Parathyroid Hormone Secretion is Controlled by Both Ionised Calcium and Phosphate During Exercise and Recovery in Men

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Abstract

The mechanism by which PTH is controlled during and after exercise is poorly understood due to insufficient temporal frequency of measurements.

Objective

To examine the temporal pattern of PTH, PO_4, ACa and Ca^{2+} during and after exercise.

Design and setting

A laboratory-based study with a cross-over design, comparing 30 min of running at 55%, 65% and 75%VO_{2max}, followed by 2.5-h of recovery. Blood was obtained at baseline, after 2.5, 5, 7.5, 10, 15, 20, 25 and 30 min of exercise and after 2.5, 5, 7.5, 10, 15, 20, 25, 30, 60, 90 and 150 min of recovery

Participants

Ten men (age 23±1 y, height 1.82±0.07 m, body mass 77.0±7.5 kg) participated.

Main Outcome Measures

PTH, PO_4, ACa and Ca^{2+}

Results

Independent of intensity, PTH concentrations decreased with the onset of exercise (-21 to -33%; $P \leq 0.001$), increased thereafter and were higher than baseline by the end of exercise at 75%VO_{2max} (+52%; $P \leq 0.001$). PTH peaked transiently after 5–7.5 min of recovery (+73 to +110%; $P \leq 0.001$). PO_4 followed a similar temporal pattern to PTH and Ca^{2+} followed a similar but inverse pattern to PTH. PTH was negatively correlated with Ca^{2+} across all intensities (r=-0.739 to -0.790; $P \leq 0.001$). When PTH was increasing, the strongest cross-correlation was with Ca^{2+} at 0 lags (3.5 min) (r=-0.902 to -0.950); during recovery, the strongest cross-correlation was with PO_4 at 0 lags (8 min) (r=0.987 to 0.995).

Conclusions

PTH secretion during exercise and recovery is controlled by a combination of changes in Ca^{2+} and PO_4 in men.

Abbreviations

ACa, albumin-adjusted calcium; Ca, calcium; Ca^{2+}, ionised calcium; CV, coefficient of variation; PO_4, phosphate; PTH, parathyroid hormone; VO_{2max}, maximal oxygen consumption.

Introduction

At rest, PTH secretory activity is regulated by serum ionised calcium (Ca^{2+}), which is detected by the calcium-sensing receptor on the chief cells of the parathyroid gland (1). When Ca^{2+} decreases from the homeostatic set point, PTH is synthesised and secreted, increasing serum calcium (Ca) through mobilisation of the bone reservoir via bone resorption, and by increasing renal tubular reabsorption and intestinal Ca absorption (2–4). PTH has a dual effect on bone that appears to be dependent on the signalling mechanism and the length of time that concentrations remain elevated for (5). Prolonged elevations in PTH, that are seen with endurance type exercise, and that can also result in the loss of the circadian rhythm of PTH, might cause an increase in bone resorption, whereas, transient spikes in PTH, that are seen with high intensity interval type training, might cause an increase in bone formation (6), provided that the magnitude of the increase is sufficient. Chronic elevations in PTH concentrations have been associated with increased fracture risk (7, 8). Complete fractures and stress fractures are also debilitating injuries for elite athletes (9), therefore understanding how PTH is regulated during exercise and recovery may have implications for both the general population and athletes who are at risk of chronically elevated PTH concentrations, as a positive calcium balance is necessary for bone adaptation to mechanical loading (10).

Exercise increases PTH concentrations (11–20), although studies have used different exercise modes, durations and intensities. Exercise intensity is important, given that Scott *et al.* (17) have shown that 60 min of running at 55%, 65% and 75% of maximal oxygen consumption (VO_{2max}) results in different PTH responses during and after exercise. Any study investigating the underlying mechanisms responsible for the changes in PTH during exercise and recovery should examine the effects of exercise intensity.

During exercise, reductions in circulating Ca do not explain the increase in PTH, as the concentration of albumin-adjusted calcium (ACa) – a surrogate for Ca^{2+} – is either increased (12, 15, 17) or unchanged

(14, 18, 19) concomitantly with PTH. Barry *et al*. (16) showed that Ca ingestion before exercise attenuated, but did not abolish the increase in PTH, suggesting that some other mechanism contributed to the increase. This could involve phosphate (PO_4), as an increase in PO_4 increases PTH in rested individuals (21). Following exercise, PO_4 concentrations decrease and the timing and magnitude of these decreases reflect those in PTH (17, 18, 20), also suggesting that PO_4 may be involved in PTH regulation with exercise.

The hypothesis that decreased Ca^{2+} triggers increased PTH during exercise has not yet been proven (16). PTH is secreted within seconds of a decrease in Ca^{2+} and subsequent increases in Ca^{2+} take only minutes to occur in response to increased PTH, highlighting a dynamic relationship (1, 22). Despite this, no studies have measured PTH and other markers of Ca metabolism until 20 minutes of exercise has been completed, by which time PTH is elevated. Most studies have started taking measurements at 30 min post-exercise, by which time PTH has returned to near pre-exercise levels (15–19, 23). Single or infrequent measurements of PTH, ACa and PO_4 during and after exercise might fail to capture the dynamic nature of Ca regulation with exercise (16). Using repeated measurements with a high frequency, we examined the temporal pattern of PTH, PO_4, ACa and Ca^{2+} during and after 30 minutes of treadmill running at three exercise intensities.

Materials and Methods

Participants

Ten healthy, physically active men ([mean±SD] age 23±1 y, height 1.82±0.07 m, body mass 77.0±7.5 kg) volunteered for the study, which was approved by the Institutional Ethics Committee. Participants were non-smokers, had not suffered a fracture in the past 12 months, were free from musculoskeletal injury and were not taking any medication or experiencing any problems known to affect Ca or bone metabolism. Eligibility was confirmed during the initial session, when participants provided written informed consent.

Experimental Design

Participants completed a preliminary visit for health screening, habituation and measurement of VO_{2max}. Participants then completed three randomised (Latin Square Design), three-day experimental trials, each separated by one week. On days 1–2, participants refrained from exercise, caffeine and alcohol. On day 2, participants consumed a self-selected diet that was repeated for each trial. On day 3, participants performed a 30 min bout of running at 55%, 65% and 75%VO_{2max}, followed by 2.5 h of recovery.

Trial Procedures

VO_{2max}

Participants performed an incremental treadmill test to determine lactate threshold, followed by a ramp test to determine VO_{2max}, as per Jones and Doust (24). The level running velocities corresponding to 55% (8.7±0.6 $km \cdot h^{-1}$), 65% (10.1±0.8 $km \cdot h^{-1}$) and 75%VO_{2max} (11.9±0.9 $km \cdot h^{-1}$) were calculated based on the regression of VO_2 and velocity.

120 ***Main Trials***

121 Participants arrived (09:00) following an overnight fast and after consuming 500 mL of water upon
122 awakening. After voiding, participants had their body mass measured before adopting a semi-recumbent
123 position and having a cannula inserted into a forearm vein. After 10 min rest, a baseline blood sample
124 (5 mL) was collected for measurement of PTH, PO4, ACa and Ca^{2+}. Thirty min of treadmill running at
125 55%, 65% or 75%VO_{2max} commenced thereafter. Additional blood was collected after 2.5, 5, 7.5, 10,
126 15, 20, 25 and 30 min of exercise. After exercise, participants adopted a semi-recumbent position and
127 blood was collected at 32.5, 35, 37.5, 40, 45, 50, 55, 60, 90, 120 and 180 min. Ca^{2+} was measured
128 immediately but due to equipment availability Ca^{2+} was only measured in participants 5–10. Blood
129 samples were transferred to pre-cooled standard serum tubes (Becton Dickinson Vacutainer System,
130 USA) to clot at room temperature for 60 min. Samples were centrifuged at 2000 rev·min^{-1} and 5°C for
131 10 min and the resulting serum was transferred into Eppendorf tubes and frozen at -80°C. Following
132 the last blood sample, the cannula was removed and body mass measured. Participants were given 3
133 mL·$kgBM^{-1}$·h^{-1} of water to consume throughout the trials. The timings of blood samples and exercise
134 were identical in each trial to ensure that circadian rhythms of the metabolites were controlled for.

135

136 ***Biochemical Analysis***

137 PTH was measured using ECLIA on a Modular Analytics E170 analyser (Roche Diagnostics, Burgess
138 Hill, UK). Inter-assay CV for PTH was <4% between 1–30 pmol·L^{-1} and sensitivity of 0.8 pmol·L^{-1}.
139 PO_4, total Ca and albumin were measured using standard colorimetric assays and spectrophotometric
140 methods, performed on an ABX Pentra 400 (Horiba ABX, Montpellier, France). Inter-assay CVs were
141 ≤3.6% between 0.09–7.80 mmol·L^{-1} for PO_4, ≤1.7% between 0.04–5.00 mmol·L^{-1} for total Ca and
142 ≤1.9% between 0.02–5.99 g·dL^{-1} for albumin. Because fluctuations in protein, particularly albumin,
143 may cause total Ca levels to change independently of the Ca^{2+} concentrations, total Ca concentrations
144 were corrected to give albumin-adjusted Ca values: 0.8 mg·dL^{-1} was subtracted from total Ca
145 concentrations for every 1.0 g·dL^{-1} that albumin concentrations were less than 4 g·dL^{-1} or 0.8 mg·dL^{-1}

was added to total Ca concentrations for every 1.0 $mg \cdot dL^{-1}$ that albumin concentration were greater than 4 $mg \cdot dL^{-1}$. Ca^{2+}, glucose and lactate were measured in whole blood using a blood gas analyser (Radiometer ABL90 FLEX, Copenhagen, Denmark). Ca^{2+} is estimated directly between pH 7.2-7.6 with no pH correction applied. The inter- and intra-assay CV for Ca^{2+} was ≤3% between 0.2–9.99 $mmol \cdot L^{-1}$, for glucose was ≤5% between 0–60 $mmol \cdot L^{-1}$ and for lactate was ≤26.7% between 0.1–31 $mmol \cdot L^{-1}$.

Statistical Analysis

Statistical significance was accepted at $P \leq 0.05$. Baseline concentrations were compared using one-way ANOVA. All data were analysed using repeated measures ANOVA, with *Intensity* (55% *vs* 65% *vs* 75%VO_{2max}) and *Time* (of sampling) as within subject factors. Parametric assumptions of normality and sphericity were confirmed using Shapiro-Wilks and Maulchy's tests. Tukey's HSD *post-hoc* test was used to compare timepoints against baseline and to compare exercise intensities at each timepoint, where appropriate. Pearson's correlation coefficients were calculated for PO_4, ACa and Ca^{2+} with PTH.

Cross-correlational analyses were performed to determine the temporal relationships between PTH and PO_4, ACa and Ca^{2+}. Cubic interpolation was performed to adjust for unevenly spaced data points and cross-correlational analyses were subsequently performed using R (version 3.2.2, Vienna, Austria). To determine whether one time series led another, cross-correlation functions were computed at seven lag time points for 'PEAK' (data points between baseline and peak PTH concentrations [5 min of recovery]), where each lag represented 3.5 min, and six lag time points for 'DEC' (all data points during the decrease in PTH concentrations [5 to 90 min of recovery]), where each lag represented 8 min.

Results

Baseline biochemistry

Baseline PTH, PO_4, ACa and albumin were not significantly different between trials (P=0.339 to 0.982). Baseline Ca^{2+} at 55%VO_{2max} was significantly ($P \leq 0.05$) higher than at 65%VO_{2max} and 75%VO_{2max} (Table 1).

PTH

There was no main effect of *Intensity*, but there was a main effect of *Time* ($P \leq 0.001$) and an *Intensity x Time* interaction ($P \leq 0.001$). PTH concentrations decreased with the onset of exercise and were significantly lower than baseline after 5 min of exercise at 55%VO_{2max} (-23%; $P \leq 0.05$) and 75%VO_{2max} (-33%; $P \leq 0.001$), but not at 65%VO_{2max} (-21%; P=0.305) (Fig. 1A all participants; Fig. 2A participants 5–10). Thereafter, PTH increased, becoming significantly greater than baseline at the end of exercise (30 min) at 75%VO_{2max} (+52%; $P \leq 0.001$) and after 2.5 min of recovery at 55%VO_{2max} (+43%; $P \leq 0.001$) and 65%VO_{2max} (+52%; $P \leq 0.001$). PTH concentrations peaked after 5 min of recovery at 55%VO_{2max} (+73%; $P \leq 0.001$) and 75%VO_{2max} (+110%; $P \leq 0.001$), and after 7.5 min of recovery at 65%VO_{2max} (+76; $P \leq 0.001$). PTH concentrations then decreased, but remained significantly higher than baseline until 15 min into recovery at 55%VO_{2max} and until 25 min at 65%VO_{2max} and 75%VO_{2max}. PTH concentrations decreased below baseline after 60 min of recovery in all trials (-8% to – 17%).

PTH concentrations were not significantly different at any time point between 55% and 65%VO_{2max} trials. Exercise at 75%VO_{2max} resulted in significantly higher PTH concentrations than at 55%VO_{2max} at the end of exercise ($P \leq 0.001$), and at 2.5 ($P \leq 0.001$), 5 ($P \leq 0.001$), 7.5 ($P \leq 0.05$), 10 ($P \leq 0.05$) and 15 ($P \leq 0.001$) min into recovery, and higher than exercise at 65%VO_{2max} at the end of exercise ($P \leq 0.001$), and at 2.5 ($P \leq 0.001$) and 5 ($P \leq 0.001$) min into recovery.

PO_4

There was no main effect of *Intensity*, but there was a main effect of *Time* ($P \leq 0.001$) and an *Intensity x Time* interaction ($P \leq 0.05$). PO_4 concentrations increased with the onset of exercise at all intensities, being significantly higher than baseline from 7.5 min to the end of exercise at 55%VO_{2max} (+16%; $P \leq 0.001$), and between 5 min and the end of exercise at 65%VO_{2max} (+22%) and 75%VO_{2max} (+26%) ($P \leq 0.05$ to $P \leq 0.001$) (Fig. 1B). PO_4 concentrations peaked at the end of exercise, and decreased thereafter, but remained significantly higher than baseline until 5 min into recovery at 55%VO_{2max}, 10 min at 65%VO_{2max} and 15 min at 75%VO_{2max}. PO_4 concentrations decreased below baseline at 60 min of recovery and remained so until 150 minutes of recovery at 65%VO_{2max} (-5 to -10%) and 75%VO_{2max} (-7 to -12%) ($P \leq 0.05$ to $P \leq 0.001$). Concentrations did not decrease significantly below baseline at 55%VO_{2max}.

Exercise at 65%VO_{2max} resulted in significantly higher PO_4 concentrations than exercise at 55%VO_{2max} at 10 ($P \leq 0.05$), 20 ($P \leq 0.001$) and 25 ($P \leq 0.05$) min of exercise.

ACa

There was no main effect of *Intensity*, but there was a main effect of *Time* ($P \leq 0.001$) and an *Intensity x Time* interaction ($P \leq 0.001$). ACa concentrations increased with the onset of exercise and were significantly higher than baseline between 7.5 min and the end of exercise at 65%VO_{2max} (+9%; $P \leq 0.001$) and between 2.5 min and the end of exercise at 75%VO_{2max} (+14%; $P \leq 0.001$) (Fig. 1C). ACa concentrations peaked after 20 min of exercise and decreased thereafter, but remained significantly higher than baseline until 5 min into recovery at 65%VO_{2max} and 7.5 minutes at 75%VO_{2max}. ACa concentrations decreased below baseline 15 min into recovery and remained so until 30 min of recovery at 55%VO_{2max} (-7 to -9%; $P \leq 0.05$ to $P \leq 0.001$). Concentrations decreased below baseline 25 min into

recovery and remained so until 90 min of recovery at 65%VO_{2max} (-6 to -8%; $P \leq 0.05$ to $P \leq 0.001$). ACa concentrations did not decrease significantly below baseline at 75%VO_{2max}.

Exercise at 75%VO_{2max} resulted in significantly higher ACa concentrations than exercise at 55%VO_{2max} after 20 ($P \leq 0.05$), 25 ($P \leq 0.001$) and 30 min of exercise ($P \leq 0.001$) and after 25 min of recovery ($P \leq 0.01$).

Albumin

There was no main effect of *Intensity*, but there was a main effect of *Time* ($P \leq 0.001$) and an *Intensity x Time* interaction ($P \leq 0.01$). Albumin concentrations increased with the onset of exercise and were higher than baseline between 7.5 min and the end of exercise at 65%VO_{2max} (+4%; $P \leq 0.05$) and between 5 min of exercise and the end of exercise at 75%VO_{2max} (+6%; $P \leq 0.05$) (Fig. 1D). Albumin concentrations peaked after 20 min of exercise and decreased thereafter, but remained higher than baseline until 5 min into recovery at 75%VO_{2max} ($P \leq 0.001$). Albumin concentrations decreased below baseline 25 min into recovery and remained so until 90 min of recovery at 55%VO_{2max} (-3 to -4%; $P \leq 0.01$). Concentrations decreased below baseline 20 min into recovery and remained so until 90 min of recovery at 65%VO_{2max} (-3 to -5%; $P \leq 0.05$ to $P \leq 0.001$). Albumin concentrations did not decrease below baseline at 75%VO_{2max}.

Exercise at 75%VO_{2max} resulted in significantly higher albumin concentrations than exercise at 55%VO_{2max} after 25 min of exercise ($P \leq 0.05$).

Ca^{2+}

There was no main effect of *Intensity*, but there was a main effect of *Time* ($P \leq 0.001$) and an *Intensity x Time* interaction ($P \leq 0.001$). At 55%VO_{2max}, Ca^{2+} concentrations decreased after 10 min of exercise, being significantly below baseline between 25 minutes and the end of exercise (Fig 2B) (-2%; $P \leq 0.001$).

243 Ca^{2+} concentrations continued to decrease into recovery, remaining significantly below baseline until
244 90 minutes of recovery (-2 to -6%; $P \leq 0.001$). At 65%VO_{2max} and 75%VO_{2max} Ca^{2+} concentrations
245 increased with the onset of exercise and were significantly higher than baseline between 2.5 and 10 min
246 of exercise at 65%VO_{2max} (+2 to +3%; $P \leq 0.001$) and between 2.5 and 7.5 min at 75%VO_{2max} (+2 to
247 +3%; $P \leq 0.001$). Thereafter, Ca^{2+} concentrations decreased and were significantly below baseline
248 between 2.5 and 30 min of recovery at 65%VO_{2max} (-3 to -4%; $P \leq 0.05$ to $P \leq 0.001$) and 75%VO_{2max} (-3
249 to -4%; $P \leq 0.001$).

250

251 There were no significant differences between the three trials at any time point other than at baseline
252 (Table 1), which created the significant *Intensity x Time* interaction.

253

254 ***Correlation Analyses***

255 Changes in PTH were not correlated with changes in PO_4 or ACa in any trial. Across all data points
256 PTH was significantly ($P \leq 0.001$) negatively correlated with Ca^{2+} at all intensities (Table 2).

257

258 Across PEAK data points, PO_4 was correlated with PTH at all exercise intensities (r=0.661 to 0.772)
259 (Table 3) when the PTH series was lagged by 1 time point (3.5 min) behind the PO_4 series, suggesting
260 that increases in PO_4 precede increases in PTH by 3.5 min. Ca^{2+} was most strongly correlated with PTH
261 at all exercise intensities (r=-0.902 to -0.950) when there was no time lag, suggesting that increases in
262 PTH occur within 3.5 min of a decrease in Ca^{2+}.

263

264 Across DEC data points, PO_4, ACa and Ca^{2+} were correlated with PTH at all exercise intensities. PO_4
265 was most strongly correlated with PTH at all exercise intensities (r=0.987 to 0.995) (Table 3) when
266 there was no time lag, suggesting that decreases in PTH occur within 8 min of a decrease in PO_4.

Discussion

The novel findings from this study are: 1) changes in PTH, PO_4, ACa and Ca^{2+} occur within 2.5 min of the onset of exercise; 2) there is an initial decrease in PTH concentrations at the start of exercise that coincides with a significant increase in Ca^{2+} concentrations at the two higher exercise intensities; 3) peak PTH concentrations occur within 5–7.5 min of recovery; 4) increases in PO_4 precede increases in PTH; 5) decreases in Ca^{2+} precede increases in PTH; 6) post-exercise decreases in PTH concentrations are preceded by decreases in PO_4.

The pattern of change in PTH in this study is comparable to previous studies, with PTH concentrations increasing during exercise (15, 17–20) and peaking in the first minutes of recovery (12). The pattern of change in PTH was similar across the three exercise intensities, with an initial decrease from baseline to 5 min of exercise. We are the first to observe this initial response in PTH, due to the higher temporal frequency of blood sampling at the start of exercise compared with previous studies. This response requires verification from further studies and the use of even more frequent sampling. The lack of a resting control group in the present study means that we cannot confirm whether this is a characteristic physiological response to the onset of exercise or whether this reflects the circadian rhythm of PTH at the time of sampling. The nadir in PTH occurs between 08:00 and 10:00 (25–28) and our baseline blood was taken at 08:55, with exercise commencing at 09:02. If the initial decrease in PTH were due to the circadian rhythm, however, it would be expected that the decrease would have lasted longer than 5 min into exercise. Additionally, a decrease of 33% from baseline, followed by a rapid reversal in the direction of change, as shown here, has not been reported in circadian studies. Peak PTH concentrations have previously been shown to occur 15 min after exercise (12), due to a lower sampling frequency, but the results of the present study show that the peak in PTH after exercise occurs with 5 – 7.5 min of recovery (+73 to +110% from baseline). This peak is also transient; PTH concentrations start to decrease immediately after reaching peak concentrations. Transient spikes in PTH have been shown to be anabolic for bone (5), resulting in net bone gain (29). As such, our identification of peak PTH concentrations 5 – 7.5 min after exercise could be utilised as a tool for improving bone health amongst

individuals at risk of fractures, stress fractures or poor bone health, including the development of an exercise regime involving bouts of running sufficient to cause a spike in PTH concentrations, followed by rest periods to ensure that the spike is transitory. Further work is required to determine whether the response of PTH to this type of exercise is consistent and whether the magnitude of the changes in PTH are sufficient to induce such an effect.

Cross-correlations suggested that PTH secretion during exercise and recovery is controlled by a combination of changes in Ca^{2+} and PO_4. Ca^{2+} is not routinely measured due to analytical difficulties; consequently ACa is estimated as a surrogate and has been shown clinically to be a reliable indicator of Ca metabolism at rest (30). We have shown different responses to exercise and recovery between ACa and Ca^{2+} and also different relationships with PTH; Ca^{2+} concentrations were correlated with PTH, whereas ACa was not. Albumin changes taking place during exercise will have a greater effect on the ACa estimation compared to the small effect that can occur on Ca^{2+} measurement; changes in pH were not sufficient to have a major effect on Ca^{2+} measurement by the blood gas analyser. The results support previous data (14, 15, 17–20) suggesting that changes in ACa do not explain the changes in PTH or regulation of PTH during exercise, because, as PTH is increasing, ACa either also increases (15, 17) or is unchanged (14, 18, 19). Scott *et al*. (19) argued that because both PTH and ACa were increased after 20 minutes of exercise, a decrease in Ca^{2+} could have occurred in the first few minutes of exercise, stimulating the secretion of PTH and causing serum Ca^{2+} concentrations to increase as a result of PTH-stimulated bone resorption and Ca^{2+} liberation. However, through frequent sampling, we have shown that ACa and Ca^{2+}, at 65% and 75%VO_{2max}, increase within 2.5 min of exercise, with ACa increasing and Ca^{2+} decreasing thereafter. Although it is well established that PTH responds rapidly to a reduction in Ca^{2+} at rest (1, 22), this is the first study to show that this rapid response also occurs during exercise. The lack of an initial increase in Ca^{2+} at 55%VO_{2max} is surprising and the reason for this is currently unknown. The strong negative correlation of PTH and Ca^{2+} during exercise at all three intensities with a 0 time lag (r=-0.902 to -0.950) suggests that as Ca^{2+} decreases, PTH increases within 3.5 min. This

negative cross-correlation supports the findings of Bouassida *et al*. (11) who showed that as Ca^{2+} decreased during 42 minutes of running, PTH increased.

These findings suggest that Ca^{2+} may control PTH secretion during exercise. The reasons for the initial increase in Ca^{2+} at the start of exercise in the two higher exercise intensities are unknown, although this might be important in explaining the decreased PTH concentrations with the onset of exercise. It could have been related to exercise-induced acidosis occurring in the first few minutes of exercise, before aerobic metabolism stabilises (31, 32), which can increase Ca^{2+} concentrations (33) but have minimal effects on ACa. Blood pH did not, however, decrease significantly during exercise, suggesting that exercise-induced acidosis was not the reason for the initial increase in Ca^{2+}. Further mechanistic studies are needed to identify why this initial increase occurs, but it could be from calcium being released from other binding proteins such as transferrin (34) or calcium dissociating from PO_4 (35, 36).

Changes in systemic PO_4 can influence PTH secretion, with Ahmad *et al.* (37) showing that circadian changes in PO_4 precede changes in PTH. During the increase in PTH in the present study, PO_4 and PTH were most strongly positively cross-correlated at -1 time lag, suggesting that increases in PO_4 precede those in PTH by less than 3.5 min. This cross-correlation was not as strong, however, as the cross-correlation between Ca^{2+} and PTH, which might indicate that both PO_4 and Ca^{2+} are influential during the increase in PTH. Our data do not fully support that the exercise-induced increases in PTH are driven solely by increased PO_4, as PO_4 increased with the onset of exercise despite the initial decrease in PTH. The increase in PO_4 might reflect release of PO_4 from PTH-induced bone resorption (15, 37, 38) towards the end of exercise, or that PO_4 is being released from muscle tissue, although this is speculative (39, 40). Taken together, these results suggest that Ca^{2+} is the stronger driver of PTH secretion and synthesis at the onset of exercise, however it is possible that the degree of association/dissociation between Ca^{2+} and PO_4 varies during exercise, meaning that PTH regulation might change accordingly.

With the decrease in PTH during recovery, the strongest positive cross-correlation between PO_4 and PTH occurred at a 0 time lag, suggesting that PTH decreased within 8 min of a decrease in PO_4. These findings support Scott *et al*. (15, 18–20), who showed that PO_4 followed the same response as PTH after exercise. If the decrease in PTH during recovery is explained by renal clearance (11), the strong cross-correlation may suggest that PO_4 is driving PTH clearance and over-riding Ca^{2+} regulation in recovery. Alternatively, the elevated PTH concentrations could be enhancing renal PO_4 excretion and causing a subsequent decrease in circulating PO_4 (41).

Reductions in vitamin D concentrations can contribute to an increase in PTH, as 1,25, dihydroxyvitamin D regulates the active transport of calcium and PO_4 absorption in the small intestine (42). Vitamin D status was not measured so we cannot confirm whether a change occurred during the study. The three trials were, however, completed within one month for each participant and the order of trials was randomised, meaning that, although changes in vitamin D concentrations could have occurred, they are unlikely to have influenced the results.

In conclusion, at the onset of exercise PTH transiently decreases then increases throughout exercise, peaking in the first minutes of recovery, before decreasing below the baseline concentration during ongoing recovery. Changes in Ca^{2+} and PO_4 occur in close temporal relation to changes in PTH. Cross-correlational analysis suggests that PTH secretion during exercise and recovery is controlled by a combination of changes in Ca^{2+} and PO_4 and that the mechanism might be different during exercise and recovery. ACa may not be a suitable surrogate for Ca^{2+} when investigating the rapid response to exercise, since ACa concentrations do not reflect temporal PTH responses or correlate strongly with PTH.

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Table Legends

Table 1. Baseline biochemistry across all trials.

Table 2. Pearson's correlation coefficient values for changes in PTH, with changes in PO_4, ACa and Ca^{2+}.

Table 3. Maximum cross-correlation values and corresponding lag times for PTH with PO_4, ACa and Ca^{2+}.

Figure Legends

Fig. 1. The percent change in baseline concentrations of PTH (A), PO_4 (B), ACa (C) and albumin (D) for all participants with 30 min of treadmill running at 55%VO_{2max} (open circles), 65%VO_{2max} (filled squares), 75%VO_{2max} (open triangles). Grey box denotes exercise. Data are mean±SD. [a] different ($P \leq 0.05$) from baseline (55%VO_{2max}) [b] different ($P \leq 0.05$) from baseline (65%VO_{2max}), [c] different ($P \leq 0.05$) from baseline (75%VO_{2max}). [*] 55%VO_{2max} different ($P \leq 0.05$) from 65%VO_{2max}, [α] 55%VO_{2max} different ($P \leq 0.05$) from 75%VO_{2max}, [•] 65%VO_{2max} different ($P \leq 0.05$) from 75%VO_{2max}.

Fig. 2. The percent change in baseline concentrations of PTH (A) and Ca^{2+} (B) for participants 5–10 with 30 min of treadmill running at 55%VO_{2max} (open circles), 65%VO_{2max} (filled squares), 75%VO_{2max} (open triangles). Grey box denotes exercise. Data are mean±SD. [a] different ($P \leq 0.05$) from baseline (55%VO_{2max}) [b] different ($P \leq 0.05$) from baseline (65%VO_{2max}), [c] different ($P \leq 0.05$) from baseline (75%VO_{2max}). [*] 55%VO_{2max} different ($P \leq 0.05$) from 65%VO_{2max}, [α] 55%VO_{2max} different ($P \leq 0.05$) from 75%VO_{2max}, [•] 65%VO_{2max} different ($P \leq 0.05$) from 75%VO_{2max}. Statistical analysis not reported or denoted for the PTH response in participants 5–10; data plotted for the comparison with Ca^{2+} only.

Table 1.

Measure	55%VO_{2max}	65%VO_{2max}	75%VO_{2max}
PTH ($pmol \cdot L^{-1}$)	2.62±0.88	2.51±0.50	2.63±0.60
PO_4 ($mmol \cdot L^{-1}$)	1.14±0.12	1.17±0.25	1.12±0.16
ACa ($mmol \cdot L^{-1}$)	2.83±0.21	2.83±0.23	2.78±0.22
Albumin ($g \cdot dL^{-1}$)	4.60±0.14	4.63±0.19	4.57±0.22
Ca^{2+} ($mmol \cdot L^{-1}$)	1.27±0.03 [a]	1.25±0.02	1.24±0.01

Data are mean±SD. [a] = Baseline Ca^{2+} at 55%VO_{2max} was significantly ($P \leq 0.05$) higher than at 65% and 75%VO_{2max}.

500 **Table 2.**

	r value		
Exercise intensity	PO_4	ACa	Ca^{2+}
55% VO_{2max}	0.175	-0.160	-0.739 [a]
65% VO_{2max}	0.215	-0.077	-0.769 [a]
75% VO_{2max}	0.416	0.078	-0.790 [a]

501 [a] = Significant correlation with PTH ($P \leq 0.001$).

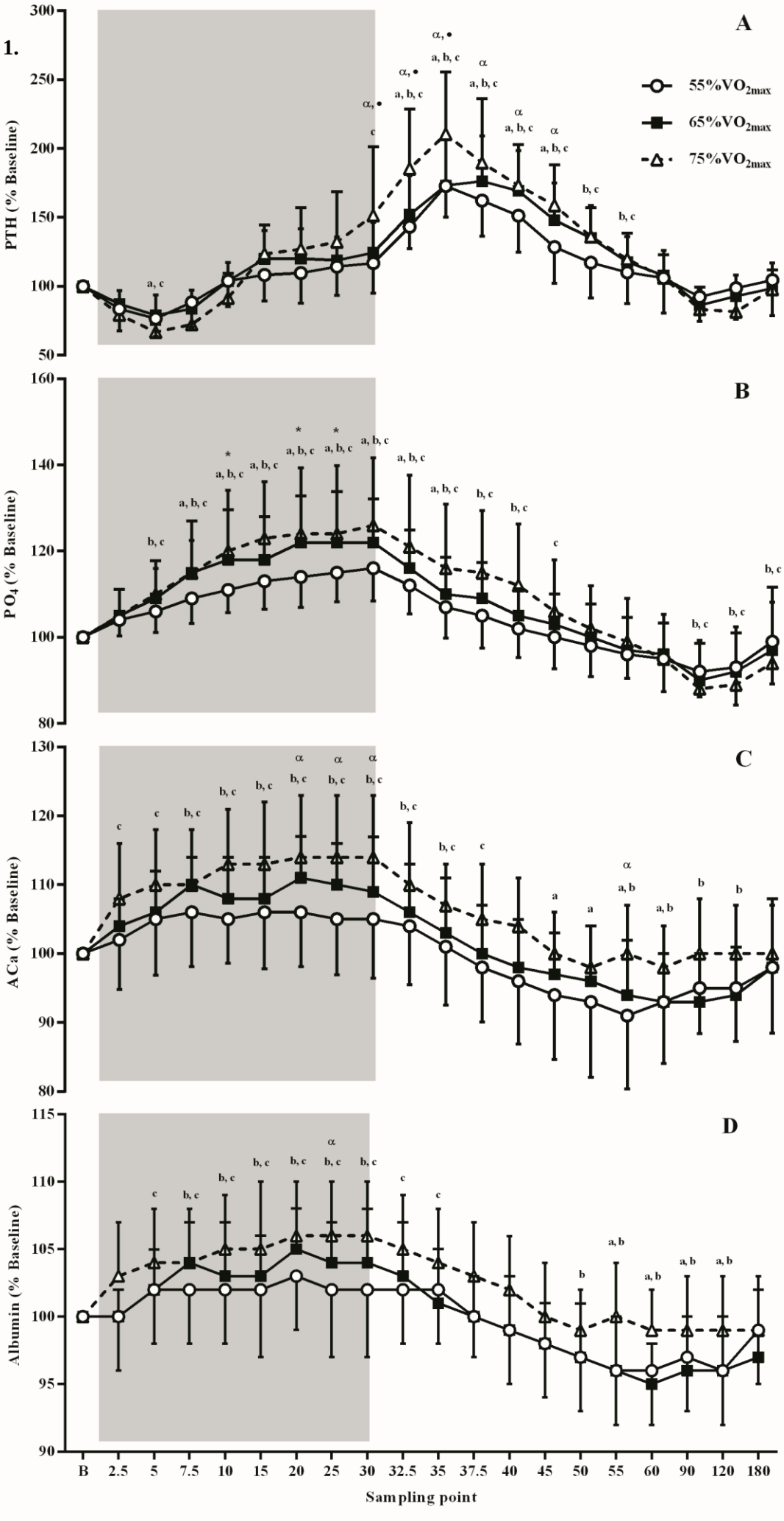
502 **Table 3.**

	PO_4		ACa		Ca^{2+}	
Exercise intensity	Time lag	*r* value	Time lag	*r* value	Time lag	*r* value
PEAK data points (baseline to 5 min of recovery)						
55%VO_{2max}	-1	0.661	0	-0.431	0	-0.902
65%VO_{2max}	-1	0.677	-2	0.550	0	-0.936
75%VO_{2max}	-1	0.772	-2	0.669	0	-0.950
DEC data points (5 to 90 min of recovery)						
55%VO_{2max}	0	0.995	0	0.761	+1	-0.794
65%VO_{2max}	0	0.987	0	0.908	0	-0.856
75%VO_{2max}	0	0.994	0	0.809	+1	-0.817

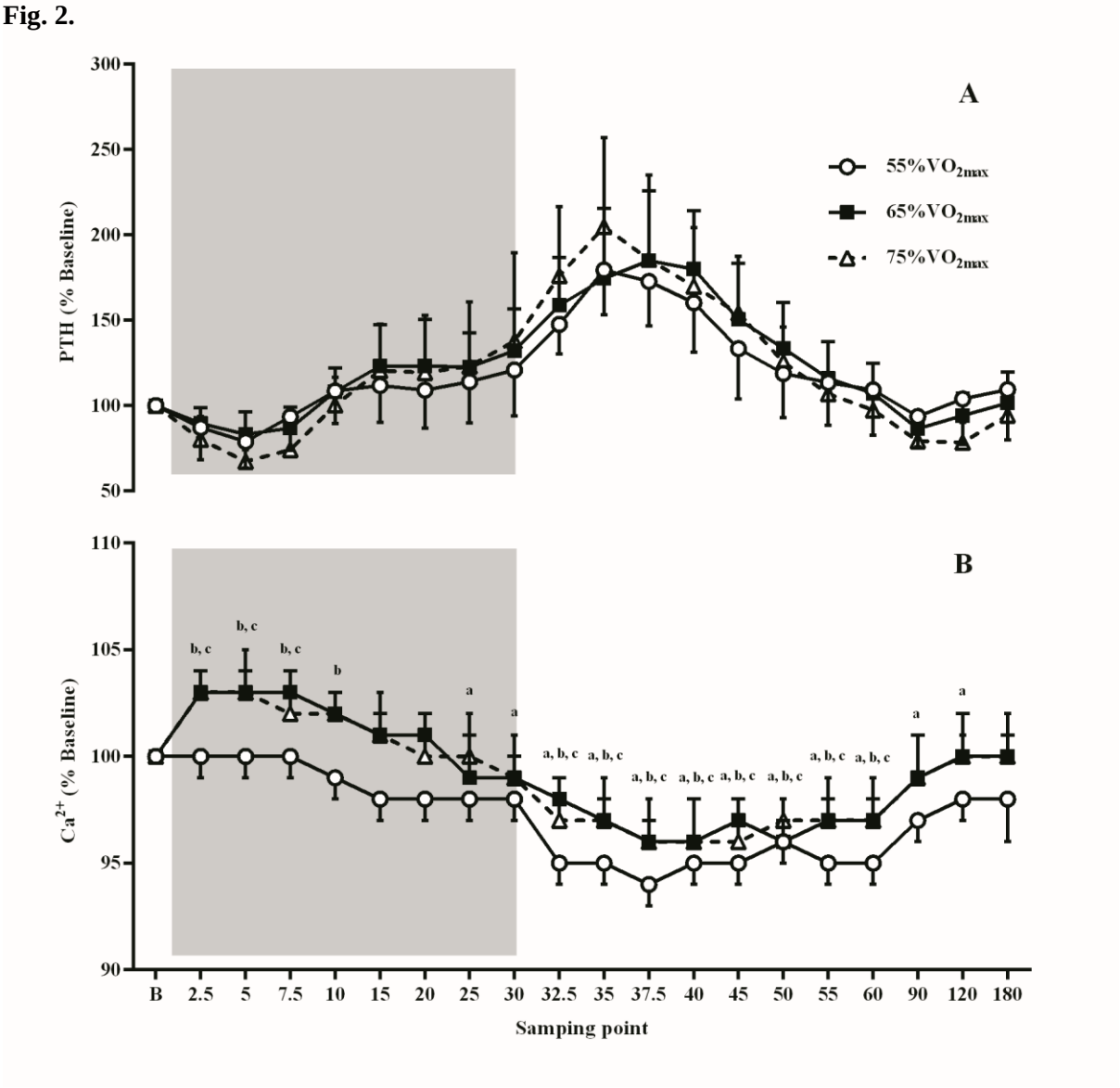
503

504

505 **Fig. 1.**


A
B
C
D
55%VO2max
65%VO2max
75%VO2max
PTH (% Baseline)
PO4 (% Baseline)
ACa (% Baseline)
Albumin (% Baseline)
Sampling point
B 2.5 5 7.5 10 15 20 25 30 32.5 35 37.5 40 45 50 55 60 90 120 180

506 **Fig. 2.**


A
B
55%VO2max
65%VO2max
75%VO2max
PTH (% Baseline)
Ca2+ (% Baseline)
Samping point
B 2.5 5 7.5 10 15 20 25 30 32.5 35 37.5 40 45 50 55 60 90 120 180