Effect of Recovery Duration between Two Bouts of Running on Bone Metabolism

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ABSTRACT

SCOTT, J. P. R., C. SALE, J. P. GREEVES, A. CASEY, J. DUTTON, and W. D. FRASER. Effect of Recovery Duration between Two Bouts of Running on Bone Metabolism. *Med. Sci. Sports Exerc.*, Vol. 45, No. 3, pp. 429–438, 2013. **Purpose**: Strenuous endurance exercise increases biochemical markers of bone resorption but not formation, although the effect of recovery duration between consecutive bouts of exercise is unknown. We examined the effect of recovery duration on the bone metabolic response to two bouts of running. **Methods**: Ten physically active men completed two 9-d trials. On days 4 and 5 (D4 and D5), participants completed two 60-min bouts of running at 65% $\dot{V}O_{2max}$ separated by either a 23-h (LONG) or a 3-h (SHORT) recovery period. Osteoprotegerin (OPG), parathyroid hormone (PTH), albumin-adjusted calcium (ACa), and phosphate (PO_4) were measured from blood samples obtained before and for 3 h after exercise and on four follow-up days (D6–D9). Markers of bone resorption (C-terminal telopeptide region of collagen type 1) and bone formation (N-terminal propeptides of procollagen type 1 and bone alkaline phosphatase) were measured in early morning fasted samples on D4–D9. **Results**: There were no significant changes in C-terminal telopeptide region of collagen type 1, N-terminal propeptides of procollagen type 1, or bone alkaline phosphatase with either protocol. OPG, PTH, ACa, and PO_4 concentrations increased with all exercise bouts, but the response to the second bout was not altered by recovery duration. **Conclusions**: Two 60-min bouts of running at 65% $\dot{V}O_{2max}$ separated by either 23 or 3 h had no effect on the markers of bone resorption or formation from 1 to 4 d after exercise. Reducing recovery duration from 23 to 3 h between two bouts of running did not alter the increase in OPG, PTH, ACa, and PO_4 to the second bout.
Key Words: REPEATED EXERCISE, RECOVERY DURATION, BONE TURNOVER MARKERS, PARATHYROID HORMONE, OSTEOPROTEGERIN

It is common for elite athletes and military recruits to perform bouts of hard exercise on consecutive days or even to perform multiple bouts of exercise on the same day, resulting in favorable training adaptations (27), including those to bone structure and quality (7,25,32). However, not all adaptations with repeated exercise are positive, with reports of reduced spinal bone mineral density in endurance runners (5,23) and stress fractures (SFx) in both runners (4) and military recruits (31). In both cases, albeit for different periods, effects on bone occur after repeated bouts of exercise and changes in bone turnover have been implicated (18,43).

Biochemical markers have been used to establish the effects of both single bouts of exercise and short periods of exercise training on bone turnover, and changes in these markers have been reported with a variety of training types lasting 3–16 wk (1,12,17,31,50). However, in the case of weight-bearing exercise, although highly strenuous acute exercise may result in pronounced changes in these markers (28,45), several studies suggest no effect of more moderate exercise that is more representative of a daily training session (46).

Studies of weight-bearing exercise over even shorter periods (3–5 d) suggest no effect of daily, acute exercise on bone turnover, provided that participants are in energy balance (24,51). To date, however, no studies have examined the time course of changes in bone metabolism after multiple bouts of acute weight-bearing exercise or the effect of recovery duration between consecutive bouts of exercise.

Compared with a single bout, performing a second bout of exercise on the same day induces more pronounced metabolic responses, including increased leukocyte, stress hormone, and cytokine (IL-6 and IL-1ra) concentrations (39–41). In some instances, these responses occur despite concentrations

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returning to baseline levels before the second bout (39,41). We (45,46) and others (20,34,42) have shown that parathyroid hormone (PTH) concentrations increase rapidly and remain increased during exercise, although they return to baseline within 30 min after exercise. There is also some evidence that the increase in PTH may be responsible for the increase in bone resorption that occurs in the first few hours after exercise (20), although the time course of changes in PTH with repeated bouts of exercise and with different recovery durations remains unknown.

As changes in bone turnover are implicated in changes to bone mass (18) and the development of SFx (43), it is important to understand how repeated bouts of exercise influence bone metabolism because such information might inform the scheduling of exercise during periods of athletic or military training and help to minimize unfavorable changes in bone health.

The aim of this study was to investigate the bone metabolic response to two consecutive bouts of exercise in young men when recovery duration was either 23 or 3 h.

METHODS

Participants

Ten healthy and physically active men (mean ± 1 SD: age = 26 ± 5 yr, height = 1.79 ± 0.05 m, body mass = 78.3 ± 5.8 kg, $\dot{V}O_{2max}$ = 57.3 ± 6.9 $mL \cdot kg^{-1} \cdot min^{-1}$) were recruited to participate in the study, which was approved by the QinetiQ Research Ethics Committee. Participants were included if they were nonsmokers, had not suffered a bone fracture in the previous 12 months, were free from musculoskeletal injury, and were not taking any medication or experiencing any condition known to affect bone metabolism. Compliance with these inclusion criteria was confirmed from a medical screening questionnaire and examination. All volunteers provided written, informed consent before participation.

Design

Participants completed two preliminary visits for medical screening, habituation, and measurement of $\dot{V}O_{2max}$. They then completed two counterbalanced 9-d experimental protocols separated by at least 1 wk. On D1–D3 of each protocol, participants refrained from physical activity and ate a prescribed diet (Fig. 1). On D4 and D5, all participants performed two 60-min bouts of treadmill running (exercise bouts A [ExA] and B [ExB]) separated by a recovery period of either 23 h (LONG) or 3 h (SHORT) (Fig. 1). In LONG, exercise was performed at 2:30 p.m. on D4 (L-ExA) and an identical bout at 2:30 p.m. on D5 (L-ExB). In SHORT, participants rested on D4 and completed both bouts of running on D5 at 10:30 a.m. (S-ExA) and 2:30 p.m. (S-ExB). On D6–D9, participants attended the laboratory for early morning follow-up analysis and continued to follow a controlled diet and refrain from physical activity. The order in which participants completed the two conditions was counterbalanced.

Preliminary Measures

Dietary analysis. Participants completed a 3-d food diary (two weekdays, one weekend day) to calculate habitual daily energy intake (MJ) and macronutrient composition (Microdiet V2; Downlee Systems Limited, UK).

Assessment of cardiorespiratory responses and aerobic power. Participants completed a 20-min submaximal run on a treadmill (XELG 70 ERGO; Woodway, Waukesha, WI), consisting of four 5-min stages. Sixty-second samples of expired air (inspiration to inspiration) were collected in the final minute of each stage. After a 30-min rest, participants

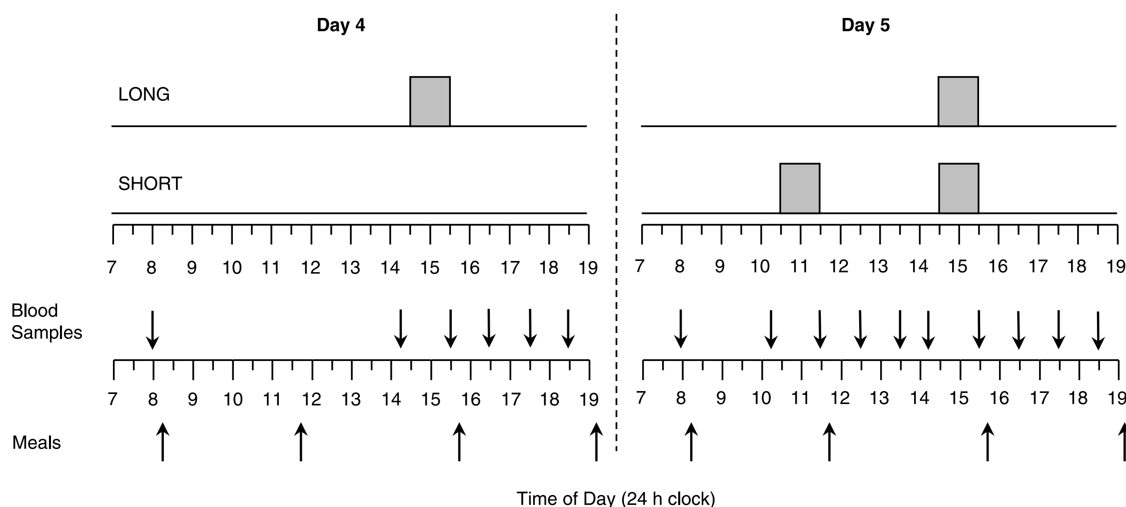

FIGURE 1—Diagram showing the exercise protocol on D4 and D5 in the LONG (top) and SHORT (bottom) experimental conditions. *Shaded boxes* indicate exercise bouts A and B (60 min at 65% $\dot{V}O_{2max}$). *Vertical arrows* indicate timings of blood samples and standardized meals.

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completed a discontinuous, incremental exercise test to volitional exhaustion to establish $\dot{V}O_{2max}$. The results of the two tests were used to estimate the treadmill velocity corresponding to 50% and 65% $\dot{V}O_{2max}$ during flat running based on the regression line of $\dot{V}O_2$ and velocity.

Experimental dietary provision. A diet containing 6 g CHO per kilogram of FFM and isocaloric with habitual diet was designed for each participant. Three menus were provided and administered in a 3-d cyclic order with menu A on D1 and D6, menu B on D2 and D7, and menu C on D3 and D8. During the experimental period, with the exception of D4 and D5, participants provided their own food but were given instructions concerning the quantity, preparation, and timings of meals.

Trial Procedures

D1–D3. All subjects refrained from physical activity and consumed their experimental diets.

D4 and D5. On D4, after an overnight fast, participants arrived at the laboratory at 7:30 a.m. and had their body mass measured (Mettler-Toledo ID7; Mettler-Toledo, Germany). Subsequently, participants adopted a semirecumbent position, and a blood sample was collected by venepuncture at 8:00 a.m. At 2:00 p.m., a cannula (18GA 1.2 × 45 mm; Becton Dickinson, Franklin Lakes, NJ) was inserted into a prominent forearm vein, with subsequent blood samples being taken at 2:15, 3:30, 4:30, 5:30, and 6:30 p.m. On D5, after an overnight fast, participants arrived at the laboratory at 7:30 a.m., had their body mass measured, and adopted a semirecumbent position on a bed. A cannula was inserted into a prominent forearm vein, with subsequent blood samples being taken at 8:00 a.m., 10:15 a.m., 11:30 a.m., 12:30 p.m., 1:30 p.m., 2:15 p.m., 3:30 p.m., 4:30 p.m., 5:30 p.m., and 6:30 p.m. (Fig. 1).

Participants exercised for 60 min at 65% $\dot{V}O_{2max}$, after a 5-min warm-up at 50% $\dot{V}O_{2max}$. Sixty-second samples of expired air (inspiration to inspiration) and RPE were collected at 14, 29, 44, and 59 min of each exercise session, and heart rate was recorded continuously. The ambient room temperature during exercise was 21.4°C ± 1.3°C in both conditions. On completion of exercise, participants toweled dry and had their nude body mass measured. The difference between pre- and postexercise body mass was calculated, and participants consumed 1.5 mL of plain water during the recovery period for every gram change in body mass.

On both days of each condition, participants consumed a standardized diet (13.3 MJ, 52% CHO, 33% FAT, 15% PRO) divided into four meals. This diet was calculated from a standard diet of 2500 kcal plus a 660-kcal supplement on D4 and D5, based on the data of Åstrand and Rodahl, (3) and the mean running speed of a group of recreationally active men at 65% $\dot{V}O_{2max}$ in a previous study from our laboratory (45). The first three meals were eaten in the laboratory at 8:15 a.m., 11:45 a.m., and 3:45 p.m., and participants took the fourth meal home to be consumed at 7:00 p.m. (Fig. 1).

D6–D9. Participants attended the laboratory at 7:45 a.m. after an overnight fast, voided, had their nude body mass measured, and had their blood sample collected at 8:00 a.m. Only water was allowed after 9:00 p.m. on the days before laboratory visits (D4–D9).

Metabolic Measurements

Samples of expired air were collected into evacuated Douglas bags. The bags were emptied through a flow controller and volume counter and analyzed for fractions of oxygen (O_2) and carbon dioxide (CO_2) (Servomex 1400, Sussex, UK). The gas analyzer was calibrated using certified reference gases (100% nitrogen, 16% O_2, and 5% CO_2; BOC Gases, Surrey, UK). To allow the conversion of gas volumes from ambient temperature and pressure saturated to body temperature and pressure saturated, measures of air temperature and pressure were also made.

Biochemical analysis. C-terminal telopeptide region of collagen type 1 (β-CTX), N-terminal propeptides of procollagen type 1 (P1NP), osteoprotegerin (OPG), and PTH were measured in EDTA plasma. Bone alkaline phosphatase (bone ALP), calcium (Ca), albumin, and phosphate (PO_4) were measured in serum. β-CTX was measured using an electrochemiluminescent immunoassay on an Elecsys 2010 immunoanalyzer (Roche, Lewes, UK). Interassay coefficient of variation (CV) was <8% from 0.2 to 1.5 $\mu g \cdot L^{-1}$, and assay sensitivity (replicates of the zero standard) was 0.01 $\mu g \cdot L^{-1}$. P1NP was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland), and assay sensitivity was 4 $\mu g \cdot L^{-1}$ established from precision profiles (22% CV of duplicates), with an interassay CV of 3.5–5.4% across the concentration range 10–250 $\mu g \cdot L^{-1}$. Bone ALP was measured using a commercial immunometric assay (Metra, Biosystems, Oxford, UK), with a sensitivity of 0.7 $U \cdot L^{-1}$ and a CV of <8% across the range 12–100 $U \cdot L^{-1}$. OPG was measured using a commercial solid phase enzyme linked immunosorbent assay (IDS Boldon, Tyne and Wear, UK), with a detection limit of 0.14 $pmol \cdot L^{-1}$ and an inter/intra-assay CV of <10% across the range 1–30 $pmol \cdot L^{-1}$. PTH was measured using a commercial immunometric assay (Nichols Institute, San Juan, Capistrano, CA), with a detection limit of 0.5 $pmol \cdot L^{-1}$ and an inter/intra-assay CV of <5% across the range 1–40 $pmol \cdot L^{-1}$. Ca (range of measurement in serum of 0.05–5.00 $mmol \cdot L^{-1}$), albumin (range of measurement in serum of 10–70 $g \cdot L^{-1}$), and PO_4 (range of measurement in serum of 0.10–6.46 $mmol \cdot L^{-1}$) were measured using standard commercial assays (Roche) performed on a Roche Modular Analytical System. As fluctuations in protein concentrations, especially albumin, may cause total Ca levels to change independently of the ionized calcium (iCa) concentration, Ca concentrations were "corrected" to give an albumin-adjusted calcium (ACa) value as follows: 0.8 $mg \cdot dL^{-1}$ was subtracted from the total Ca concentration for every 1.0 $g \cdot dL^{-1}$ by which the serum albumin concentration was >4 $g \cdot dL^{-1}$ or 0.8 $mg \cdot dL^{-1}$ was added to the total Ca concentration for every 1.0 $g \cdot dL^{-1}$ by which the

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TABLE 1. Oxygen uptake ($\dot{V}O_2$), percentage of maximal rate of oxygen uptake (%$\dot{V}O_{2max}$), heart rate (percentage of maximum; %HR_{max}), RER, lactate concentrations, and RPE during exercise bout B (E×B) in the LONG (L-ExB) and SHORT (S-ExB) experimental conditions.

Variable	L-ExB	S-ExB
$\dot{V}O_2$ (L·min^{-1})	2.80 ± 0.28	2.91 ± 0.41**
%$\dot{V}O_{2max}$	63.6 ± 2.6	65.4 ± 2.5**
HR (%HR_{max})	84 ± 3	87 ± 5*
RER	0.891 ± 0.030	0.876 ± 0.028**
Lactate (mmol·L^{-1})	0.8 ± 0.2	0.9 ± 0.4*
RPE	12.6 ± 1.1	13.3 ± 1.4***

Values are presented as mean ± 1 SD.
* Different (P < 0.05) from LONG.
** Different (P < 0.01) from LONG.
*** Different (P < 0.001) from LONG.

serum albumin concentration was <4 g·dL^{-1}. Glucose and lactate were measured immediately in whole blood in duplicate (Yellow Springs Instruments 2300 Stat Plus; YSI Inc., Yellow Springs, OH). Concentrations of analytes were not corrected for changes in plasma volume associated with exercise.

Statistical Analysis

All data are presented as mean ± 1 SD unless otherwise stated. Statistical significance was accepted at an alpha level of P < 0.05. Paired samples *t*-tests were used to compare habitual and experimental dietary data and baseline biochemistry.

All biochemical data, body mass, and variables relating to exercise were analyzed using a linear mixed model, with the factors time (of sampling) and condition (LONG vs SHORT) included and with participants as a random within-group factor. For bone turnover markers, data were analyzed from measurements taken at BASE, 8:00 a.m. on D5 and the four follow-up days (D6–D9). To examine the effect of recovery duration on responses to a second bout of exercise, data from glucose and lactate measurements from 2:15 to 6:30 p.m. on D5 were included, and data from measurements of OPG, PTH, ACa, and PO_4 were included from 2:15 p.m. on D5–D9. Assumptions of the linear mixed model were investigated by examining the distribution of residuals and the pattern of residuals versus fitted values. Where nonnormality or nonconstant variance was observed, a transformation was applied to the data so that the assumptions were satisfied. Body mass, β-CTX, P1NP, bone ALP, and ACa did not require transformation. Normality and homogeneity were achieved following log transformations for all other variables.

Where there was a significant main effect of time but no significant condition × time interaction, each subsequent time point was compared against BASE using a pooled mean using the Dunnett test. When the condition × time interaction was significant, within each group, each subsequent time point was compared against BASE using the Dunnett test, and groups were compared using the Student–Newman–Keuls test.

RESULTS

Dietary Analysis

Habitual calcium intake reported by all subjects exceeded 700 mg·d^{-1}. Experimental diets provided a smaller quantity of protein (15.6% vs 17.6%, P < 0.01) and a greater quantity of CHO as a percentage of total energy (56.8% vs 50.5%, P < 0.05), but there were no significant differences for any dietary variable, including total energy intake (10.9 ± 2.4 vs 11.4 ± 1.4 MJ, P = 0.287).

Body Mass

Measured from D4 (BASE) to D9, body mass did not change significantly in either experimental condition (P = 0.679, data not shown).

Baseline Biochemistry

There were no significant differences (range of P values = 0.202–0.975) in baseline concentrations of any biochemical marker between the LONG and the SHORT

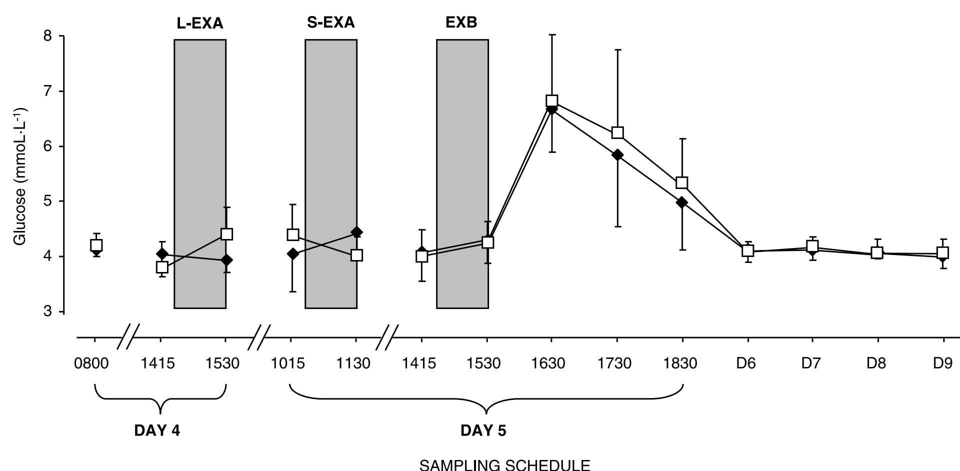

FIGURE 2—Blood glucose concentrations in the LONG (*open squares*) and SHORT (*filled diamonds*) experimental conditions at baseline (8:00 a.m. on D4); before and immediately after exercise bout A; before (2:15 p.m.), immediately after (3:30 p.m.), and for 3 h after (4:30–6:30 p.m.) exercise bout B (ExB); and on the four follow-up days (D6–D9). *Shaded boxes* denote exercise. Values are presented as mean ± SD.

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conditions (see Table, Supplemental Digital Content 1, http://links.lww.com/MSS/A196 and all values were within the normal range.

Exercise and Cardiorespiratory Variables

The mean running speed for the group was 10.1 km·h^{-1} (range = 8.7–12.3 km·h^{-1}). Comparing responses to ExB alone, $\dot{V}O_2$, and percentage $\dot{V}O_{2max}$ (P < 0.01), heart rate (P < 0.05), lactate concentrations (P < 0.05), and perceived exertion (P < 0.001) were all significantly higher in SHORT compared with LONG, whereas respiratory exchange ratio was significantly lower (P < 0.01) (Table 1).

Glucose

When data were examined from 2:15 to 6:30 p.m. on D5, there was a significant main effect of time (P < 0.001) but no significant condition × time interaction (P = 0.788) for blood glucose concentrations. Immediately after exercise, pooled mean concentrations were not significantly different from 2:15 p.m. (Fig. 2). At 4:30 p.m., concentrations were significantly increased from 2:15 p.m. (P < 0.001). Concentrations declined thereafter but remained significantly elevated from preexercise at 5:30 p.m. (P < 0.001) and 6:30 p.m. (P < 0.001).

Bone Markers

There was no significant main effect of time (β-CTX, P = 0.169; P1NP, P = 0.133; bone ALP, P = 0.289) and no significant group × time interaction (β-CTX, P = 0.892; P1NP, P = 0.919; bone ALP, P = 0.529) for β-CTX, P1NP, and bone ALP (Fig. 3A–C).

Osteoprotegerin

When data were examined from 2:15 p.m. on D5 to D9, there was a significant main effect of time (P < 0.05) but no significant group × time interaction (P = 0.949) for OPG concentrations. Pooled mean concentrations were increased by 10% from BASE immediately after exercise and decreased by 20% at D8 and D9, but no individual time points were significantly different from BASE (Fig. 4).

Calcium Metabolism

Parathyroid hormone. When data were examined from 2:15 p.m. on D5 to D9, there was a significant main effect of time (P < 0.01) but no significant group × time interaction (P = 0.543) for PTH. Pooled mean concentrations were significantly (P < 0.001) increased immediately after exercise where concentrations were 55% ± 30% and 69% ± 40% higher than at 2:15 p.m. in LONG and SHORT (Fig. 5A). PTH declined rapidly thereafter and were significantly (P < 0.05) reduced by 10–15% at 4:30 p.m. compared with 2:15 p.m. This reduction was transient, with concentrations not significantly different from 2:15 p.m. at 5:30 p.m. or 6:30 p.m. Although mean concentrations were significantly (P < 0.01) increased from 2:15 p.m. at D7 and D8, concentrations at this time point (3.3 and 3.6 pmol·L^{-1}) were similar to those at BASE (3.5 and 3.5 pmol·L^{-1}) in LONG and SHORT.

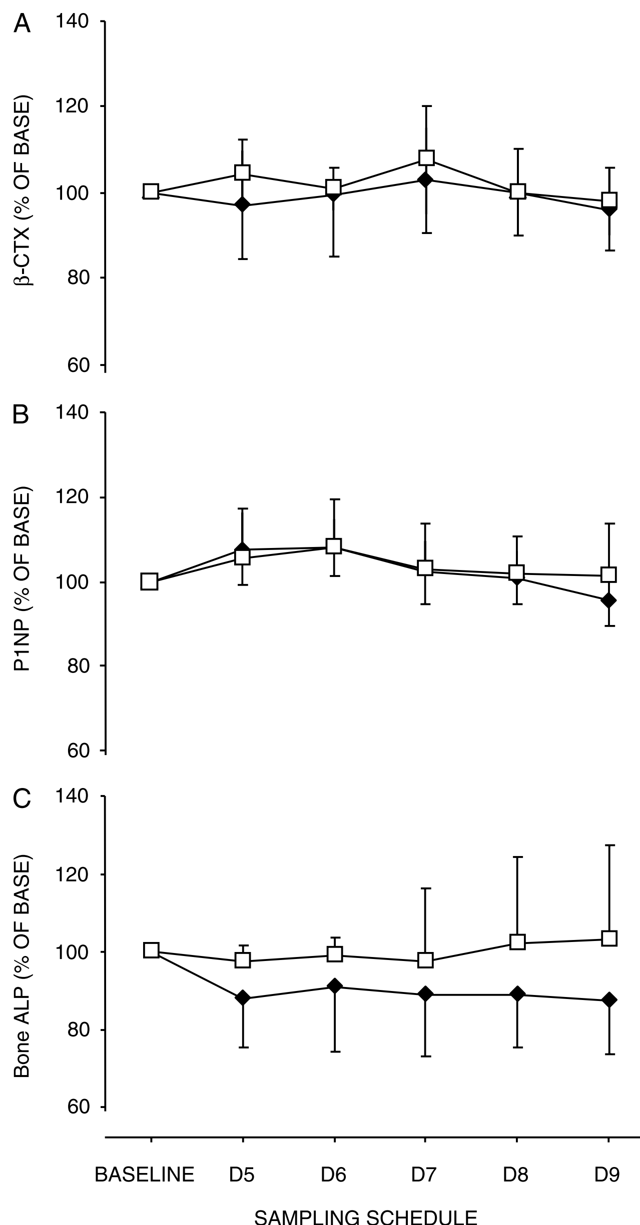

FIGURE 3—Percentage change from baseline (8:00 a.m. on D4) concentrations of β-CTX (A), P1NP (B), and bone ALP (C) on D5 and on the four follow-up days (D6–D9) in the LONG (*open squares*) and SHORT (*filled diamonds*) experimental conditions. All measurements were made from blood samples collected at 8:00 a.m. after an overnight fast. Values are presented as mean ± SD.

ACa. When data were examined from 2:15 p.m. on D5 to D9, there was a significant main effect of time (P < 0.001)

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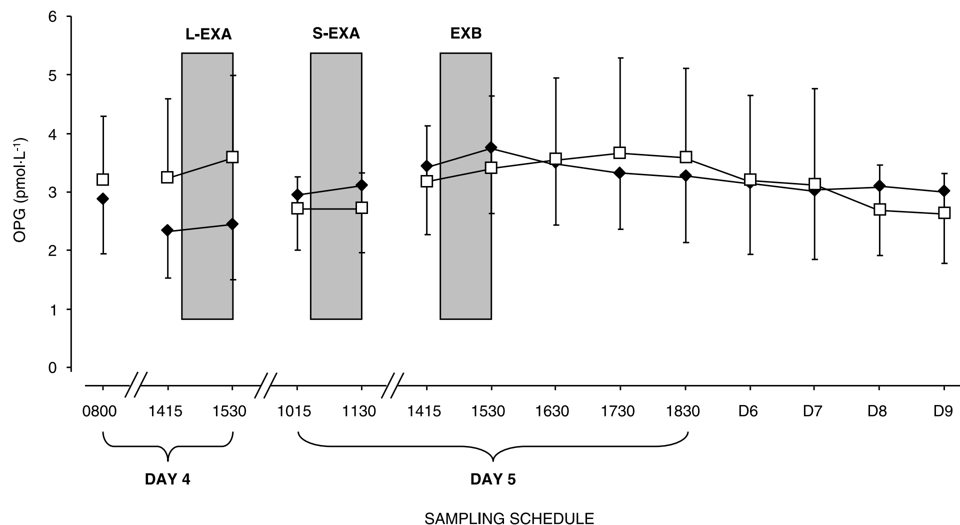

FIGURE 4—OPG concentrations in the LONG (*open squares*) and SHORT (*filled diamonds*) experimental conditions at baseline (8:00 a.m. on D4); before and immediately after exercise bout A; before (2:15 p.m.), immediately after (3:30 p.m.), and for 3 h after (4:30–6:30 p.m.) exercise bout B (ExB); and on the four follow-up days (D6–D9). *Shaded boxes* denote exercise. Values are presented as mean ± SD.

but no significant group × time interaction (P = 0.334) for ACa concentrations. Immediately after exercise, pooled mean concentrations were significantly (P < 0.001) increased from 2:15 p.m. with values of 2.43 ± 0.09 mmol·L^{-1} and 2.48 ± 0.11 mmol·L^{-1} in LONG and SHORT (Fig. 5B). Concentrations were not significantly different from 2:15 p.m. at 4:30 p.m. or any other time point thereafter.

PO_4. When data were examined from 2:15 p.m. on D5 to D9, there was a significant main effect of time (P < 0.001) but no significant group × time interaction (P = 0.703) for PO_4. Immediately after exercise, pooled mean concentrations were significantly (P < 0.001) increased from 2:15 p.m. with values of 1.60 ± 0.17 and 1.64 ± 0.27 mmol·L^{-1} in LONG and SHORT (Fig. 5C). PO_4 declined rapidly thereafter and were not significantly different from 2:15 p.m. at 4:30 p.m., 5:30 p.m., or 6:30 p.m. Although mean concentrations were significantly (P < 0.01) decreased from 2:15 p.m. at D6 to D9, concentrations at these time points (1.02 and 1.04 mmol·L^{-1}) were similar to those at BASE (1.04 and 1.08 pmol·L^{-1}) in LONG and SHORT.

DISCUSSION

The main findings from this study were that 1) when measured from 1 to 4 d after exercise, two 60-min bouts of running at 65% $\dot{V}O_{2max}$ separated by either 23 or 3 h had no significant effect on circulating markers of bone resorption and formation, and 2) reducing the recovery duration from 23 to 3 h between two bouts of exercise had no significant effect on the increase in OPG, PTH, ACa, or PO_4 with the second bout.

This is the first study to examine the effect of recovery duration between two bouts of exercise on bone turnover markers. Previous studies have examined responses to repeated exercise on several consecutive days (24,51) and report that when dietary intake is sufficient to account for the increase in energy expenditure with exercise, bone marker levels are unchanged with either 60 min of running (65%–85% $\dot{V}O_{2max}$) on three consecutive days (51) or with expenditure of 15 kcal·kg^{-1} of lean body mass per day for 5 d (24). However, when energy availability is reduced by 33%–56%, bone formation decreases (24,51) and, in the case of extreme reductions (78%), bone resorption is also increased (24). We provided a standard energy supplement of 660 kcal·d^{-1} on D4 and D5, and neither experimental condition was associated with a significant change in body mass, suggesting that our dietary provision, although not individualized, was sufficient to prevent a marked energy deficit. As such, our findings are broadly consistent with these previous studies by showing no measurable effect of short periods of repeated exercise on bone turnover when energy intake is sufficient (24,51).

Strenuous endurance exercise can increase β-CTX on the days after exercise (22,28,45), and we have previously observed increased β-CTX from 1 to 4 d after exhaustive running lasting approximately 2 h, when no deliberate attempt was made to keep participants in energy balance (45). As such, the lack of any effect of two 1-h bouts of running, performed on the same day, on β-CTX was unexpected. That said, the factors that determine the time course of changes in β-CTX with endurance exercise remain incompletely understood. For example, β-CTX increases in the hours after acute endurance exercise lasting up to 60 min, performed at high exercise intensities including 80% $\dot{V}O_{2max}$ (20), 110% of anaerobic threshold (22), and 115% of ventilatory threshold (VT) (34) but not 85% VT (22), and exercise intensity may be an important factor in this response (22,34,46). It is possible that 65% $\dot{V}O_{2max}$ was not a sufficient intensity to stimulate β-CTX. With that said, however, we have recently observed a transient increase in β-CTX after 60 min of running at 65% $\dot{V}O_{2max}$ under identical conditions to those on D5 in

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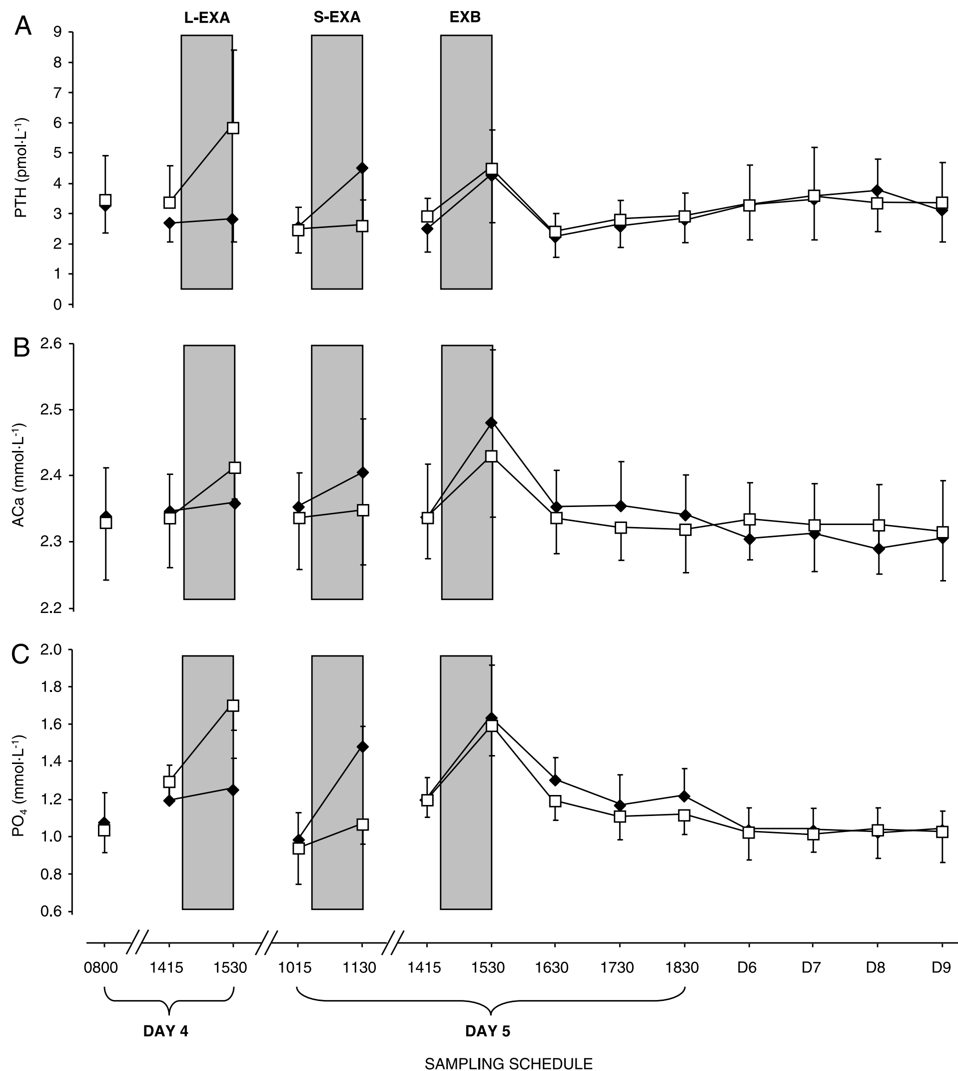

FIGURE 5—PTH (A), ACa (B), and PO_4 (C) concentrations in the LONG (*open squares*) and SHORT (*filled diamonds*) experimental conditions at baseline (8:00 a.m. on D4); before and immediately after exercise bout A; before (2:15 p.m.), immediately after (3:30 p.m.), and for 3 h after (4:30–6:30 p.m.) exercise bout B (ExB); and on the four follow-up days (D6–D9). *Shaded boxes* denote exercise. Values are presented as mean ± SD.

SHORT (exercise at 10:30 a.m. in participants fed at 8:00 a.m.) that was no longer evident at 24 h after exercise (47). It is also possible, therefore, that β-CTX did increase with the exercise bouts, but the response was not sustained through to the following morning.

When studying the response of the biochemical markers of bone turnover to exercise, it is important to acknowledge that markers are a systemic measure reflecting full-body bone metabolism. As such, the absence of significant changes in their concentrations does not necessarily reflect the lack of a local effect at the tissue level. It is possible that bone turnover markers are not sufficiently sensitive to detect local effects of exercise, and there is evidence in both animals (29) and humans (48,49), suggesting that mechanical loading and exercise can induce structural alterations in bone without concomitant changes in systemic markers. With responses to a single loading session ranging from an increase in c-fos mRNA (38) to new bone formation (13), given the duration and intensity of the bouts of running in the present study, the failure of either protocol to have any effect on bone metabolism seems unlikely, and a local effect on bone that went undetected by the markers measured cannot be ruled out.

This is the first study to measure the OPG response to repeated bouts of exercise and shows similar OPG responses to consecutive bouts of running separated by either 23 or 3 h. The approximately 10% increase in OPG is somewhat less than we have observed previously (~25%) during 60 min of running at 65% $\dot{V}O_{2max}$ (45,46) and markedly less than that reported (100%–150%) during much longer duration running (28,52). The reasons for these different responses to exercise are, as yet, unknown. As three of the four exercise bouts in the current study were performed in the afternoon, any increase in OPG with exercise might have been attenuated by declining concentrations in the afternoon due to its circadian

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rhythm (26), although Ziegler et al. (52) reported no change in OPG during a running race of greater duration (1.26 ± 0.19 h). A system including OPG, in conjunction with receptor activator of NF-κB ligand (RANKL) and its receptor RANK, may be a common link between bone and immune and cardiovascular function (10), and to what extent systemic concentrations reflect the biological activity of OPG in the bone microenvironment remains a key question in understanding its relationship with bone turnover. As exercise stimulates both immune and cardiovascular responses, differing changes in OPG with exercise might reflect the contribution of tissues in addition to bone to its circulating concentrations. In addition, as it is the OPG-to-RANKL ratio that determines osteoclastic activity, the interpretation of changes in systemic OPG is further complicated by RANKL currently being undetectable in at least 50% of healthy adults (21).

This study also examined the effect of recovery duration between two bouts of exercise on PTH and shows that the response to a second bout of exercise was not altered by a prior bout performed either 23 or 3 h earlier. One previous study has examined the PTH response to two bouts of endurance running performed on the same day (6), reporting increased PTH after 21 min of running at 70% $\dot{V}O_{2max}$ but no increase during 21 min of running at 85% $\dot{V}O_{2max}$ performed 40 min later (6). In comparison, when the two bouts were performed consecutively, the increase during the second "half" was considerably greater (85% vs 15%) than that in the first half, suggesting an attenuation of the PTH response by previous exercise. This attenuation is particularly surprising as higher exercise intensities are associated with greater increases in PTH (34,46). The reason for the different findings from our study and that of Bouassida et al. (6) might be related to the considerably shorter rest period in the latter study (6). There may be a minimal time for recovery that allows the parathyroid gland to respond to stimuli from a second bout of exercise (greater than 40 min but may be less than 3 h).

Consistent with two previous studies (45,46), ACa concentrations were elevated at the end of exercise. Rather than total Ca, it is the iCa concentration that is sensed by the Ca-sensing receptor in the parathyroid gland (8), and PTH secretion occurs in response to small decrements in iCa (9). Owing to the methodological difficulties in measuring iCa, we calculated ACa as an estimation of the iCa concentration. Changes in blood pH can influence the albumin–iCa binding complex; however, previous studies (37) report no decrease in blood pH during endurance exercise with lactate levels greater (4–5 mmol·L^{-1}) than those observed in the present study, suggesting that ACa remains a valid estimation of the iCa concentrations. The present results might suggest that the increase in PTH with exercise is not mediated by calcium concentrations. However, we cannot exclude the possibility of a decrease in ACa early in exercise stimulating PTH, which in turn may have contributed to the increase in ACa observed at the end of exercise by stimulating Ca reabsorption in the kidney and then by osteoclastic bone resorption (15). With that said, any decrease in Ca must occur in the first few minutes of exercise, as in previous studies using similar exercise protocols, we have reported that both ACa and PTH are increased after only 20 min (45,46).

In circadian rhythm studies, changes in serum PO_4 precede changes in PTH (2), whereas acute exogenous PO_4 results in increased PTH (11). Whether the increase in PO_4 occurred before and might therefore have stimulated the increase in PTH in the present study is unknown. Previous data, which show that both PTH and PO_4 are already increased after 20 min similar exercise (45,46), are not informative in this regard. It should be noted, however, that in a recent study of exercise intensity (46), PTH was increased during 60 min of treadmill running at 75%, but not 65% $\dot{V}O_{2max}$ despite similar changes in PO_4, suggesting that factors other than PO_4 regulate the PTH response.

As in previous studies of acute, endurance exercise (20,42,45,46), increases in PTH in the present study were transient. Transient increases in PTH seen with daily PTH injections result in bone formation (35,36) and increases in the markers of bone formation (P1NP) within 3 d (19). However, previous studies of acute exercise provide no convincing evidence of an anabolic effect of PTH, with bone markers either unchanged (20,42,45) or transiently increased during exercise only (42,46). In the present study, despite two transient increases in PTH during both experimental conditions, with mean values ranging from 55% to 85%, there was no significant effect on bone formation markers measured up to 4 d after the second bout of exercise.

The maximum absolute PTH concentration in the current study was 5.9 pmol·L^{-1}, and the maximum relative increase was 4.2-fold. These figures are considerably lower than those reported after injections of PTH 1–84 and 1–34, where mean peak concentrations were 79 and 39 pmol·L^{-1} (44), with relative increases of approximately 10-fold (33). Alone, this might suggest that the magnitude of the increase in PTH with running would be insufficient to elicit an anabolic effect in bone. That said, however, rather than absolute concentration, the primary determinate of whether PTH has anabolic effects might be the length of time concentrations remain above baseline (16). In animals, modest (three to sixfold) endogenous (EDTA-induced) increases in PTH produce area under the curve values similar to those seen with a 5-μg·kg^{-1} injection of PTH, the latter of which results in bone formation (14), whereas a short-acting calcium-sensing receptor antagonist, which produces endogenous increases in PTH, has been shown to increase bone strength in animals (30). In humans, these antagonists produce modest (3- to 5.5-fold) and transient (several hours) increases in PTH, consistent with those observed after exogenous PTH 1–34, which results in bone formation (30). It is also possible, therefore, that PTH increases during acute running might produce anabolic effects, but as described earlier, these effects are not detectable in the peripheral markers of bone formation, at least in the hours and days after acute endurance exercise.

In conclusion, in young, physically active men with sufficient energy intake, two 60-min bouts of running at 65%

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$\dot{V}O_{2max}$ separated by either 23 or 3 h had no effect on the markers of bone resorption or formation from 1 to 4 d after exercise. OPG, PTH, ACa, and PO_4 all increased during running, but reducing recovery duration from 23 to 3 h between the two consecutive bouts did not affect the response to the second bout.

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