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Original Article

Acute responses of bone specific and related markers to maximal eccentric exercise of the knee extensors and flexors in young men

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Abstract

Objectives: The present study determined time-course changes in plasma bone-specific and -related markers following a bout of maximal eccentric contractions (MaxEC) of bilateral knee extensors (KE) and flexors (KF). **Methods**: Sedentary young men (n=30) performed a bout of 10 sets of 10 MaxEC (30°/s) of KE and KF with each leg, respectively. Maximal voluntary isometric contraction (MVC) torque, muscle soreness (SOR), plasma creatine kinase (CK) activity, insulin, leptin, tumor necrosis factor-α (TNF-α), undercarboxylated-osteocalcin (ucOCN), carboxy-terminal crosslinking telopeptide of type I collagen (CTX-1) and procollagen type I N-terminal propeptide (P1NP) concentrations were measured from before to 7 days after MaxEC. **Results**: Significant changes in MVC (KE: -28%, KF: -38%), SOR and plasma CK activity (peak: 39,163 IU/L) following MaxEC were evident (P<0.05) compared to baseline. Plasma leptin (17%) concentrations decreased at 1 day after MaxEC. In bone related markers, plasma ucOCN concentrations (20%) increased at 7 days after MaxEC, and plasma CTX-1 concentrations decreased at 2, 4 and 7 days after MaxEC (6~7%; P<0.05). **Conclusion**: These results demonstrate that a lean effect of bone generation and an enhanced energy anabolism can be induced by a single bout of MaxEC.

Keywords: Bone Metabolism, Delayed Onset Muscle Soreness, Lengthening Contractions, Muscle Damage, Plasma Creatine Kinase Activity

Introduction

It is well-established that a single bout of unaccustomed eccentric exercise could result in significant and transient muscle damage[1,2], which can be categorized as eccentric exercise-induced muscle damage (EMID). Typically, EIMD is manifested by development of delayed onset muscle soreness (DOMS), prolonged loss in muscle function [e.g., maximal voluntary isometric contraction (MVC) strength], limb swelling, increased blood levels of intramuscular proteins [e.g., plasma creatine kinase (CK) activity], and abnormalities in muscular ultrasound images[1,3-7]. In terms of its pathological course, EMID begins with mechanical damage in muscles, which is followed by acute inflammatory responses, resulting in secondary damage[1,8], and a full recovery from EMID generally takes about 10-14 days[1,8]. Although EIMD is unfavorable, EIMD is indeed a prerequisite factor for launching repair and stimulating muscle hypertrophy[9].

In addition to EIMD and inflammation, two previous studies[10,11] have reported that eccentric exercise could specifically stimulate bone metabolism and induce significant responses in the endocrine system. For example, Tsuchiya et al.[11] observed that one bout of 60 maximal eccentric contraction-induced muscle damage of the elbow flexors (EF) using the non-dominant arm caused significant increases in bone remodeling markers such as plasma osteocalcin (OC) and tartrate-resistant acid phosphatase 5b (TRACP-5b), suggesting that bone remodeling was induced by EIMD. They

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also found that this effect was greater (peak TRACP-5b: 19%) and lasted longer (5 days post-exercise) after 60 maximal eccentric contractions (MaxEC) than after 30 MaxEC (9%, immediately post-exercise), suggesting that the magnitude of bone remodeling can be related to the total number of eccentric contractions performed. However, following conventional resistance training, which combined both concentric and eccentric muscle contractions, changes in bone metabolism markers demonstrated different patterns. Blood and urinary bone markers showed a bone formation favored or a down-regulated bone remodeling following a single bout of resistance exercise[12-14]. Taken together, a typically eccentric exercise seems to have stronger effects than general resistance exercise for stimulating bone metabolism.

It has been reported that a single bout of MaxEC induced different magnitudes of muscle damage in different muscle groups (e.g., arms vs legs muscles)[15-19]. For example, Chen et al.[15] reported that the magnitude of EIMD for the unilateral elbow flexors was significantly greater than for the unilateral knee extensors and flexors. It appears that differences in muscle characteristics (e.g., muscle architecture, muscle fibers composition) and exposure to eccentric exercise during daily activities could possibly explain the varying extents of EIMD between arm and leg muscles[15,16]. Thus, the results in these aforementioned elbow flexor studies[10,11] may not be generalizable to other muscles, such as knee extensors and flexors, which support the whole body weight and generate massive muscular contractions during daily activities (e.g., walking, jogging, running), and no previous studies have investigated the effects of EIMD on bone metabolism using knee extensors and flexors.

It is also important to note that bone metabolism has been verified to be closely associated with energy metabolism. Specifically, under-carboxylated osteocalcin (ucOCN), which is directly produced by osteoblasts or indirectly released from the bone matrix by bone resorption activity, plays a role in the down-regulation of blood glucose[20-22]. Several studies have shown that serum levels of ucOCN were significantly increased after either an acute bout of aerobic exercise[23,24] or a period of endurance exercise training[25,26], and this increased ucOCN was found to be associated with enhanced insulin sensitivity[23-26]. However, only Mera and colleagues compared the effects of aerobic exercise and power exercise on serum ucOCN and energy metabolism related markers before and 2 hours after the respective exercise bouts[24]. In this study, power exercise, comprised of leg presses and jumping exercises, did not show effects on serum ucOCN, while the 45-minute aerobic cycling exercise increased serum ucOCN. Interestingly, it has also been demonstrated that mechanisms potentially related to muscle repair during the state of EIMD could possibly result in the prolonged elevation of resting energy metabolism[27,28]. Since the maximal eccentric exercise-induced severe muscle damage response (e.g., MVC, CK) normally lasts for over 7 days, a delayed adaptation in energy and bone metabolism could be shown. Hence, it is also valuable to investigate post-exercise effects on ucOCN and energy metabolism during the recovery from EIMD.

Therefore, the purpose of the present study was to investigate the time-course changes in bone-specific and -related metabolic markers induced by a single bout of maximal isokinetic eccentric exercise of knee extensors and flexors in sedentary young men. It was hypothesized that a bout of unaccustomed lower-limb eccentric exercise would generate interference on bone metabolism.

Materials and methods

Participants

Thirty healthy sedentary young men (age: 21.3±2.1 yrs, height: 172.5±4.6 cm, body mass: 67.2±8.9 kg), who had not regularly performed any resistance, aerobic or flexibility training in the past year, were recruited for the present study. All participants had no history of injuries in the muscles, joints or bones of the lower extremities, and signed an informed consent to participate in this study that was approved by a research ethics committee at Chinese Culture University (Approval No.10009). The present study was conducted in conformity with the policy statement regarding the use of human subjects by the Declaration of Helsinki. All participants were asked and reminded to refrain from unaccustomed exercises and/or vigorous physical activities, to maintain their normal dietary habits, and not to take any anti-inflammatory drugs or nutritional supplements during the 2 weeks before the eccentric exercise bout through the end of the subsequent blood sampling period. The participants were instructed and asked to drink enough water (more than 1 L a day) after MaxEC to avoid the possible risk of acute renal injury due to rhabdomyolysis, and not to have any physical treatments (e.g., massage, stretching, cryotherapy) of the exercised muscles during the experimental period. Previous studies have suggested that ingesting protein together with carbohydrates is more effective for the recovery and regeneration of damaged muscles after exercise, and drinking plain water is likely not enough protective measure for rhabdomyolysis after exercise[29,30]. However, the purpose of the present study was not to investigate the effects of supplemental nutrition on eccentric exercise-induced rhabdomyolysis, and we did not consider the role of supplemental nutrition in protection from eccentric exercise-induced rhabdomyolysis in order to avoid interference with the final results of muscle damage and bone-related markers. Moreover, in order to minimize the interference from individual diets and lifestyles, participants' meals were provided by the investigator from the time of baseline testing through the last day of blood sampling.

Experimental design

The experimental period included a familiarization session scheduled 3-5 days before the MaxEC bout, which allowed the participants to familiarize themselves with the measurements of the dependent variables mentioned below. For establishing a reliable baseline reference, MVC torque and various plasma

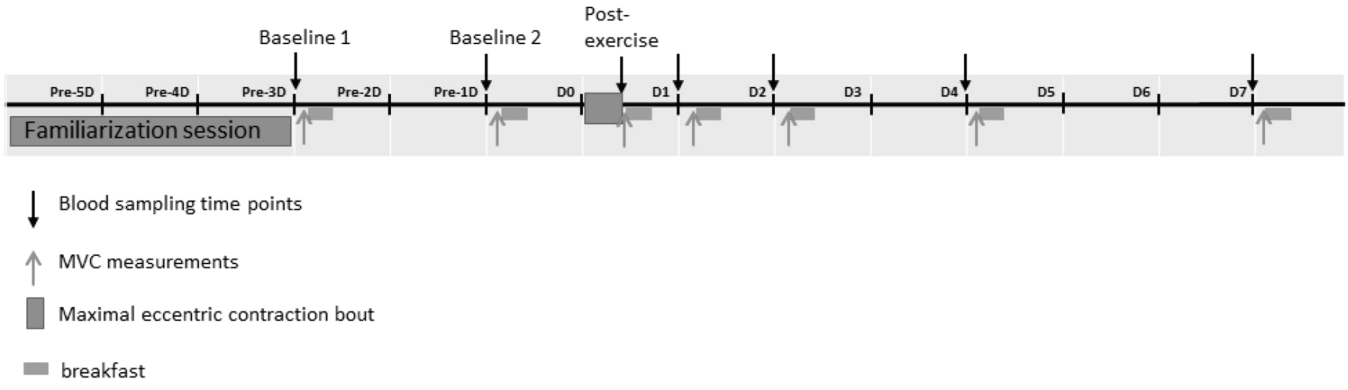

Figure 1. Experimental time schedule of the present study. d: day; MVC, Maximal voluntary isometric contraction strength.

markers were the average of the measurements taken 3 and 1 days before the MaxEC. Similarly, MVC measurement and blood sampling were conducted immediately after and 1, 2, 4 and 7 days after exercise (Figure 1) (See below for detailed description of MaxEC and criterion measures).

Maximal eccentric contractions (MaxEC)

All participants performed a bout of 10 sets of 10 MaxEC knee extensors (KE) and flexors (KF), respectively, on each leg with a counterbalanced order; thus, the total number of maximal eccentric contractions was 400 for each participant. In detail, each participant laid prone on the platform of the dynamometer with the upper and lower back regions, while the upper region of the left leg was strapped to the platform. The knee joint of the exercising leg was aligned with the rotation axis of the dynamometer, and the ankle of the leg was strapped to the pad connected to the dynamometer level arm. In this position, a gravity correction for the limb mass was made in accordance with the manufacturer's instructions. MaxECs of KE and KF were performed on each leg on an isokinetic dynamometer (Biodex System 3 Pro; Biodex Medical Systems, Shirley, NY, USA). The participants were instructed to contract the KF maximally to resist the knee extending action of the dynamometer that moved the knee joint from a flexed (100°; 0°=a full knee extension) to an extended position (0°) at an angular velocity of 30°·s^{-1}. In contrast, the knee joint was forcibly flexed from an extended position (0°) to a flexed position (100°) in 3.3 s at 30°·s^{-1} for KE immediately after MaxEC of KF. Each participant performed this consecutive MaxEC of KF-to-KE for 10 consecutive contractions as a set, and a 2-min rest was given between sets to complete ten sets. The participants received strong verbal encouragement during MaxEC to generate maximal voluntarily force. Torque and displacement signals of each contraction were stored in a computer (Model P4P800-TAYZ; ASUSTeK Computer, Inc., Taiwan) connected to the same isokinetic dynamometer, and peak torque and work (the area under the torque curve) of each contraction were calculated using the Biodex software[31-33].

Criterion measures

The criterion measures consisted of MVC and muscle soreness for each exercised muscle and blood marker assays including plasma creatine kinase (CK) activity, insulin, leptin, TNF-alpha, undercarboxylated-osteocalcin (ucOCN), carboxy-terminal crosslinking telopeptide of type I collagen (CTX-1) and procollagen type I N-terminal propeptide (P1NP) concentrations. All measures were performed at time points of 3 and 1 days before, immediately after (except for muscle soreness) and 1, 2, 4 and 7 days post-exercise. The reliability and coefficient of variation (CV, %) of MVC, muscle soreness and CK have been established and found in our previous studies[15,34].

Maximal voluntary isometric contraction (MVC) torque

MVC was measured at the knee flexion angle of 80° for each KE and 30° for each KF, respectively, with the same set up and position as the MaxEC using the same dynamometer after gravity correction[15,18,34]. The order of the testing muscles and legs used a counterbalanced method. The participants were instructed to generate maximal force for 3 s at each angle 3 times with a 45-s rest between trials, a 2-min rest between muscles, and a 5-min rest between limbs. Verbal encouragement was provided in a consistent manner during all tests. The highest value of the three trials of each muscle was used for further analysis[35-37].

Muscle soreness

Muscle soreness level was quantified using a visual analog scale (VAS) of a 100-mm continuous line with "not sore at all" on one side (0 mm) and "very, very sore" on the other side (100 mm). The investigator asked each participant to rate his perceived soreness on the VAS when the muscles were passively extended for KF and flexed for KE for the ROM between 120° to 0° and 0° to 120° of the knee extension angles for each leg[15,34,38,39].

Blood sampling and measurements

Because some plasma markers (e.g., CTX-1 or insulin) are relatively sensitive to diet as well as circadian rhythm, we collected all blood samples after ten-hours of fasting. Blood samples were collected between 8:00-9:00 am except for the time point of immediately after MaxEC (Figure 1). The time points for blood sample collection were selected according to previous studies based on the needs of monitoring bone turnover status as well as muscle damage recovery status[14]. Approximately 7-ml of venous blood was drawn using a standard venipuncture technique with an ethylenediaminetetraacetic acid (EDTA) contained vacutainer tube (Becton Dickinson and Company, Plymouth, UK) from the cubital fossa region of the arm, and centrifuged at 3000 rpm for 10 minutes[40]. Plasma samples were then collected, aliquoted and stored at -80°C. In the current study, muscle damage (i.e., CK), energy metabolic and bone turnover marker assays were conducted within 3 months following blood sample collection and related assay procedures were described as below.

Plasma CK activity was analyzed by an automated clinical chemistry analyzer (Model 7080, Hitachi, Co. Ltd., Tokyo, Japan) using a commercial test kit (CK: ACN8057, Roche Diagnostics, Indianapolis, Indiana, USA) [within assay coefficient of variation (CV)=1.50%]. Bone turnover markers of ucOCN, CTX-1 and P1NP were estimated using commercial ELISA kits, which were Glu-OC EIA kit (ng/mL) (TaKaRa Bio Inc., Japan) (CV=4.22%), CTX-1 Elisa kit (ng/mL) (Bluegene Biotech, China) (CV=5.37%), and Human P1NP ELISA kit (pg/mL) (MyBioSource, USA) (CV=5.01%). A Luminex® multiplexing instrument (Luminex 200®, Merck Millipore, USA) was used for measured plasma insulin (ng/mL) (CV=6.03%), leptin (ng/mL) (CV=6.59%) and TNF-α (ng/mL) (CV=5.77%) (HBNMAG-51K, Milliplex map Human Bone Magnetic Bead Panel-Bone Metabolism Multiplex Assay, Merck Millipore, USA).

Statistical analyses

Changes in MVC and muscle soreness of the right and left KE and KF before MaxEC were analyzed using a pair-wise t-test. Changes in MVC and muscle soreness after MaxEC were compared over time for both right and left of KE and KF, respectively, by repeated measure method. When the repeated measure method found a significant main effect of time, the Least Significant Difference (LSD) of paired t-test was used to find the significant difference of measurement means. Values in each of muscle damage (MVC, muscle soreness, CK), energy and bone metabolism indices over time points subsequent to MaxEC were respectively compared to their corresponding baseline values by a repeated measure method, and LSD of paired t-test was used to find the significant difference of measurement means between baseline and subsequent time points. A significant level was set at $P \leq 0.05$. The data are presented as mean ± SEM.

In addition to conventional statistical methods, reference change values (RCV) for each plasma marker were calculated to serve as another objective reference for evaluating clinically significant change. The calculation is conducted according to the previous tutorial publication of Fraser[41]:

$$RCV = 2^{1/2} \cdot Z \cdot (CV_A^2 + CV_I^2)^{1/2}$$

where the Z score to be used is 1.96 for $P<0.05$, CV_A of each biomarker is the means of assay coefficient of variation from duplicated tests of various standard samples; CV_I values for various plasma markers are found in online database[42,43]. Because the CVI of plasma leptin and ucOCN were not available from a reliable online database, we averaged the within-subject CVs of time serial assays as CV_I for leptin and ucOCN.

Results

Peak torque and work during MaxEC

The average peak torque of both legs was significantly ($P<0.05$) greater in KE (255±8 Nm) compared to KF (113±3 Nm), and the total work of each leg was also significantly ($P<0.05$) greater in KE (25,541±771 J) compared to KF (11,310±329 J). No significant ($P>0.05$) difference was shown between right and left KE and KF, respectively.

Changes in criterion measures after MaxEC

Muscle damage markers

MVC

Before the MaxEC bout, no significant differences ($P>0.05$) in MVC were observed between the right (R) and left (L) legs in either KE (R: 266±9 Nm, L: 271±10 Nm) or KF (R: 89±4 Nm, L: 86±4 Nm). Changes in MVC of KE and KF shown between L and R were similar following the MaxEC bout (Figure 2A). In detail, the average MVC of bilateral KE and KF ($P<0.001$) decreased 28% and 38%, respectively, immediately after eccentric exercise. Though the MVC of KE and KF both showed a tendency toward recovery the following day, only the MVC of KE returned to the baseline level at 7 days after exercise. When comparing KE and KF for each leg, the recovery of MVC after eccentric exercise was faster ($P<0.05$) for KE than for KF. No significant differences ($P>0.05$) were shown between the right and left limbs on KE and KF, respectively.

Muscle soreness

No muscle soreness (0±0 mm) was reported prior to eccentric exercise of each muscle. Significant changes in the VAS representing muscle soreness were shown following eccentric exercise (Figure 2B). The development of muscle soreness in both KE and KF after the MaxEC bout was observed with peak values at 2 days post-exercise ($P<0.001$). VAS nearly returned to the baseline level 7 days after exercise. The extent of muscle soreness was significantly smaller ($P<0.05$) for KE than for KF, but no significant differences ($P>0.05$) were evident between the right and left limbs on KE and KF, respectively.

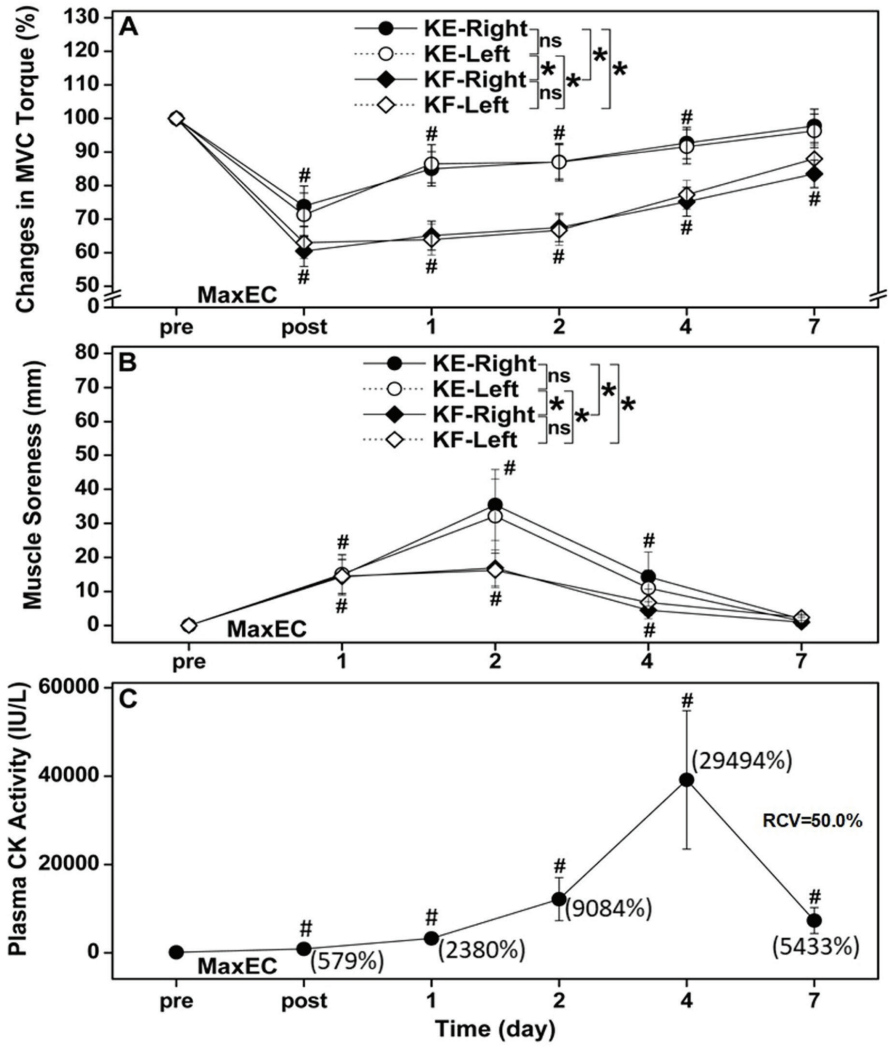

Figure 2. Normalized changes (mean ± SEM) in maximal voluntary isometric strength (MVC, A) and change in muscle soreness (B) and plasma creatine kinase (CK) activity (C) at time points before, immediately after, and 1, 2, 4 and 7 days after maximal eccentric contractions (MaxEC). *: a significant (P<0.05) difference from knee flexors (KF); #: a significant (P<0.05) difference from baseline; RCV: reference change value (%) in parentheses.

CK

Plasma CK activity before MaxEC was 132.3±8.4 IU/L. Significant increases (P<0.001) in plasma CK activity after MaxEC were observed, and peaked at 4 days post-exercise, and had not returned to baseline levels 7 days after MaxEC (RCV=15.8%) (Figure 2C).

Energy and bone metabolism markers

Figure 3 shows normalized changes in various plasma markers at time points prior to, immediately after, and 1, 2, 4 and 7 days after MaxEC. Plasma leptin concentrations were significantly lower (P=0.032) at 1 day after MaxEC (Figure 3B). In bone-specific metabolism markers, plasma ucOCN concentration was higher at 7 days (P=0.001) (Figure 3D). Though P1NP as a bone formation marker did not show significant changes (Figure 3E), plasma CTX-1 as an indicator of bone resorption was lower at 2 days (P=0.006), 4 days (P=0.016) and 7 days (P=0.042) after MaxEC (Figure 3F). RCVs for each of the markers were shown in Figures 3A to 3F as another objective reference for evaluating clinical significance.

Discussion

The main findings of the current study showed that: 1) the MaxEC bout of KE and KF induced significant muscle damage as demonstrated by prolonged loss of muscle strength, development of muscle soreness and elevation of plasma CK activity (Figure 2), and 2) simultaneously, plasma energy metabolic (e.g., leptin; Figure 3B) and bone metabolic markers

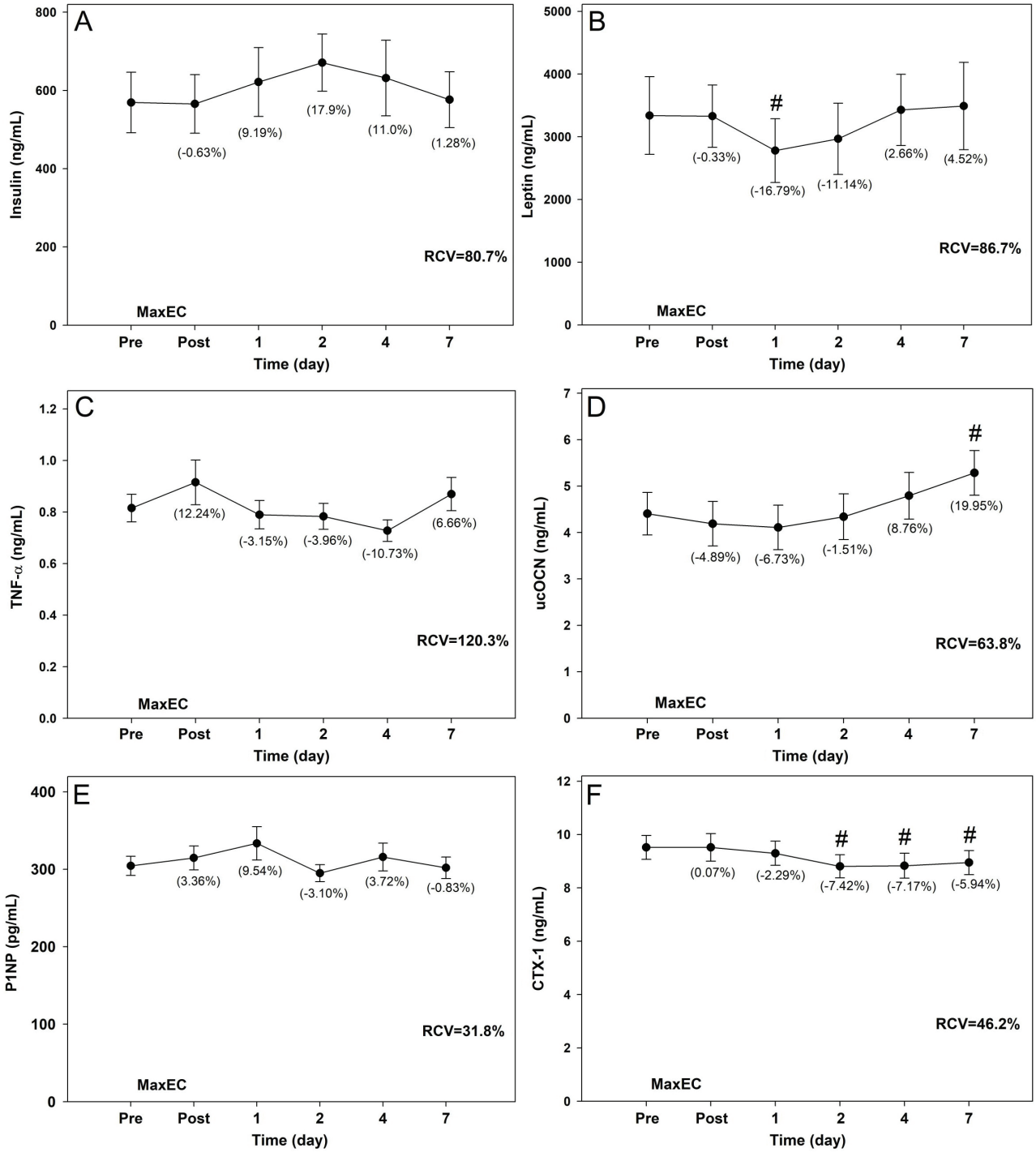

Figure 3. Changes (mean ± SEM) in plasma energy metabolism markers [e.g., insulin, leptin and tumor necrosis factor-α (TNF-α)] (A-C) and bone metabolism markers [e.g., undercarboxylated-osteocalcin (ucOCN), procollagen type I N-terminal propeptide (P1NP), and type 1 C-terminal telopeptide (CTX-1)] (D-F) at time points of before, immediately after, and 1, 2, 4 and 7 days after maximal eccentric contractions (MaxEC). #: a significant (P<0.05) difference from baseline; RCV: reference change value (%) in parentheses.

(e.g., ucOCN, CTX-1; Figures 3D & 3F) following MaxEC were significantly changed but showed different shift patterns. These results supported our hypothesis and suggested that eccentric exercise can induce a series of responses, which might further generate cross-talk among muscles, skeleton, and energy metabolism.

In the present study, the changes in the criterion measures (e.g., peak CK: ~40,000 IU/L) after eccentric

exercise of bilateral KE and KF (Figures 2) were greater than those reported in previous studies, in which a different eccentric exercise of the unilateral knee extensors and flexors (1,500 – 6,000 IU/L)[15,34] or unilateral EF (~4,800 IU/L)[10,11] was performed. It is possible that the difference in total contractions between the present (400 contractions) and previous studies (30-150 contractions)[10,11,15,34] could partially explain the difference in magnitude of EIMD. With such a relatively higher EIMD, a significant physiological impact and, subsequently, significant changes in energy and bone metabolism markers are possible.

For the past decades, numerous studies have used a single-bout of resistance exercise as a stimulus to investigate the interference on bone metabolism caused by physical activity[10-14,44,45]. The findings from those different studies are inconsistent and somewhat controversial; this can be attributed to differences in exercise load (e.g., intensity, duration, and repetition number), involvement of muscle groups (e.g., arms, legs), the conditions of participants (e.g., trained/untrained, age) or exercise modes (e.g., eccentric or concentric exercise). Among different types of resistance exercise, unaccustomed eccentric exercise shows a unique impact on muscle dysfunction (e.g., extreme elevations of blood CK)[1,8,18,46] and energy metabolism (e.g., elevation of resting energy expenditure)[17,27,28], which could impact bone metabolism through different channels and is deserving of further investigation.

In the past, only two studies have investigated the effects of a bout of eccentric exercise of a unilateral EF on bone metabolism[10,11]; these studies found up-regulated serum bone markers (e.g., peak osteocalcin and TRACP-5b: 19% at 1 and 5 days post, respectively) and muscle damage markers (e.g., peak CK: ~4,800 IU/L at 4 days post) were significantly changed after eccentric exercise. In the present study, conversely, MaxEC of KE and KF resulted in a minor but significant down-regulation of bone resorption activity (CTX-1: 6-7%; Figure 3F), despite the fact that peak changes in muscle damage markers were higher than in these studies. For example, peak CK following exercise was 8.3 folds higher (~40,000 IU/L)(Figure 2) than the peak CK in those prior two studies[10,11]. One plausible reason for the different findings among the current and Tsuchiya et al.'s studies[10,11] could be inherent differences between the exercise limbs adopted by their (non-dominant arm) and our (bilateral lower limbs) studies. From the mechanotransduction aspect, the minimal effective strains or stimuli (MES) to initiate bone remodeling would be different among bone regions with different mechanical environments[47]. Thus, a relatively lower mechanical loading is needed to surpass the MES for bone remodeling in the non-dominant arm due to its relatively lower involvement in daily activity. In contrast to the unilateral elbow flexors of MaxEC[10,11], the MaxEC in the current study might not generate a mechanical impact reaching the MES for the weight-bearing lower-limb bones, though it actually induced a tremendous increase in plasma CK activity, significant muscle soreness and prolonged loss in MVC (Figure 2). Thus, the magnitude of EIMD might not be a dominant factor in influencing changes in bone metabolism markers, but the MES plays a determinant factor to stimulate bone markers. A controlled trial study that includes non-dominant-limb and dominant-limb exercise is needed to clarify the effects of eccentric muscular contraction on bone metabolism.

In addition to the local effects of mechanical loading, exercise can potentially generate systemic effects to regulate bone metabolism, and further, the cross-talk between bone and energy metabolism. Since osteoblastic-sourced ucOCN has been rigorously investigated and reported as a modulator to enhance insulin sensitivity and glucose regulation[20-22], several studies have further verified that short-term or acute exercise interventions could enhance insulin sensitivity and down-regulate blood glucose through upregulating ucOCN[23-26]. Our current study, using eccentric exercise as one type of muscular exercise, showed no effects on levels of ucOCN immediately after the MaxEC bout (Figure 3), which is similar to the findings of Levinger et al.[24]. Briefly, Levinger and colleagues compared the effects of a 45-minute bout of aerobic exercise and a bout of power exercise (leg press at 75% of 1RM plus jumping sequence) on serum ucOCN. While aerobic exercise demonstrated efficacy in up-regulating ucOCN, participants in the power exercise group showed no change in serum ucOCN. The difference in effects between aerobic and power exercises has been attributed to the difference in total energy expenditure in the participants of the aerobic exercise group, who performed a continuous 45-minute cycling bout at 75% of $\dot{V}O_{2max}$. Participants in the power exercise group took only about 7 min[24]. However, in the current study, we found no change in ucOCN immediately after the MaxEC (Figure 3B), even though the total amount of active time was increased to ~22 minutes (3.3 sec/repetition * 400 repetition). Instead, the MaxEC demonstrated effects on bone/energy related markers during the later phase of EIMD, as shown by different timing changes of CTX-1, leptin and and ucOCN between 1-7 days after the MaxEC (Figure 3). The MaxEC first induced a short period of down-regulation on the adipose tissue derived leptin (Figure 3B), then a consistent down-regulation of CTX-1 (Figure 3F) and a slow but progressively up-regulated ucOCN (Figure 3D). Although insulin, as an anabolic index, did not show significant statistical differences, there were 15 participants who showed percentage changes in plasma insulin higher than RCV (80.7%) during time points subsequent to MaxEC (Table 1). Taken together, the whole profile of plasma indices suggests an enhancement in energy metabolic sensitivity as well as in bone anabolic activity.

Though the mechanisms for this later-phase shift of bone resorption and ucOCN are currently unknown, it can be speculated that a unique effect of these above markers is caused by eccentric exercise. According to the results of numerous previous studies[17,18], it normally takes a relatively long period (e.g., 7-14 days) for damaged muscles to completely recover from an intensive eccentric exercise induced muscle damage, which could probably be associated with the later-phase shift of bone metabolism in the current study. Studies using molecular biology and transgenic mice

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Table 1. Fifteen participants showed up-regulated insulin in relative to the Reference Change Value (RCV).

	Original insulin level (ng/mL)						Percentage change (%)					
	pre	post	1d	2d	4d	7d	pre	post	1d	2d	4d	7d
subject 2	290.7	213.6	354.9	693.0	290.7	384.0	100%	-26.54%	22.09%	**138.38%**	0.00%	32.09%
subject 4	354.9	462.8	2615.5	2054.4	746.3	907.1	100%	30.40%	**636.92%**	**478.82%**	**110.28%**	**155.59%**
subject 6	290.7	290.7	596.6	487.0	554.6	354.9	100%	0.00%	**105.21%**	67.51%	**90.80%**	22.09%
subject 9	213.6	290.7	994.3	437.7	290.7	411.5	100%	36.12%	**365.57%**	**104.96%**	36.12%	92.68%
subject 10	462.8	104.8	780.4	1076.2	1298.8	411.5	100%	-77.37%	68.62%	**132.52%**	**180.61%**	-11.09%
subject 12	861.2	1827.5	674.5	411.5	290.7	290.7	100%	**112.20%**	-21.68%	-52.22%	-66.25%	-66.25%
subject 14	411.5	1496.7	1102.5	1153.8	636.4	674.5	100%	**263.73%**	**167.92%**	**180.39%**	54.66%	63.92%
subject 15	354.9	636.4	290.7	674.5	290.7	411.5	100%	79.31%	-18.10%	**90.04%**	-18.10%	15.94%
subject 17	290.7	290.7	845.5	829.6	1178.8	845.5	100%	0.00%	**190.85%**	**185.38%**	**305.51%**	**190.85%**
subject 18	290.7	411.5	324.0	907.1	462.8	290.7	100%	41.55%	11.45%	**212.05%**	59.21%	0.00%
subject 24	354.9	746.3	510.3	510.3	674.5	462.8	100%	**110.28%**	43.77%	43.77%	**90.04%**	30.40%
subject 26	616.7	876.7	636.4	965.9	1128.3	1881.6	100%	42.16%	3.19%	56.61%	**82.96%**	**205.09%**
subject 27	104.8	213.6	290.7	554.6	636.4	354.9	100%	**103.87%**	**177.51%**	**429.47%**	**507.53%**	**238.82%**
subject 28	290.7	1275.4	411.5	1035.8	411.5	510.3	100%	**338.74%**	41.55%	**256.31%**	41.55%	75.53%
subject 29	354.9	462.8	510.3	845.5	411.5	876.7	100%	30.40%	43.77%	**138.22%**	15.94%	**147.02%**

Notes: CV_A= 6.03%; CV_I=28.47% (from https://biologicalvariation.eu/search?q=insulin); RCV=80.66%; data marked in **bold** were higher than the RCV value (80.66%).

may provide critical information to explain ucOCN following exercise[25]. According to the most recent evidence, G protein-coupled receptor family C group 6 members A (Gprc6a) of myofibers act as receptors for osteocalcin signaling, through which osteocalcin enhances uptake and catabolism of both glucose and fatty acids during exercise[25]. In addition, osteoblastic *osteocalcin*$^{-/-}$ mice's overexpression of *Gprc6a* as well as *Gprc6a* deficient mice's reduced expression of *osteocalcin* further proved the cross-talk between bone and muscle metabolism[25,48]. Given that EIMD causes a dysfunction of membrane receptors[49], the current late up-regulation of ucOCN (Figure 3D) may have been an effect corresponding to the recovery of muscular membrane receptors (e.g., Gprc6a) and their interactions with bone metabolism. Further animal studies would be valuable to clarify: 1) whether the expression of muscular *Gprc6a* synchronizes with the recovery timing of EIMD, and 2) the relationship among Gprc6a, ucOCN and the uptake/catabolism of energy substrate during EIMD. Moreover, the down-regulation of CTX-1 and unchanged P1NP imply a bone remodeling slow-down and bone accumulation favoring status. Since bone remodeling is a high energy cost process[20-22], this bone metabolism status could further preserve the energetic cost for muscle rebuilding after EIMD.

In conclusion, the present study demonstrated that MaxEC of the bilateral knee extensors and flexors effectively induced severe muscle damage, leading to significant changes in bone and energy metabolism markers. The changes in bone metabolism markers after the MaxEC-induced muscle damage in leg muscles seems smaller than in arm muscles[10,11], which might be due to the inherent difference in mechanical environments. Further, the different response timing in bone and energy related indices could be due to a corresponding phenomenon of post-EIMD muscle rebuilding. Further studies are necessary to investigate: 1) the role of eccentric exercise induced mechanical stress on bone metabolism and 2) the dynamic of this bone-to-muscle loop on the processes of EIMD and its role on the following adaptations in energy metabolism.

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