The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise

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Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. J Appl Physiol 110: 423–432, 2011. First published December 2, 2010; doi:10.1152/japplphysiol.00764.2010.—We compared the effects of exercise intensity (EI) on bone metabolism during and for 4 days after acute, weight-bearing endurance exercise. Ten males [mean ± SD maximum oxygen uptake (VO2max): 56.2 ± 8.1 ml·min⁻¹·kg⁻¹] completed three counterbalanced 8-day trials. Following three control days, on day 4, subjects completed 60 min of running at 55%, 65%, and 75% VO2max. Markers of bone resorption [COOH-terminal telopeptide region of collagen type 1 (β-CTX)] and formation [NH2-terminal propeptides of procollagen type 1 (P1NP), osteocalcin (OC), bone-alkaline phosphatase (ALP)], osteoprotegerin (OPG), parathyroid hormone (PTH), albumin-adjusted calcium (ACa), phosphate (PO4), and cortisol were measured during and for 3 h after exercise and on four follow-up days (FU1–FU4). At 75% VO2max, β-CTX was not significantly increased from baseline by exercise but was higher compared with 55% (17–19%, P < 0.01) and 65% (11–13%, P < 0.05) VO2max in the first hour postexercise. Concentrations were decreased from baseline in all three groups by 39–42% (P < 0.001) at 3 h postexercise but not thereafter. P1NP increased (P < 0.001) during exercise only, while bone-ALP concentrations were increased (P < 0.01) at FU3 and FU4, but neither were affected by EI. PTH and cortisol increased (P < 0.001) with exercise at 75% VO2max only and were higher (P < 0.05) than at 55% and 65% VO2max during and immediately after exercise. The increases (P < 0.001) in OPG, ACa, and PO4 with exercise were not affected by EI. Increasing EI from 55% to 75% VO2max during 60 min of running resulted in higher β-CTX concentrations in the first hour postexercise but had no effect on bone formation markers. Increased bone-ALP concentrations at 3 and 4 days postexercise suggest a beneficial effect of this type of exercise on bone mineralization. The increase in OPG was not influenced by exercise intensity, whereas PTH was increased at 75% VO2max only, which cannot be fully explained by changes in serum calcium or PO4 concentrations.

treadmill running; bone turnover markers; osteoprotegerin; parathyroid hormone

EXERCISE is associated with improvements in bone mineral density (BMD), particularly at load-bearing sites (9, 11, 22, 60), and might play a role in reducing fragility fractures associated with osteoporosis (19, 24), either by increasing the accumulation of bone mass during childhood growth (9, 68) or by decreasing the rate of bone loss following the attainment of peak bone mass (37, 60). Despite this, the specific mechanisms by which exercise exerts its effects on bone remain to be fully determined, as does the most effective form of exercise to increase BMD.

Mechanical loads imposed by muscle contractions and gravity that are in excess of those habitually encountered can positively influence the size, shape, and internal structure of the skeleton (20). This osteogenic response saturates during prolonged loading and is improved by rest periods and the addition of more exercise sessions rather than by increasing the duration of individual sessions (64). Animal studies show that mechanical strain induced by loading must also reach a specified magnitude before an osteogenic effect is initiated. Once this threshold is exceeded, the bone formation response is correlated positively with peak strain magnitude (13, 55, 63).

Consistent with these findings, it has been suggested that high-impact activities are effective in improving femoral neck BMD in premenopausal women (71) while intervention studies of brief but regular impact exercise such as jumping and hopping can also have positive effects at the hip (3, 4, 32).

Since it is impractical to directly assess bone strain in free-living humans, it has been suggested that peak acceleration might be a reasonable estimate of peak bone strain, thus acting as a surrogate measure (66). Studies in humans that have examined the long-term effects of physical activity at different acceleration levels indicate that, like in animals, osteogenic effects are evident only above a certain threshold (65, 66).

Despite the apparent benefits of short-duration, high-impact exercise on bone, many people partake in longer-duration endurance exercise for recreational, competitive, or occupational (e.g., the military) purposes. Such activities are also characterized in terms of their “intensity” using relative measures of cardiovascular strain, such as percentage of maximum oxygen uptake (VO2max), lactate threshold (LT), or heart rate reserve (HRR). All of these modes of exercise will, to a varying degree, impose mechanical loads on bone either by muscle contraction or gravity, although such measures of cardiovascular exercise intensity cannot be used interchangeably with those used in relation to mechanical strain on bone.

Although weight bearing in nature, endurance running exercise has been associated with deleterious effects on bone in some populations, including reduced spinal BMD in endurance runners (7, 31) and stress fractures (SFx) in runners (6) and military recruits (35). Both endurance runners and recruits are regularly exposed to high-intensity exercise, while military recruits also frequently perform “common” physical training sessions during which individual relative exercise intensities range from 53% to 73% of HRR (52). It is recruits with the lowest aerobic fitness—who will experience the higher relative exercise intensities during these activities—that have an increased risk of SFx (58, 67). These findings suggest that high...
cardiovascular intensity itself might, in part, contribute to a deleterious effect of exercise on bone.

Changes in BMD result from the net effects of bone resorption and bone formation, a process termed bone turnover. Using biochemical markers of bone turnover, several studies indicate that acute endurance exercise induces a burst of osteoclastic activity that, at least in the short term, is not accompanied by an associated increase in osteoblastic activity (25, 33, 57). In one study of well-trained triathletes (25), the COOH-terminal telopeptide region of collagen type 1 (β-CTX), a specific marker of bone resorption, was increased by 50% 1 h after 60 min of stationary cycling at 80% VO2max. As cycling imposes negligible mechanical loading due to the effects of gravity, and this population is well accustomed to this type of exercise, this finding might suggest that a high cardiovascular strain itself could result in increased bone resorption and a negative bone remodeling balance, at least in the short term. As many endurance training sessions are structured according to relative cardiovascular exercise intensities and the American College of Sports Medicine recommendations for improving cardiorespiratory fitness use similar measures (49), it is of interest to understand how the cardiovascular intensity of endurance exercise might influence bone metabolism.

Few studies have attempted to directly compare variations in exercise intensity in cardiovascular terms on bone turnover markers. These studies indicate that there might be a threshold above which bone markers (29, 38), as well as parathyroid hormone (PTH) concentrations (38) are stimulated. However, both studies used cycling as the mode of exercise and examined only two different exercise intensities. Additionally, one study took no samples for analysis beyond 15 min postexercise (38) while the other failed to standardize the time of day of the trials and the nutritional status of subjects (29), both of which are known to affect bone turnover markers, particularly β-CTX (14, 15, 21, 70).

The aim of the present study, therefore, was to compare the effects of three different cardiovascular exercise intensities on changes in bone turnover markers during and for 4 days following acute endurance running under highly standardized conditions.

MATERIALS AND METHODS

Subjects

Ten men were recruited to the study, which was approved by the QinetiQ Research Ethics Committee. Subjects were nonsmokers, had not suffered a bone fracture of any type in the previous 12 mo, were included team sports players, recreational runners, and one “club”-level runner. All subjects performed at least one bout of endurance running per week.

Design

Subjects completed two preliminary visits for medical screening, habituation with trial procedures, and measurement of VO2max. Subjects then completed three counterbalanced 8-day experimental conditions, separated by a minimum of 1 wk, during which they refrained from unspecified physical activity and ate a controlled diet. On days 1–3, subjects refrained from all physical activity and followed their prescribed diet. On day 4, subjects performed a single 60-min bout of treadmill running at 55%, 65% and 75% of VO2max, followed by 3 h of recovery. On days 5–8, subjects refrained from all physical activity, followed their prescribed diet, and attended the laboratory for follow-up analysis (FU1-FU4). Subjects were asked to report any symptoms of illness or fever in the days leading up to the study. If such an episode occurred, the trial was postponed until subjects had been clear of symptoms for a minimum of 5 days.

Pretrial Measurements

Dietary analysis. Subjects completed a 3-day food diary consisting of two weekdays and one weekend day to calculate habitual daily energy intake and macronutrient composition. Subjects were issued with a set of calibrated weighing scales to measure food intake and received both verbal and written instructions. Food diaries were analyzed using nutritional analysis software (Microdiet V2, Downlee Systems).

Assessment of cardiorespiratory responses to level running and aerobic power. To establish the association between oxygen uptake and running velocity during level running, subjects completed a 20-min submaximal run on a treadmill (XELG 70 ERGO, Woodway), 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, subjects completed a discontinuous, incremental exercise test to exhaustion to establish VO2max using a modified Taylor protocol (23). Heart rate was monitored continuously throughout the test (Vantage NV, Polar Electro Oy, Kempele, Finland). The results of the two tests were used to estimate the treadmill velocity corresponding to 50%, 55%, 65%, and 75% VO2max during level running based on the regression line of VO2 and velocity.

Experimental dietary provision. A diet consisting of ~55% carbohydrate (CHO), 30% fat, and 15% protein, and isocaloric with their habitual diet, was designed for each subject based on individual dietary habits. Subjects were provided with three menus that were given in a 3-day cyclic order with menu A on days 1 and 5, menu B on days 2 and 6, and menu C on days 3 and 7 (see Trial Procedures for details of diet on day 4). During the experimental period, subjects provided their own food and were given both verbal and written instructions concerning the quantity and preparation of their meals and timings. Subjects received a set of calibrated weighing scales to aid them with food preparation.

Trial Procedures

Day 4. Following an overnight fast, subjects arrived at the laboratory at 0730, voided, and had their nude body mass measured (ID7, Mettler-Toledo, Germany). Subjects subsequently adopted a semi-recumbent position on a bed and had a cannula (18GA 1.2 × 45 mm, Becton Dickinson) inserted into a prominent forearm vein, where it remained until the final blood sample was collected. The cannula was kept patent with 5 ml of an isotonic saline solution (0.9% NaCl).

A fasting baseline blood sample was collected at 0800 for measurement of all biochemical markers and exercise commenced at 0815 (Fig. 1). Exercise bouts consisted of 60 min of treadmill running preceded by a 5-min warm-up at 50% VO2max, separated by 5 min for volitional stretching. Sixty-second samples of expired air and ratings of perceived exertion (RPE) (46) were collected after 18, 38, and 58 min of exercise. Heart rate was recorded continuously and water was consumed ad libitum during exercise (0.27 ± 0.14, 0.28 ± 0.10, and
Biochemical Analysis

β-CTX was measured using an electrochemiluminescent immunoassay (ECLIA) on an Elecsys 2010 immunoanalyzer (Roche, Lewes, UK). The interassay coefficient of variation (CV) was <8% from 0.2 to 1.5 µg/l. The assay sensitivity (replicates of the zero standard) was 0.01 µg/l. P1NP was measured by radioimmunoassay (RIA) supplied by Orion Diagnostica (Espoo, Finland). This assay has a sensitivity of 4 µg/l established from precision profiles (22% coefficient of variation of duplicates) and an interassay CV of 3.5–5.4% from 10–250 µg/l. Plasma N-MID OC was measured using an ECLIA on a Modular Analytics E170 analyzer (Roche Diagnostics, Lewes, UK). The interassay CV was <5% from 2 to 200 µg/l. The assay sensitivity (replicates of the zero standard) was 0.6 µg/l. Bone-ALP was measured using a commercial immunometric assay (Metra Biosystems, Oxford, UK) with a sensitivity of 0.7 U/l and a CV of <8% from 12 to 100 U/l. Cortisol was measured in plasma using ECLIA on a Roche Modular E170. The assay has a sensitivity of 8 nmol/l established from precision profiles (22% coefficient of variation of duplicates) and a CV of <6% from 16 to 1,750 nmol/l. OPG was measured using a commercial solid-phase enzyme-linked immunosorbent assay (ELISA) that measures both free OPG and that complexed with RANK-L (IDS Boldon Tyne and Wear UK). The assay has a detection limit of 0.14 pmol/l and an inter- and intra-assay CV of <10% from 1 to 30 pmol/l.

PTH was measured using a commercial immunometric assay (Nichols Institute, San Juan Capistrano, CA) with a detection limit of 0.5 pmol/l and inter- and intra-assay CV of <5% from 1 to 40 pmol/l. Ca (range of measurement in serum of 0.05–5.00 mmol/l), albumin (range of measurement in serum of 10–70 g/l), and PO4 (range of measurement in serum of 0.10–6.46 mmol/l) were measured using standard commercial assays supplied by Roche (Lewes, UK) performed on a Roche Modular Analytical System. 25-OH vitamin D2 and 25-OH vitamin D3 were quantified after extraction using straight-phase HPLC employing 1,5-trans-vitamin D3 as an internal standard. The lower and upper detection limits for both 25-OH molecules are 2,000 pmol/l and 5°C for 10 min, samples separated and aliquots stored at −70°C until analysis. For the measurement of glucose and lactate, whole blood was transferred into precooled tubes containing fluoride-oxalate. Tubes were gently inverted 8–10 times and analyzed in duplicate immediately (Yellow Springs Instruments, 2300 Stat Plus, YSI).

Statistical Analysis

All data are presented as means ± SD unless otherwise stated. Statistical significance was accepted at an α-level of P < 0.05. Paired samples t-tests were used to compare habitual with experimental dietary data. A one-way ANOVA was used to compare baseline biochemistry and variables relating to exercise between the 55%, 65%, and 75% VO2max conditions, with Student-Newman-Keuls tests used to compare habitual with experimental conditions.
pared against BASE from a pooled mean using Dunnett’s test with BASE as the “control.” When the condition \( \times \) time interaction was significant, within each condition, each subsequent time point was compared against BASE using Dunnett’s test with BASE as the “control” and conditions were compared with each other at all time points using the SNK test. All statistical analysis was performed with the SPSS v15 (SPSS, Chicago, IL) with the exception of the Dunnett’s and SNK tests, which were performed with Statistica (StatSoft, Tulsa, OK).

### RESULTS

**Subject Characteristics**

Subject characteristics are shown in Table 1.

**Dietary Analysis**

Results of food dairy analysis are shown in Table 2. Daily calcium intake exceeded 700 mg/day for all subjects. The energy content of the experimental diets was not significantly different from habitual energy intake (\( P = 0.440 \)).

**Body Mass**

There was no effect of exercise intensity on changes in body mass (condition \( \times \) time interaction, \( P = 0.970 \)) during the experimental conditions measured from day 4 (BASE) to day 8 (FU4). Pooled, mean data from the three conditions showed a decrease (~0.5%) in body mass from BASE that was significant (\( P < 0.05 \)) at FU4 only.

**Exercise Variables**

Measured exercise intensities in the 55%, 65%, and 75% \( V\dot{O}_{2\text{max}} \) conditions were 55 \( \pm \) 3%, 63 \( \pm \) 3%, and 75 \( \pm \) 3% \( V\dot{O}_{2\text{max}} \). Oxygen uptake, heart rate, and RPE all increased (\( P < 0.001 \)) with increasing exercise intensity. Respiratory exchange ratio (RER) was not significantly different between 55% and 65% \( V\dot{O}_{2\text{max}} \) but was significantly higher at 75% \( V\dot{O}_{2\text{max}} \) compared with both 55% (\( P < 0.001 \)) and 65% (\( P < 0.01 \)) \( V\dot{O}_{2\text{max}} \).

**Glucose, Lactate, and Cortisol**

There was no effect of exercise intensity on blood glucose concentrations (condition \( \times \) time interaction, \( P = 0.449 \)). Pooled, mean concentrations were significantly increased (\( P < 0.001 \)) from BASE throughout exercise (Fig. 2A). Concentrations increased from 4.3 mmol/l at BASE to 4.5 mmol/l at EX60 with exercise at 55% and 65% \( V\dot{O}_{2\text{max}} \) and 5.3 \( \pm \) 0.8 mmol/l at 75% \( V\dot{O}_{2\text{max}} \). Following exercise, glucose concentrations were decreased from BASE at R1.0 (\( P < 0.001 \)) and R3.0 (\( P < 0.01 \)).

Analysis of blood lactate concentrations showed a main effect of time (\( P < 0.001 \)) and a condition \( \times \) time interaction (\( P < 0.001 \)). Concentrations were significantly increased (\( P < 0.001 \)) from BASE at EX20 in the 55% \( V\dot{O}_{2\text{max}} \) condition,
throughout exercise at 65% \( \dot{V}O_{2\text{max}} \) \((P < 0.001)\), and throughout exercise and up to R1.0 at 75% \( \dot{V}O_{2\text{max}} \) \((P < 0.01)\) (Fig. 2B). The increase at 65% \( \dot{V}O_{2\text{max}} \) was greater than that at 55% \( \dot{V}O_{2\text{max}} \) resulting in higher \((P < 0.001)\) concentrations at EX20, EX40, and EX60, while lactate concentrations at 75% \( \dot{V}O_{2\text{max}} \) were higher \((P < 0.001)\) than at both 55% and 65% \( \dot{V}O_{2\text{max}} \) throughout exercise and up to R1.0.

There were no significant differences between baseline cortisol concentrations in the three conditions \((P = 0.618)\). Analysis of cortisol concentrations showed a main effect of time \((P < 0.001)\) and a condition \(\times\) time interaction \((P < 0.001)\). Cortisol concentrations at 55% \( \dot{V}O_{2\text{max}} \) were significantly decreased from BASE throughout exercise \((P < 0.05)\), with a trend being shown at R0.5 \((P = 0.06)\) (Fig. 2C). Subsequently, cortisol concentrations decreased further and were significantly decreased from BASE at R1.0 \((P < 0.001)\), R2.0 \((P < 0.001)\), and at R3.0 \((P < 0.001)\), where concentrations were reduced to 65 ± 17% of BASE levels. At 65% \( \dot{V}O_{2\text{max}} \), concentrations were not significantly different from BASE during exercise but were significantly lower than BASE at R0.5 \((P < 0.05)\), R1.0 \((P < 0.001)\), and R3.0 \((P < 0.001)\), where concentrations were reduced to 51 ± 18% of BASE levels. At 75% \( \dot{V}O_{2\text{max}} \), cortisol concentrations increased during exercise and, by EX60, were significantly increased from BASE \((+30 ± 37\%; P < 0.01)\), resulting in higher cortisol concentrations compared with 55% \( \dot{V}O_{2\text{max}} \) at EX20 \((P < 0.05)\) and EX40 \((P < 0.001)\), and with both 55% \( \dot{V}O_{2\text{max}} \) \((P < 0.001)\) and 65% \( \dot{V}O_{2\text{max}} \) \((P < 0.001)\) at EX60. Concentrations were not significantly different from BASE from R0.5 to R2.0 but remained higher than both 55% \( \dot{V}O_{2\text{max}} \) and 65% \( \dot{V}O_{2\text{max}} \) at R0.5 \((P < 0.001)\), R1.0 \((P < 0.001)\), and R2.0 \((P < 0.01)\). At R3.0, concentrations remained lower \((P < 0.001)\) than BASE but were no longer significantly different from 55% \( \dot{V}O_{2\text{max}} \) or 65% \( \dot{V}O_{2\text{max}} \). At FU1, concentrations were not different from BASE in any condition and there were no further changes in cortisol thereafter.

**Bone Turnover Markers**

There were no significant differences between baseline \(\beta\)-CTX concentrations in the three conditions \((55% \dot{V}O_{2\text{max}}, 0.71 ± 0.22; 65% \dot{V}O_{2\text{max}}, 0.69 ± 0.25; 75% \dot{V}O_{2\text{max}}, 0.69 ± 0.22 \mu g/l; P = 0.998)\). Analysis of \(\beta\)-CTX showed a main effect of time \((P < 0.001)\) and a condition \(\times\) time interaction \((P < 0.05)\). At 55% \( \dot{V}O_{2\text{max}} \), \(\beta\)-CTX concentrations decreased by 16% during exercise and were significantly lower than BASE at EX60 \((P < 0.05)\) (Fig. 3A). \(\beta\)-CTX remained lower than BASE at R0.5 \((P < 0.01)\), R1.0 \((P < 0.05)\), R2.0 \((P < 0.001)\), and at R3.0 \((P < 0.001)\), where concentrations were decreased by 39%. At 65% \( \dot{V}O_{2\text{max}} \), \(\beta\)-CTX concentrations decreased by 10% during exercise, although this difference did not reach the assigned level of significance. \(\beta\)-CTX concentrations decreased further during recovery and were significantly decreased from BASE at R2.0 \((P < 0.001)\) and at R3.0 \((P < 0.001)\), where concentrations were reduced by 40%. At 75% \( \dot{V}O_{2\text{max}} \), \(\beta\)-CTX concentrations were not significantly different from BASE during exercise. At EX60 concentrations were 3% above BASE values and significantly higher than at 55% \((+19\%, P < 0.01)\) and 65% \((+14\%, P < 0.05)\) \( \dot{V}O_{2\text{max}} \). The magnitude of this difference was maintained in the first hour postexercise, with concentrations at 75% \( \dot{V}O_{2\text{max}} \) remaining higher than at 55% \( \dot{V}O_{2\text{max}} \) at R0.5 \((P < 0.01)\), and higher than both 55% \( \dot{V}O_{2\text{max}} \) \((P < 0.01)\) and 65% \( \dot{V}O_{2\text{max}} \) \((P < 0.01)\) at R1.0. Concentrations subsequently decreased and were lower \((P < 0.001)\) than BASE at R2.0 but no longer significantly different from 55% \( \dot{V}O_{2\text{max}} \) or 65% \( \dot{V}O_{2\text{max}} \). At R3.0, concentrations were reduced \((P < 0.001)\) 42% from BASE and lower \((P < 0.05)\) than in 55% \( \dot{V}O_{2\text{max}} \). At FU1, concentrations were not significantly different from BASE in any condition and there were no further changes in \(\beta\)-CTX thereafter.

There were no significant differences between baseline P1NP concentrations in the three conditions \((55% \dot{V}O_{2\text{max}}, 55 ± 26; 65% \dot{V}O_{2\text{max}}, 56 ± 28; 75% \dot{V}O_{2\text{max}}, 56 ± 22 \mu g/l; P = 0.998)\). There was no effect of exercise intensity on P1NP concentrations \((P = 0.477)\). Pooled, mean concentrations were significantly increased from BASE at EX20 \((P < 0.01)\), EX40 \((P < 0.001)\), and at EX60 \((P < 0.001)\), where concentrations were increased by 10–31% (Fig. 3B). P1NP concentrations decreased rapidly in recovery
and were not significantly different from BASE at R0.5 and there were no further changes thereafter.

There were no significant differences between baseline OC concentrations in the three conditions (55% \( \dot{V}O_{2\text{max}} \), 28.9 ± 9.3; 65% \( \dot{V}O_{2\text{max}} \), 28.2 ± 8.3; 75% \( \dot{V}O_{2\text{max}} \), 28.5 ± 7.8 µg/l; \( P = 0.983 \). There was no effect of exercise intensity on OC concentrations (condition \( \times \) time interaction, \( P = 0.515 \)). Pooled, mean concentrations were significantly decreased (\( P < 0.001 \)) by 5% at EX20 compared with BASE (Fig. 3C) although had returned back toward BASE values by EX40. Following exercise OC concentrations increased slightly up to R1.0 before decreasing again and being lower (\( P < 0.001 \)) than BASE at R3.0. Concentrations were not different from BASE at FU1 or thereafter.

There were no significant differences between baseline bone-ALP concentrations in the three conditions (55% \( \dot{V}O_{2\text{max}} \), 29 ± 10; 65% \( \dot{V}O_{2\text{max}} \), 28 ± 11; 75% \( \dot{V}O_{2\text{max}} \), 27 ± 9 U/l; \( P = 0.968 \). There was no effect of exercise intensity on bone-ALP concentrations (condition \( \times \) time interaction, \( P = 0.722 \)). Pooled, mean concentrations were not significantly different from BASE at FU1 and FU2 but were significantly increased from BASE at FU3 (\( P < 0.01 \)) and FU4 (\( P < 0.01 \)) by 1–7% (Fig. 4).

**OPG**

There were no significant differences between baseline OPG concentrations in the three conditions (\( P = 0.922 \)). There was no effect of exercise intensity on OPG concentrations (condition \( \times \) time interaction, \( P = 0.753 \)). Pooled, mean concentrations were significantly increased from BASE at EX20 (\( P < 0.001 \)) by 15–41% and remained increased throughout exercise and also during the first 3 h of recovery (\( P < 0.001 \)), where concentrations in the three conditions ranged from 107% to 122% of BASE values (Fig. 5). Concentrations were not different from BASE at FU1, FU2, or FU4 but were increased (\( P < 0.01 \)) at FU3 by 5–19%.

**Calcium Metabolism**

There were no significant differences between baseline PTH concentrations in the three conditions (\( P = 0.666 \)). Analysis of PTH showed a main effect of time (\( P < 0.001 \)) and a condition \( \times \) time interaction (\( P < 0.001 \)). There was no significant effect of exercise or recovery on PTH concentrations with exercise at 55% (Fig. 6A). At 65% \( \dot{V}O_{2\text{max}} \), PTH was unchanged during exercise but was significantly decreased from BASE between R1.0 and R3.0 (\( P < 0.05 \)), with concentrations reduced by 19–21% from BASE. At 75% \( \dot{V}O_{2\text{max}} \), there was a progressive increase in PTH during exercise, with concentrations significantly increased (\( P < 0.001 \)) from BASE at EX20 (48 ± 44%)

**Fig. 4.** Bone-ALP concentrations, expressed as a percentage of baseline (BASE) values on the 4 follow-up days (FU1–FU4) in the 55% (○), 65% (□), and 75% (■) \( \dot{V}O_{2\text{max}} \) conditions. Values are means ± SD.

**Fig. 5.** Osteoprotegerin (OPG) concentrations at baseline (BASE), during exercise (EX20–EX40), during 3 h of recovery (EX60–R3.0), and on 4 follow-up days (FU1–FU4) in the 55% (○), 65% (□), and 75% (■) \( \dot{V}O_{2\text{max}} \) conditions. Shaded box denotes exercise. Values are means ± SD.

**Fig. 6.** Parathyroid hormone (PTH) (A), albumin-adjusted calcium (ACa) (B), and PO4 (C) concentrations at baseline (BASE), during exercise (EX20–EX40), during 3 h of recovery (EX60–R3.0), and on 4 follow-up days (FU1–FU4) in the 55% (○), 65% (□), and 75% (■) \( \dot{V}O_{2\text{max}} \) conditions. Shaded box denotes exercise. Values are means ± SD. *75% \( \dot{V}O_{2\text{max}} \) different (\( P < 0.05 \)) from 55% \( \dot{V}O_{2\text{max}} \); †75% \( \dot{V}O_{2\text{max}} \) different (\( P < 0.001 \)) from 55% \( \dot{V}O_{2\text{max}} \); ‡75% \( \dot{V}O_{2\text{max}} \) different (\( P < 0.001 \)) from 65% \( \dot{V}O_{2\text{max}} \).
and EX40 (65 ± 56%). Concentrations at EX20 were higher (P < 0.05) than at 55% \( \dot{V}O_{2\text{max}} \), and at EX40, higher than at both 55% and 65% \( \dot{V}O_{2\text{max}} \) (P < 0.001). Peak concentrations (9.0 ± 4 pmol/l) occurred at EX60, when they were increased (P < 0.001) from BASE by 86 ± 47%, and higher than at 55% and 65% \( \dot{V}O_{2\text{max}} \) (P < 0.001). At R0.5, PTH concentrations were not significantly different from BASE, or from 55% and 65% \( \dot{V}O_{2\text{max}} \), but were decreased (P < 0.001) from BASE at R1.0 (18–30%), R2.0 (16–37%), and R3.0 (11–30%), with no significant differences between the three conditions. Concentrations were not significantly different from BASE at FU1 or thereafter in any condition.

There were no significant differences between baseline ACa concentrations in the three conditions (P = 0.896). There was no effect of exercise intensity on ACa concentrations (condition × time interaction, P = 0.585). Pooled, mean concentrations were significantly increased (P < 0.001) from BASE by 5% throughout exercise and remained increased (P < 0.001) at R0.5 through to R3.0 (Fig. 6B). Concentrations were not significantly different from BASE at FU1 or thereafter.

There were no significant differences between baseline PO4 concentrations in the three conditions (P = 0.972). There was no effect of exercise intensity on PO4 concentrations (condition × time interaction, P = 0.236). Pooled, mean concentrations were significantly increased from BASE by 15% at EX20 (P < 0.001) and remained so at EX40 (P < 0.001) and EX60 (P < 0.001) (Fig. 6C). Concentrations decreased rapidly in recovery and were lower (P < 0.001) than BASE between R1.0 and R3.0. The lowest PO4 concentrations occurred at R2.0 in all conditions. At this point, concentrations were decreased by 7% in the 55% and 65% \( \dot{V}O_{2\text{max}} \) conditions and 17% in the 75% \( \dot{V}O_{2\text{max}} \) condition compared with BASE. Concentrations were not significantly different from BASE at FU1 or thereafter.

**DISCUSSION**

\( \beta \)-CTX concentrations were not significantly increased from baseline with exercise at 75% \( \dot{V}O_{2\text{max}} \), but were significantly higher than at 55% and 65% \( \dot{V}O_{2\text{max}} \) in the first hour postexercise. From 2–3 h of recovery, at all exercise intensities, \( \beta \)-CTX concentrations were decreased by 39–42%. Zittermann et al. (75) observed a 45% reduction in \( \beta \)-CTX in a 4-h period (0830 to 1330) that included either a 60-min run at 70% of lactate threshold or no exercise. This reduction in \( \beta \)-CTX is comparable to that seen in our study (~40% decrease from 0800 to 1215 across all conditions) and is consistent with the circadian rhythm of serum \( \beta \)-CTX in fasted humans (8).

Only two previous studies have examined the effects of exercise intensity on bone turnover markers. Herrmann et al. (29) showed no increase in \( \beta \)-CTX 3 h after 60 min of cycling at 75% individual anaerobic threshold (IAT). Increases were observed 3 h after exercise at 95%, and at 3 and 24 h after exercise at 110% IAT. However, the time of day when exercise was performed and the nutritional status of the subjects were not standardized. Maimoun et al. (38) showed a 16% increase in \( \beta \)-CTX immediately after exercise at 115% but not 85% of ventilatory threshold (VT) that was no longer evident at 15 min postexercise. In the present study, \( \beta \)-CTX concentrations were 12–19% higher in the 75% \( \dot{V}O_{2\text{max}} \) condition compared with the 55% and 65% \( \dot{V}O_{2\text{max}} \) conditions in the first hour postexercise, which is comparable to that reported by Maimoun et al. (38), albeit over a longer time frame. However, as in the study of Maimoun et al. (38), the impact of exercise intensity on \( \beta \)-CTX concentrations in our study was both modest and transient.

At 75% \( \dot{V}O_{2\text{max}} \), \( \beta \)-CTX concentrations were 103% of baseline levels during the first hour postexercise. Guillemant et al. (25) used a similar exercise intensity (80% \( \dot{V}O_{2\text{max}} \)) and sampling period to the present study but showed an increase of 45–50% in \( \beta \)-CTX concentrations following 60 min of cycling. This difference in study findings might be explained by the mode of exercise, although a direct comparison of the effects of running and cycling on \( \beta \)-CTX has yet to be performed. Alternatively, Guillemant et al. (25) provided a standardized meal 3 h before exercise that would have suppressed preexercise \( \beta \)-CTX concentrations (28). In contrast to our study, by decreasing \( \beta \)-CTX to its natural daytime nadir before exercise, the meal provided by Guillemant et al. (25) may have allowed the full stimulatory effects of exercise to be evident.

\( \beta \)-CTX was not different from baseline on the recovery days in the present study, which is in contrast with previous results showing a significant increase in \( \beta \)-CTX for 4 days following exhaustive running (57) and at 3 days after the start of a 246-km run (33). One possible reason for the discrepancies in these findings is that the exercise performed in the present study was less strenuous (i.e., not exhaustive) and of shorter duration. As such, the interactions between exercise intensity and duration warrant further investigation in relation to the impact of weight-bearing exercise on bone resorption. Due to the modest and transient nature of the effect of exercise intensity on \( \beta \)-CTX observed in the present study and by Maimoun et al. (38), the clinical significance of these changes is unclear.

This is the first study to make frequent measures of PINP during and in recovery from acute exercise and indicates a transient increase in PINP during exercise, which tended to be greater during high-intensity exercise. Previous studies have shown no change in PINP concentrations immediately after 30 min of walking (62) or cycling at 95% VT (50), while Herrmann et al. (29) report a reduction in PINP at 3 h following 60 min of cycling at 75% IAT. Basal PINP concentrations decrease in the late morning (1), while the magnitude of the increase during exercise (10–41%) in our study exceeds the CV of the assay, together pointing to a genuine effect of exercise.

Whether the marked increase in PINP in our study reflects an increase in type 1 collagen formation in bone is unclear, as it occurs rapidly following the onset of exercise and appears to precede any effects of exercise on \( \beta \)-CTX. Several factors might affect concentrations of bone turnover markers during exercise. Although under resting conditions PINP is considered a specific marker of bone formation, PINP is also upregulated in both skeletal muscle (18) and tendon tissue (26, 42) with exercise. Thus we cannot completely exclude a contribution from these tissues to the increase in PINP during exercise. As PINP is cleared via scavenger liver receptors (40), the increase is unlikely to be explained by the accumulation of PINP in the circulation due to reduced renal function with exercise. However, clearance rates might have contributed to the different responses during exercise of PINP and OC, with the smaller OC molecule being cleared more rapidly by the kidney compared with the larger PINP molecule (40).
As only a brief period of loading produces an osteogenetic response, which quickly saturates (53, 54), and bone formation markers can respond rapidly to other physiological stimuli (15, 16), the possibility that the increase in P1NP in our study does indeed reflect new bone formation cannot at this stage be excluded.

Consistent with our previous study on acute exhaustive exercise (57), we did not show any effect of exercise on P1NP concentrations when measured from 1 to 4 days postexercise, although others have shown changes in P1NP concentrations at 24 h after acute endurance cycling (29).

The increase in pooled, mean bone-ALP at 3 and 4 days postexercise suggests an increase in bone mineralization with a single bout of running independent of exercise intensity. These results suggest that 60 min of running, similar to that performed with typical daily running training, can have a positive effect on bone mineralization in the days following exercise. However, this finding should be interpreted with caution as the magnitude of the change (1–7%) falls within the CV of the assay. Previous studies that have measured bone-ALP on the days following weight-bearing exercise report contrasting results with unchanged concentrations following walking (69) and strenuous running (10, 39, 57) and decreases following an ultraendurance run (44).

Other than a decrease during the first 20 min, the magnitude of which (5%) was within the CV for the assay, exercise did not alter OC concentrations. This is in contrast to previous studies of high-intensity endurance exercise (29, 38) and some (61) but not all (56, 62) studies of low- to moderate-intensity exercise. The decrease in daytime OC concentrations is associated with the nocturnal increase in cortisol that precedes it (30, 45), although we observed no decrease in OC with exercise at 75% VO_{2max} despite higher cortisol concentrations compared with 55% and 65% VO_{2max}. These findings suggest that, unlike at rest, acute changes in cortisol during exercise do not influence OC concentrations or that any effect is overridden by exercise intensity, which necessitates further study.

This is the first study to examine the effect of exercise intensity on serum (s)OPG concentrations and shows no effect of increasing cardiovascular intensity from 55% to 75% VO_{2max}. Basal sOPG concentrations do increase in the late morning (34) but as the magnitude (15–41%) of the increase in our study was greater than the CV of the assay and occurred over a short time scale (BASE to EX20 = 35 min), this suggests that much of the increase in sOPG is a genuine response to exercise. The increase in sOPG with exercise is in line with our previous investigation (57) and with other studies of endurance (33, 73) and resistance (12) exercise. Previously we showed an increase in sOPG with acute, exhaustive running that was sustained for 2 h postexercise (57), with sOPG increased 25% after 60 min running at 65% VO_{2max}, which corresponds to the magnitude of the increase (23%) seen in the 65% VO_{2max} condition in the present study.

Increased sOPG concentrations associated with bone loss have been interpreted as a compensatory response to increased bone resorption (43, 72). In the present study, the increase in sOPG during and after exercise was of a similar magnitude in all conditions, which is in contrast to the effect, albeit a modest one, of exercise intensity on β-CTX responses. It is difficult to interpret the impact of altered sOPG concentrations on bone (59) during exercise without a measure of RANKL. Previous studies have shown that RANKL is undetectable in 70% of healthy controls (27) and 55% of postmenopausal women (41), making the true RANKL response to exercise impossible to determine with current assay technology. The interpretation of sOPG in relation to bone metabolism is further complicated by its production by a range of cells other than osteoblasts including endothelial and smooth muscle cells (17). As such, we cannot exclude a possible contribution of these cells to the increase in sOPG as part of an acute, inflammatory response to exercise-induced muscle damage (48).

PTH concentrations were only increased in the 75% VO_{2max} condition, suggesting that cardiovascular exercise intensity influenced the PTH response to exercise. This supports findings from Maumoun et al. (38) who reported increased PTH at 115% but not 85% VT, although others have shown increases in PTH with exercise conducted at much lower intensities (4). Basal PTH concentrations decrease in the late morning (1), and the magnitude (48–86%) of the increase during exercise in our study exceeds the CV of the assay, together pointing to a genuine effect of exercise.

The increase in PTH with exercise at 75% VO_{2max} was transient, peaking at the end of exercise and rapidly returning to baseline values thereafter. Sustained elevations in PTH are normally associated with increased bone resorption (2) whereas daily injections that produce transient “spikes” in PTH are associated with bone formation (51). Despite the potentially anabolic profile of PTH in the 75% VO_{2max} condition, there were no discernable effects of exercise intensity on bone formation markers. However, the increase in PTH during exercise preceded the significantly higher β-CTX concentrations at 75% VO_{2max} compared with 55% and 65% VO_{2max} and the apparent lag of 30–60 min is similar to that seen with an EDTA infusion (74). Although the effect of exercise intensity on β-CTX was modest, this might suggest that changes in PTH may be mediating some of the β-CTX response.

The increase in PTH concentrations with exercise at 75% VO_{2max} was not mediated by calcium concentrations or increased PO_{4}, as ACa concentrations were increased and the magnitude of the increase in PO_{4} was not significantly different from that in the 55% and 65% VO_{2max} conditions. Metabolic acidosis can stimulate PTH concentrations (36), although we did not measure blood pH. However, previous studies (47) report no decrease in blood pH during endurance exercise with lactate levels greater (4–5 mmol/l) than those observed in our study. Metabolic acidosis, therefore, is unlikely to explain the increase in PTH in the 75% VO_{2max} condition.

A limitation of our study is that increasing cardiovascular exercise intensity during treadmill running, by increasing running speed, will likely increase absolute mechanical strains on the skeleton. Therefore, we cannot rule out an independent effect of increasing mechanical strain in our findings. However, increasing cardiovascular exercise intensity during other common forms of non-weight-bearing endurance exercise, such as cycling, rowing, and swimming, will likely have the same effect, although mediated mainly through increased muscular activity. Standardizing the mechanical stresses during endurance running would require the consideration of both the magnitude and the number of loads and would result in a markedly different type of study, which would likely vary considerably between subjects in terms of exercise duration and cardiovascular intensity. The resulting exercise sessions...
might also bear little resemblance to a typical endurance-training session.

In conclusion, increasing cardiovascular exercise intensity from 55% to 75% \( \dot{V}O_{2\text{max}} \) during 60 min of running resulted in higher \( \beta\)-CTX concentrations, but only in the first hour postexercise, and had no effect on bone formation markers in recreationally active men. Bone-ALP concentrations increased at 3 and 4 days postexercise, suggesting a beneficial effect of a single bout of this type of exercise on bone mineralization. Running results in a rapid increase in sOPG concentrations, the magnitude of which is not influenced by exercise intensity. PTH concentrations were transiently increased at 75% \( \dot{V}O_{2\text{max}} \) only, which cannot be explained in this instance by changes in serum calcium or \( PO_4 \) concentrations.

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**REFERENCES**


EXERCISE INTENSITY AND BONE METABOLISM


