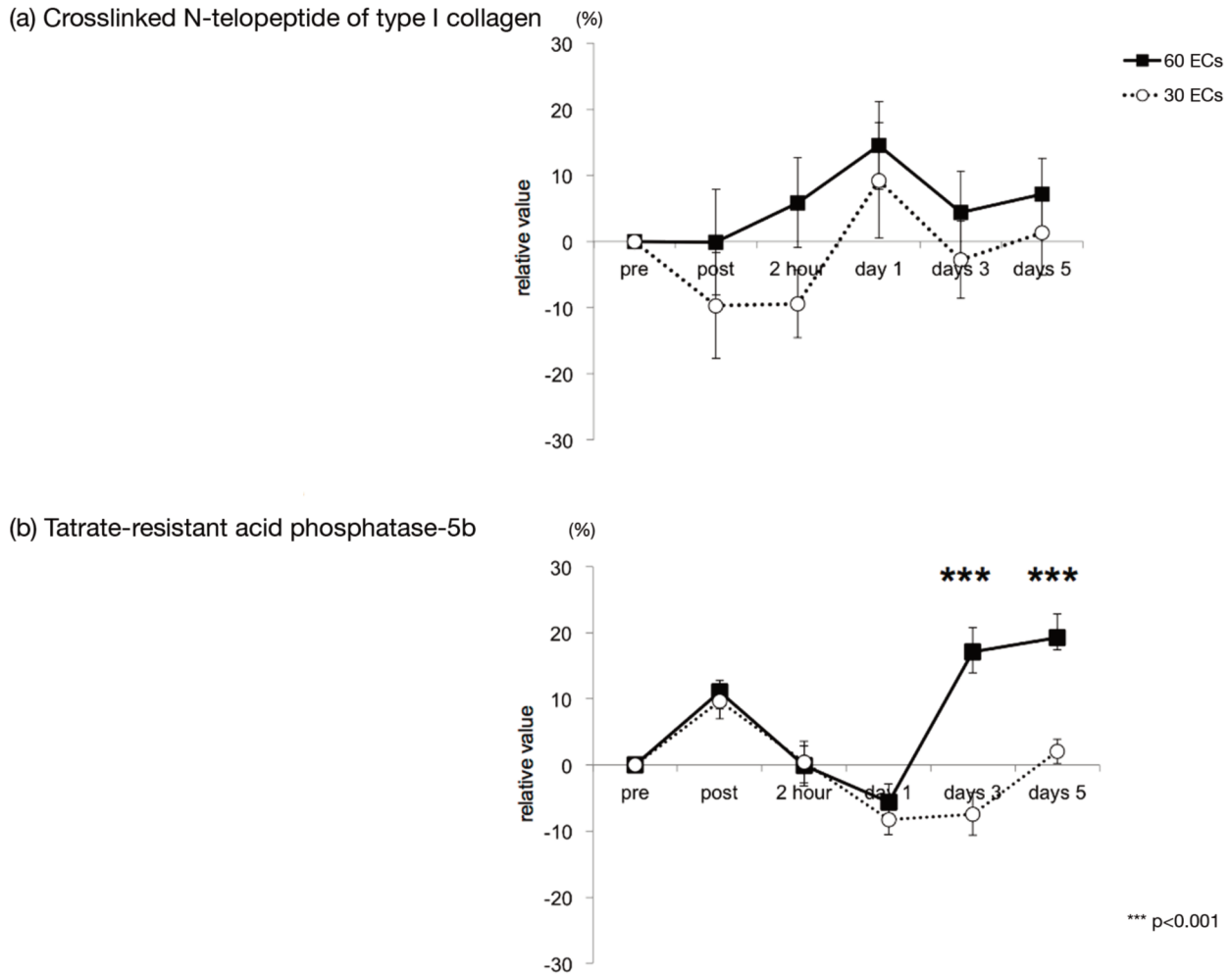


Figure 3. The time course changes in crosslinked N-telopeptide of type I collagen (NTx; a) and tartrate-resistant acid phosphatase-5b (TRACP-5b; b) was significantly higher with 60 ECs than 30 ECs on day 3 and 5 ($p<0.001$). Values are means \pm S.E.

ences were observed between the 60 ECs and 30 ECs groups for age (60 ECs: 20.1 ± 0.5 years, 30 ECs: 20.0 ± 0.4 years), height (60 ECs: 171.8 ± 1.9 cm, 30 ECs: 168.4 ± 1.9 cm), weight (60 ECs: 65.0 ± 2.8 kg, 30 ECs: 64.9 ± 1.9 kg), body fat (60 ECs: $15.1\pm 1.5\%$, 30 ECs: $15.9\pm 1.0\%$), or lean body mass (60 ECs: 55.0 ± 1.8 kg, 30 ECs: 54.5 ± 1.7 kg). Total work output was 50.5 ± 1.8 Nm/BW in the 60 ECs group and 32.9 ± 1.4 Nm/BW in the 30 ECs group ($p<0.001$).

Time-course changes in blood lactate, CK, and Mb are shown in Table 2. Blood lactate concentrations after ECs were 3.8 ± 0.3 mmol/l in the 60 ECs group and 2.3 ± 0.2 mmol/l in the 30 ECs group ($p<0.001$). CK was significantly higher on day 3 and 5 (day 3, 2417.3 ± 678.9 IU/l; day 5, 4792.7 ± 649.2 IU/l) than on day 3 and 5 in the 30 ECs group (day 3, 837.1 ± 394.5 IU/l; day 5, 1197.0 ± 436.8 IU/l). Furthermore, Mb levels showed similar results (day 3, $p<0.05$; day 5, $p<0.001$). Comparisons of the percentage changes in GH (a) and IGF-1 (b) are shown in Fig-

ure 1. Both GH and IGF-1 changed more significantly after exercise in the 60 ECs group than in the 30 ECs group (GH, $p<0.01$; IGF-1, $p<0.05$).

Time-course changes in bone formation markers are shown in Figure 2 (a) and (b). No significant difference in BAP was observed at any time in either group, but OC was significantly higher in the 60 ECs group than in the 30 ECs group at 2 hours, day 1, and day 5 (2 hours, $p<0.05$; day 1 and day 5, $p<0.01$). On the other hand, no significant difference in NTx was observed in either group, but the change in TRACP-5b was significantly higher in the 60 ECs group than in the 30 ECs group on day 3 and 5 (Figure 3b; $p<0.001$).

The association between post-exercise values minus pre-exercise values of GH and IGF-1 (Δ GH and Δ IGF-1) and bone metabolism markers was also assessed. No significant correlations were observed between Δ GH or Δ IGF-1 and OC, BAP, TRACP-5b, or NTx in the 30 ECs group. Moreover, no significant correlations were observed between Δ GH or Δ IGF-1

Table 3. Pearson's correlation coefficient between insulin growth factor - 1 and osteocalcin, crosslinked N-telopeptide of type I collagen.

	post	2 hours	day 1	day 3	day 5
IGF-1 and OC					
60ECs (n=9)	0.48	0.68 *	0.79 *	0.65	0.72 *
30ECs (n=8)	0.26	0.01	-0.01	0.31	-0.27
IGF-1 and NTx					
60ECs (n=9)	0.65	0.81 **	0.67 *	0.64	0.51
30ECs (n=8)	-0.39	-0.29	-0.38	-0.16	-0.19

means \pm S.E. * $p < 0.05$, ** $p < 0.01$

IGF-1: insulin growth factor-1

OC: osteocalcin

ECs: eccentric contractions

NTx: crosslinked N-telopeptide of type1 collagen

and BAP, TRACP-5b in the 60 ECs group. However, significant positive correlations were observed between Δ IGF-1 and OC at 2 hours, day 1, and day 5 in the 60 ECs group ($p < 0.05$, Table 3). In addition, a significant positive correlation was observed between Δ IGF-1 and NTx at 2 hours and day 1 (2 hours, $p < 0.01$; day 1, $p < 0.05$, Table 3). No significant correlations were observed between blood lactate and bone metabolism markers.

Discussion

The bone metabolism response to quantitative exercise volume remains unclear. Because, previous studies did not demonstrate the relationship between bone metabolism markers and mechanical load (total work)⁶⁻⁸. Furthermore, no study has examined the effect of exercise-induced GH/IGF-1 on bone metabolism markers. Thus, the purpose of this study was to investigate changes in bone metabolism markers after ECs and to determine the association between these markers and GH and IGF-1 in young males. Our results showed that ECs of the local biceps muscle resulted in an increase in bone turnover with greater work output. The results also suggested that ECs-induced IGF-1 may enhance bone turnover.

The total work output in the 60 ECs group was apparently higher than that in the 30 ECs group ($p < 0.001$). Furthermore, circulating post-exercise concentrations of lactate, CK, and Mb were significantly higher in the 60 ECs group than in the 30 ECs group (Table 2). These findings are similar to those from previous studies^{21,22}. Although we did not investigate other muscle damage markers, which are TNF- α , IL-1, and IL-6²⁶, these results suggest that the 60 ECs group had more bicep damage than the 30 ECs group.

Although no significant changes in BAP levels were observed, the percentage changes in OC were significantly higher in the 60 ECs group at 2 hours, day 1, day 3, and day 5 than in the 30 ECs group. NTx was not significantly different between the groups but did show increases at 2 hours and day 1. TRACP-5b was significantly higher in the 60 ECs group on

day 3 and 5 than in the 30 ECs group. Ashizawa et al. found that BAP and TRACP levels decrease significantly after high-intensity resistance training⁷. A contradicting study by Whipple et al. reported that BAP significantly increases and NTx decreases after moderate-intensity resistance training⁸. There is no consensus regarding bone formation or resorption, even for experiments involving running and pedaling exercises^{10,20,27}. The reasons for the controversy may be the use of multiple movements and the inability to quantitatively calculate the work output during exercise. Therefore, in the present study, we used ECs in elbow flexors because it is a simple movement with a clearly definable work output. Our results showed that the changes in OC and TRACP-5b were significantly higher for the 60 ECs group with a larger work output than for the 30 ECs group. These findings suggest that when ECs generate a large work output, bone turnover increases for at least 5 days after exercise. Because high bone turnover increases the risk of a bone stress fracture in long-distance runners and osteoporosis patients^{5,28}, the high work output of ECs may cause a temporary negative effect on bone metabolism.

No significant correlations between blood lactate concentrations and bone metabolism markers were observed in either group. Previous studies have revealed that exercise-induced oxidation suppresses bone formation and increases resorption^{17,18}. However, Lin et al. showed no significant correlation between lactic acid and OC/TRACP after high-intensity jumping and interval running¹⁹. Similarly, Herrmann et al. reported these issues using pedaling exercise at ventilation thresholds of 75%, 90%, and 110% for 60 min. Their results showed that lactic acid levels increased only in the highest intensity group (110%), although there was still no significant association between lactic acid levels and bone metabolism markers²⁰. The results of our study support these previous findings^{19,20}. Therefore, we believe that blood lactate concentration, which is temporarily increased by ECs, has no effect on bone metabolism markers.

We also investigated the association between GH and IGF-1 and bone metabolism markers. We found significant positive correlations between Δ IGF-1 and OC/NTx in the 60 ECs group.

Ehrnborg et al. showed that 117 athletes had significantly increased IGF-1 and bone resorption markers immediately after exercise²⁹. In addition, the previous study on elite rowers found significant positive correlations between IGF-1 and OC before and 6 months after chronic training³⁰. Because these studies did not show any apparent correlations between acute exercise-induced GH/IGF-1 and bone metabolism markers, we hypothesized an association between these parameters. Interestingly our results showed that IGF-1 levels, which temporarily increase by exercise, may be associated with bone metabolism markers. We speculate that OC and NTx might depend on IGF-1 secretion and that TRACP-5b may be independent of IGF-1. However since parathyroid hormone (PTH)³¹, estrogen³², androgens³³, and vitamins D³⁴ and K³⁵ also regulate bone cellular activities, further research is also needed on these factors.

Recent study has reported that GH/IGF-1 secreted from skeletal muscle regulates bone metabolism¹². Although it remains to be determined whether IGF-1 produced by skeletal muscle has a direct effect on bone metabolism balance, we believe there is a crosstalk in which exercise-stimulated skeletal muscle interacts closely with bone. Future studies will need to clarify whether skeletal muscle-derived hormones and cytokines (myokines) increase bone formation and/or resorption to a greater extent.

There are three limitations to our study. First, a non-exercise control group was not included. Since bone metabolism markers are influenced by food¹³ and show intra- and inter-day variations³⁶, a control group would have helped to monitor these influences. However, we are confident of our findings of differential expression of bone specific markers (OC and TRACP-5b) with 2 ECs modes, as well as the IGF-1/OC interaction. Secondly, we did not have information on reactive oxygen species, inflammatory responses, and hormones such as testosterone, cortisol, and PTH. Since these factors influence bone cellular activities^{13,37}, studies on these factors are needed to establish a causal link between bone metabolism markers and the exercise intervention. Lastly, ECs was employed because this contraction mode induces higher muscle damage and soreness than other contractions³⁸. Although the main purpose of the present study was to understand the effect of mechanical stress on bone metabolism markers, ECs should be compared with concentric and isometric contractions to fully understand contraction-induced hormonal and metabolic responses.

Based on our findings, we conclude that high force ECs increase OC and TRACP-5b production (enhance bone turnover) for up to 5 days after exercise. In addition, we showed that ECs-induced IGF-1 may be associated with OC and NTx. However, further studies are needed on the responses to aging and different muscle contractions. In future, the mechanism of high bone turnover suggested by our result must be clarified in the context of the association between skeletal muscle and bone.

Acknowledgements

We would like to thank Hiroshi Kiyota and staff of laboratory of health and sports science, Meiji Gakuin University.

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