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The reliability of measuring gross efficiency during high intensity cycling exercise

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Keywords:	anaerobic capacity, excess post-exercise oxygen consumption, pacing strategy, exercise performance, maximal exercise

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1 Abstract

2 **Purpose:** To evaluate the reliability of calculating gross efficiency (GE) conventionally and using a
3 back extrapolation (BE) method during high intensity exercise (HIE).

4 **Methods:** 12 trained participants completed two HIE bouts (P1 = 4-min 80% Maximal Aerobic
5 Power (MAP); P2 = 4-min at 100%_{MAP}). GE was calculated conventionally in the last 3 min of
6 submaximal (50%_{MAP}) cycling bouts performed before and after HIE (Pre50%_{MAP} and Post 50%_{MAP}).
7 To calculate GE using BE (BE_{GE}), a linear regression of $\dot{V}O_2$ -GE submaximal values post-HIE were
8 back extrapolated to the end of the HIE bout.

9 **Results:** BE_{GE} was significantly correlated with Post50%_{MAP} GE in P1 ($r = 0.64$; $P = 0.01$), and in
10 P2 ($r = 0.85$; $P = 0.002$). Reliability data for P1 and P2 BE_{GE} demonstrate a mean CV of 7.8% and
11 9.8% with limits of agreement of 4.3% and 4.5% in relative GE units respectively. P2 BE_{GE} was
12 significantly lower than P2 Post50%_{MAP} GE ($18.1 \pm 1.6\%$ vs $20.3 \pm 1.7\%$; $P = < 0.01$). Using a
13 declining GE from the BE method, there was a 44% greater anaerobic contribution compared to
14 assuming a constant GE during 4 min HIE at 100%_{MAP}.

15 **Conclusion:** HIE acutely reduced BE_{GE} at 100%_{MAP}. A greater anaerobic contribution to exercise
16 as well as excess post oxygen consumption at 100%_{MAP} may contribute to this decline in efficiency.
17 The BE method may be a reliable and valid tool in both estimating GE during HIE and calculating
18 aerobic and anaerobic contributions.

19
20 **Keywords:** anaerobic capacity, excess post-exercise oxygen consumption, pacing strategy,
21 performance, maximal exercise

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38 Introduction

39 Gross efficiency (GE) is defined as the ratio of work generated to the metabolic energy cost, and has
40 been shown to be a key component of cycling performance.^{1,2} The calculation of GE is
41 conventionally determined from steady state measures where energy expenditure from purely aerobic
42 processes can be accounted for via expired gases measured at the mouth.

43 However, during high-intensity exercise (HIE) with a significant anaerobic energy contribution, VO_2
44 measured at the mouth cannot be used to estimate the total energy expenditure. Therefore,
45 conventional measurement of GE during submaximal exercise may not be a valid estimate of GE
46 during HIE. A novel approach to estimate GE during high intensity exercise has recently been
47 proposed.³ This method uses linear regression of post-HIE GE values and back extrapolates these
48 values to estimate GE at the end of the HIE-bout. Using this back-extrapolation (BE) method, de
49 Koning et al.³ found GE declined by 2.5% during 4 minutes of cycling at 100% $_{MAP}$. As a result, the
50 calculated anaerobic contribution to their HIE bout was 32% larger when assuming a declining vs
51 constant GE (23.7kJ vs 17.9kJ).³

52 The BE method has previously been used to investigate the impact of GE on high intensity cycling
53 performance.⁴ It has been demonstrated that the estimated anaerobic contribution to cycling time trial
54 performance is 30% larger during time trials of less than 4000m when a declining rather than constant
55 GE is assumed.⁴ A declining GE and a higher anaerobic contribution could therefore have important
56 implications for both pacing strategy and performance. However, the reliability and validity of the
57 BE method in estimating GE during HIE has not previously been assessed.

58 There is also debate in the literature regarding possible physiological factors that might be influential
59 in the reduction of GE seen following both prolonged submaximal^{5,6,7,8} and high intensity^{9,10} bouts of
60 exercise. Near-infrared spectroscopy (NIRS) has previously been used to investigate changes in
61 muscle oxygen consumption during a bout of prolonged constant load cycling. It has been shown that
62 90 mins of cycling at 60% maximal minute power resulted in an increased muscle oxygen
63 consumption, and reduction in whole body GE.⁸ Whether the same relationships are seen during short
64 duration high intensity cycling is yet to be elucidated.

65 The purpose of this study is: 1) to assess the reliability and validity of measuring whole body GE in
66 HIE calculated using the BE method, compared to traditional submaximal methods 2) to investigate
67 the relationship between changes in whole body GE and NIRS parameters from high intensity
68 exercise.

69

70 Methods

71 *Subjects*

72 Thirteen trained male (mean \pm SD: age 35 ± 5 yr, mass 75 ± 7 kg, VO_{2max} 63 ± 7 ml \cdot kg⁻¹ \cdot min⁻¹,
73 Maximal Aerobic Power (MAP) 389 ± 46 W) and 2 female (age 25 ± 5 yr, mass 60 ± 1 kg, VO_{2max}
74 50 ± 2 ml \cdot kg⁻¹ \cdot min⁻¹, MAP 272 ± 39 W) participants who trained for a minimum of 6 hours per week
75 volunteered to participate in the study. The study was conducted with full university ethical approval
76 and after obtaining informed written consent from all participants.

77 *Experimental design*

78 Participants attended the exercise testing laboratory on four separate occasions. Visit 1 consisted of
79 a maximal incremental exercise test to determine $\text{VO}_{2\text{max}}$ and MAP. On ~~23~~ subsequent visits
80 participants completed 2 high intensity bouts in the same order; ~~one bout at 80% MAP , 30-min rest,~~
81 ~~followed by a bout at 100% MAP .~~ Each HIE bout was preceded and followed with a submaximal
82 exercise bout at 50% MAP . One HIE was performed at 80% MAP , and the second HIE bout at 100% MAP .
83 All tests were performed on an electromagnetically braked ergometer (Schoberer Rad Messtechnik,
84 Germany). Handlebar and saddle height were adjusted for each individual during visit 1, and then
85 replicated for each subsequent visit. Participants used their own clipless pedals. Respiratory
86 exchange data was collected on a breath-by-breath basis during each visit using an online gas analyser
87 (Metalyser 3B; CORTEX Biophysik GmbH, Germany).

88

89 *Maximal Incremental Exercise Test*

90 Upon reporting to the laboratory, body mass was measured to the nearest 0.1 kg using beam balance
91 scales (Seca, Germany), and stature was measured to the nearest 0.5 cm using a stadiometer (Seca,
92 Germany). Participants undertook a maximal incremental cycling test to ascertain their $\text{VO}_{2\text{max}}$ and
93 MAP. The test started with a 10-min warm up at 100W. After a 1-min rest the cycling output increased
94 by 5 W every 15 s until the participant reached volitional exhaustion (defined as a cadence of <60
95 revolutions/minute despite strong verbal encouragement). $\text{VO}_{2\text{max}}$ was determined as the highest
96 measured 60 s VO_2 achieved during the incremental test. MAP was calculated as the average power
97 output over the final minute of the ramp test.

98

99 *High Intensity Exercise Tests*

100 Participants completed two HIE 4-min bouts of exercise in the same order per visit, the first HIE bout
101 at 80% MAP (P1) and the second HIE bout at 100% MAP (P2) (see Figure 1). ~~Bouts were separated by~~
102 ~~30-minutes recovery.~~ Each HIE bout was preceded by 6-min cycling at 50% MAP and 2-min at 25
103 W, and followed by 1-min at 25 W and 10-min at 50% MAP . P1 and P2 were separated by 30 minute
104 rest. Participants were instructed to maintain a cadence of 80 $\text{rev}\cdot\text{min}^{-1}$ throughout the testing
105 protocols.

106

107 ***INSERT FIGURE 1 HERE***

108

109 Muscle oxygenation was measured continuously throughout the trials via near-infrared spatially
110 resolved dual-wavelength spectrometry (Portamon, Artinins Medical Systems, Netherlands), emitting
111 light at 760 nm and 850 nm wavelengths. The device was placed on the right thigh over the belly of
112 the vastus lateralis muscle, 10cm proximal to the knee joint. Relative concentration changes in
113 deoxygenated haemoglobin (HHb) were calculated from an arbitrary baseline value taken for 2-min
114 prior to the start of each exercise protocol. An absolute measure of tissue oxygen saturation (TSI%)
115 was also recorded throughout the exercise trial. Skinfold thickness was measured at the site of the
116 NIRS device over the vastus lateralis muscle in the seated position using Harpenden skinfold calipers
117 (British Indicators Ltd, Burgess Hill, UK). Adipose tissue thickness (ATT) was calculated by taking
118 the median of three skinfold measurements and dividing the skinfold thickness by two with a mean
119 value of 6.8 ± 2.5 mm.

120 Blood lactate was taken via a finger-prick blood sample immediately prior to the start and
 121 immediately at the end of the trial (Biosen C-Line analyser, EKF diagnostics, Wales).

122

123 Data analysis

124 Expired gas data measured at steady state during the last 3-min of the 50%_{MAP} bouts¹¹ prior to, and
 125 after, HIE (Pre50%_{MAP} and Post50%_{MAP} respectively) was used to calculate GE conventionally with
 126 equations 1 and 2:

$$127 \quad GE (\%) = (Power\ input\ (W) / Energy\ expenditure\ (W)) \times 100 \quad (1)$$

128

$$129 \quad Energy\ expenditure = (VO_2) \times (RER \times 4904) + 16040 / 60 \quad (2)$$

130 VO₂ in equation 2 is expressed in L·min⁻¹. VO₂ data points in the last 3 minutes that had an RER
 131 >1.0 were excluded.

132 BEGE was calculated by fitting a linear regression to the GEVO₂ data points in the last 8-min of the
 133 Post50%_{MAP} bout; only data points with an RER < 1.0 were included in this 8-min period. These
 134 values were then back-extrapolated to the end of the HIE bout to give an estimation of the change of
 135 GE.³ The decline in GE during the HIE bout was subsequently calculated by plotting a linear
 136 relationship between GE during Pre50%_{MAP} and calculated BEGE values. Whilst, Mulder et al. (2015)
 137 found that a linear relationship can be used for short bouts of HIE, an exponential relationship
 138 should be used for HIE of longer duration.⁴ Total work, aerobic work and anaerobic work were
 139 estimated by calculating the total power output, aerobic power and anaerobic power over time during
 140 HIE in P1 and P2 as previously described elsewhere.^{3,4,12}

141

142 Statistical analysis

143 Shapiro-Wilk tests were conducted to assess for normality of distribution. To assess validity of
 144 BEGE, the relationship between submaximal GE and BEGE was assessed using a partial correlation
 145 controlling for Pre50% efficiency. The magnitude thresholds to assess the strength of the validity
 146 correlation are based on Cohen's effect sizes.¹³ To assess the reliability of GE, BEGE, HHb and TSI,
 147 data from all three visits were used. Within-subject variation across the three repeated visits was
 148 calculated using Coefficient of Variation (CV) and 95% limits of agreement.¹⁴ A repeated measures
 149 ANOVA was conducted to assess differences between the repeated visits in terms of GE, BEGE, TSI
 150 and HHb. Statistical significance was set at P < 0.05. All values are presented as mean ± SD unless
 151 otherwise stated.

152

153 Results

154 All participants completed the P1 HIE bouts at all visits. Three participants failed to complete the P2
 155 HIE bout on two or all three visits; therefore, their P2 GE data were excluded from the analyses.

156 Reliability of conventional and back-extrapolation GE methods

157 A mean CV of 7.8% (95% CL: 5.9 – 11.7%) for P1 and 9.8% (95% CL: 7.3 – 15.1%) in P2 BEGE
 158 was found. For GE Pre50%_{MAP} there was a mean CV of 7.6% (95% CL: 5.8 – 11.6%) for P1 and
 159 8.8% (95% CL: 6.8%– 12.9%) in P2. For GE Post50%_{MAP} a mean CV of 6.2% (95% CL: 4.4 – 10.8%)
 160 for P1 and 6.8% (95% CL: 5.3 – 10.6%) in P2 was found. The mean limits of agreement in relative

161 GE percentage point units were $\pm 3.6\%$ for Pre50%MAP and $\pm 3.74\%$ for BEGE in P1, and $\pm 4.2\%$
162 for Pre50%MAP and $\pm 4.1\%$ for BEGE in P2.

163 Figure 2 illustrates the limits of agreement between the three repeated visits for P1 and P2 using
164 Pre50%_{MAP} and BEGE.

165

166 ***INSERT FIGURE 2 HERE***

167

168 *Reliability of GE change*

169 There was a mean CV of 0.86% (95% CL: 0.66 -1.23%) for the change in GE observed between
170 Pre50%MAP and BGE in P1 and 0.99% (95% CL: 0.74 – 1.59%) for the change observed between
171 Pre50%MAP and BEGE in P2.

172

173 *Reliability of anaerobic contribution to high-intensity exercise*

174 The mean CV for the anaerobic contribution using a constant GE in P1 were 3.5% (95% CL: 2.6-5.5
175 %) vs 2.9% (95% CL: 2.2-4.4%) using BEGE. The mean CV for the anaerobic contribution using a
176 constant GE in P2 were 6.8% (95% CL: 5.2-10.8%) vs 5.0% (95% CL: 3.9-7.1%) using BEGE.

177

178 *NIRS reliability analysis*

179 Mean CVs were calculated for the 4-min HIE bout at both intensities. A mean CV of 6.9% (95% CL:
180 5.5 – 9.8%) for P1 TSI and 9.7% (95% CL: 7.8 – 13.9%) in P2 TSI was found. For HHb there was a
181 mean CV of 19.4% (95% CL: 15.5 – 27.11%) for P1 and 17.3% (95% CL: 13.8% – 23.8%) in P2.

182

183 *Physiological responses to high-intensity exercise*

184 Mean blood lactate concentration was significantly different pre- vs post-HIE (1.49 ± 1.05 vs. $3.06 \pm$
185 0.57 mmol·L⁻¹; $P < 0.05$) in P1, and also in P2 (2.06 ± 0.73 vs. 5.52 ± 1.73 mmol·L⁻¹; $P < 0.05$) There
186 was a significant interaction effect between intensity and timepoint ($P = < 0.006$).

187 Figure 3 shows the calculated GE Pre50%_{MAP}, BEGE and GE Post50%_{MAP} in both P1 and P2. BEGE
188 was significantly correlated with GE Post50%_{MAP} in P1 ($r = 0.98$; $P = 0.01$) and in P2 ($r = 0.80$; $P =$
189 0.01) (figure 4a and 4b). Repeated measures ANOVA demonstrated that there was no significant
190 difference between P1 GE Pre50%_{MAP} and P1 BEGE (21.1% vs 20.9%; $P = 0.29$). P1 GE Pre50%_{MAP}
191 and GE Post50%_{MAP} were not significantly different (21.1% vs. 21.0%; $P = 0.65$). A greater reduction
192 in BEGE is seen following HIE from P2 compared to P1 (-3.0% vs 0.27% , absolute GE units, figure
193 5a). HIE in P2 resulted in a significantly lower BEGE compared to P2 GE Pre50%_{MAP} ($18.1 \pm 1.6\%$
194 vs $21.1 \pm 2.2\%$; $P = 0.01$), and P2 GE Post50%_{MAP} ($20.3 \pm 1.7\%$ $P = 0.01$). P2 Post50%_{MAP} GE was
195 also significantly different than P2 Pre50%_{MAP} GE (20.3 ± 1.7 vs $21.1\% \pm 2.2\%$)

196

197 ***INSERT FIGURE 3 HERE***

198 ***INSERT FIGURE 4 HERE***

199

200 Mean total energy expenditure during the 4-min HIE bouts were 72.8kJ in P1 and 92.5 kJ in P2.
201 Calculated anaerobic contribution at the end of the 4min HIE bout in both P1 and P2 were higher at
202 the end of the HIE bout using the BE method compared to assuming a constant GE from the
203 Pre50%_{MAP} bout (P1 = 6.8 kJ vs 6.1kJ, $P = <0.8905$; P2 = 20.9kJ vs 11.8kJ, $P = <0.0345$). This
204 resulted in a 9% and 44% difference in anaerobic work contributions at the end of the 80%_{MAP} (P1)
205 and 100%_{MAP} (P2) bouts respectively (figures 5c and d).

206

207 ***INSERT FIGURE 5 HERE***

208 ***INSERT FIGURE 6 HERE***

209

210 A typical NIRS trace for TSI and HHb is shown in figure 6a and 6b, respectively. Table 1 presents
211 mean data and changes from baseline for TSI and HHb for both P1 and P2 before the HIE, during
212 HIE, at 5-min after HIE (post5), and at 10-min after HIE (post10). There were no significant
213 differences across all TSI timepoints in P1. However, there was a significant difference in TSI
214 between 4-min HIE vs Post5 ($P = 0.02$), and Post5 vs Post10 ($P = 0.02$) in P2. There were no
215 significant differences across all HHb timepoints in P1. However, there was a significant difference
216 between Post5 vs Post10 ($P = 0.009$) in P2.

217

218 ***INSERT TABLE 1 HERE***

219

220 Discussion

221 The main findings of this study are 1) the BE method is valid and reliable to estimate GE during HIE;
222 2) using the BE method, GE declines during HIE accompanied by a significant reduction in TSI ~~and~~
223 ~~increase in HHb~~; 3) assuming a declining GE during HIE, resulted in a larger calculated anaerobic
224 contribution compared to using an assumed constant GE.

225 BEGE was significantly correlated with GE Post50%_{MAP} in both P1 ($r = 0.63$; $P = 0.01$), and in P2 (r
226 $= 0.85$; $P = 0.002$). The “r medium-large” correlation¹³ between conventional GE and BEGE
227 measured in P1 and P2 suggests that BEGE is a valid measure of GE during high intensity exercise
228 such as that used in the current study.

229 The total within-subject variation in GE using the Douglas bag method has previously been reported
230 to be as low as 1.5% during submaximal exercise.¹⁵ However, in line with the current study, previous
231 research using breath-by-breath online gas analysers have reported higher mean CVs of 3.2- 6.4%;
232 closer to that of the current study.^{11,16} Moreover, the mean bias of all trials using Pre50%_{MAP} GE and
233 BEGE were almost zero, indicating a similar level of repeatability for both methods. By assessing
234 reliability, the smallest important difference (SID) in GE can also be ascertained. This is calculated
235 by square rooting the difference between the observed standard deviation of the difference scores, by
236 the typical error.¹⁷ This measure is useful in monitoring any beneficial or detrimental changes in an
237 individual's GE. SID values for GE Post50%_{MAP} compared to BEGE were 0.29 and 0.27 relative
238 absolute GE units in P1, and 0.30 and 0.10 relativeabsolute GE units and P2, respectively. This
239 suggests that BEGE may be a more sensitive method in detecting important differences from high
240 intensity work rates.

241

242 The decline in GE of 2.5% seen during 4-min at 100%_{MAP} in de Koning et al.³ is comparable to the
243 3% seen at the same intensity (P2) in the present study. As illustrated in Figure 3, the differential
244 effects of the two different HIE protocols on GE (using both conventional and the BE methods)
245 suggests that its determining mechanisms are intensity dependent. Indeed, GE has been shown to be
246 decreased as a result of both HIE^{3,4,9}, and prolonged exercise.^{8,18} However, this study is the first to
247 demonstrate that the relative intensity of HIE may play a major role in the observed decrease in GE.
248 Specifically, the present study demonstrated that BEGE was significantly lower in P2, but not P1
249 where GE was not different across the measurement time points (Figure 3).

250 In agreement with previous work using BEGE,^{3,4} the current study demonstrates a larger anaerobic
251 work contribution during the 4-min HIE bout at 100%_{MAP} results in a reduction in GE. However, as
252 indicated by similar calculated energetic contributions using conventional and BEGE methods,
253 cycling for 4-min at an intensity of 80%_{MAP} does not appear to have a large reliance on anaerobic
254 metabolism (figure 5c and 5d), and hence does not result in a reduction in GE. Moreover, the current
255 study demonstrates a greater decrease in the TSI in P2 compared to P1 (Table 1), accompanied by a
256 corresponding significant reduction in BEGE. It is likely that these changes in TSI when cycling at
257 an intensity of 100%_{MAP} arise from the greater metabolic demand than oxidative supply, and therefore
258 greater anaerobic energy contribution to power production, compared to 80%_{MAP}.

259 It is important to note that part of the estimated GE using BE may be affected by recovery processes
260 that increase VO₂ post-HIE. Whilst there were no significant differences in P1 GE or NIRS
261 parameters, the reduction of BEGE in P2 was accompanied by significant differences in P2 GE and
262 TSI and HHb at both Post5 and Post10 timepoints (see table 1). This suggests that exercise at
263 100%_{MAP} may create a larger oxygen deficit as well as a greater excess post-exercise oxygen
264 consumption where there is an increase in adenosine triphosphate and creatine phosphate re-synthesis,
265 as well as increased lactate removal. Thus, at supramaximal intensities, or longer durations of HIE¹⁷,
266 there may be greater reductions in calculated BEGE leading to greater calculated anaerobic
267 contribution and a prolonged period of recovery. ~~From a practical perspective, our data suggests that
268 performing short duration HIE during a bicycle race may involve a large anaerobic energy
269 expenditure, which will subsequently reduce GE. However, should the intensity drop post-HIE
270 sufficiently for the oxygen deficit to be repaid, then GE will likely recover. The mechanisms causing
271 reductions in GE after short duration HIE seem to be driven by a transient recovery related process.
272 By comparison, previous research has suggested changes in GE during prolonged submaximal
273 exercise may be driven by progressive reductions in mitochondrial/contractile efficiency of the
274 working muscle.⁸ Therefore, in bicycle races combining high-intensity and prolonged duration (e.g.
275 criteriums, stage races) there may be reductions in GE through a combination of both recovery related
276 processes and reductions in muscle contraction-coupling efficiency. Consequently, optimal pacing
277 strategy may have to be modified to ensure that GE declines at a rate that doesn't negatively affect
278 performance, or to allow GE to recover adequately between HIE efforts.~~

279 Practical applications

280 ~~From a practical perspective, our data suggests that performing short duration HIE during a bicycle
281 race may involve a large anaerobic energy expenditure, which will subsequently reduce GE.
282 However, should exercisethe intensity drop post-HIReduce sufficiently for the oxygen deficit to be
283 repaid, then GE will likely recover. Therefore, in bicycle races combining high-intensity exercise
284 and prolonged duration (e.g. criteriums, stage races) there may be reductions in GE through a
285 combination of both recovery related processes and reductions in muscle contraction-coupling
286 efficiency.⁸ (Rønnestad et al., 2011, Hopker et al., 2013). Consequently, optimal pacing
287 strategycompetition or pacing strategy may have to be modified to minimize the negative impact of~~

288 ensure that reductions in GE declines at a rate that does not negatively affecton performance, or to
289 allow GE to recover adequately between HIE efforts.

290

291

292 **Conclusion**

293 This study demonstrates that the BE method to estimate GE during HIE is a valid and reliable
294 measure. Assuming a declining GE during short duration HIE at an intensity of 100%_{MAP} there is a
295 significantly greater anaerobic contribution compared to cycling at 80%_{MAP}, leading to a larger O₂
296 deficit, and thus contributing to a reduction in GE. Following short-duration HIE, GE may recover
297 if the intensity is sufficiently low enough to allow the oxygen debt to be repaid. Further work is
298 needed to characterise changes in GE during prolonged exercise interspersed with HIE.

299

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302

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Figure Legends:

Figure 1. High intensity exercise protocols with 4-min HIE at 80%_{MAP} during P1 and 100%_{MAP} during P2

Figure 2. Bland Altman plots with 95% LOA for GE across all three trials for each HIE protocol a) P1 Pre50%_{MAP} GE b) P1 BEGE c) P2 Pre50%_{MAP} GE d) P2 BEGE

Figure 3. Mean GE changes (with 95% CIs) at 80%_{MAP} (solid line, circle) and 100%_{MAP} (dotted line, square) calculated Pre and Post 4 min HIE and using the back-extrapolation method to the end of the 4min-HIE bout. α = significant difference from Pre50%_{MAP} ($P < 0.05$). β = significant difference from BEGE ($P < 0.05$).

Figure 4. Linear partial correlation using GE residuals between Post50 and BEGE in (a) P1 and (b) P2

Figure 5. (a) Mean change in GE calculated using back extrapolation during the 4 min HIE bout at 80% MAP (solid line, squares) and 100% MAP (dotted line, circles). (b) Mean BE values from the end of P1 (solid line) and P2 (dotted line) from the end of the 10 min post HIE recovery to the end of the 4 min HIE bout. Mean aerobic (Paer) and anaerobic (Pan) power contributions during 4 min HIE bout at (c) 80%_{MAP} and (d) 100%_{MAP} using a constant Pre50% MAP GE (solid line, circle) and a variable BE50 GE (dotted line, triangle).

Figure 6. (a) A typical TSI (%) and (b) HHb concentration changes for P1 (dotted line) and P2 (solid line)

	Pre50	Δ baseline	4min HIE	Δ baseline	Post5	Δ baseline	Post10	Δ baseline
P1								
TSI(%)	68.6 (\pm 1.0)	-3.8 (\pm 0.4)	68.5 (\pm 3.8)	-8.7 (\pm 0.8)	69.2 (\pm 1.2)	-5.9 (\pm 0.3)	68.6 (\pm 1.0)	-5.1 (\pm 0.3)
HHb (μm)	10.2 (\pm 1.1)	9.1 (\pm 0.6)	9.9 (\pm 4.9)	11.8 (\pm 1.2)	9.9 (\pm 1.0)	4.9 (\pm 0.3)	9.7 (\pm 1.4)	6.7 (\pm 0.4)
P2								
TSI(%)	67.8 (\pm 1.0)	-3.5 (\pm 0.2)	66.6 (\pm 4.4)	-10.3 (\pm 1.3)	70.0 (\pm 2.0) *	-7.9 (\pm 0.6)	68.6 (\pm 2.0) §	-8.1 (\pm 0.8)
HHb (μm)	9.7 (\pm 1.2)	10.3 (\pm 0.3)	10.8 (\pm 4.6)	10.4 (\pm 0.7)	11.0 (\pm 1.7)	8.9 (\pm 0.3)	9.8 (\pm 1.7) §	7.2 (\pm 0.2)

Table 1. TSI and HHb NIRS response during Pre50, 4-min HIE, Post5 and Post10. Values are mean response and Δ baseline. *significantly different to 4-min HIE (P = 0.02). [§] significantly different to Post5 (P = 0.009)

For Peer Review

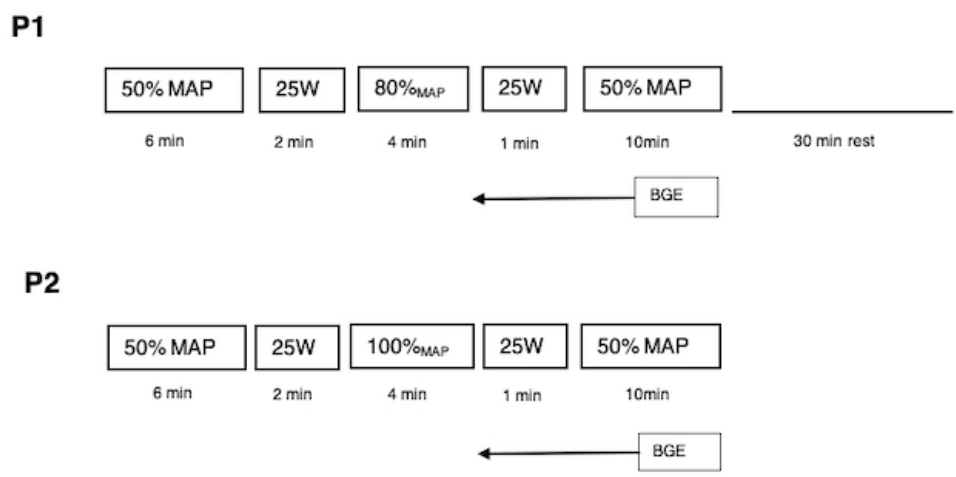


Figure 1. High intensity exercise protocols with 4-min HIE at 80%MAP during P1 and 100%MAP during P2
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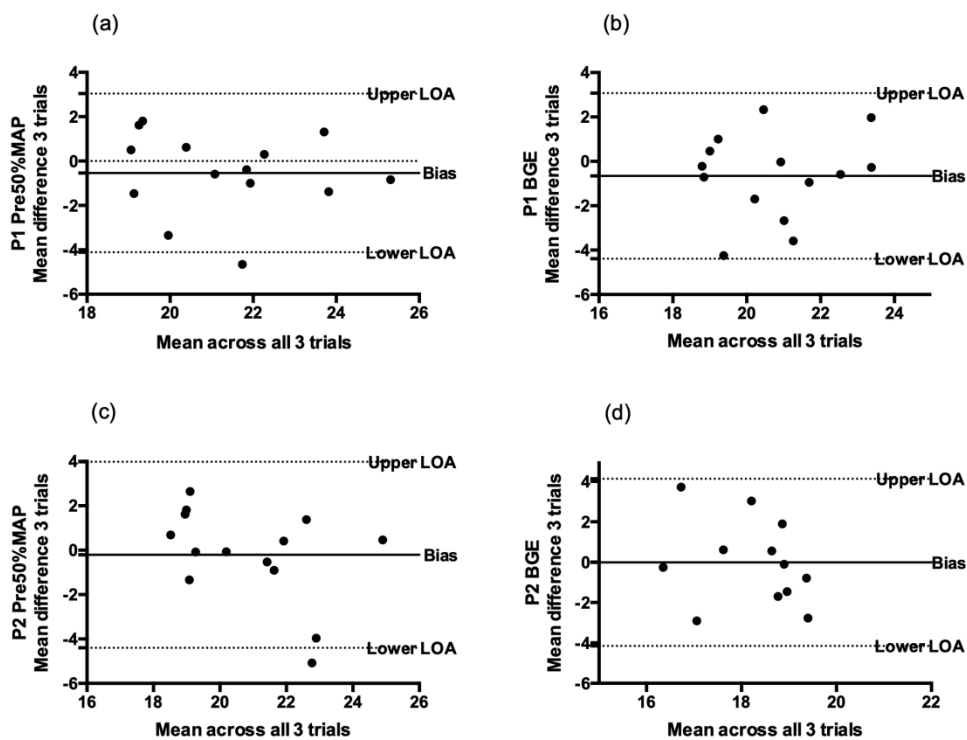


Figure 2. Bland Altman plots with 95% LOA for GE across all three trials for each HIE protocol a) P1 Pre50%MAP GE b) P1 BEGE c) P2 Pre50%MAP GE d) P2 BEGE

165x127mm (300 x 300 DPI)

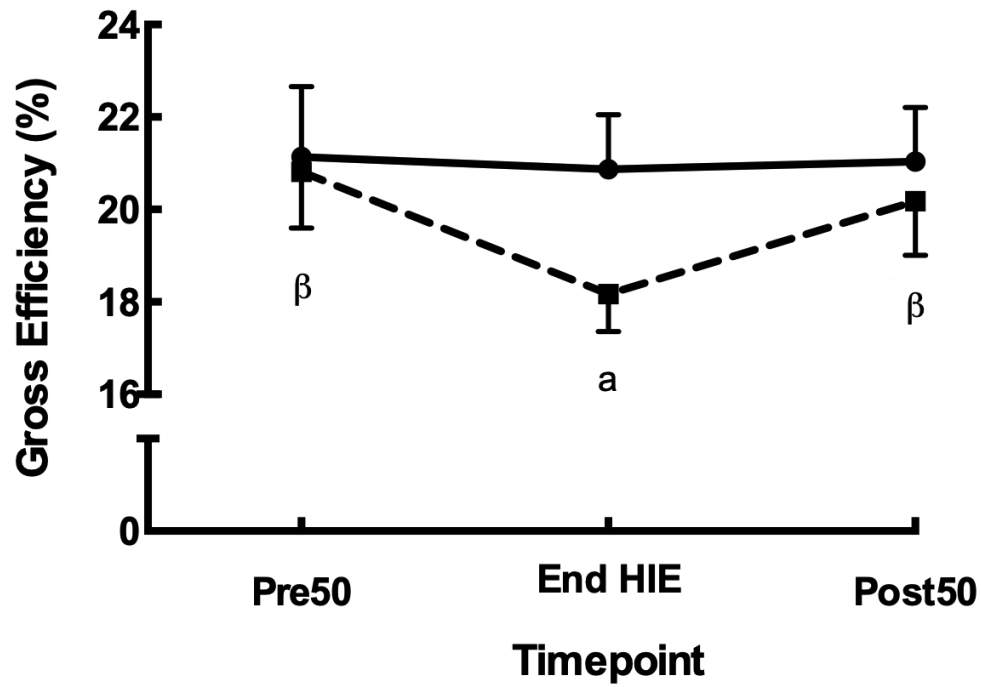


Figure 3. Mean GE changes (with 95% CIs) at 80% MAP (solid line, circle) and 100% MAP (dotted line, square) calculated Pre and Post 4 min HIE and using the back-extrapolation method to the end of the 4min-HIE bout. a = significant difference from Pre50%MAP ($P < 0.05$). B = significant difference from BEGE ($P < 0.05$).

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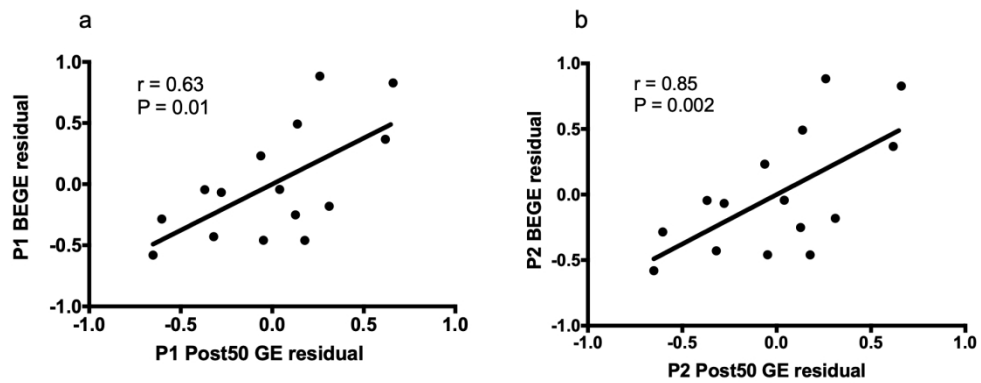


Figure 4. Linear partial correlation using GE residuals between Post50 and BEGE in (a) P1 and (b) P2

165x66mm (300 x 300 DPI)

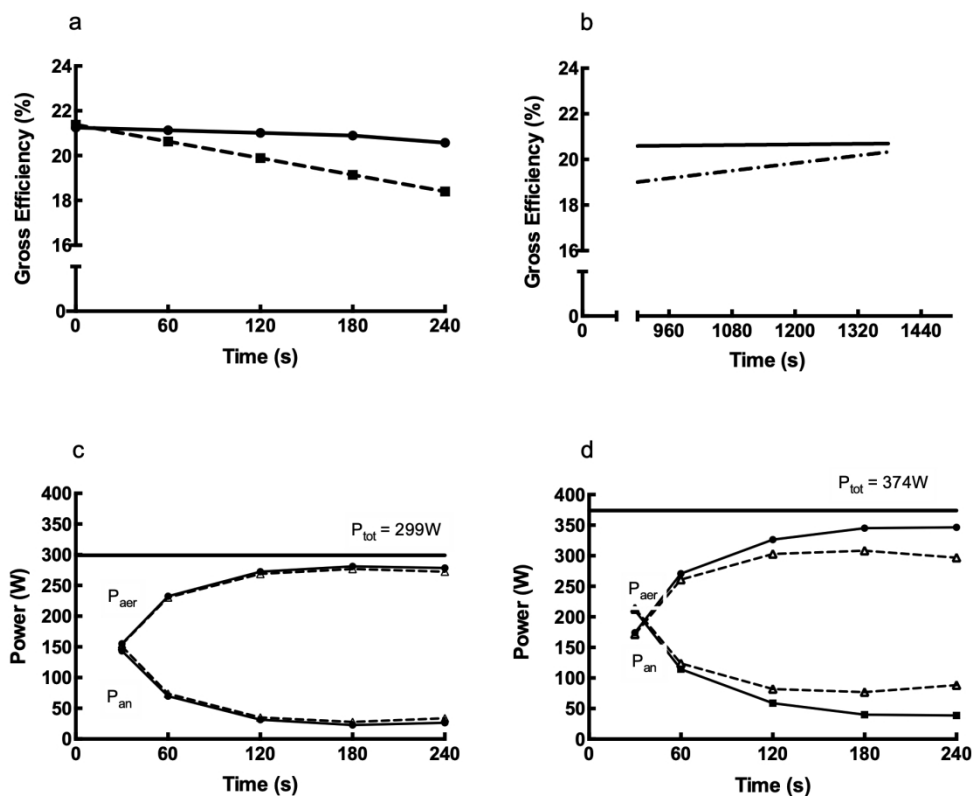


Figure 5. (a) Mean change in GE calculated using back extrapolation during the 4 min HIE bout at 80% MAP (solid line, squares) and 100% MAP (dotted line, circles). (b) Mean BE values from the end of P1 (solid line) and P2 (dotted line) from the end of the 10 min post HIE recovery to the end of the 4 min HIE bout. Mean aerobic (P_{aer}) and anaerobic (P_{an}) power contributions during 4 min HIE bout at (c) 80%MAP and (d) 100%MAP using a constant Pre50% MAP GE (solid line, circle) and a variable BE50 GE (dotted line, triangle).

166x138mm (300 x 300 DPI)

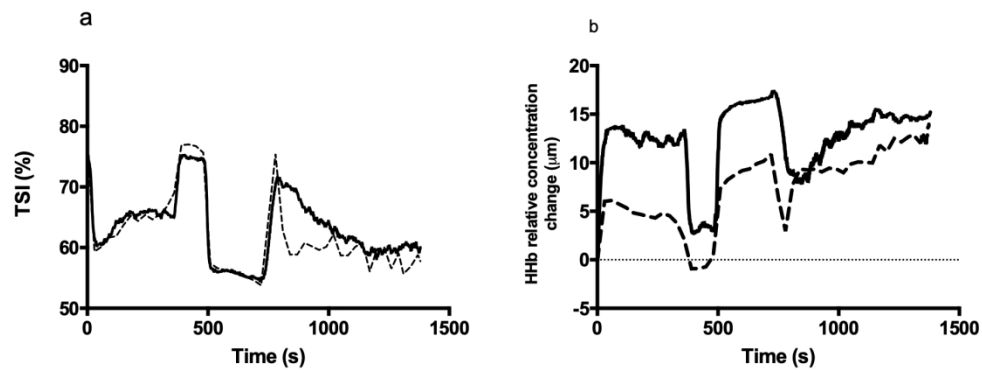


Figure 6. (a) A typical TSI (%) and (b) HHb concentration changes for P1 (dotted line) and P2 (solid line)

166x66mm (300 x 300 DPI)