Title: Effect of fasting versus feeding on the bone metabolic response to running

Authors: Jonathan PR Scott[a*], Craig Sale[b], Julie P Greeves[c], Anna Casey[a], John Dutton[d], William D Fraser[e]

Affiliations

[a] Human Sciences, QinetiQ Ltd., Farnborough, UK;

[b] Biomedical, Life and Health Sciences Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK; Department of Occupational Medicine;

[c] HQ Army Recruiting and Training Division, Upavon, UK;

[d] Department of Musculoskeletal Biology, University of Liverpool, Liverpool, UK;

[e] Norwich Medical School, University of East Anglia, Norwich, UK.

* **Corresponding author:** Jonathan PR Scott, Room 2022, Building A5, QinetiQ Ltd., Cody Technology Park, Ively Road, Farnborough, Hampshire, UK, GU14 0LX; Tel: +44 (0)1252 519591; Fax +44 (0)1252 519591; Email: jscott1@qinetiq.com.

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Abbreviations: ACa, albumin-adjusted calcium; Bone ALP, bone alkaline phosphatase; BMD, bone mineral density; β-CTX, C-terminal telopeptide region of collagen type 1; CHO, carbohydrate; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GIP, glucose-dependent insulinotropic polypeptide; LMM, linear mixed model; OC, osteocalcin; OPG, osteoprotegerin; P1NP, N-terminal propeptides of procollagen type 1; PTH, parathyroid hormone; PO_4, phosphate; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; VO_2, oxygen uptake; VO_{2max}, maximal rate of oxygen uptake.

ABSTRACT

Individuals often perform exercise in the fasted state, but the effects on bone metabolism are not currently known. We compared the effect of an overnight fast with feeding a mixed meal on the bone metabolic response to treadmill running. Ten, physically-active males aged 28 ± 4 y (mean ± 1SD) completed two, counterbalanced, 8 d trials. After 3 d on a standardised diet, participants performed 60 min of treadmill running at 65% VO_{2max} on Day 4 following an overnight fast (FAST) or a standardised breakfast (FED). Blood samples were collected at baseline, before and during exercise, for 3h after exercise, and on four consecutive follow-up days (FU1–FU4). Plasma/serum were analysed for the c-terminal telopeptide region of collagen type 1 (β-CTX), n-terminal propeptides of procollagen type 1 (P1NP), osteocalcin (OC), bone alkaline phosphatase (bone ALP), parathyroid hormone (PTH), albumin-adjusted calcium, phosphate, osteoprotegerin (OPG), cortisol, leptin and ghrelin. Only the β-CTX response was significantly affected by feeding. Pre-exercise concentrations decreased more in FED compared with FAST (47% *vs* 26%, $P < 0.001$) but increased during exercise in both groups and were not significantly different from baseline at 1 h post-exercise. At 3 h post-exercise, concentrations were decreased (33%, $P < 0.001$) from baseline in FAST and significantly lower ($P < 0.001$) than in FED. P1NP and PTH increased, and OC decreased during exercise. Bone markers were not significantly different from baseline on FU1–FU4. Fasting had only a minor effect on the bone metabolic response to subsequent acute, endurance exercise, reducing the duration of the increase in β-CTX during early recovery, but having no effect on changes in bone formation markers. The reduced duration of the β-CTX response with fasting was not fully explained by changes in PTH, OPG, leptin or ghrelin.

Key words: fasting; feeding; endurance running; bone resorption; parathyroid hormone.

1.0 INTRODUCTION

1.1 Feeding, specifically the energy content and macronutrient composition of meals, modulates the metabolic responses to prolonged exercise [1]. Provision of carbohydrate before and during exercise is essential to maintain glucose availability to the contracting skeletal muscle [2–4], whilst fat ingestion [5] or intravenous infusion with fatty acids [6,7] stimulates fat oxidation and reduces carbohydrate utilisation, offering potentially beneficial adaptations for the endurance athlete or athletic individual. To promote the same response, endurance athletes exercise in the fasted state [1], which increases lipolysis in adipose tissue and subsequent fat oxidation [8], and promotes an adaptation to fat metabolism. The potential for increased fat oxidation, might also make this practice worthy of consideration for the purposes of weight management [9].

1.2 The adaptations to fuel supply and oxidation with fasting are well studied, however, there is little consideration of the effect of these practices on other biological responses to exercise and training, including bone metabolism. Fasting attenuates the circadian variation in the C-terminal telopeptide region of collagen type 1 (β-CTX), a specific marker of bone resorption, while feeding suppresses resting β-CTX within 1 h and, after 2–3 h, concentrations are decreased by ~50% [10–13]. In contrast, bone formation is largely unresponsive to nutrient ingestion [10–12,14].

1.3 Prolonged, endurance exercise increases β-CTX [15–19] but again, bone formation markers are largely unresponsive [15,16,18,19], suggesting that prolonged exercise might result in a transient negative remodelling balance. This acute response may be of significance to athletic populations as the net effects of changes in bone resorption and formation have

been implicated in both stress fractures [20] and changes in bone mineral density (BMD) [21]. Additionally, the acute response of bone markers to exercise has been reported with both running [19] and cycling [15], and reduced spinal BMD (running and cycling) and an increased incidence of stress fractures (running) have been observed in some groups who regularly perform both types of activity [22–26].

1.4 Most studies of responses to endurance exercise are conducted after either an overnight fast or in the post-absorptive state, but it remains unknown how these practices mediate the effects of exercise on bone metabolism. Calcium ingestion has been shown to attenuate the increase in β-CTX with subsequent endurance cycling [15] but, to date, only one study has compared the effect of fasting and feeding [27]. This study reported no effect of a liquid replacement meal on responses to resistance or plyometric exercise, although the choice of bone markers was surprising [28], as only tartrate-resistant acid phosphatase 5b (TRAP5b), which mainly reflects prevailing osteoclast number [29], and bone alkaline phosphatase (ALP), which does not to respond to feeding [14], were measured. The exclusion of β-CTX was also surprising, as it is reported to be highly responsive to nutrient ingestion [10,11,14]. In addition, bone markers levels are reported to vary between individuals who participate in different types of activity [see Ref. 30], which might indicate that changes in bone markers with acute exercise might also differ with exercise type itself. As such, responses to acute endurance exercise cannot necessarily be inferred from studies of other forms of physical activity.

1.5 Several biochemical responses may link feeding, acute exercise and bone metabolism. Changes in parathyroid hormone (PTH) might be one such link, with concentrations being decreased following nutrient ingestion [13] and increased promptly with endurance exercise

[15,19,31]. Similarly, both leptin and ghrelin concentrations change markedly following feeding, with ghrelin decreasing [32] and leptin increasing [33], and both respond to exercise [34,35]. These hormones both play a role in the regulation of bone metabolism [36,37], and, in the case of leptin, might also be involved in a pathway linking bone metabolism and energy metabolism homeostasis [38].

1.6 Understanding the bone metabolic response to exercise following fasting and feeding is important, since the practice of periodic fasting, be it for training, health or indeed cultural or religious purposes [39], might influence the risk of skeletal injury and longer-term bone health. As such, we aimed to investigate the effect of an overnight fast versus feeding of a single mixed meal, on the bone metabolic response to an acute bout of treadmill running.

2.1 Methods

2.2 Participants

Ten physically-active men (age: 28 ± 4 y, height: 1.78 ± 0.06 m, body mass: 82.3 ± 6.4 kg, maximal rate of oxygen uptake [VO_{2max}]: 52.2 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$, mean ± 1SD) were studied. All participants were in good physical condition, with a history of weight-bearing exercise and average to above average levels of fitness. The group was a mixture of team sports players and recreational runners, and included one 'club' level runner and one recreational mountaineer. All participants performed at least bout of endurance running per week. Health screening and a medical examination confirmed that participants were non-smokers, had not suffered a fracture in the previous 12 months, were free from musculoskeletal injury and did not suffer from any condition known to affect bone metabolism. Additional inclusion criteria were a fasting vitamin D concentration of >30 $nmol \cdot L^{-1}$ and/or a fasting PTH concentration of <6.9 $pmol \cdot L^{-1}$. All participants provided written informed consent and the study was approved by the QinetiQ Research Ethics Committee.

2.3 Design

Participants completed two preliminary visits for medical screening, habituation and measurement of VO_{2max}. They then completed two, counterbalanced, 8 d experimental protocols separated by at least one week. On Days 1 to 3 (D1–D3) of each protocol, participants refrained from physical activity and ate a prescribed diet. At 10:15 on Day 4 (D4), participants performed 60 min of treadmill running at 65% VO_{2max} after a standardised breakfast at 08:15 (FED) or no breakfast (FAST), followed by 3 h recovery. On Days 5 to 8 (D5–D8) participants continued to follow a controlled diet and refrain from physical activity,

and attended the laboratory at 07:45 for follow-up analysis.

2.4 Pre-Trial Measurements

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

2.4.2 Assessment of cardiorespiratory responses and aerobic power: To establish the association between oxygen uptake (VO_2) and running velocity, participants completed a 20 min submaximal run on a treadmill (XELG 70 ERGO, Woodway, USA), 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, VO_{2max} was determined using a discontinuous, incremental exercise test to exhaustion using a modified Taylor protocol [40]. Participants completed a series of 3 min runs separated by 5 min rest periods. The treadmill gradient was increased by 2.5 % with each subsequent stage until volitional exhaustion was reached. Sixty second samples of expired air were collected (Douglas bag technique) in the final minute of each stage (1 min 45 sec to 2 min 45 sec) and during the final 30 sec of the last stage. Heart rate was monitored continuously throughout the test. In addition to volitional exhaustion, the criteria for attaining VO_{2max} were an increase in oxygen uptake (VO_2) of less than 0.15 $L \cdot min^{-1}$ with an increase in exercise intensity, a respiratory exchange ratio (RER) of >1.05, a heart rate of 220 – age (± 10 $beats \cdot min^{-1}$) and a blood lactate concentration at 1–2 min post-exercise of > 9 $mmol \cdot L^{-1}$. The results of the two tests were used to estimate the treadmill velocity corresponding to 50% and 65% VO_{2max} during flat running based on the regression line of VO_2 and velocity.

2.4.3 Experimental dietary provision: A diet consisting of 55% carbohydrate (CHO), 30% fat and 15% protein and isocaloric with habitual diet was designed for each participant. Three menus were provided and administered in a three-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. During the experimental period, with the exception of Day 4, participants provided their own food but were given instructions concerning the quantity, preparation and timing of meals.

2.5 Trial Procedures

2.5.1 Day 4: Following an overnight fast, participants arrived at 07:30, voided and had their nude body mass measured. Subsequently, participants adopted a semi-recumbent position and a cannula was inserted into a vein in the forearm, where it remained until after the final blood sample. A baseline blood sample was collected at 08:00. In FED, but not FAST, participants received a standardised breakfast (2.3 MJ, 60% CHO, 32% fat, 8% protein, 116 mg calcium) at 08:15.

Exercise bouts commenced at 10:30 and consisted of 60 min of treadmill running at 65% VO_{2max}. Expired air (for the determination of VO_2 and RER and ratings of perceived exertion (RPE) were collected at 18, 38 and 58 min. Heart rate was recorded continuously. On completion of exercise, nude body mass was measured and participants rested in a semi-recumbent position on a bed for a further 3 h consuming 1.5 mL of water per gram of body mass lost during exercise. Blood samples were collected at identical time points in the two conditions; at 08:00 (BASE), 09:00, 09:30, and 10:15 (Pre-exercise; PRE), after 30 (EX30) and 60 (EX60) min of exercise, and after 1 h (R1.0), 2 h (R2.0) and 3 h (R3.0) of recovery.

Participants consumed a standardised diet (13.2 MJ, 53% CHO, 32% fat, 15 % protein, 767 mg calcium), which was given as four meals (08:15 [the standardised breakfast], 14:30, 17:30 and 20:00) in FED and as three meals in FAST (14:30, 17:30 and 20:00). Only water was allowed after 21:00.

2.5.2 Days 5 to 8 (FU1–FU4): Participants attended the laboratory at 07:45 following an overnight fast, voided, had their nude body mass measured and a blood sample at 08:00. Only water was allowed after 21:00 on the days prior to laboratory visits (Day 5–7).

2.5.3 Metabolic measurements

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

2.5.4 Treatment and storage of blood samples

For measurement of β-CTX, n-terminal propeptides of procollagen type 1 (P1NP), N mid-terminal osteocalcin (OC), osteoprotegerin (OPG) and cortisol, blood was transferred into pre-cooled tubes containing 15%, 0.12 ml of K_3E EDTA (Becton Dickinson Vacutainer System, USA) generating plasma. Tubes were gently inverted 8–10 times and centrifuged immediately at 2000 rpm and 5°C for 10 min. Plasma was separated and stored at -70°C until analysis. For measurement of bone ALP, PTH, albumin-adjusted calcium (ACa), phosphate (PO_4), leptin and ghrelin, blood was transferred into pre-cooled standard tubes

(Becton Dickinson Vacutainer System, USA) and left to clot at room temperature for 60 min generating serum. Tubes were subsequently centrifuged at 2000 rpm and 5°C for 10 min and serum was separated and stored at -70°C until analysis. For measurement of glucose and lactate, whole blood was transferred into pre-cooled tubes containing fluoride-oxalate generating plasma. Tubes were gently inverted 8–10 times and analysed in duplicate immediately (Yellow Springs Instruments, 2300 Stat Plus; YSI Inc., Yellow Springs, USA).

2.5.5 Biochemical Analysis

β-CTX was measured using an electrochemiluminescent immunoassay (ECLIA) on an Elecsys 2010 immunoanalyser (Roche, Lewes, UK). Inter-assay coefficient of variation (CV) was <8% from 0.2–1.5 $ug \cdot L^{-1}$. The assay sensitivity (replicates of the zero standard) was 0.01 $ug \cdot L^{-1}$. P1NP was measured by radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland). Assay sensitivity was 4 $ug \cdot L^{-1}$ established from precision profiles (22% CV of duplicates) and an inter-assay CV of 3.5–5.4% across the concentration range 10–250 $ug \cdot L^{-1}$. N-MID OC was measured using ECLIA on a Modular Analytics E170 analyser (Roche Diagnostics, Lewes, UK). Inter/intra assay CVs were <5% from 2–200 $ug \cdot L^{-1}$. Assay sensitivity (replicates of the zero standard) was 0.6 $ug \cdot L^{-1}$. Bone ALP was measured using an enzyme immunometric assay (EIA) (Metra, Biosystems, Oxford, UK) with a sensitivity of 0.7 $U \cdot L^{-1}$ and a CV of <8% across the range 12–100 $U \cdot L^{-1}$. Cortisol was measured using ECLIA on a Roche Modular E170. The assay has a sensitivity of 8 $nmol \cdot L^{-1}$ established from precision profiles (22% CV of duplicates) and a CV of <6% across the range 16–1750 $nmol \cdot L^{-1}$. OPG was measured using a solid phase enzyme linked immunosorbent assay (ELISA) that measures free OPG and that complexed with receptor activator of NF-kB ligand (RANKL) (IDS Boldon, Tyne and Wear, UK). The assay has a detection limit of 0.14 $pmol \cdot L^{-1}$ and inter/intra assay CVs of <10% across the range 1–30 $pmol \cdot L^{-1}$.

PTH was measured using an EIA (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, Lewes, UK) performed on a Roche Modular Analytical System. 25 OH vitamin D_2 and 25 OH vitamin D_3 were quantified after extraction using straight phase high performance liquid chromatography (HPLC) employing 1,5 trans vitamin D_3 as an internal standard. The lower detection limit for both 25 OH vitamin D molecules is 2 $ng \cdot mL^{-1}$ and upper assay limit is 100 $ng \cdot mL^{-1}$. Intra-assay CV is less than 10% and inter-assay CV less than 12% across the concentration range 10–40 $ng \cdot mL^{-1}$.

Leptin was measured using an EIA (IDS Boldon, Tyne and Wear, UK). The assay has a sensitivity of 16 $ng \cdot mL^{-1}$ established from precision profiles (22% CV of duplicates) and a CV of <8% across the range 30–500 $ng \cdot mL^{-1}$. Total (acylated and de-acylated) ghrelin was measured using an RIA (Phoenix Pharmaceuticals, Belmont, CA). Inter/intra assay CVs were less than 10% across the working range of the assay. Blood glucose and lactate were analysed automatically using the Yellow Springs Instruments, 2300 Stat Plus (YSI Ltd, UK).

Concentrations of analytes were not corrected for changes in plasma volume associated with exercise.

2.5.5 Statistical Analysis

All data are presented as mean ± 1SD and statistical significance was accepted at $P < 0.05$. Paired samples t-tests were used to compare habitual and experimental dietary data, and baseline biochemical data and variables relating to exercise performance in the two experimental conditions. Body mass and biochemical data were analysed using a linear, mixed model (LMM), with factors 'condition' and 'time point' as fixed effects and 'participants' as a random, within-group effect. The assumptions of the LMM (homogeneity of variance and normality) were investigated by examining the distribution of residuals and the pattern of residuals *versus* fitted values. Where non-normality or non-constant variance was observed, a transformation was applied to the data so that the assumptions were satisfied. Body mass, bone ALP, glucose, ACa and PO_4 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* x *Time* interaction, each subsequent time point was compared against BASE using a pooled mean from the two groups using Dunnett's test, with BASE as the 'Control'. When the *Condition* x *Time* interaction was significant, within each group, each time point was compared against BASE using Dunnett's test with BASE as the 'Control' and groups were compared to each other at all time points using the Student Newman-Keuls (SNK) test.

3.0 RESULTS

3.1 Baseline biochemical data for the FAST and FED conditions, and a summary of the LMM outcomes are shown in Table 1.

3.2 Dietary analysis and experimental dietary provision

Compared with habitual intake, experimental diets provided more energy (10.5 ± 2.5 *vs* 10.2 ± 2.8 MJ; $P < 0.05$), a greater quantity of carbohydrate (CHO) relative to fat-free mass (363 ± 83 *vs* 337 ± 97 g CHO·kg FFM^{-1}; $P < 0.05$) and a smaller quantity of protein (16.0 % *vs* 18.7%, $P < 0.05$), but there were no other significant differences. Body mass did not change from Day 4 to Day 8 in either experimental condition (FED, 80.7 ± 8.5 to 80.5 ± 8.6 kg; FAST, 81.0 ± 8.5 to 80.5 ± 8.4 kg, $P = 0.275$).

3.3 Exercise variables

There was no difference in VO_2, lactate concentrations or RPE between the FED and FAST Conditions (Table 2). Heart rate (percentage of maximum heart rate [HR_{max}]), was lower in FAST compared with FED (79 ± 8 *vs* 84 ± 5%, $P < 0.001$), as was RER (0.881 ± 0.033 *vs* 0.920 ± 0.042, $P < 0.001$).

3.4 Glucose and cortisol

3.4.1 Glucose: In FED, glucose was increased ($P < 0.001$) from BASE at 09:00 only (Fig. 1, Panel A). In FAST, glucose concentrations were not significantly different from BASE before or during exercise, but were lower ($P < 0.05$) at R3.0. Compared with FED, concentrations in FAST were lower ($P < 0.001$) at 09:00 and higher ($P < 0.05$) at the end of exercise.

3.4.2 Cortisol: Pooled, mean concentrations were lower than BASE at 09:00 ($P < 0.05$), 09:30 ($P < 0.001$) and PRE ($P < 0.001$), where they were decreased approximately 30% from BASE (Fig. 1, Panel B). Cortisol increased during exercise and was not significantly different from BASE at EX30 and EX60. Concentrations decreased thereafter and were lower than BASE at R1.0 ($P < 0.01$), R2.0 ($P < 0.001$) and R3.0 ($P < 0.001$), but were not different from BASE at FU1–FU4.

3.5 Bone turnover markers

3.5.1 β-CTX: In FED, concentrations were lower than BASE at 09:00 ($P < 0.05$), 09:30 ($P < 0.001$) and at PRE (-47%, $P < 0.001$) (Fig. 2, Panel A). Concentrations increased during exercise and by 1 h post-exercise (R1.0), were not significantly different from BASE, having increased 86 ± 40% from PRE. Concentrations were not significantly different from BASE thereafter. In FAST, β-CTX concentrations were decreased at PRE (-26%, $P < 0.001$), and were higher than in FED at 09:30 and at PRE ($P < 0.001$). Concentrations remained higher than in FED at EX30 ($P < 0.001$) and EX60 ($P < 0.05$), but were no longer significantly different from FED or BASE at R1.0 (increased 22 ± 18% from PRE). In contrast to FED, concentrations subsequently decreased and were lower than BASE at R2.0 ($P < 0.05$), and both BASE (-33%, $P < 0.001$) and FED ($P < 0.001$) at R3.0. However, as with FED, concentrations were not significantly different from BASE at FU1–FU4.

3.5.2 P1NP: Pooled, mean P1NP concentrations were unchanged prior to exercise, were increased by 20% from BASE during exercise ($P < 0.001$), but were not significantly different from BASE at R1.0 (Fig. 2, Panel B). Concentrations were increased ($P < 0.05$) at R3.0, but were not different from BASE at FU1–FU4.

3.5.3 OC: Pooled, mean concentrations were significantly lower than BASE at 09:30 and PRE ($P < 0.01$) and remained lower ($P < 0.001$) at EX30, but then increased and were not significantly different from BASE at EX60 or thereafter (Fig. 2, Panel C).

3.5.4 Bone ALP: There was no effect of either experimental condition on bone ALP concentrations ($P = 0.222$, data not shown).

3.6 OPG

Pooled, mean OPG concentrations were unchanged at 09:00 and 09:30, but were significantly increased ($P < 0.01$) from BASE at PRE (FED, 11%; FAST, 33%) (Fig. 3). OPG remained increased from BASE during exercise and the first 3 h of recovery ($P < 0.001$), but not at FU1–FU4.

3.7 Calcium Metabolism

3.7.1 PTH: Pooled, mean PTH concentrations were lower ($P < 0.05$) than BASE at 09:00 but not at 09:30 or PRE (Fig. 4, Panel A). Concentrations increased during exercise and at EX60, were 87% and 71% higher ($P < 0.001$) in FAST and FED compared with BASE. PTH was not different from BASE in the first 3 h of recovery, but was higher ($P < 0.05$) than BASE at FU1, although not thereafter.

3.7.2 ACa: There was no significant effect of either experimental condition on ACa concentrations ($P = 0.387$) (Fig. 4, Panel B).

3.7.3 *PO_4*: Pooled, mean PO_4 concentrations were lower ($P < 0.01$) than BASE at 09:30 and PRE, but increased during exercise and were higher ($P < 0.001$) than BASE at EX30 and at EX60 (27% and 22% from BASE in FED and FAST) (Fig. 4, Panel C). Concentrations were not significantly different from BASE at R1.0 or thereafter.

3.8 *Leptin and Ghrelin*

3.8.1 *Leptin*: Leptin was undetectable in 3 participants. Pooled, mean concentrations were unchanged before and during exercise, but were lower than BASE at R1.0 ($P < 0.01$), R2.0 ($P < 0.05$) and R3.0 ($P < 0.01$) (Fig. 5, Panel A). Concentrations were not significantly different from BASE at FU1–FU4.

3.8.2 *Ghrelin*: Despite the significant main effect of time, pooled, mean concentrations were not significantly different from BASE at any time point (Fig. 5, Panel B).

4.0 DISCUSSION

4.1 Individuals may exercise in the fasted state whether out of choice or for training, health, cultural or religious reasons. To date, the effects of exercise on bone metabolism have been examined with participants in both fasted and post-absorptive states, which might, in part, explain the wide range of responses reported. For these reasons, we compared the effects of an overnight fast *versus* feeding on the bone metabolic response to acute exercise. Prior to exercise, feeding reduced β-CTX compared with fasting, but this difference was not maintained during exercise, with β-CTX increasing with both feeding (86%) and fasting (22%), resulting in similar concentrations at 1 h post-exercise. Only with feeding was this increase sustained, however, resulting in lower concentrations with fasting at 3 h post-exercise, although not thereafter. With no difference in changes in markers of bone formation, fasting appeared to have only a minor effect on bone turnover with acute exercise. These results are, however, suggestive of a novel interaction between feeding, exercise and bone resorption that warrants further study. These findings also suggest that pre-exercise nutritional status and the timing of the baseline sample might be important factors in the interpretation of the effects of acute, endurance exercise on β-CTX.

4.2 Similar to previous studies [10,14], we showed an effect of a single meal on resting β-CTX. Calcium ingestion also decreases resting β-CTX [41,42], although the mechanism for this effect is different, since changes in PTH do not explain the reduction in β-CTX following macronutrient ingestion [13]. Our meal contained 116 mg of calcium so we cannot exclude an effect on resting β-CTX, although this was only one third of the quantity (344 mg) shown to suppress both PTH and β-CTX [41] and we showed no significant effect of feeding on PTH. This is in contrast to Rogers et al. [27], although their liquid replacement meal contained 500 mg of calcium, which would be sufficient to decrease β-CTX and PTH

[41,42], making it difficult to directly link the effects of feeding itself to subsequent changes with exercise.

4.3 In the current study, PTH increased before β-CTX during exercise, suggesting that changes in PTH might partly mediate the β-CTX response. The relative increase in PTH in our study (70–90%) was similar to that shown during exercise with calcium ingestion [15], where there was no increase in β-CTX. However, peak PTH (7.5 $pmol \cdot L^{-1}$) was higher than reported by Guillemant *et al.* [15], with (3.8 $pmol \cdot L^{-1}$) and without (6.6 $pmol \cdot L^{-1}$) calcium ingestion, indicating that the increase in β-CTX with exercise might be dependent on absolute PTH concentration. It is possible that the failure of a mixed meal to prevent the subsequent increase in β-CTX with exercise in FED was the result of the stimulatory effect of PTH on β-CTX overriding the effect of feeding. Differences in the β-CTX response between studies might also be explained by different ingestion schedules, as Barry *et al.* [43] report that, despite attenuating the PTH response, pre-exercise calcium ingestion alone did not affect the increase in β-CTX with exercise.

4.4 Nutrient ingestion changes the circulating concentrations of intestinal peptides, pancreatic beta cell peptides and members of the neuropeptide Y family, which could influence bone metabolism [44]. Although not measured in the current study, changes in these hormones are unlikely to explain the more sustained increase in β-CTX in FED, as their responses to feeding favour decreased bone resorption [11,12]. In addition, the available evidence relating to the responses of these hormones to exercise, suggests either no change [45] or a similar direction of change to that with feeding [46,47]. Changes in cortisol, PTH, leptin and ghrelin were also not significantly different between the two conditions.

4.5 No effect of feeding on OC is consistent with previous studies [10–12], although others report decreases following the ingestion of glucose [13] or a meal [14]. Similar decreases in P1NP occur after a meal [14] and glucose [13] ingestion, which may explain why P1NP was approximately 10% lower in FED at 10:15. Consistent with a previous study [48], OC increased during exercise in FED and FAST despite increased cortisol, which has been associated with a decrease in OC at rest [48,49], suggesting that changes in OC during exercise are not mediated by cortisol. Although P1NP is unchanged with walking [50] and cycling [51], the transient increase in P1NP during running also confirms previous findings [31]. Whether increased P1NP reflects an increase in type 1 collagen formation in bone is unclear, since P1NP is also up-regulated in muscle [52] and tendon [53] with exercise. Clearance rates might contribute to changes during exercise, with slower clearance of the larger P1NP molecule by the liver [54] compared with the smaller OC molecule by the kidney. However, only a brief period of loading is required for an osteogenic response [55], so it is possible that increased P1NP does reflect bone formation. Bone resorption is expected to precede bone formation, although we have shown the opposite. This might be explained by exercise initiating the osteogenic response, with P1NP reflecting the formation, and β-CTX the degradation of the collagen matrix, and the subsequent bone resorption being initiated by osteoclasts via RANKL signalling from osteoblasts.

4.6 We showed no effect of feeding on OPG, but others report decreases following glucose ingestion [56], although this is likely due to hyperinsulinemia rather than hyperglycaemia itself [57]. Thus, glucose might be a more potent suppressor of OPG. Increased OPG has been reported with running [17,19,31,58], but in the present study, OPG was increased before exercise and remained so into recovery, suggesting that OPG was not related to the different β-CTX responses. If RANKL were to increase, a greater relative

increase in β-CTX could result. However, RANKL is undetectable in at least 50% of healthy adult controls [59], meaning that information relating to the true RANKL response to exercise is unavailable.

4.7 With similar changes in bone formation markers, but differing β-CTX responses, our data indicates distinctive changes in bone metabolism with exercise following feeding and fasting. The more prolonged increase in β-CTX in FED might suggest a less favourable response to exercise. Alternatively, we cannot rule out the possibility that the increase in β-CTX with acute running is actually the early phase of a PTH-mediated, longer-term anabolic response [60], which went undetected in bone formation markers due to a short follow-up period. The significance of this difference in β-CTX responses is uncertain, however, as it was short lived and no longer evident the following morning or up to 4 d post-exercise.

4.8 Participants exercised for 60 min at 65% VO_{2max}, which is representative of daily exercise undertaken by many individuals and by those who exercise as part of their occupation (*e.g.* military recruits). Previously, we [19] have shown that exhaustive running in the fasted state increases bone resorption from 1–4 d post-exercise although, as in the present investigation, a further study [31] suggested no effect of more moderate (60 min of running at up to 75% VO_{2max}) exercise over the same period. As such, whilst the current study suggests that fasting does not affect the response of bone to exercise of moderate intensity and duration, how this practice affects more prolonged and strenuous exercise remains unknown. Thus, future studies incorporating longer follow up periods are required to better understand the significance of the transient differences in bone metabolism to feeding and fasting shown here. In addition, these findings might be particular to endurance running. Although, no previous studies have directly compared the response of bone markers to

different forms of exercise, basal bone marker levels reportedly vary between sports [30], suggesting type-specific responses to exercise. One previous study [27] suggests no effect of fasting on responses to plyometric exercise, but different bone markers were measured so comparisons cannot be drawn. As such, our findings should be confirmed with different types of physical activity.

4.9 There are several limitations to our study. Firstly, a non-exercising control condition was not included in this study, so it is not possible to confirm that observed changes were a result of exercise alone and not, in part, due to other factors such as circadian rhythms. However, the primary purpose of this study was to establish the impact of fasting prior to exercise on the response of bone. As such, in this instance we would consider the FED group to represent a control condition (*i.e.* normal practice) with fasting as the intervention. Additionally, should we have included non-exercising controls, for thoroughness, we would have to have included two groups: one where participants were fed and one where they remained fasted. This would have been beyond the scope of the current investigation due to the prohibitive doubling of the cost of biochemical analysis and the time commitment required from our volunteers. A further limitation is the prescription of exercise as a percentage of VO_{2max}. Whilst this method attempted to standardise the cardiovascular intensity of exercise, it did not control for the total mechanical strain on bone, which would likely have varied markedly between participants due to differences in factors such as bone shape and size, body mass and running speed. Although total mechanical strain would have been near identical between the two experimental conditions, differences between participants may have introduced increased variability into our data.

4.10 In conclusion, fasting had only a minor effect on the bone metabolic response to subsequent acute, endurance exercise. Prior to exercise, feeding was associated with lower β-CTX concentrations, but did not attenuate the increase during subsequent running. Fasting had no effect on changes in bone formation markers with exercise, but reduced the duration of the increase in β-CTX during early recovery, which was not explained by changes in PTH, leptin or ghrelin. OPG concentrations did not reflect changes in β-CTX with feeding or subsequent exercise.

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REFERENCES

[1] K. Van Proyen, K. Szlufcik, H. Nielens, M. Ramaekers, P. Hespel. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. J. Appl. Physiol. 110 (2011) 236–245.

[2] E.F. Coyle, A.R. Coggan, M.K. Hemmert, J.L. Ivy. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. J. Appl. Physiol. 61 (1986) 165–172.

[3] M.J. Arkinstall, C.R. Bruce, V. Nikolopoulos, A.P Garnham, J.A. Hawley. Effect of carbohydrate ingestion on metabolism during running and cycling. J. Appl. Physiol. 91 (2001) 2125–2134.

[4] G.R. Cox, S.A. Clark, A.J Cox, S.L. Halson, M. Hargreaves, J.A. Hawley, N. Jeacocke, R.J. Snow, W.K. Yeo, L.M. Burke. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. J. Appl. Physiol. 109 (2010) 126–134.

[5] J.W. Helge, P.W. Watt, E.A. Richter, M.J. Rennie, B. Kiens. Fat utilization during exercise: adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. J. Physiol. 537 (2001) 1009–1020.

[6] L.M. Odland, G.J Heigenhauser, L.L Spriet. Effects of high fat provision on muscle PDH activation and malonyl-CoA content in moderate exercise. J. Appl. Physiol. 89 (2000) 2352–2358.

[7] L.M. Odland, G.J. Heigenhauser, D. Wong, M.G. Hollidge-Horvat, L.L Spriet. Effects of increased fat availability on fat-carbohydrate interaction during prolonged exercise in men. Am. J. Physiol. 274 (1998) R894–902.

[8] J.F. Horowitz, R. Mora-Rodriguez, L.O. Byerley, E.F. Coyle. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am. J. Physiol. 273 (1997) E768–775.

[9] R.J. Maughan, J. Fallah, E.F. Coyle. The effects of fasting on metabolism and performance. Brit. J. Sp. Med. 44 (2010) 490–494.

[10] N.H. Bjarnason, E.E. Henriksen, P. Alexandersen, S. Christgau, D.B. Henriksen, C. Christiansen. Mechanism of circadian variation in bone resorption. Bone. 30 (2002) 307–313.

[11] D.B. Henriksen, P. Alexandersen, N.H. Bjarnason, T. Vilsbøll, B. Hartmann, E.E. Henriksen, I. Byrjalsen, T. Krarup, J.J. Holst, C. Christiansen. Role of gastrointestinal hormones in postprandial reduction of bone resorption. J. Bone. Miner. Res. 18 (2003) 2180–2189.

[12] J.J Holst, B. Hartmann, I.B. Gottschalck, P.B. Jeppesen, J. Miholic, D.B. Henriksen. Bone resorption is decreased postprandially by intestinal factors and glucagon-like peptide-2 is a possible candidate. Scand. J. Gastroenterol. 42 (2007) 814–820.

[13] J.A. Clowes, H.C. Allen, D.M. Prentis, R. Eastell, A. Blumsohn. Octreotide abolishes the acute decrease in bone turnover in response to oral glucose. J. Clin. Endocrinol. Metab. 88 (2003) 4867–4873.

[14] J.A. Clowes, T.S. Yap, J. Li, N. Hoyle, A. Blumsohn, R.A.Hannon, R. Eastell. The effect of feeding on bone turnover markers and its impact on biological variability of measurements. Bone. 30 (2002) 886–890.

[15] J. Guillemant, C. Accarie, G. Peres, S. Guillemant. Acute effects of an oral calcium load on markers of bone metabolism during endurance cycling exercise in male athletes. Calcif. Tissue. Int. 74 (2004) 407–414.

[16] M. Herrmann, M. Müller, J. Scharhag, M. Sand-Hill, W. Kindermann, W. Herrmann. The effect of endurance exercise-induced lactacidosis on biochemical markers of bone turnover. Clin. Chem. Lab. Med. 45 (2007) 1381–1389.

[17] K. Kerschan-Schindl, M. Thalmann, G.H Sodeck, K. Skenderi, A.L. Matalas, S. Grampp, C. Ebner, P. Pietschmann. A 246-km continuous running race causes significant changes in bone metabolism. Bone. 45 (2009) 1079–1083.

[18] L. Maïmoun, J. Manetta, I. Couret, A.M. Dupuy, D. Mariano-Goulart, J.P. Micallef, E. Peruchon, M. Rossi. The intensity level of physical exercise and the bone metabolism response. Int. J. Sports. Med. 27 (2006) 105–111.

[19] J.P.R. Scott, C. Sale, J.P Greeves, A. Casey, J. Dutton, W.D. Fraser. The effects of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. J. Clin. Endocrinol. Metab. 95 (2010) 3918–3925.

[20] M.B. Schaffler, E.L. Radin, D.B. Burr. Long-term fatigue behaviour of compact bone at low strain magnitude and rate. Bone. 11 (1990) 321–326.

[21] P. Garnero, E. Sornay-Rendu, M.C. Chapuy, P.D. Delmas. Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. J. Bone. Miner. Res. 11 (1996) 337–349.

[22] K. Bennell, S. Malcolm, S. Thomas, J. Wark, P. Brukner. The incidence and distribution of stress fractures in competitive track and field athletes: A twelve-month prospective study. Am. J. Sports. Med. 24 (1996) 211–217.

[23] A.D. Stewart, J. Hannan. Total and regional bone density in male runners, cyclists, and controls. Med. Sci. Sports. Exerc. 32 (2000) 1373–1377.

[24] K. Hind, J.G. Truscott, J.A. Evans. Low lumbar spine bone mineral density in both male and female endurance runners. Bone. 39 (2006) 880–885.

[25] J. Lappe, D. Cullen, G. Haynatzki, R. Recker, R. Ahlf, K. Thompson. Calcium and vitamin d supplementation decreases incidence of stress fractures in female navy recruits. J. Bone Miner. Res. 23 (2008) 741–749.

[26] D.W. Barry, W.M. Kohrt. BMD decreases over the course of a year in competitive male cyclists. J. Bone. Miner. Res. 23 (2008) 484–491.

[27] R.S. Rogers, A.W. Dawson, Z. Wang, J.P Thyfault, P.S Hinton. Acute response of plasma markers of bone turnover to a single-bout of resistance-training or plyometrics. J. Appl. Physiol. 111 (2011) 1353–1360.

[28] J.P.R. Scott, C. Sale, J.P Greeves, A. Casey, J. Dutton, W.D. Fraser. Comment on Rogers et al. Acute response of plasma markers of bone turnover to a single-bout of resistance-training or plyometrics. J. Appl. Physiol. 112 (2012) 328–329.

[29] J.P. Rissanen, M.I. Suominen, Z. Peng, J.M. Halleen. Secreted tartrateresistant acid phosphatase 5b is a marker of osteoclast number in human osteoclast cultures and the rat ovariectomy model. Calcif. Tissue. Int. 82 (2008) 108–115.

[30] G. Banfi, G. Lombardi, A. Colombini, G. Lippi G. Bone metabolism markers in sports medicine. Sports. Med. 40 (2010) 697–714.

[31] J.P.R. Scott, C. Sale, J.P. Greeves, A. Casey, J. Dutton, W.D. Fraser. The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. J. Appl. Physiol. 110 (2011) 423–432.

[32] J. Erdmann, F. Lippl, S. Wagenpfeil, V. Schusdziarra. Differential association of basal and postprandial plasma ghrelin with leptin, insulin, and type 2 diabetes. Diabetes. 54 (2005) 1371–1378.

[33] J. Dallongeville, B. Hecquet, P. Lebel, J-L. Edmé, C. Le Fur, J-C. Fruchart, J. Auwerx, M. Romon. Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. Int. J. Obesity. 22 (1998) 728–733.

[34] J.L. Olive, G.D. Miller. Differential effects of maximal- and moderate-intensity runs on plasma leptin in healthy trained subjects. Nutrition. 17 (2001) 365–9.

[35] E.T. Vestergaard, R. Dall, K.H. Lange, M. Kjaer, J.S. Christiansen, J.O. Jorgensen. The ghrelin response to exercise before and after growth hormone administration. J. Clin. Endocrinol. Metab 92 (2007) :297–303.

[36] N. Fukushima, R. Hanada, H. Teranishi, Y. Fukue, T. Tachibana, H. Ishikawa, S. Takeda, Y. Takeuchi, S. Fukumoto, K. Kangawa, K. Nagata, M. Kojima. J. Bone. Miner. Res. 20 (2005) 790–8.

[37] T. Thomas. The complex effects of leptin on bone metabolism through multiple pathways. Curr. Opin. Pharmacol. 4 (2004) 295–300.

[38] P. Ducy. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. Diabetologia. 54 (2011) 1291–7.

[39] R.J. Maughan. Fasting and sport: an introduction. Brit. J. Sp. Med. 44 (2010) 473–474.

[40] V.F. Jr Froelicher, H. Brammell, G. Davis, I. Noguera, A. Stewart, M.C. Lancaster. A comparison of three maximal treadmill exercise protocols. J. Appl. Physiol. 36 (1974) 720–725.

[41] J. Guillemant, H.T. Le, C. Accarie, S.T. du Montcel, A.M. Delabroise, M.J. Arnaud, S. Guillemant. Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. Am. J. Clin. Nutr. 71 (2000) 999–1002.

[42] V. Zikán, T. Haas, J.J. Stepan. Acute effects in healthy women of oral calcium on the calcium-parathyroid axis and bone resorption as assessed by serum beta-CrossLaps. Calcif. Tissue. Int. 68 (2001) 352–357.

[43] D.W. Barry, R.E. Van Pelt, K.C. Hansen, M. Witten, P. Wolfe, W. M. Kohrt. Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. Med. Sci. Sports. Exerc. 43 (2011) 617–623.

[44] J.S. Walsh, D.B. Henriksen. Feeding and bone. Arch. Biochem. Biophys. 503 (2010) 11–19.

[45] A.M. O'Connor, S. Pola, B. M. Ward, D. Fillmore, K.D. Buchanan, J.P. Kirwan. The gastroenteroinsular response to glucose ingestion during postexercise recovery. Am. J. Physiol. 290 (2006) E1155–1161.

[46] S.Y. Ueda, T. Yoshikawa, Y. Katsura, T. Usui, S. Fujimoto. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. J. Endocrinol. 203 (2009a) 357–364.

[47] S.Y. Ueda, T. Yoshikawa, Y. Katsura, T. Usui, H. Nakao, S. Fujimoto. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. J. Endocrinol. 201 (2009b) 151–159.

[48] H.K. Nielsen, K. Brixen, M. Kassem, P. Charles, L. Mosekilde. Inhibition of the morning cortisol peak abolishes the expected morning decrease in serum osteocalcin in normal males: evidence of a controlling effect of serum cortisol on the circadian rhythm in serum osteocalcin. J. Clin. Endocrinol. Metab. 74 (1992) 1410–1414.

[49] H.M. Heshmati, B.L. Riggs, M.F. Burritt, C.A. McAlister, P.C. Wollan, S. Khosla. Effects of the circadian variation in serum cortisol on markers of bone turnover and calcium homeostasis in normal postmenopausal women. J. Clin. Endocrinol. Metab. 83 (1998) 751–756.

[50] A. Tosun, N. Bölükbaşi, E. Cingi, M. Beyazova, M. Unlü. Acute effects of a single session of aerobic exercise with or without weight-lifting on bone turnover in healthy young women. Mod. Rheumatol. 16 (2006) 300–304.

[51] T. Pomerants, V. Tillmann, K. Karelson, J. Jürimäe, T. Jürimäe. Impact of acute exercise on bone turnover and growth hormone/insulin-like growth factor axis in boys. J. Sports. Med. Phys. Fitness. 48 (2008) 266–271.

[52] R.M. Crameri, H. Langberg, B. Teisner, P. Magnusson, H.D. Schrøder, J.L. Olesen, C.H. Jensen, S. Koskinena, C. Suetta, M. Kjaer. Enhanced procollagen processing in skeletal muscle after a single bout of eccentric loading in humans. Matrix. Biol. 23 (2004) 259–264.

[53] B.F. Miller, M. Hansen, J.L. Olesen, P. Schwarz, J.A. Babraj, K. Smith, M.J. Rennie, M. Kjaer. Tendon collagen synthesis at rest and after exercise in women. J. Appl. Physiol. 102 (2007) 541–546.

[54] J. Melkko, T. Hellevik, L. Risteli, J. Risteli, B. Smedsrod. Clearance of NH2-terminal propeptides of types I and III procollagen is a physiologic function of the scavenger receptor in liver endothelial cells. J. Exp. Med. 179 (1994) 405–412.

[55] A.G. Robling, F.M. Hinant, D.B. Burr, C.H. Turner. Shorter, more frequent mechanical loading sessions enhance bone mass. Med. Sci. Sports. Exerc. 34 (2002) 196–202.

[56] L.O. Chailurkit, S. Chanprasertyothin, R. Rajatanavin, B. Ongphiphadhanakul. Reduced attenuation of bone resorption after oral glucose in type 2 diabetes. Clin. Endocrinol. (Oxf). 68 (2008) 858–862.

[57] G.M. Jørgensen, B. Vind, M. Nybo, L.M. Rasmussen, K. Højlund. Acute hyperinsulinemia decreases plasma osteoprotegerin with diminished effect in type 2 diabetes and obesity. Eur. J. Endocrinol. 161 (2009) 95–101.

[58] S. Ziegler, A. Niessner, B. Richter, S. Wirth, E. Billensteiner, W. Woloszczuk, J. Slany, G. Geyer. Endurance running acutely raises plasma osteoprotegerin and lowers plasma receptor activator of nuclear factor kappa B ligand. Metabolism. 54 (2005) 935–938.

[59] D. Hegedus, V. Ferencz, P.L. Lakatos, S. Meszaros, P. Lakatos, C. Horvath, F. Szalay. Decreased bone density, elevated serum osteoprotegerin, and beta-cross-laps in Wilson disease. J. Bone. Miner. Res. 17 (2002) 1961–1967.

[60] V. Zikan, J.J. Stepan. Marker of bone resorption in acute response to exogenous or endogenous parathyroid hormone. Biomark. Insights. 25 (2008) 19–24.

FIGURE LEGENDS

Fig. 1. Glucose (Panel A) and cortisol (Panel B) concentrations at baseline, before and during exercise, during 3 h of recovery, and on 4 follow-up days in the FED (open squares) and FAST (filled diamonds) conditions. Values are mean ± 1SD. [a] FAST different ($P < 0.05$, Student Newman-Keuls test) from FED; [c] FAST different ($P < 0.001$) from FED. Vertical arrow denotes provision of meal in the FED condition; grey box denotes exercise BASE, baseline; EX30, 30 min of exercise; EX60, 60 min of exercise; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; FU1–FU4, follow-up days 1–4; PRE, immediately pre-exercise; R1.0–R3.0, 1–3 h of recovery.

Fig. 2. β-CTX (Panel A), P1NP (Panel B) and OC (Panel C) concentrations, expressed as a percentage of baseline, before and during exercise, during 3 h of recovery, and on 4 follow-up days in the FED (open squares) and FAST (filled diamonds) conditions. Values are mean ± 1SD. [a] FAST different ($P < 0.05$, Student Newman-Keuls test) from FED; [c] FAST different ($P < 0.001$) from FED. Vertical arrow denotes provision of meal in the FED condition. Grey box denotes exercise. BASE, baseline; EX30, 30 min of exercise; EX60, 60 min of exercise; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; FU1–FU4, follow-up days 1–4; PRE, immediately pre-exercise; R1.0–R3.0, 1–3 h of recovery.

Fig. 3. OPG concentrations at baseline, before and during exercise, during exercise, during 3 h of recovery, and on 4 follow-up days in the FED (open squares) and FAST (filled diamonds) conditions. Values are mean ± 1SD. Linear Mixed Model *Group x Time* interaction was not significant. Vertical arrow denotes provision of meal in the FED

condition; grey box denotes exercise. BASE, baseline; EX30, 30 min of exercise; EX60, 60 min of exercise; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; FU1–FU4, follow-up days 1–4; PRE, immediately pre-exercise; R1.0–R3.0, 1–3 h of recovery.

Fig. 4. PTH (Panel A), ACa (Panel B) and PO_4 (Panel C) concentrations at baseline (BASE), before exercise (0900–PRE), during exercise (EX30), during 3 h of recovery (EX60–R3.0) and on 4 follow-up days (FU1–FU4) in the FED (open squares) and FAST (filled diamonds) conditions. Values are mean ± 1SD. Linear Mixed Model *Group x Time* interactions were not significant. Vertical arrow denotes provision of meal in the FED condition; grey box denotes exercise. ACa, albumin-adjusted calcium; BASE, baseline; EX30, 30 min of exercise; EX60, 60 min of exercise; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; FU1–FU4, follow-up days 1–4; OPG, osteoprotegerin; PO_4, phosphate; PRE, immediately pre-exercise; PTH, parathyroid hormone; R1.0–R3.0, 1–3 h of recovery.

Fig. 5. Leptin (Panel A) and ghrelin (Panel B) concentrations at baseline, before and during exercise, during 3 h of recovery, and on 4 follow-up days in the FED (open squares) and FAST (filled diamonds) conditions. Values are mean ± 1SD. Leptin was undetectable in 3 participants and analysis was performed on the remaining participants (n = 7). Linear Mixed Model *Group x Time* interactions were not significant. Vertical arrow denotes provision of meal in the FED condition; grey box denotes exercise. BASE, baseline; EX30, 30 min of exercise; EX60, 60 min of exercise; FAST, overnight fast from 21:00; FED, a standardised breakfast consumed at 08:15; FU1–FU4, follow-up days 1–4; PRE, immediately pre-exercise; R1.0–R3.0, 1–3 h of recovery.

Figure 1

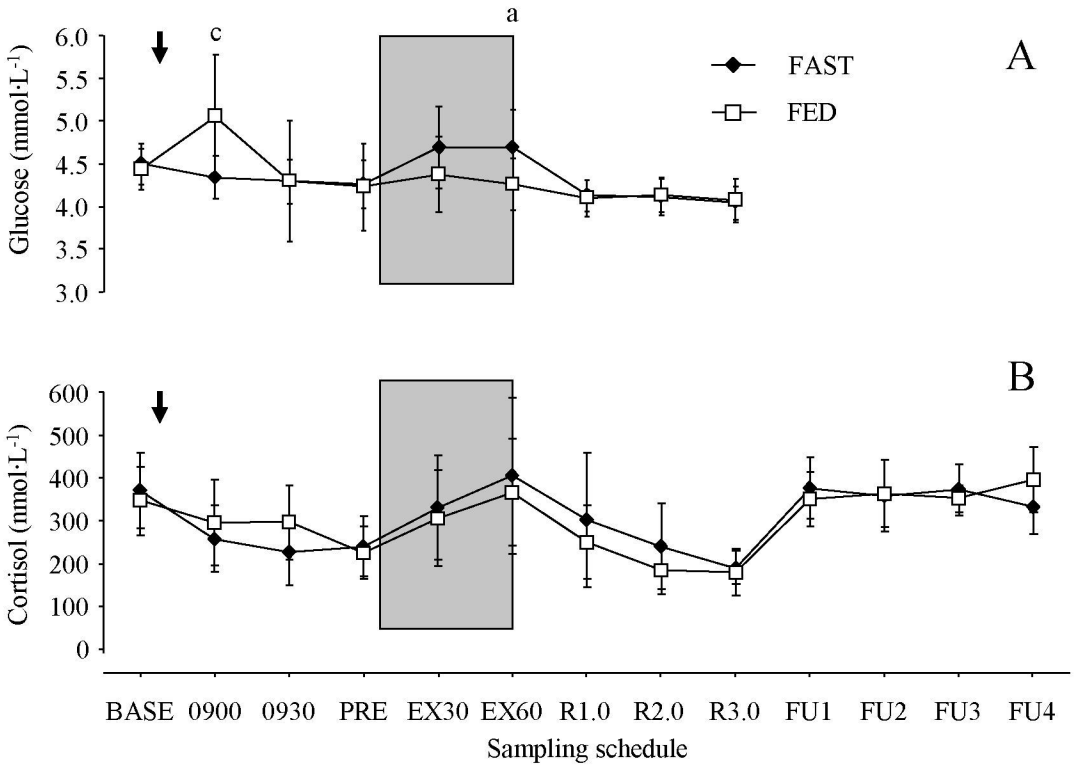
a
c
A
FAST
FED
Glucose (mmol·L-1)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
B
Cortisol (nmol·L-1)
600
500
400
300
200
100
0
BASE 0900 0930 PRE EX30 EX60 R1.0 R2.0 R3.0 FU1 FU2 FU3 FU4
Sampling schedule

Figure 2

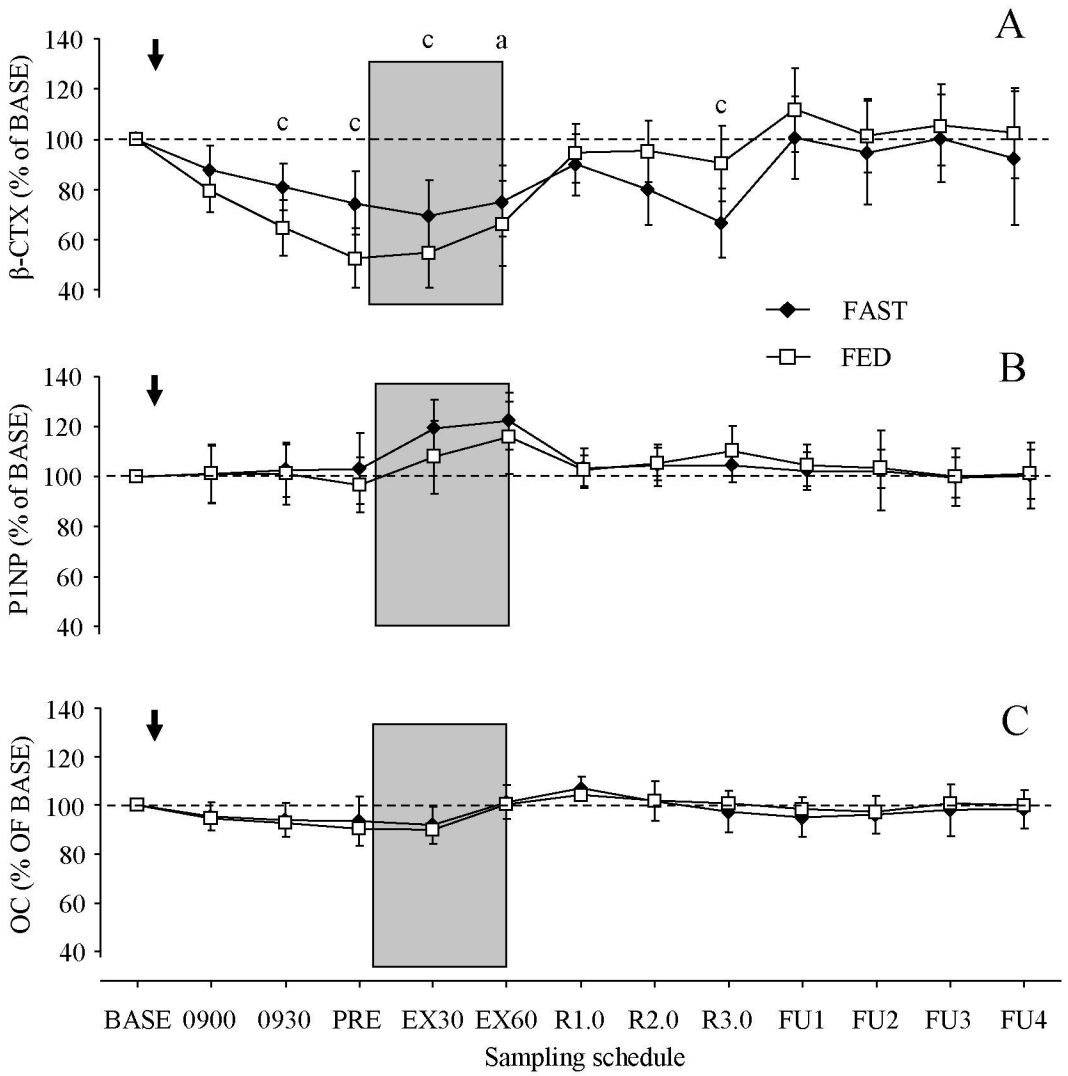
A
B
C
β-CTX (% of BASE)
P1NP (% of BASE)
OC (% OF BASE)
140
120
100
80
60
40
c
c
c
a
FAST
FED
BASE 0900 0930 PRE EX30 EX60 R1.0 R2.0 R3.0 FU1 FU2 FU3 FU4
Sampling schedule

Figure 3

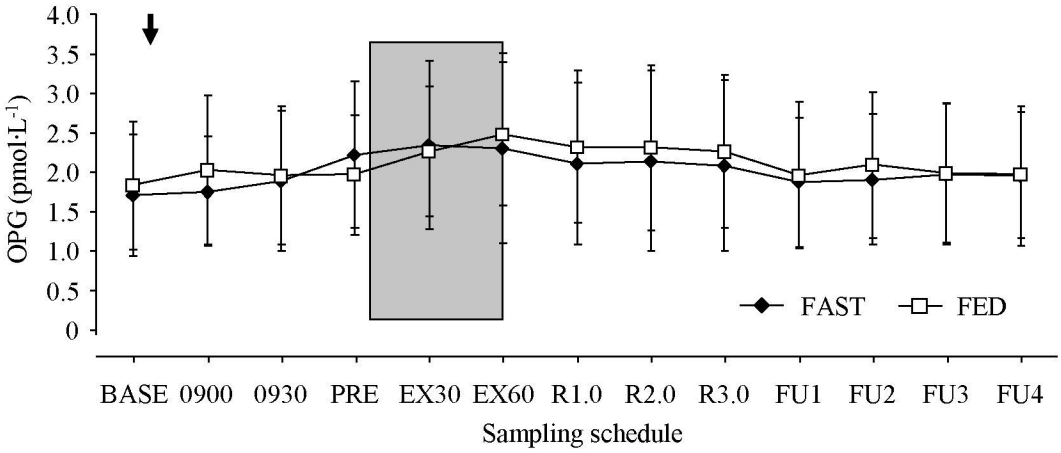
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
OPG (pmol·L-1)
FAST
FED
BASE 0900 0930 PRE EX30 EX60 R1.0 R2.0 R3.0 FU1 FU2 FU3 FU4
Sampling schedule

Figure 4

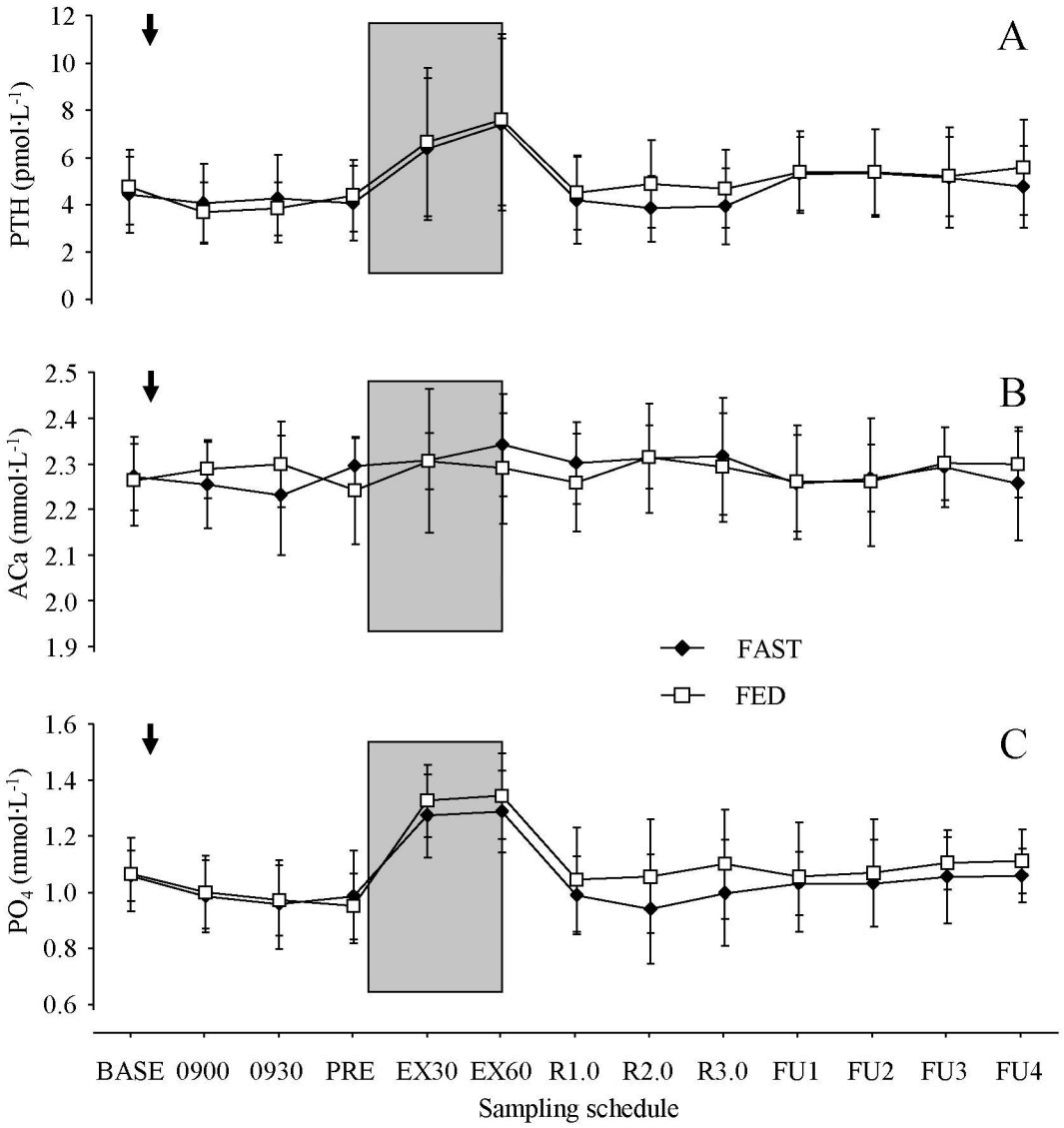
A
B
C
PTH (pmol·L-1)
ACa (mmol·L-1)
PO4 (mmol·L-1)
FAST
FED
BASE 0900 0930 PRE EX30 EX60 R1.0 R2.0 R3.0 FU1 FU2 FU3 FU4
Sampling schedule

Figure 5

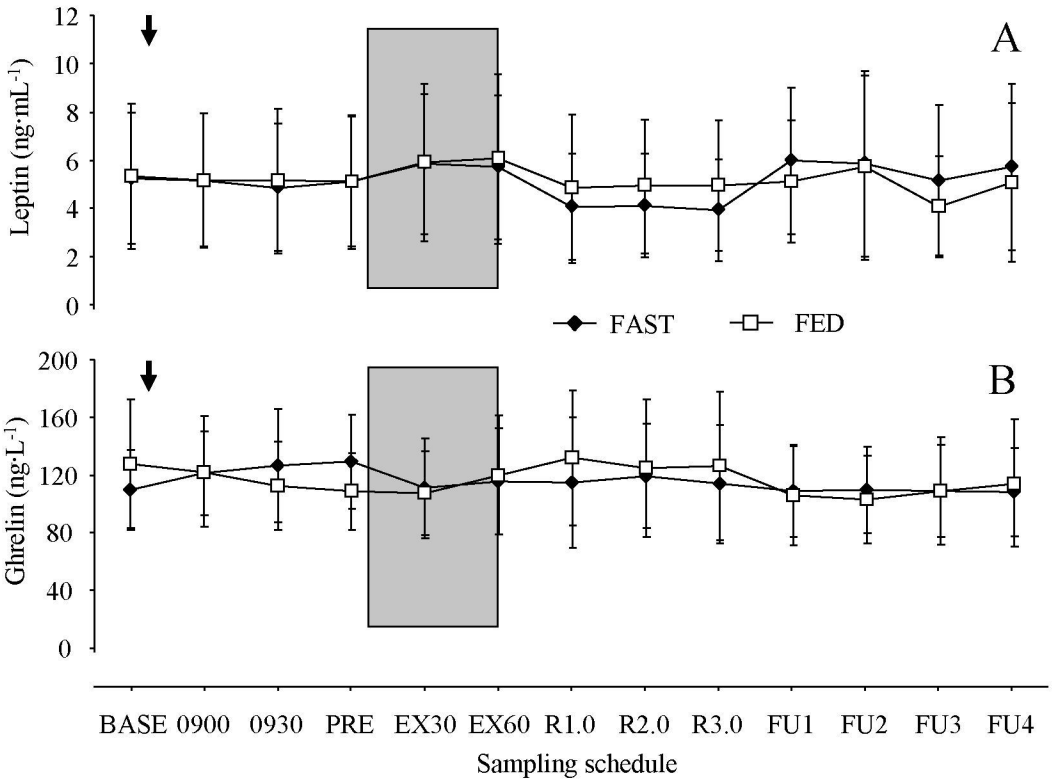
A
B
Leptin (ng·mL-1)
Ghrelin (ng·L-1)
FAST
FED
BASE 0900 0930 PRE EX30 EX60 R1.0 R2.0 R3.0 FU1 FU2 FU3 FU4
Sampling schedule

Table 1

Baseline biochemical data and linear mixed model outcomes.

Marker	Baseline data			LMM Outcome[a]	
	FAST	FED	*P* Value[b]	*Time*	*Group* x *Time* Interaction
Glucose ($mm \cdot L^{-1}$)	4.5 ± 0.2	4.4 ± 0.2	0.111	$P < 0.001$	$P < 0.01$
Lactate ($mm \cdot L^{-1}$)	0.5 ± 0.1	0.5 ± 0.1	0.859	$P < 0.001$	$P < 0.001$[c]
Cortisol ($nmol \cdot L^{-1}$)	370 ± 89	348 ± 80	0.507	$P < 0.001$	$P = 0.140$[c]
β-CTX ($ug \cdot L^{-1}$)	0.56 ± 0.24	0.52 ± 0.23	0.118	$P < 0.001$	$P < 0.001$[c]
P1NP ($ug \cdot L^{-1}$)	53 ± 24	52 ± 22	0.672	$P < 0.001$	$P = 0.742$[c]
OC ($ug \cdot L^{-1}$)	26.7 ± 8.7	26.6 ± 8.4	0.881	$P < 0.001$	$P = 0.978$[c]
Bone ALP ($U \cdot L^{-1}$)	19 ± 7	21 ± 7	0.133	$P = 0.222$	-
OPG ($pmol \cdot L^{-1}$)	1.7 ± 0.8	1.8 ± 0.8	0.096	$P < 0.001$	$P = 0.403$[c]
PTH ($pmol \cdot L^{-1}$)	4.4 ± 1.6	4.7 ± 1.6	0.334	$P < 0.001$	$P = 0.130$[c]
ACa ($mmol \cdot L^{-1}$)	2.27 ± 0.07	2.26 ± 0.10	0.788	$P = 0.387$	-
PO_4 ($mmol \cdot L^{-1}$)	1.06 ± 0.09	1.07 ± 0.13	0.860	$P < 0.001$	$P = 0.680$
Leptin ($ng \cdot mL^{-1}$)[d]	5.3 ± 2.7	5.3 ± 3.0	0.872	$P < 0.001$	$P = 0.520$[c]
Ghrelin ($ng \cdot L^{-1}$)	110 ± 28	128 ± 45	0.053	P < 0.001	$P = 0.158$[c]

All values are mean ± SD. [a] Statistical analysis was performed using a linear mixed model (LMM) with factors 'condition' and 'time point' as fixed effects and 'participants' as a random, within-group effect. [b] Student's t-test for paired samples. [c] Raw values reported but data were log transformed to achieve normality before statistical analysis. [d] Leptin was undetectable in 3 participants and statistical analysis performed on remaining 7 participants.

Table 2

Oxygen uptake (VO_2), heart rate (percentage of maximum heart rate, $\%HR_{max}$), lactate concentration, respiratory exchange ratio (RER) and ratings of perceived exertion (RPE) during exercise in the FAST and FED conditions.

Variable	FED	FAST
VO_2 ($L \cdot min^{-1}$)	2.71 ± 0.30	2.70 ± 0.29
Lactate ($mmol \cdot L^{-1}$)*	1.1 ± 0.5	1.1 ± 0.4
HR (% HR_{max})	84 ± 5 [c]	79 ± 8
RER	0.920 ± 0.042	0.881 ± 0.033 [c]
RPE	12 ± 1	12 ± 2

* Average of concentrations measured in blood samples drawn after 30 min of exercise (EX30) and immediately post exercise (EX60). All values are mean ± SD. [c] different ($P < 0.001$, Student's t-test for paired samples) from FED.