

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

3

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23

24 **Abstract**

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

27 ***Objective***

28 To examine the temporal pattern of PTH, PO<sub>4</sub>, ACa and Ca<sup>2+</sup> during and after exercise.

29 ***Design and setting***

30 A laboratory-based study with a cross-over design, comparing 30 min of running at 55%, 65% and  
31 75% VO<sub>2max</sub>, followed by 2.5-h of recovery. Blood was obtained at baseline, after 2.5, 5, 7.5, 10, 15, 20,  
32 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

33 ***Participants***

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

35 ***Main Outcome Measures***

36 PTH, PO<sub>4</sub>, ACa and Ca<sup>2+</sup>

37 ***Results***

38 Independent of intensity, PTH concentrations decreased with the onset of exercise (-21 to -33%;  
39  $P\leq 0.001$ ), increased thereafter and were higher than baseline by the end of exercise at 75%VO<sub>2max</sub>  
40 (+52%;  $P\leq 0.001$ ). PTH peaked transiently after 5–7.5 min of recovery (+73 to +110%;  $P\leq 0.001$ ). PO<sub>4</sub>  
41 followed a similar temporal pattern to PTH and Ca<sup>2+</sup> followed a similar but inverse pattern to PTH.  
42 PTH was negatively correlated with Ca<sup>2+</sup> across all intensities ( $r=-0.739$  to  $-0.790$ ;  $P\leq 0.001$ ). When  
43 PTH was increasing, the strongest cross-correlation was with Ca<sup>2+</sup> at 0 lags (3.5 min) ( $r=-0.902$  to -  
44 0.950); during recovery, the strongest cross-correlation was with PO<sub>4</sub> at 0 lags (8 min) ( $r=0.987$  to  
45 0.995).

46 ***Conclusions***

47 PTH secretion during exercise and recovery is controlled by a combination of changes in Ca<sup>2+</sup> and PO<sub>4</sub>  
48 in men.

49

50 **Abbreviations**

51 ACa, albumin-adjusted calcium; Ca, calcium; Ca<sup>2+</sup>, ionised calcium; CV, coefficient of variation;

52 PO<sub>4</sub>, phosphate; PTH, parathyroid hormone; VO<sub>2max</sub>, maximal oxygen consumption.

53 **Introduction**

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

69

70 Exercise increases PTH concentrations (11–20), although studies have used different exercise modes,  
71 durations and intensities. Exercise intensity is important, given that Scott *et al.* (17) have shown that  
72 60 min of running at 55%, 65% and 75% of maximal oxygen consumption ( $\text{VO}_{2\text{max}}$ ) results in different  
73 PTH responses during and after exercise. Any study investigating the underlying mechanisms  
74 responsible for the changes in PTH during exercise and recovery should examine the effects of exercise  
75 intensity.

76

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

79 (14, 18, 19) concomitantly with PTH. Barry *et al.* (16) showed that Ca ingestion before exercise  
80 attenuated, but did not abolish the increase in PTH, suggesting that some other mechanism contributed  
81 to the increase. This could involve phosphate (PO<sub>4</sub>), as an increase in PO<sub>4</sub> increases PTH in rested  
82 individuals (21). Following exercise, PO<sub>4</sub> concentrations decrease and the timing and magnitude of  
83 these decreases reflect those in PTH (17, 18, 20), also suggesting that PO<sub>4</sub> may be involved in PTH  
84 regulation with exercise.

85

86 The hypothesis that decreased Ca<sup>2+</sup> triggers increased PTH during exercise has not yet been proven (16).  
87 PTH is secreted within seconds of a decrease in Ca<sup>2+</sup> and subsequent increases in Ca<sup>2+</sup> take only minutes  
88 to occur in response to increased PTH, highlighting a dynamic relationship (1, 22). Despite this, no  
89 studies have measured PTH and other markers of Ca metabolism until 20 minutes of exercise has been  
90 completed, by which time PTH is elevated. Most studies have started taking measurements at 30 min  
91 post-exercise, by which time PTH has returned to near pre-exercise levels (15–19, 23). Single or  
92 infrequent measurements of PTH, ACa and PO<sub>4</sub> during and after exercise might fail to capture the  
93 dynamic nature of Ca regulation with exercise (16). Using repeated measurements with a high frequency,  
94 we examined the temporal pattern of PTH, PO<sub>4</sub>, ACa and Ca<sup>2+</sup> during and after 30 minutes of treadmill  
95 running at three exercise intensities.

## 96 **Materials and Methods**

### 97 *Participants*

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

104

### 105 *Experimental Design*

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

112

### 113 *Trial Procedures*

#### 114 *$VO_{2max}$*

115 Participants performed an incremental treadmill test to determine lactate threshold, followed by a ramp  
116 test to determine  $VO_{2max}$ , as per Jones and Doust (24). The level running velocities corresponding to  
117 55% ( $8.7\pm 0.6$  km·h<sup>-1</sup>), 65% ( $10.1\pm 0.8$  km·h<sup>-1</sup>) and 75%  $VO_{2max}$  ( $11.9\pm 0.9$  km·h<sup>-1</sup>) were calculated based  
118 on the regression of  $VO_2$  and velocity.

119

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, PO<sub>4</sub>, ACa and Ca<sup>2+</sup>. Thirty min of treadmill running at  
125 55%, 65% or 75% VO<sub>2max</sub> 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<sup>2+</sup> was measured  
128 immediately but due to equipment availability Ca<sup>2+</sup> 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<sup>-1</sup> 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<sup>-1</sup>·h<sup>-1</sup> 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<sup>-1</sup> and sensitivity of 0.8 pmol·L<sup>-1</sup>.  
139 PO<sub>4</sub>, 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<sup>-1</sup> for PO<sub>4</sub>, ≤1.7% between 0.04–5.00 mmol·L<sup>-1</sup> for total Ca and  
142 ≤1.9% between 0.02–5.99 g·dL<sup>-1</sup> for albumin. Because fluctuations in protein, particularly albumin,  
143 may cause total Ca levels to change independently of the Ca<sup>2+</sup> concentrations, total Ca concentrations  
144 were corrected to give albumin-adjusted Ca values: 0.8 mg·dL<sup>-1</sup> was subtracted from total Ca  
145 concentrations for every 1.0 g·dL<sup>-1</sup> that albumin concentrations were less than 4 g·dL<sup>-1</sup> or 0.8 mg·dL<sup>-1</sup>

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

152

153

#### 154 *Statistical Analysis*

155 Statistical significance was accepted at  $P \leq 0.05$ . Baseline concentrations were compared using one-way  
156 ANOVA. All data were analysed using repeated measures ANOVA, with *Intensity* (55% vs 65% vs  
157 75% VO<sub>2max</sub>) and *Time* (of sampling) as within subject factors. Parametric assumptions of normality and  
158 sphericity were confirmed using Shapiro-Wilks and Mauchly's tests. Tukey's HSD *post-hoc* test was  
159 used to compare timepoints against baseline and to compare exercise intensities at each timepoint,  
160 where appropriate. Pearson's correlation coefficients were calculated for PO<sub>4</sub>, A Ca and Ca<sup>2+</sup> with PTH.

161

162 Cross-correlational analyses were performed to determine the temporal relationships between PTH and  
163 PO<sub>4</sub>, A Ca and Ca<sup>2+</sup>. Cubic interpolation was performed to adjust for unevenly spaced data points and  
164 cross-correlational analyses were subsequently performed using R (version 3.2.2, Vienna, Austria). To  
165 determine whether one time series led another, cross-correlation functions were computed at seven lag  
166 time points for 'PEAK' (data points between baseline and peak PTH concentrations [5 min of recovery]),  
167 where each lag represented 3.5 min, and six lag time points for 'DEC' (all data points during the  
168 decrease in PTH concentrations [5 to 90 min of recovery]), where each lag represented 8 min.

169 **Results**

170

171 **Baseline biochemistry**

172 Baseline PTH, PO<sub>4</sub>, A Ca and albumin were not significantly different between trials ( $P=0.339$  to  $0.982$ ).

173 Baseline Ca<sup>2+</sup> at 55% VO<sub>2max</sub> was significantly ( $P\leq 0.05$ ) higher than at 65% VO<sub>2max</sub> and 75% VO<sub>2max</sub>

174 (Table 1).

175

176 **PTH**

177 There was no main effect of *Intensity*, but there was a main effect of *Time* ( $P\leq 0.001$ ) and an *Intensity x*

178 *Time* interaction ( $P\leq 0.001$ ). PTH concentrations decreased with the onset of exercise and were

179 significantly lower than baseline after 5 min of exercise at 55% VO<sub>2max</sub> (-23%;  $P\leq 0.05$ ) and 75% VO<sub>2max</sub>

180 (-33%;  $P\leq 0.001$ ), but not at 65% VO<sub>2max</sub> (-21%;  $P=0.305$ ) (Fig. 1A all participants; Fig. 2A participants

181 5–10). Thereafter, PTH increased, becoming significantly greater than baseline at the end of exercise

182 (30 min) at 75% VO<sub>2max</sub> (+52%;  $P\leq 0.001$ ) and after 2.5 min of recovery at 55% VO<sub>2max</sub> (+43%;  $P\leq 0.001$ )

183 and 65% VO<sub>2max</sub> (+52%;  $P\leq 0.001$ ). PTH concentrations peaked after 5 min of recovery at 55% VO<sub>2max</sub>

184 (+73%;  $P\leq 0.001$ ) and 75% VO<sub>2max</sub> (+110%;  $P\leq 0.001$ ), and after 7.5 min of recovery at 65% VO<sub>2max</sub> (+76;

185  $P\leq 0.001$ ). PTH concentrations then decreased, but remained significantly higher than baseline until 15

186 min into recovery at 55% VO<sub>2max</sub> and until 25 min at 65% VO<sub>2max</sub> and 75% VO<sub>2max</sub>. PTH concentrations

187 decreased below baseline after 60 min of recovery in all trials (-8% to -17%).

188

189 PTH concentrations were not significantly different at any time point between 55% and 65% VO<sub>2max</sub>

190 trials. Exercise at 75% VO<sub>2max</sub> resulted in significantly higher PTH concentrations than at 55% VO<sub>2max</sub>

191 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

192 ( $P\leq 0.001$ ) min into recovery, and higher than exercise at 65% VO<sub>2max</sub> at the end of exercise ( $P\leq 0.001$ ),

193 and at 2.5 ( $P\leq 0.001$ ) and 5 ( $P\leq 0.001$ ) min into recovery.



194

195 ***PO<sub>4</sub>***

196 There was no main effect of *Intensity*, but there was a main effect of *Time* ( $P \leq 0.001$ ) and an *Intensity x*  
197 *Time* interaction ( $P \leq 0.05$ ).  $PO_4$  concentrations increased with the onset of exercise at all intensities,  
198 being significantly higher than baseline from 7.5 min to the end of exercise at 55%  $VO_{2max}$  (+16%;  
199  $P \leq 0.001$ ), and between 5 min and the end of exercise at 65%  $VO_{2max}$  (+22%) and 75%  $VO_{2max}$  (+26%)  
200 ( $P \leq 0.05$  to  $P \leq 0.001$ ) (Fig. 1B).  $PO_4$  concentrations peaked at the end of exercise, and decreased  
201 thereafter, but remained significantly higher than baseline until 5 min into recovery at 55%  $VO_{2max}$ , 10  
202 min at 65%  $VO_{2max}$  and 15 min at 75%  $VO_{2max}$ .  $PO_4$  concentrations decreased below baseline at 60 min  
203 of recovery and remained so until 150 minutes of recovery at 65%  $VO_{2max}$  (-5 to -10%) and 75%  $VO_{2max}$   
204 (-7 to -12%) ( $P \leq 0.05$  to  $P \leq 0.001$ ). Concentrations did not decrease significantly below baseline at  
205 55%  $VO_{2max}$ .

206

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

209

210 ***ACa***

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

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

221

222 Exercise at 75%  $\text{VO}_{2\text{max}}$  resulted in significantly higher A Ca concentrations than exercise at 55%  $\text{VO}_{2\text{max}}$   
223 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 ( $P \leq 0.01$ ).

224

### 225 *Albumin*

226 There was no main effect of *Intensity*, but there was a main effect of *Time* ( $P \leq 0.001$ ) and an *Intensity x*  
227 *Time* interaction ( $P \leq 0.01$ ). Albumin concentrations increased with the onset of exercise and were higher  
228 than baseline between 7.5 min and the end of exercise at 65%  $\text{VO}_{2\text{max}}$  (+4%;  $P \leq 0.05$ ) and between 5 min  
229 of exercise and the end of exercise at 75%  $\text{VO}_{2\text{max}}$  (+6%;  $P \leq 0.05$ ) (Fig. 1D). Albumin concentrations  
230 peaked after 20 min of exercise and decreased thereafter, but remained higher than baseline until 5 min  
231 into recovery at 75%  $\text{VO}_{2\text{max}}$  ( $P \leq 0.001$ ). Albumin concentrations decreased below baseline 25 min into  
232 recovery and remained so until 90 min of recovery at 55%  $\text{VO}_{2\text{max}}$  (-3 to -4%;  $P \leq 0.01$ ). Concentrations  
233 decreased below baseline 20 min into recovery and remained so until 90 min of recovery at 65%  $\text{VO}_{2\text{max}}$   
234 (-3 to -5%;  $P \leq 0.05$  to  $P \leq 0.001$ ). Albumin concentrations did not decrease below baseline at 75%  $\text{VO}_{2\text{max}}$ .

235

236 Exercise at 75%  $\text{VO}_{2\text{max}}$  resulted in significantly higher albumin concentrations than exercise at  
237 55%  $\text{VO}_{2\text{max}}$  after 25 min of exercise ( $P \leq 0.05$ ).

238

### 239 $\text{Ca}^{2+}$

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

243  $\text{Ca}^{2+}$  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% $\text{VO}_{2\text{max}}$  and 75% $\text{VO}_{2\text{max}}$   $\text{Ca}^{2+}$  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% $\text{VO}_{2\text{max}}$  (+2 to +3%;  $P \leq 0.001$ ) and between 2.5 and 7.5 min at 75% $\text{VO}_{2\text{max}}$  (+2 to  
247 +3%;  $P \leq 0.001$ ). Thereafter,  $\text{Ca}^{2+}$  concentrations decreased and were significantly below baseline  
248 between 2.5 and 30 min of recovery at 65% $\text{VO}_{2\text{max}}$  (-3 to -4%;  $P \leq 0.05$  to  $P \leq 0.001$ ) and 75% $\text{VO}_{2\text{max}}$  (-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  $\text{PO}_4$  or ACa in any trial. Across all data points  
256 PTH was significantly ( $P \leq 0.001$ ) negatively correlated with  $\text{Ca}^{2+}$  at all intensities (Table 2).

257

258 Across PEAK data points,  $\text{PO}_4$  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  $\text{PO}_4$  series, suggesting  
260 that increases in  $\text{PO}_4$  precede increases in PTH by 3.5 min.  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ .

263

264 Across DEC data points,  $\text{PO}_4$ , ACa and  $\text{Ca}^{2+}$  were correlated with PTH at all exercise intensities.  $\text{PO}_4$   
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  $\text{PO}_4$ .

267 **Discussion**

268 The novel findings from this study are: 1) changes in PTH, PO<sub>4</sub>, ACa and Ca<sup>2+</sup> occur within 2.5 min of  
269 the onset of exercise; 2) there is an initial decrease in PTH concentrations at the start of exercise that  
270 coincides with a significant increase in Ca<sup>2+</sup> concentrations at the two higher exercise intensities; 3)  
271 peak PTH concentrations occur within 5–7.5 min of recovery; 4) increases in PO<sub>4</sub> precede increases in  
272 PTH; 5) decreases in Ca<sup>2+</sup> precede increases in PTH; 6) post-exercise decreases in PTH concentrations  
273 are preceded by decreases in PO<sub>4</sub>.

274

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

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

299

300 Cross-correlations suggested that PTH secretion during exercise and recovery is controlled by a  
301 combination of changes in  $\text{Ca}^{2+}$  and  $\text{PO}_4$ .  $\text{Ca}^{2+}$  is not routinely measured due to analytical difficulties;  
302 consequently ACa is estimated as a surrogate and has been shown clinically to be a reliable indicator of  
303 Ca metabolism at rest (30). We have shown different responses to exercise and recovery between ACa  
304 and  $\text{Ca}^{2+}$  and also different relationships with PTH;  $\text{Ca}^{2+}$  concentrations were correlated with PTH,  
305 whereas ACa was not. Albumin changes taking place during exercise will have a greater effect on the  
306 ACa estimation compared to the small effect that can occur on  $\text{Ca}^{2+}$  measurement; changes in pH were  
307 not sufficient to have a major effect on  $\text{Ca}^{2+}$  measurement by the blood gas analyser. The results support  
308 previous data (14, 15, 17–20) suggesting that changes in ACa do not explain the changes in PTH or  
309 regulation of PTH during exercise, because, as PTH is increasing, ACa either also increases (15, 17) or  
310 is unchanged (14, 18, 19). Scott *et al.* (19) argued that because both PTH and ACa were increased after  
311 20 minutes of exercise, a decrease in  $\text{Ca}^{2+}$  could have occurred in the first few minutes of exercise,  
312 stimulating the secretion of PTH and causing serum  $\text{Ca}^{2+}$  concentrations to increase as a result of PTH-  
313 stimulated bone resorption and  $\text{Ca}^{2+}$  liberation. However, through frequent sampling, we have shown  
314 that ACa and  $\text{Ca}^{2+}$ , at 65% and 75%  $\text{VO}_{2\text{max}}$ , increase within 2.5 min of exercise, with ACa increasing  
315 and  $\text{Ca}^{2+}$  decreasing thereafter. Although it is well established that PTH responds rapidly to a reduction  
316 in  $\text{Ca}^{2+}$  at rest (1, 22), this is the first study to show that this rapid response also occurs during exercise.  
317 The lack of an initial increase in  $\text{Ca}^{2+}$  at 55%  $\text{VO}_{2\text{max}}$  is surprising and the reason for this is currently  
318 unknown. The strong negative correlation of PTH and  $\text{Ca}^{2+}$  during exercise at all three intensities with  
319 a 0 time lag ( $r=-0.902$  to  $-0.950$ ) suggests that as  $\text{Ca}^{2+}$  decreases, PTH increases within 3.5 min. This

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

322

323 These findings suggest that  $\text{Ca}^{2+}$  may control PTH secretion during exercise. The reasons for the initial  
324 increase in  $\text{Ca}^{2+}$  at the start of exercise in the two higher exercise intensities are unknown, although this  
325 might be important in explaining the decreased PTH concentrations with the onset of exercise. It could  
326 have been related to exercise-induced acidosis occurring in the first few minutes of exercise, before  
327 aerobic metabolism stabilises (31, 32), which can increase  $\text{Ca}^{2+}$  concentrations (33) but have minimal  
328 effects on ACa. Blood pH did not, however, decrease significantly during exercise, suggesting that  
329 exercise-induced acidosis was not the reason for the initial increase in  $\text{Ca}^{2+}$ . Further mechanistic studies  
330 are needed to identify why this initial increase occurs, but it could be from calcium being released from  
331 other binding proteins such as transferrin (34) or calcium dissociating from  $\text{PO}_4$  (35, 36).

332

333 Changes in systemic  $\text{PO}_4$  can influence PTH secretion, with Ahmad *et al.* (37) showing that circadian  
334 changes in  $\text{PO}_4$  precede changes in PTH. During the increase in PTH in the present study,  $\text{PO}_4$  and PTH  
335 were most strongly positively cross-correlated at -1 time lag, suggesting that increases in  $\text{PO}_4$  precede  
336 those in PTH by less than 3.5 min. This cross-correlation was not as strong, however, as the cross-  
337 correlation between  $\text{Ca}^{2+}$  and PTH, which might indicate that both  $\text{PO}_4$  and  $\text{Ca}^{2+}$  are influential during  
338 the increase in PTH. Our data do not fully support that the exercise-induced increases in PTH are driven  
339 solely by increased  $\text{PO}_4$ , as  $\text{PO}_4$  increased with the onset of exercise despite the initial decrease in PTH.  
340 The increase in  $\text{PO}_4$  might reflect release of  $\text{PO}_4$  from PTH-induced bone resorption (15, 37, 38) towards  
341 the end of exercise, or that  $\text{PO}_4$  is being released from muscle tissue, although this is speculative (39,  
342 40). Taken together, these results suggest that  $\text{Ca}^{2+}$  is the stronger driver of PTH secretion and synthesis  
343 at the onset of exercise, however it is possible that the degree of association/dissociation between  $\text{Ca}^{2+}$   
344 and  $\text{PO}_4$  varies during exercise, meaning that PTH regulation might change accordingly.

345

346 With the decrease in PTH during recovery, the strongest positive cross-correlation between PO<sub>4</sub> and  
347 PTH occurred at a 0 time lag, suggesting that PTH decreased within 8 min of a decrease in PO<sub>4</sub>. These  
348 findings support Scott *et al.* (15, 18–20), who showed that PO<sub>4</sub> followed the same response as PTH  
349 after exercise. If the decrease in PTH during recovery is explained by renal clearance (11), the strong  
350 cross-correlation may suggest that PO<sub>4</sub> is driving PTH clearance and over-riding Ca<sup>2+</sup> regulation in  
351 recovery. Alternatively, the elevated PTH concentrations could be enhancing renal PO<sub>4</sub> excretion and  
352 causing a subsequent decrease in circulating PO<sub>4</sub> (41).

353

354 Reductions in vitamin D concentrations can contribute to an increase in PTH, as 1,25, dihydroxyvitamin  
355 D regulates the active transport of calcium and PO<sub>4</sub> absorption in the small intestine (42). Vitamin D  
356 status was not measured so we cannot confirm whether a change occurred during the study. The three  
357 trials were, however, completed within one month for each participant and the order of trials was  
358 randomised, meaning that, although changes in vitamin D concentrations could have occurred, they are  
359 unlikely to have influenced the results.

360

361 In conclusion, at the onset of exercise PTH transiently decreases then increases throughout exercise,  
362 peaking in the first minutes of recovery, before decreasing below the baseline concentration during  
363 ongoing recovery. Changes in Ca<sup>2+</sup> and PO<sub>4</sub> occur in close temporal relation to changes in PTH. Cross-  
364 correlational analysis suggests that PTH secretion during exercise and recovery is controlled by a  
365 combination of changes in Ca<sup>2+</sup> and PO<sub>4</sub> and that the mechanism might be different during exercise and  
366 recovery. A Ca may not be a suitable surrogate for Ca<sup>2+</sup> when investigating the rapid response to exercise,  
367 since A Ca concentrations do not reflect temporal PTH responses or correlate strongly with PTH.

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474 **Table Legends**

475

476 **Table 1.** Baseline biochemistry across all trials.

477 **Table 2.** Pearson's correlation coefficient values for changes in PTH, with changes in PO<sub>4</sub>, ACa and

478 Ca<sup>2+</sup>.

479 **Table 3.** Maximum cross-correlation values and corresponding lag times for PTH with PO<sub>4</sub>, ACa and

480 Ca<sup>2+</sup>.

481 **Figure Legends**

482

483 **Fig. 1.** The percent change in baseline concentrations of PTH (A), PO<sub>4</sub> (B), ACa (C) and albumin (D)  
484 for all participants with 30 min of treadmill running at 55% VO<sub>2max</sub> (open circles), 65% VO<sub>2max</sub> (filled  
485 squares), 75% VO<sub>2max</sub> (open triangles). Grey box denotes exercise. Data are mean±SD. <sup>a</sup> different  
486 ( $P\leq 0.05$ ) from baseline (55% VO<sub>2max</sub>) <sup>b</sup> different ( $P\leq 0.05$ ) from baseline (65% VO<sub>2max</sub>), <sup>c</sup> different  
487 ( $P\leq 0.05$ ) from baseline (75% VO<sub>2max</sub>). \* 55% VO<sub>2max</sub> different ( $P\leq 0.05$ ) from 65% VO<sub>2max</sub>, <sup>a</sup> 55% VO<sub>2max</sub>  
488 different ( $P\leq 0.05$ ) from 75% VO<sub>2max</sub>, • 65% VO<sub>2max</sub> different ( $P\leq 0.05$ ) from 75% VO<sub>2max</sub>.

489

490 **Fig. 2.** The percent change in baseline concentrations of PTH (A) and Ca<sup>2+</sup> (B) for participants 5–10  
491 with 30 min of treadmill running at 55% VO<sub>2max</sub> (open circles), 65% VO<sub>2max</sub> (filled squares), 75% VO<sub>2max</sub>  
492 (open triangles). Grey box denotes exercise. Data are mean±SD. <sup>a</sup> different ( $P\leq 0.05$ ) from baseline  
493 (55% VO<sub>2max</sub>) <sup>b</sup> different ( $P\leq 0.05$ ) from baseline (65% VO<sub>2max</sub>), <sup>c</sup> different ( $P\leq 0.05$ ) from baseline  
494 (75% VO<sub>2max</sub>). \* 55% VO<sub>2max</sub> different ( $P\leq 0.05$ ) from 65% VO<sub>2max</sub>, <sup>a</sup> 55% VO<sub>2max</sub> different ( $P\leq 0.05$ ) from  
495 75% VO<sub>2max</sub>, • 65% VO<sub>2max</sub> different ( $P\leq 0.05$ ) from 75% VO<sub>2max</sub>. Statistical analysis not reported or  
496 denoted for the PTH response in participants 5–10; data plotted for the comparison with Ca<sup>2+</sup> only.

497 **Table 1.**

Measure	55% VO <sub>2max</sub>	65% VO <sub>2max</sub>	75% VO <sub>2max</sub>
PTH (pmol·L <sup>-1</sup> )	2.62±0.88	2.51±0.50	2.63±0.60
PO <sub>4</sub> (mmol·L <sup>-1</sup> )	1.14±0.12	1.17±0.25	1.12±0.16
ACa (mmol·L <sup>-1</sup> )	2.83±0.21	2.83±0.23	2.78±0.22
Albumin (g·dL <sup>-1</sup> )	4.60±0.14	4.63±0.19	4.57±0.22
Ca <sup>2+</sup> (mmol·L <sup>-1</sup> )	1.27±0.03 <sup>a</sup>	1.25±0.02	1.24±0.01

498 Data are mean±SD. <sup>a</sup> = Baseline Ca<sup>2+</sup> at 55% VO<sub>2max</sub> was significantly ( $P\leq 0.05$ ) higher than at 65% and

499 75% VO<sub>2max</sub>.

500 **Table 2.**

Exercise intensity	<i>r</i> value		
	PO <sub>4</sub>	ACa	Ca <sup>2+</sup>
55% VO <sub>2max</sub>	0.175	-0.160	-0.739 <sup>a</sup>
65% VO <sub>2max</sub>	0.215	-0.077	-0.769 <sup>a</sup>
75% VO <sub>2max</sub>	0.416	0.078	-0.790 <sup>a</sup>

501 <sup>a</sup> = Significant correlation with PTH ( $P \leq 0.001$ ).



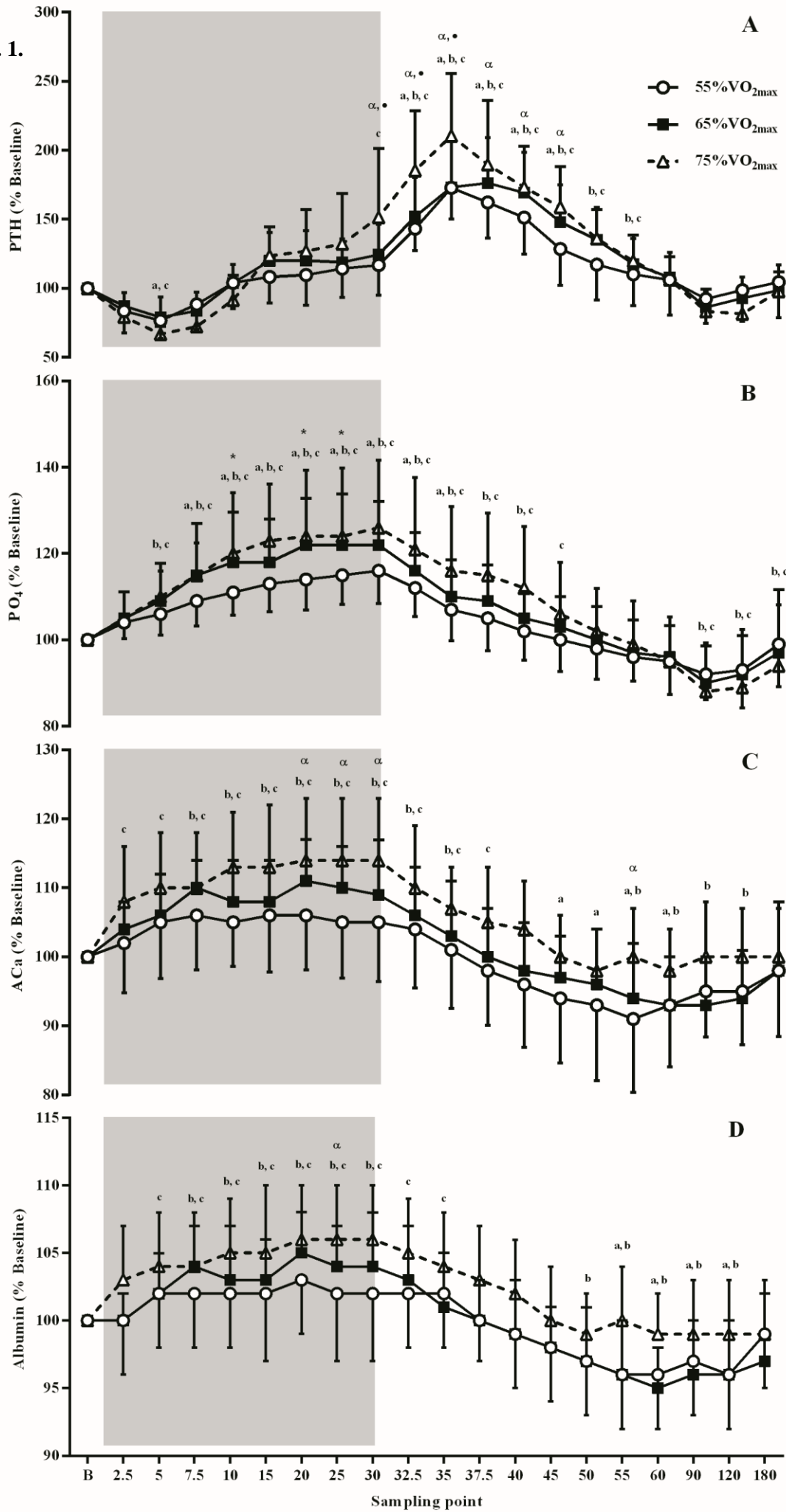
502 **Table 3.**

Exercise intensity	PO <sub>4</sub>		ACa		Ca <sup>2+</sup>	
	Time lag	<i>r</i> value	Time lag	<i>r</i> value	Time lag	<i>r</i> value
<i>PEAK data points (baseline to 5 min of recovery)</i>						
55% VO <sub>2max</sub>	-1	0.661	0	-0.431	0	-0.902
65% VO <sub>2max</sub>	-1	0.677	-2	0.550	0	-0.936
75% VO <sub>2max</sub>	-1	0.772	-2	0.669	0	-0.950
<i>DEC data points (5 to 90 min of recovery)</i>						
55% VO <sub>2max</sub>	0	0.995	0	0.761	+1	-0.794
65% VO <sub>2max</sub>	0	0.987	0	0.908	0	-0.856
75% VO <sub>2max</sub>	0	0.994	0	0.809	+1	-0.817

503

504

Fig. 1.



506 Fig. 2.

