1   **The Effects of Sodium Phosphate Supplementation on Physiological Responses to Submaximal**

2   **Exercise and 20 km Cycling Time-Trial Performance**

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17   **Running title**: Physiology, performance, and sodium phosphate

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25 **ABSTRACT**

26   The aim of this study was to examine the effects of sodium phosphate (SP) supplementation on

27 physiological responses to submaximal exercise and 20 km cycling time-trial performance. Using a

28 randomised, double-blind, crossover design, 20 endurance-trained male cyclists (age: 31 ± 6 years;

29 height: 1.82 ± 0.07 m; body mass: 76.3 ± 7.0 kg; maximal oxygen uptake [$\dot{V}O_{2max}$]: 57.9 ± 5.5 mL·kg^{-}

30 ^{1}·min^{-1}) completed two supplementation trials separated by a 14-day washout period. The trials

31 consisted of 10 minutes of cycling at 65% $\dot{V}O_{2max}$ followed by a 20 km time-trial. Expired air was

32 monitored throughout each trial for the evaluation of $\dot{V}O_2$, minute ventilation ($\dot{V}_E$), and respiratory

33 exchange ratio (RER). Heart rate was monitored during each trial along with ratings of perceived

34 exertion (RPE) and blood lactate concentration. For four days before each trial, participants ingested 50

35 mg·kg fat-free-mass^{-1}·day^{-1} of either SP or placebo. There were no effects ($p \geq 0.05$) of supplementation

36 on physiological responses during cycling at 65% $\dot{V}O_{2max}$. There were also no effects of

37 supplementation on time-trial performance (placebo: 32.8 ± 2.2 mins; SP: 32.8 ± 2.3 mins).

38 Nevertheless, relative to placebo, SP increased $\dot{V}_E$ (mean difference: 3.81 L·min^{-1}; 95% likely range:

39 0.16-7.46 L·min^{-1}), RER (mean difference: 0.020; 95% likely range: 0.004-0.036), and RPE (mean

40 difference: 0.39; 95% likely range: 0.04-0.73) during time-trials; as well as post time-trial blood lactate

41 concentration (mean difference: 1.06 mmol·L^{-1}; 95% likely range: 0.31-1.80 mmol·L^{-1}). In conclusion,

42 SP supplementation has no significant effects on submaximal physiological responses or 20 km time-

43 trial performance.

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45 **Key Words:** Ergogenic; endurance; serum phosphate; 2, 3-diphosphoglycerate

INTRODUCTION

Sodium phosphate (SP) is a legal nutritional supplement that has been suggested to improve athletic performance (Currell et al., 2012). Several mechanisms have been proposed to explain this potential ergogenic effect, including an increase in resting erythrocyte 2, 3-diphosphoglycerate (2, 3-DPG) concentration (promoting oxygen offloading at the muscle via a reduction in oxyhaemoglobin affinity) (Bremner et al., 2002; Cade et al., 1984), an enhancement of myocardial contractility (Kreider et al., 1992), an increase in extracellular hydrogen phosphate (HPO_4^-) concentration (facilitating hydrogen ion buffering) (Buck et al., 2015; Kopec et al., 2016), and an increase in the activity of various oxidative enzymes, such as phosphofructokinase and glyceraldehyde 3-phosphate dehydrogenase (Buck et al., 2013).

Given the above mechanisms, it has been hypothesised that SP supplementation may increase aerobic metabolism, and thus enhance endurance performance (Buck et al., 2013; Fukuda et al., 2010). In corroboration, despite one conflicting report (West et al., 2012), research into SP supplementation has consistently shown an increase in maximal oxygen uptake ($\dot{V}O_{2max}$) (Brewer et al., 2013; Cade et al., 1984; Czuba et al., 2009; Kreider et al., 1992; Kreider et al., 1990; Stewart et al., 1990). Nevertheless, research examining the effects of SP supplementation on endurance performance has produced conflicting results, with some studies reporting significant improvements (Folland et al., 2008; Kreider et al., 1992), while others report no effect (Brewer et al., 2013, 2014; Buck et al., 2014; Kreider et al., 1990). It is also difficult to determine the effects of SP supplementation on physiological responses during endurance exercise, possibly because most investigations have evaluated those responses during self-paced time-trials or incremental tests rather than fixed-intensity bouts of exercise. As such, while some studies have shown a significant increase in oxygen uptake ($\dot{V}O_2$) following SP supplementation (Czuba et al., 2009; Kreider et al., 1990, 1992), others have observed no effect (Brewer et al., 2014; Folland et al., 2008). Similarly, some investigations have demonstrated a SP-induced

71   decrease in heart rate (Czuba et al., 2009), whereas others have reported no effect (Brewer et al., 2013,

72   2014; Folland et al., 2008; Kreider et al., 1990, 1992; West et al., 2012).

73

74   It is difficult to attribute the conflicting responses of SP supplementation to methodological

75   differences between studies, since discrepant findings exist regardless of differences in dosing

76   strategies, mode of exercise, or participant training status. Indeed, despite some differences in the

77   duration of supplementation (typically 6 days) the dose range used in previous research is very small

78   (3.3-4.0 $g \cdot day^{-1}$; Buck et al., 2013). Moreover, all studies into SP supplementation have used trained

79   participants; though between-study differences in $\dot{V}O_{2max}$ (50-75 $mL \cdot kg^{-1} \cdot min^{-1}$) support clear

80   differences in levels of ability. The issue of training status is important since well-trained athletes have

81   been shown to have already elevated resting erythrocyte 2, 3-DPG levels (Brodthagen et al., 1985),

82   likely due to an adaptive training response (Mairbäurl, 2013). However, the suggestion that well-trained

83   athletes may be less responsive to the potential ergogenic benefits of SP supplementation seems unlikely

84   considering that previous research has demonstrated that SP can improve time-trial performance in

85   those individuals (Folland et al., 2008; Kreider et al., 1992).

86

87   The factor that explains most likely the discrepant findings regarding the effects of SP

88   supplementation on endurance performance is statistical error associated with the use of small sample

89   sizes (Button et al., 2013). Small sample sizes reduce the chances of finding a true effect as well as

90   reducing the likelihood that a statistically significant finding reflects a real effect (Button et al., 2013).

91   Indeed, of those studies that have investigated the effects of SP supplementation on endurance

92   performance, the largest sample size was 13 (Buck et al., 2014), with most using sample sizes ≤ 10

93   (Brewer et al., 2013, 2014; Folland et al., 2008; Kreider et al., 1990, 1992). The principal aim of this

94   study was therefore to address the issue of sample size in order to examine the effects of SP

95   supplementation on endurance (20 km cycling time-trial) performance. In addition, by examining the

96   effects of SP supplementation on physiological responses during both fixed-intensity submaximal

97   cycling and time-trial performance, the study also aimed to provide insight into the potential

98   mechanisms behind any ergogenic effect of SP supplementation.

99

100   **METHODS**

101   **Participants**

102   Twenty endurance-trained (De Pauw et al., 2013) male cyclists and triathletes volunteered for

103   the study, which was approved by St. Mary's University Ethics Committee. Sample size calculations

104   were performed based on the results of previous investigations into the effects of SP supplementation

105   on endurance performance (Brewer et al., 2013, 2014; Buck et al., 2014; Folland et al., 2008; Kreider

106   et al., 1990, 1992). Using the associated effect sizes, a power of 0.8, and a p value of 0.05, the analyses

107   produced sample sizes ranging from 2 to 20,000. Given the practical limitations associated with

108   recruiting trained participants, a sample size of 20 was chosen as it fell within the range determined

109   from the calculations and was, with one exception ($n = 13$), at least double the sample size used in

110   previous investigations. Before testing, participants received written and verbal instructions regarding

111   the nature of the investigation and completed a training history questionnaire, which indicated that all

112   had been actively involved in road cycling for at least one year. Time spent training each week was

113   reported as 9.4 ± 3.9 hours. Before commencement of the study, all participants completed a health-

114   screening questionnaire and provided written informed consent. Means $\pm$ standard deviation for age,

115   height, body mass, fat-free-mass (FFM), and $\dot{V}O_{2max}$ of the participants were: 31 ± 6 years, 1.82 ± 0.07

116   m, 76.3 ± 7.0 kg, 67.3 ± 6.3 kg, and 57.9 ± 5.5 mL·kg^{-1}·min^{-1}, respectively. Participants were instructed

117   to maintain a consistent training volume throughout the study and to follow the same diet for 24 hours

118   before all trials. Participants were also instructed to avoid food and drink for 1 hour before all trials and

119   to abstain from caffeine, alcohol, and strenuous exercise for 24 hours before all trials.

120

121   **Experimental overview**

122   Participants were required to complete one preliminary trial followed by two experimental

123   trials. The preliminary trial was used to provide descriptive data and to determine the fixed-intensity

124   submaximal cycling workloads employed during the first part of the experimental trials. The

125   experimental trials were performed in a crossover, randomised, counterbalanced, and double-blinded

126   manner, separated by 14 days to allow for the washout period of SP (Cade et al., 1984). In line with

127   strategies used in previous research (Buck et al., 2013), for four consecutive days before each

128   experimental trial, participants ingested 50 mg·kg FFM^{-1}·day^{-1} of either tribasic SP dodecahydrate (Iron

129   Power, Melbourne, Australia) or placebo (maltodextrin; My Protein, Manchester, United Kingdom).

130   Daily amounts were divided into four equal doses, with each dose administered in an opaque gelatine

131   capsule (My Protein, Manchester, United Kingdom). As in previous research (Brewer et al., 2013, 2014;

132   Buck et al., 2014), doses were ingested at ~4 hour intervals with a meal and ~300 mL of water to prevent

133   gastrointestinal discomfort. Exercise, other than the time trials, was performed on an

134   electromagnetically-braked cycle ergometer (Lode Excalibur Sport, Lode BV, Groningen, The

135   Netherlands). Time trials were performed on a racing bicycle (San Remo, Claud Butler, Brigg, United

136   Kingdom) seated on a motor-braked turbo trainer (Tacx Genius, Aardenburg, the Netherlands).

137   Ergometers of this type have previously been shown to have very good test-retest reliability for 20 km

138   time-trial performance (Peiffer & Losco, 2011). The cycle ergometer and the racing bicycle were fitted

139   with clipless pedals and the participants cycled using their own cycling shoes. Saddle height and

140   handlebar position for each participant were determined during the preliminary trial to enable

141   replication in subsequent trials. Prior to all trials equipment was calibrated in accordance with

142   manufacturer instructions.

143

144   **Procedures**

145   *Preliminary trial*

146   All trials were performed at the same time of day (± 2 hours) in an air-conditioned laboratory

147   maintained at a temperature of 18°C. The preliminary trial began with the calculation of participant

148   FFM using air-displacement plethysmography (device for measuring volume changes within a body)

149   (BOD-POD, Life Measurement Inc., Concord, CA, USA). Subsequently, participants performed an

150   incremental exercise test which began at 120 W and increased by 20 W every 3 minutes. Participants

151   were given 30 s during the first stage to achieve a comfortable cadence and were instructed to maintain

152   this throughout the remainder of the incremental tests. Each stage was followed by 30 s of passive rest,

153   during which 20 μL of capillary blood was obtained from the earlobe and analysed for blood lactate

154   concentration using an automated analyser (Biosen C-Line, EKF Diagnostic, Barleben, Germany). The

155   test was terminated when a blood lactate concentration ≥ 4 mmol·L^{-1} was attained. After 5 minutes of

156   passive rest, the maximal phase of the incremental exercise test began at 160 W and increased by 20 W

157   every minute. The test was terminated when participants reached volitional exhaustion, at which time a

158   final blood lactate concentration measurement was obtained. Throughout both phases of the incremental

159   exercise test, participants breathed room air through a facemask (Hans Rudolph, Kansas City, MO,

160   USA) that was secured in place by a head-cap assembly (Hans Rudolph, Kansas City, MO, USA).

161   Expired air was monitored continuously using an online gas analyser (Oxycon Pro, Jaeger, Hoechberg,

162   Germany). The analyser was calibrated before each trial using oxygen and carbon dioxide gases of

163   known concentrations (Cryoservice, Worcester, UK), and the flowmeter was calibrated using a 3 L

164   syringe (Viasys Healthcare GmbH, Hoechberg, Germany). All $\dot{V}O_2$ data were filtered to eliminate

165   values that were outside four standard deviations of the local mean (the two breaths preceding and

166   following the breath of interest). Oxygen demand for each of the submaximal incremental stages was

167   determined as the average $\dot{V}O_2$ during the final 30 s of each 3-minute stage. $\dot{V}O_{2max}$ was determined as

168   the highest 30 s average $\dot{V}O_2$ recorded during the maximal phase of the test provided that at least two

169   of the following criteria had been met: (a) a plateau in $\dot{V}O_2$, as determined by an increase of less than 2

170   mL·kg^{-1}·min^{-1} over the previous stage, (b) a heart rate within 10 b·min^{-1} of age-predicted maximum, (c)

171   a respiratory exchange ratio (RER) ≥ 1.15, and (d) a blood lactate concentration ≥ 8 mmol·L^{-1}. Linear

172   regression and individual power output-$\dot{V}O_2$ relationships were used to calculate the fixed-intensity

173   submaximal cycling workloads required to elicit 65% of $\dot{V}O_{2max}$, to be employed during the

174   experimental trials. After a 10-minute recovery period, where participants cycled at a self-selected low

175   intensity, a familiarisation 20 km time-trial (same protocol as in the experimental trials) was performed

176   to minimise any potential changes in performance due to learning.

177

178   *Experimental trials*

179   Prior to each experimental trial, participants rested in a seated position for 5 minutes, after

180   which 300 µL of capillary blood was collected from the earlobe. Blood samples were left to clot at room

181   temperature for 60 minutes before being centrifuged at 4000 rpm for 10 minutes at 4°C. Subsequently

182   decanted serum samples were frozen at -80°C until analysed for serum phosphate concentration using

183   an automated analyser (Monza, Randox, London, UK). Participants then performed 10 minutes of

184   cycling at 65% of the power output required to elicit $\dot{V}O_{2max}$, maintaining the same cadence as in the

185   submaximal incremental test during the preliminary trial. Oxygen uptake, minute ventilation ($\dot{V}_E$), RER,

186   and heart rate (RCX3, Polar Electro Oy, Kempele, Finland) were monitored continuously during cycling

187   at 65% $\dot{V}O_{2max}$ and averaged over the final 30 s of each 5-minute split to provide mean responses at 5

188   minutes and 10 minutes. Blood lactate concentration and rating of perceived exertion (RPE; 15-point

189   scale; Borg, 1970) were also determined at 5 minutes and 10 minutes during cycling at 65% $\dot{V}O_{2max}$.

190   After 10 minutes of passive rest, participants completed a 20 km time-trial on the turbo trainer with the

191   bicycle rear tyre pressure at 100 psi. The time-trial was performed against a resistance designed to

192   replicate outdoor, level-gradient cycling conditions. No verbal encouragement was provided and all

193   measures of elapsed time were removed from the environment. The only pertinent information visible

194   to participants throughout each time-trial was the distance completed. Participants were free to change

195   cadence and gears throughout each time-trial; however, the gearing chosen during the familiarisation

196   time-trial was noted and used to standardise the starting intensity for the experimental time-trials.

197   Distance completed, power output, and cadence were recorded at 1 Hz throughout each experimental

198   time-trial. Expired air was monitored continuously throughout each experimental time-trial for the

199   evaluation of $\dot{V}O_2$, $\dot{V}_E$, and RER. Heart rate was monitored continuously throughout each experimental

200   time-trial and RPE was recorded at 5 km intervals. Blood lactate concentration was determined 1 minute

201   before and immediately after each experimental time-trial.

202

203   **Statistical analyses**

204   All data were analysed using the Statistical Package for the Social Sciences (version 22, IBM

205   SPSS, Armonk, NY, USA). Data are presented as means ± standard deviation, and 95% confidence

206   intervals are provided for all estimates. A paired samples *t*-test was used to determine the effects of

207   supplementation on resting serum phosphate concentration. Two-way (supplement × time) analyses of

208   variance (ANOVAs) were used to determine the effects of supplementation and time on physiological

209   responses ($\dot{V}O_2$, $\dot{V}_E$, RER, heart rate, RPE, and blood lactate concentration) during exercise at 65%

210     $\dot{V}O_{2max}$. Two-way (supplement × 5 km split) ANOVAs were used to determine the effects of

211   supplementation and 5 km splits on 20 km time-trial performance measures (completion time, power

212   output, and cadence) and physiological responses ($\dot{V}O_2$, $\dot{V}_E$, RER, heart rate, and RPE). A two-way

213   (supplement × time) ANOVA was used to determine the effects of supplementation and time on blood

214   lactate concentration prior to and immediately following time-trial performance. Violations to

215   assumptions of sphericity were adjusted using the Greenhouse-Geisser correction factor (Field, 2013).

216   Significant effects were followed up using *post hoc* tests with Bonferroni adjustments (Field, 2013).

217   The significance level was set at $p < 0.05$ for all analyses.

218

219   **RESULTS**

220   **Serum phosphate**

221   Placebo supplementation resulted in a resting serum phosphate concentration of 0.77 ± 0.18

222   mmol·L^{-1}, whereas SP supplementation resulted in a resting serum phosphate concentration of 0.76 ±

223   0.15 mmol·L^{-1}. There was no significant effect of supplementation on resting serum phosphate

224   concentration ($p = 0.762$).

225

226   **Physiological responses to submaximal fixed-intensity exercise**

227   The mean power output during the submaximal exercise bouts was 186 ± 34 W. There was no

228   significant effect of supplementation on $\dot{V}O_2$ ($p = 0.694$), $\dot{V}_E$ ($p = 0.950$), RER ($p = 0.298$), heart rate (p

229 $= 0.885$), RPE ($p = 0.650$), or blood lactate ($p = 0.375$) during cycling at 65% of $\dot{V}O_{2max}$ (Table 1).

230   There was also no significant effect of time on $\dot{V}O_2$ ($p = 0.766$) or RER ($p = 0.656$). However, there

231   was a significant effect of time on $\dot{V}_E$ ($p = 0.001$), heart rate ($p < 0.001$), RPE ($p < 0.001$), and blood

232   lactate ($p = 0.033$). *Post hoc* tests revealed that from 5 min to 10 min, $\dot{V}_E$, heart rate, and RPE increased

233   significantly, whereas blood lactate decreased significantly. There were no significant supplement ×

234   time interactions for $\dot{V}O_2$ ($p = 0.982$), $\dot{V}_E$ ($p = 0.777$), RER ($p = 0.495$), heart rate ($p = 0.641$), RPE (p

235 $= 0.095$), or blood lactate ($p = 0.573$).

236

237   **Time-trial performance**

238   There was no significant effect of supplementation on completion time ($p = 0.975$), power

239   output ($p = 0.777$), or cadence ($p = 0.503$) during the time-trials (Table 2). However, there was an effect

240   of 5 km split on completion time ($p < 0.001$), with significant differences between all comparisons apart

241   from that between the 5-10 and 10-15 km splits. Similarly, there was a significant effect of 5 km split

242   on power output ($p < 0.001$). *Post hoc* tests revealed that participants produced a significantly higher

243   power output in the final 5 km of each time-trial in comparison with each of the other 5 km splits. There

244   was also an effect of 5 km split on cadence ($p = 0.001$), with significantly increased values in the 5-10

245   and 15-20 km splits, when compared with the 0-5 km split. There were no significant supplement × 5

246   km split interactions for completion time ($p = 0.505$), power output ($p = 0.512$), or cadence ($p = 0.566$).

247

248   **Time-trial physiological responses**

249   Participants completed the placebo and SP experimental time-trials at a mean intensity of 83 $\pm$

250 8% of $\dot{V}O_{2max}$. There was no significant effect of supplementation on $\dot{V}O_2$ ($p = 0.944$) or heart rate ($p =$

251 0.141) during the time-trials (Table 3). However, there was an effect of 5 km split on $\dot{V}O_2$ ($p = 0.012$),

252 with significantly increased values in the 15-20 km split, when compared with the 0-5 and 10-15 km

253 splits. There was also an effect of 5 km split on heart rate ($p < 0.001$), with mean values increasing

254 throughout the time-trials and with *post hoc* tests revealing significant differences between all

255 comparisons apart from that between the 5-10 and 10-15 km splits. There were no significant

256 supplement $\times$ 5 km split interactions for $\dot{V}O_2$ ($p = 0.701$) or heart rate ($p = 0.111$).

257

258   There was an effect of supplementation on $\dot{V}_E$ ($p = 0.042$) during the time-trials (Table 3), with

259 SP resulting in significantly higher values than placebo (mean difference: 3.81 L·min^{-1}; 95% likely

260 range: 0.16-7.46 L·min^{-1}). There was also an effect of 5 km split on $\dot{V}_E$ ($p < 0.001$), with mean values

261 increasing throughout the time-trials and with *post hoc* tests revealing significant differences between

262 all comparisons apart from that between the 5-10 and 10-15 km splits. However, there was no significant

263 supplement $\times$ 5 km split interaction for $\dot{V}_E$ ($p = 0.103$). There was also an effect of supplementation on

264 RER ($p = 0.020$) during the time-trials (Table 3), with SP resulting in significantly higher values than

265 placebo (mean difference: 0.020; 95% likely range: 0.004-0.036). However, there was no significant

266 effect of 5 km split on RER ($p = 0.095$) and no supplement $\times$ 5 km split interaction ($p = 0.978$).

267

268   There was an effect of supplementation on RPE ($p = 0.030$) during the time-trials (Table 3),

269 with SP resulting in significantly higher values than placebo (mean difference: 0.39; 95% likely range:

270 0.04-0.73). There was also a significant effect of 5 km split on RPE ($p < 0.001$). *Post hoc* tests revealed

271 a progressive increase in RPE throughout the time-trials with significant differences between all

272 comparisons. However, there was no significant supplement $\times$ 5 km split interaction for RPE ($p =$

273 0.632).

274

275   There was a significant effect of supplementation on blood lactate concentration ($p = 0.003$;

276   Figure 1). Blood lactate concentration also significantly increased from pre-time-trial to post-time-trial

277   ($p < 0.001$). Moreover, there was a significant supplement × time interaction ($p = 0.006$). *Post hoc* tests

278   revealed that there was no significant effect of supplementation on pre-time-trial blood lactate

279   concentration ($p = 0.738$); however, relative to placebo, post-time-trial blood lactate concentration was

280   significantly increased with SP (mean difference: 1.06 mmol·L^{-1}; 95% likely range: 0.31-1.80 mmol·L^{-}

281   ^{1}; $p = 0.004$).

282

283   **DISCUSSION**

284   The principal aim of this study was to examine the effects of SP supplementation on 20 km

285   cycling time-trial performance. The key finding was that SP supplementation had no significant effect

286   on time-trial completion time. Supplementation with SP also had no significant effect on power output

287   or cadence during the time-trials. The absence of any significant effect of SP supplementation on time-

288   trial performance is consistent with some reports (Brewer et al., 2013, 2014; Buck et al., 2014; Kreider

289   et al., 1990), but not others (Brewer et al., 2015; Folland et al., 2008; Kreider et al., 1992). However, it

290   is worth noting that the small sample sizes associated with previous research increase the risk of false

291   positives and reduce the likelihood that findings reflect a true effect (Button et al., 2013). Given that

292   the present study was the first to examine the effects of SP supplementation on time-trial performance

293   using a relatively large sample size, the findings of the present investigation add considerable weight to

294   the argument that SP supplementation provides no ergogenic benefit during time-trial performance.

295

296   Resting serum phosphate concentrations in the present study were slightly lower than

297   anticipated, but were within the normal range for adults (Buck et al., 2013). Nevertheless, relative to

298   placebo, the present study demonstrated no SP-induced increase in resting serum phosphate

299   concentration. Apart from one exception (Czuba et al., 2009), previous research has also reported no

300   change in serum phosphate concentration following SP supplementation (Brewer et al., 2013; Buck et

301   al., 2015; Kopec et al., 2016; Kreider et al., 1990; Stewart et al., 1990). Given similarities in the dosing

302   strategies used in these investigations, it seems, as highlighted by others (Buck et al., 2015; Kopec et

303   al., 2016; Kreider et al., 1992; Stewart et al., 1990), that the measure may not be the best indicator of

304   SP loading effects.

305   A secondary aim of the present study was to investigate the potential mechanisms behind the

306   ergogenic effects of SP supplementation. If, as hypothesised, SP supplementation improves oxygen

307   offloading at the muscle via an increase in erythrocyte 2, 3-DPG levels (Bremner et al., 2002; Cade et

308   al., 1984), then an enhancement of aerobic metabolism would be expected. However, given that there

309   was not only no SP-induced improvement in time-trial performance, but also no increase in $\dot{V}O_2$ during

310   either fixed-intensity submaximal cycling or time-trial performance, the findings of the present study

311   fail to provide support for the above mechanism. Given that the period of fixed-intensity submaximal

312   cycling was performed at a lower intensity than the time-trials, it seems unlikely that the absence of an

313   effect of SP on $\dot{V}O_2$ could be due to exercise intensity. Although resting erythrocyte 2, 3-DPG levels

314   were not measured in the present study, an alternative explanation for the lack of any increase in $\dot{V}O_2$

315   is that SP supplementation may not increase 2, 3-DPG concentration in red blood cells. Indeed, of those

316   studies that have measured 2, 3-DPG concentrations following SP supplementation, Cade et al. (1984)

317   and Stewart et al. (1990) reported significant increases, Buck et al. (2015), Czuba et al. (2008), Kopec

318   et al. (2016), and Kreider et al. (1992) reported no change, and Kreider et al. (1990) reported a

319   significant decrease. Once again, the use of a small sample sizes may have influenced these findings

320   along with the fact that erythrocyte 2, 3-DPG levels are already elevated in endurance trained

321   individuals (Brodthagen et al., 1985; Buck et al., 2013) and can change rapidly post-sampling (Llohn

322   et al., 2005).

323

324   An alternative mechanism by which SP supplementation has been suggested to increase aerobic

325   metabolism is via an enhancement of myocardial contractility (Buck et al., 2013; Fukuda et al., 2010).

326   Indeed, using cardiac ultrasound and colour flow Doppler technology, Kreider et al. (1992) reported

327   SP-induced increases in various measures of cardiac function in well-trained athletes, albeit

328   concomitant with increases in time-trial performance (Kreider et al., 1992). Then again, there were few

329   effects of SP supplementation on those same measures when compared at each intensity during an

330   incremental test, or when compared at the same relative intensity (anaerobic threshold). Moreover,

331   although heart rate values alone are unlikely to be a reliable indicator of cardiac function; it is worth

332   highlighting that the present study, and several others have reported no SP-induced change in heart rate

333   during time-trial performance (Brewer et al., 2013, 2015; Folland et al., 2008; Kreider et al., 1990).

334   Overall, considering that the present study found no effect of SP supplementation on $\dot{V}O_2$ or time-trial

335   performance, it appears that any effect of SP on cardiac function does not translate into any ergogenic

336   benefit.

337

338   It is difficult to explain how SP increased RER during the time-trial in the present study, but

339   had no effect on RER during fixed-intensity submaximal cycling. Although the latter is supported by

340   research showing no effect of SP supplementation on RER during submaximal incremental exercise

341   (Kreider et al., 1992); previous research investigating the effects of SP supplementation on RER during

342   time-trial performance has contrastingly reported no effect, despite a SP-induced increase in power

343   output (Folland et al., 2008; Kreider et al., 1992). In the present study, a SP-induced increase in RER,

344   in the absence of any change in $\dot{V}O_2$ during the time-trial, would support the corresponding increase in

345      $\dot{V}_E$, as a result of an associated increase in $\dot{V}CO_2$. Then again, it is difficult to reconcile that response in

346   the absence of any change in performance. To add to the confusion; of those studies that observed no

347   SP-induced change in RER despite an increase in time-trial performance, Folland et al. (2008) reported

348   no corresponding change in $\dot{V}_E$, while Kreider et al. (1992) reported an increase. Moreover, Brewer et

349   al. (2014) reported no change in performance and no change in $\dot{V}_E$.

350

351   As with the above, it is difficult to reconcile how, in the absence of any change in performance,

352   blood lactate concentrations in the present study increased following SP supplementation in the time

353   trial, but not during fixed-intensity submaximal exercise. Previous research has shown that SP

354   supplementation has no significant effect on post-time-trial blood lactate concentration regardless of

355   whether there was an increase in performance (Folland et al., 2008; Kreider et al., 1992) or not (Brewer

356   et al., 2013, 2014; Buck et al., 2014). In the present study, the increase in post-time-trial blood lactate

357   concentration is consistent with the corresponding increase in RER and $\dot{V}_E$ resulting from the need to

358   buffer associated hydrogen ions. However, an increase in blood lactate concentration generally indicates

359   an enhancement of anaerobic energy provision (Maughan & Gleeson, 2004) which, in the absence of

360   any change in $\dot{V}O_2$, would normally suggest an increase in performance. Similar contradictions in the

361   present study exist concerning RPE, with significant SP-induced increases during the time-trial, despite

362   no change in performance, contrasting with no effect of SP during fixed-intensity submaximal cycling.

363   Moreover, the result is in contrast with previous studies showing that SP has no effect on RPE during

364   (Folland et al., 2008; Kreider et al., 1990) or immediately after time-trial performance (Brewer et al.,

365   2014); though differential effects on performance add to the confusion. Given that the RPE scale was

366   initially validated against heart rate (Borg, 1970), it was unsurprising that the absence of an effect of

367   SP supplementation on heart rate during fixed-intensity submaximal cycling coincided with no SP-

368   induced change in RPE. However, it is unclear as to why the same response was not reflected during

369   the time-trials. One potential explanation for the increase in RPE during the time trials is that it was

370   induced by the corresponding increase in $\dot{V}_E$. In partial support, it has recently been shown that

371   breathing frequency correlates very strongly (r = 0.89) with RPE during time-trial performance (Nicolò

372   et al., 2016).

373

374   In conclusion, the results of the present study indicate that SP supplementation has no

375   significant effect on time-trial performance. Indeed, the associated increase in RPE suggests that SP

376   supplementation may result in endurance athletes having to work subjectively harder to achieve the

377   same level of time-trial performance. Given that SP has been proposed to improve endurance

378   performance primarily via aerobic mechanisms, the absence of any SP-induced change in $\dot{V}O_2$ or heart

379   rate provides further support for this lack of an ergogenic benefit. Notably, SP supplementation

380 increased RER and $\dot{V}_E$ during the time-trials and resulted in a higher post-time-trial blood lactate

381 concentration all of which are difficult to explain; particularly given the absence of a corresponding

382 effect during submaximal exercise or any effect on time trial performance.

383

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387

388 **Declaration of Interest**

389 The authors declare no conflicts of interest. The authors alone are responsible for the content and writing

390 of the article.

391

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461 Table 1. The effects of sodium phosphate supplementation on various physiological responses during

462 10 minutes of cycling at 65% of the power output required to elicit maximum oxygen uptake ($N = 20$).

463 Values are means ± standard deviation.

Supplement	Time (min)	$\dot{V}O_2$ (L·min^{-1})	$\dot{V}_E$ (L·min^{-1})	RER	Heart rate (b·min^{-1})	RPE	BLC (mmol·L^{-1})
Placebo	5	3.02 ± 0.47	69.76 ± 12.25	0.88 ± 0.06	128.1 ± 9.0	11.5 ± 1.4	1.76 ± 0.99
	10	3.02 ± 0.40	72.01 ± 11.35	0.88 ± 0.05	130.9 ± 10.2	12.2 ± 1.1	1.55 ± 1.16
SP	5	3.01 ± 0.47	69.99 ± 11.19	0.89 ± 0.06	128.2 ± 11.5	11.1 ± 1.4	1.83 ± 1.01
	10	3.00 ± 0.52	71.93 ± 11.56	0.89 ± 0.04	131.3 ± 12.5	12.3 ± 1.6	1.65 ± 1.33

464 Note: SP = sodium phosphate; $\dot{V}O_2$ = oxygen uptake; $\dot{V}_E$ = minute ventilation; RER = respiratory exchange ratio;

465 RPE = rating of perceived exertion; BLC = blood lactate concentration.

466 Table 2. The effects of sodium phosphate supplementation on completion time, power output, and

467 cadence during a 20 km cycling time-trial ($N = 20$). Values are means ± standard deviation.

Supplement	Distance (km)	Completion time (min)	Power output (W)	Cadence (rpm)
Placebo	0-5	8.40 ± 0.58	285 ± 56	96 ± 9
	5-10	8.18 ± 0.56	293 ± 55	97 ± 8
	10-15	8.21 ± 0.58	290 ± 57	97 ± 8
	15-20	7.97 ± 0.55	317 ± 58	98 ± 9
	0-20	*32.76 ± 2.20*	*296 ± 54*	*97 ± 8*
Sodium phosphate	0-5	8.43 ± 0.61	284 ± 62	96 ± 10
	5-10	8.19 ± 0.58	292 ± 59	98 ± 10
	10-15	8.19 ± 0.61	293 ± 60	98 ± 10
	15-20	7.95 ± 0.60	321 ± 64	99 ± 11
	0-20	*32.77 ± 2.31*	*297 ± 58*	*98 ± 10*

468 Note: rpm = revolutions per minute.

469 Table 3. The effects of sodium phosphate supplementation on various physiological responses during

470 a 20 km cycling time-trial ($N = 20$). Values are means ± standard deviation.

Supplement	Distance (km)	$\dot{V}O_2$ (L·min^{-1})	Heart rate (b·min^{-1})	$\dot{V}_E$ (L·min^{-1})	RER	RPE
Placebo	0-5	3.58 ± 0.61	144.4 ± 11.6	95.4 ± 20.5	0.91 ± 0.06	14.2 ± 1.3
	5-10	3.67 ± 0.55	153.7 ± 12.0	103.0 ± 22.6	0.91 ± 0.05	15.6 ± 1.3
	10-15	3.64 ± 0.56	155.8 ± 13.2	105.0 ± 24.7	0.90 ± 0.05	16.2 ± 1.5
	15-20	3.79 ± 0.56	162.0 ± 13.0	117.0 ± 29.5	0.92 ± 0.06	17.9 ± 1.5
	0-20	*3.67 ± 0.55*	*154.1 ± 11.7*	*105.1 ± 22.7*	*0.91 ± 0.05*	*-*
Sodium phosphate	0-5	3.55 ± 0.72	144.7 ± 14.9	96.8 ± 22.3	0.93 ± 0.06	14.5 ± 1.2
	5-10	3.67 ± 0.67	155.2 ± 14.0	105.6 ± 23.3	0.93 ± 0.05	15.9 ± 1.1
	10-15	3.65 ± 0.62	158.9 ± 13.9	109.6 ± 24.5	0.92 ± 0.05	16.7 ± 1.3
	15-20	3.79 ± 0.62	164.9 ± 13.1	123.8 ± 28.7	0.95 ± 0.06	18.3 ± 1.3
	0-20	*3.67 ± 0.64*	*156.0 ± 13.1*	*108.9 ± 22.9*	*0.93 ± 0.05*	*-*

471 Note: $\dot{V}O_2$ = oxygen uptake; $\dot{V}_E$ = minute ventilation; RER = respiratory exchange ratio; RPE = rating of perceived
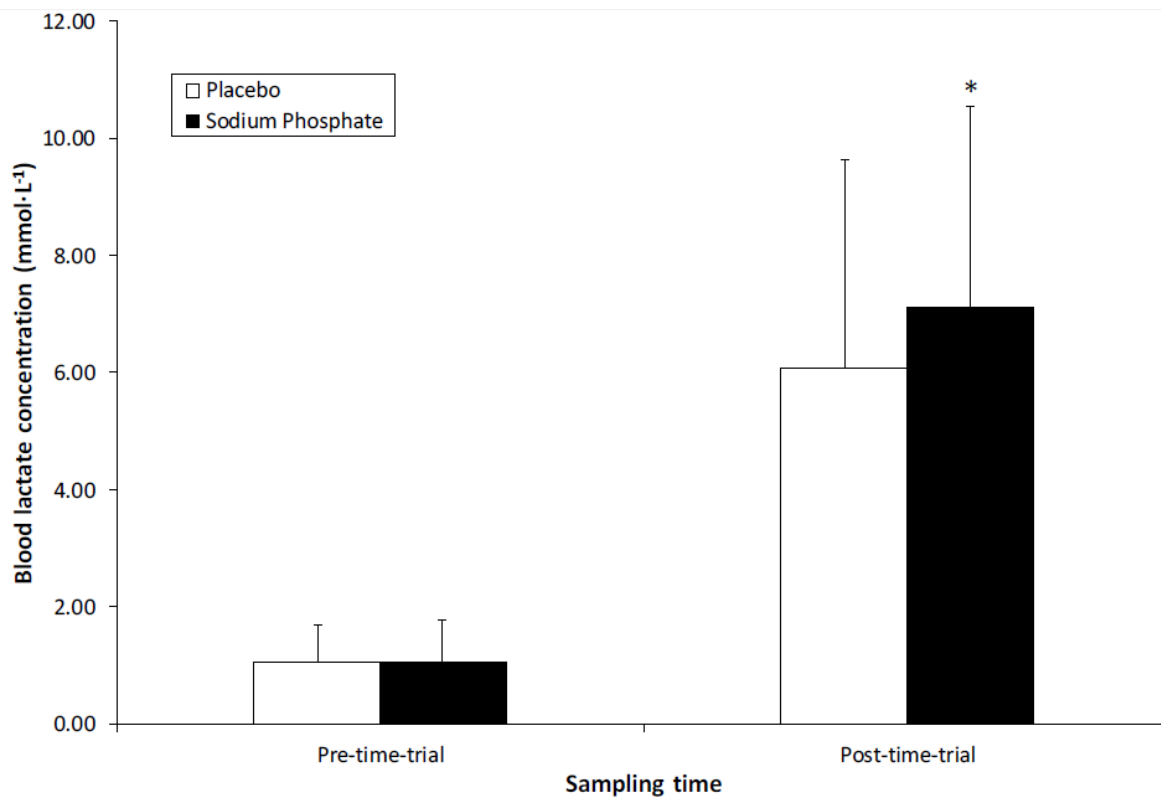
472 exertion.

Figure 1. The effects of sodium phosphate supplementation on blood lactate concentration prior to and immediately following a 20 km cycling time-trial ($N = 20$). Values are means ± standard deviation. * indicates significantly different ($p < 0.05$) from placebo.