1 **Significance of the research**

2 The research investigated the impact of two polyphenol supplements
3 on high intensity cycling performance. To date, little research has
4 been undertaken with antioxidant polyphenol supplements regarding
5 exercise performance, particularly CherryActive and Pycnogenol®.
6 This is the first study to investigate the supplementation of either
7 CherryActive or Pycnogenol® on gross efficiency and 20 km cycling
8 time trial performance. Findings suggest that the Pycnogenol®
9 supplement may have a small effect on performance during the final
10 stages of a 20 km TT, however further research with greater dosages
11 are needed to clarify the ergogenic potential of the supplements.

12 **Agreement of authors for publication**

13 The authors agree to the publication of this research.

14 **Figures and tables**

15 This manuscript contains 2 tables and 2 figures.

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26 The influence of different sources of polyphenols on
27 sub-maximal cycling and time trial performance

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49 **Abstract**

50 The primary purpose of the study was to establish the effects of
51 commercially available polyphenol-rich antioxidant supplements,
52 Pycnogenol® with added bioflavonoids (PYC-B) and CherryActive
53 (CHA), on 20 km cycling performance. Using a double-blind
54 counterbalanced, repeated-measures design, nine male cyclists or
55 triathletes (32.1 ± 11.2 years; maximal aerobic capacity 4.2 ± 0.7
56 L·min⁻¹; maximal power output 391.7 ± 39.5 watts) consumed 200
57 mg of CHA, 120 mg of PYC-B, or 200 mg of placebo (PLA) capsules,
58 2 days before and on the day of each experimental trial. The
59 experimental trials consisted of four 5 minute stages at 40%, 50%,
60 60%, and 70% maximal power output ($W_{\max}$), followed by a 20 km
61 time trial (TT). Statistical analyses revealed no significant differences
62 between trials for heart rate, respiratory exchange ratio, gross
63 mechanical efficiency, oxygen consumption, or blood lactate, at any
64 of the intensities completed during the initial 20 minute phase of the
65 trial ($p > .05$). Final 20 km TT times were not significantly different
66 between trials ($p = .115$), but, compared to PLA, PYC-B did
67 significantly increase power output by 6.2% over the final 5 km of the
68 TT ($p = .022$). The study suggests that PYC-B could be beneficial
69 towards the end of an intense bout of cycling exercise. However, as
70 total 20 km time was not significantly different between trials the
71 doses used are unlikely to benefit 20 km cycling time trial
72 performance.

73

74

75 **Key words**

76 Pycnogenol[®], free radicals, antioxidants, vasodilation, cherries.

77 **Abbreviations**

78 PYC = Pycnogenol[®]

79 CH = Cherries

80 PYC-B = Pycnogenol with added bioflavonoids.

81 CHA = CherryActive

82 FR = Free radicals

83 AO = Antioxidants

84 VO₂ = Oxygen uptake

85 HI = High intensity

86 O₂ = Oxygen

87 NO = Nitric oxide

88 TT = Time trial

89 MAP = Maximal aerobic power test

90 PLA = Placebo

91 Cm = Centimetres

92 Kg = Kilograms

93 RER = Respiratory exchange ratio

94 B_{lac} = Blood lactate

95 W_{max} = Maximal power output

96 GME = Gross mechanical efficiency

97 ES = Effect size

98

99

100

101 **Introduction**

102 When undertaking endurance exercise at a high intensity (HI), the
103 exercising muscles produce free radicals (FR) causing cellular
104 damage [1, 2]. Free radicals accumulate due to the greater oxygen
105 uptake (VO_2) associated with HI exercise, and appear to impair
106 skeletal muscle contraction, accelerating fatigue and diminishing
107 performance [1, 3, 4]. Fatigue in HI exercise may also arise from
108 circulatory limitations as the exercising muscles fail to receive a
109 sufficient oxygen (O_2) supply in order for HI performance to be
110 sustained [5, 6]. To offset fatigue during HI exercise, recent research
111 has suggested that performance could be improved through
112 nutritional interventions which simultaneously combat FR damage
113 and promote blood flow [4]. Various polyphenols appear to stimulate
114 these antioxidant (AO) and vasodilatory effects [7, 4].

115 Adding AO-rich polyphenols to the diet may assist the body's own
116 AO system to counteract FR damage and the associated muscular
117 fatigue [8-10]. Furthermore, polyphenols stimulate nitric oxide (NO)
118 production within the cell walls, encouraging blood vessels to dilate
119 [7, 12]. This could be beneficial during HI performance as NO
120 enhanced vasodilation has been shown to enhance blood flow to the
121 working muscles, which is essential if a high level of exercise
122 performance is to be maintained [13, 6]. The combination of AO
123 activity and enhanced vasodilation may be particularly beneficial in
124 situations where a high level of FR damage is possible, such as
125 when undertaking HI exercise [8, 11].

126 It has been suggested that consuming nutrients that exert containing
127 a variety of AOs are more beneficial in an attempt to counteract the
128 FR damage caused by exercise-induced oxidative stress compared
129 to singular AO nutrients (e.g. Vitamin C), potentially aiding HI
130 exercise performance [9, 11]. Two such mixtures are Pycnogenol®
131 (PYC) and Cherries (CH). There are numerous polyphenols in PYC,
132 including several proanthocyanidins, which produce a powerful
133 mixture displaying AO and vasodilatory effects [12, 14, 15]. It has
134 been shown that even a single dose of PYC (≥ 100 mg) has
135 significant effects on blood flow [16]. Three studies have reported
136 that PYC supplementation can offset muscular fatigue and prolong
137 time to exhaustion in exercise trials involving HI running [17] and
138 cycling [18, 19]. However, whether or not PYC supplementation can
139 lead to improvements in time trial (TT) tests, which are similar to race
140 performance and are therefore considered superior tests, has not
141 been investigated [20]. Similarly, the numerous polyphenols present
142 in CH including anthocyanins, catechin and kaempferol have AO
143 effects [21]. Anthocyanins in particular are found in large quantities in
144 CH and have been shown to have powerful vasodilatory effects [22].

145 Due to cherries' AO capabilities, two recent studies found that
146 supplementing with CH juice for 5 days can alleviate muscle damage
147 following intense running exercise and boosts total antioxidant
148 capacity [23, 24]. However, neither study measured the effect of CH
149 juice on race time, therefore whether CH can directly enhance HI
150 exercise performance remains unclear.

151 The majority of similar studies have employed longer protocols
152 ranging from 5-30 days [2, 9, 17, 23, 24] whereas elite cyclists seem
153 to prefer shorter protocols. Therefore the purpose of this study is to
154 determine whether the acute ingestion of two polyphenol mixtures,
155 Pycnogenol® (with added bioflavonoids; PYC-B) and/or CherryActive
156 (CHA) for 3 days will significantly improve physiological measures
157 during sub-maximal cycling and enhance subsequent 20 km TT
158 performance in moderately trained cyclists and triathletes. The
159 effects on power output during the final 5 of the TT are of particular
160 interest as skeletal muscle fatigue will likely be more evident at this
161 point, partly due to increased FR production, acidosis and increased
162 demand on the circulatory system [3, 6 11]. It is hypothesized that
163 both PYC-B and CHA will improve gross mechanical efficiency,
164 enhance lactate efflux during sub-maximal cycling and improve 20
165 km TT performance.

166

167 **Method**

168 *Participant characteristics*

169 Nine moderately trained cyclists and triathletes completed the study
170 (Age: 32.1 ± 11.2 years, Height; 179.7 ± 5.9 cm, weight: 80.2 ± 8.6
171 kg, VO_{2max} : 4.2 ± 0.7 L·min⁻¹, maximal power output: 391.7 ± 39.5
172 watts). All participants were male, >3 years competitive cycling
173 experience and were familiar with time trials and cycling distances of
174 ≥ 20 km. The study was reviewed by the University of Portsmouth's
175 BioSciences Research Ethics Committee.

176

177 *Study design*

178 A double blind repeated measures crossover design was employed
179 with the participants ingesting one of three supplements, CHA, PYC-
180 B or a placebo (PLA) in three separate trials. CHA contained dried
181 montmorency cherries which provided 216 mg of polyphenols
182 (CherryActive, Twickenham, UK), PYC-B contained 120 mg of
183 Pycnogenol® and 600 mg of citrus bioflavonoids (Horphag Research
184 Ltd, NBTY, Warks, UK) and the placebo contained 1g of maltodextrin
185 (PSP22 orange, Science in Sport Ltd. Lancashire, UK). The trial
186 orders were allocated using a partial counter-balance with 6
187 participants fully counterbalanced and the remaining 3 partially
188 counter-balanced. The participants visited the University on 4
189 occasions, firstly, to record their physical characteristics and to
190 complete a maximal aerobic power test (MAP). The participants
191 completed three experimental trials at a similar time of day, each
192 separated by ≥ 5 days at the University of Portsmouth's exercise
193 laboratory. Each trial consisted of 20 minutes of progressive intensity
194 cycling and a 20 km TT on an electronically braked cycle ergometer
195 (Velotron, Racermate Inc. Seattle).

196

197 *Procedures*198 *Preliminary measures*

199 The participants' first trial at the laboratory was a MAP test. Initially,
200 participants' height (cm) (Harpender portable stadiometer, Holtain,
201 UK) and body mass (kg) (770, Seca, Germany) were recorded.

202 Participants were fitted with a heart rate monitor (T31, Polar, UK) and
203 a face mask (7400 Series, Hans Rudolph, USA) attached to an
204 breath-by-breath expired gas analyser (Cosmed, Italy). This same
205 procedure and equipment was used in all MAP tests and
206 experimental trials.

207 An initial workload of 50 watts was set on the ergometer using their
208 preferred cadence. The workload was increased by 25 watts every
209 60 seconds until the participant voluntarily stopped or was unable to
210 continue cycling at $>70 \text{ rev}\cdot\text{min}^{-1}$ [25]. Participants were deemed to
211 have reached $\text{VO}_{2\text{max}}$ when they could not maintain cycling at >70
212 $\text{rev}\cdot\text{min}^{-1}$ for 15 seconds and HR was $\geq 90\%$ of age-predicted
213 maximum. The mean VO_2 attained over the final 60 seconds of the
214 trial was considered as $\text{VO}_{2\text{max}}$ and the highest power output attained
215 for a given 60 second increment was considered the participants'
216 W_{max} [26].

217

218 *Experimental trials and supplements*

219 Three experimental trials were completed with a different supplement
220 consumed prior to each. For three days (two days before and on the
221 day of the trial) participants consumed one of three supplements;
222 PYC-B, CHA or a PLA, consuming the other two supplements in the
223 subsequent two trials. The supplements were provided in capsule
224 form of the same size and the contents were of similar colour to
225 maintain the blinding of the study. The participants were instructed to
226 consume two capsules together with a meal. Timing of intake was

227 standardized across trials by instructing participants to consume the
228 supplements at the same time of day equivalent to two to three hours
229 prior to each trial.

230 Prior to testing, participants were provided with food diaries and
231 instructed to detail their food and fluid intake on the two days before
232 and on the day of their first experimental trial. They were then told to
233 repeat this diet over the same time period prior to the subsequent
234 trials. A list detailing foods with a high quantity of polyphenols were
235 provided to participants so that these were not consumed in the 24
236 hours leading up to the experimental trials. Participants were
237 instructed to avoid undertaking strenuous exercise and consuming
238 caffeine, alcohol or any vitamin supplement in the 48 hours prior to
239 each experimental trial. Participants were asked to arrive at the
240 laboratory in a fed state having eaten a meal of similar macronutrient
241 content two to three hours prior to each trial.

242 The initial phase of the experimental trial involved cycling at four 5-
243 minute incremental stages, each corresponding to 40, 50, 60 and
244 70% of the participants' W_{max} . The Cosmed system recorded heart
245 rate (HR), expired gas concentrations (oxygen uptake [VO_2] and
246 carbon dioxide production [VCO_2]) continuously. A 20 μ l blood
247 sample was obtained via a finger-prick sample before the end of
248 each stage to determine blood lactate (B_{lac}) concentrations (Biosen
249 C_line Sport, EFK-Diagnostics GmbH, UK). Gross mechanical
250 efficiency (GME) was calculated for each stage to determine if the

251 supplements affected the energy cost of sub-maximal cycling using
252 the following equation:

253 $\text{Mechanical power (kcal} \cdot \text{min}^{-1}) / \text{metabolic power (kcal} \cdot \text{min}^{-1}) \times 100 =$
254 GME %. Efficiency was measured.

255 Once the 20 minutes was completed the participants had a 10 minute
256 break before starting the 20 km TT. The TT was self-paced and
257 participants were instructed to complete the course as quickly as
258 possible. The only feedback the participants received was distance,
259 which was projected onto a screen in front of them. Expired gases
260 and HR were recorded continuously throughout the TT, along with
261 power output (Watts), speed ($\text{km} \cdot \text{h}^{-1}$), cadence ($\text{rev} \cdot \text{min}^{-1}$) and time
262 (milliseconds) using the Velotron computer software. A final B_{lac}
263 measure was taken in the last 0.5 km of the TTs.

264 *Data analysis*

265 Statistical analyses were performed using PASW statistics version 18
266 for Microsoft Windows, and results are presented as mean $\pm$ SD.
267 Coefficient of variation (CV) was established for time to complete 20
268 km. All data were checked for normality and one and two way
269 analyses of variance (ANOVA) with repeated measures were
270 applied. If the ANOVA found significant differences, paired samples t-
271 tests with bonferroni correction were applied to determine which
272 conditions were significantly different. Statistical power was
273 calculated and effect sizes were calculated using partial eta and
274 Cohens d [27]. The alpha was set at $p < .05$.

275

276 **Results**

277 *Sub-maximal data*

278 Due to equipment failure, data for the sub-maximal portion of the trial
279 was only collected for 6 participants. Table 1 displays the descriptive
280 data for the 5 parameters assessed. There were no significant
281 differences in GME at any of the intensities (40, 50, 60, 70% $W_{\max}$)
282 during between trials ($p = .952$, eta-squared = .05, power = .11).
283 Mean VO_2 for the 5 minute stages was also not significantly different
284 between trials ($p = .966$, eta-squared = .04, power = .10). There were
285 no differences in RER (average value over the 5 minute stage) at any
286 of the intensities completed during the trials ($p = .562$, eta-squared =
287 .14, power = .27), or for HR at the end of each stage ($p = .064$, eta
288 squared = .21, power = .71). The B_{lac} data was collected from eight
289 participants and there were no differences in B_{lac} at any of the
290 intensities between trials ($p = .742$, eta-squared = .08, power = .21).

291 Table 1. HR, B_{lac} , GME, $\dot{V}O_2$ and RER data collected for each 5 minute stage (mean $\pm$ SD).

Measure	% of W_{max}											
	40			50			60			70		
	PLA	CHA	PYC-B	PLA	CHA	PYC-B	PLA	CHA	PYC-B	PLA	CHA	PYC-B
HR (b $\cdot$ min $^{-1}$)	119.4 $\pm$ 14.1	117.6 $\pm$ 11.9	120.9 $\pm$ 12.9	134.2 $\pm$ 16.6	132.6 $\pm$ 13.7	134.2 $\pm$ 15.4	151.1 $\pm$ 17.2	148.2 $\pm$ 14.9	157.8 $\pm$ 16.4	164.9 $\pm$ 17.7	161.9 $\pm$ 16.2	162.4 $\pm$ 15.3
B_{lac} (mmol $\cdot$ l $^{-1}$)	2.1 $\pm$ 0.8	1.8 $\pm$ 0.3	2.2 $\pm$ 0.7	2.6 $\pm$ 1.2	2.2 $\pm$ 0.9	3.1 $\pm$ 1.4	3.7 $\pm$ 1.7	2.9 $\pm$ 0.8	3.4 $\pm$ 1.4	5.6 $\pm$ 2.3	4.8 $\pm$ 1.0	5.6 $\pm$ 1.7
GME (%)	21.1 $\pm$ 1.9	20.9 $\pm$ 1.6	21 $\pm$ 1.1	21.6 $\pm$ 1.8	21.4 $\pm$ 1.0	21.3 $\pm$ 1.1	22 $\pm$ 1.3	22.9 $\pm$ 0.9	22 $\pm$ 1.2	22.4 $\pm$ 1.5	22.3 $\pm$ 1.0	22.3 $\pm$ 1.2
$\dot{V}O_2$ (L $\cdot$ min $^{-1}$)	2.18 $\pm$ 0.29	2.19 $\pm$ 0.17	2.19 $\pm$ 0.15	2.65 $\pm$ 0.38	2.64 $\pm$ 0.20	2.67 $\pm$ 0.24	3.10 $\pm$ 0.39	3.14 $\pm$ 0.28	3.08 $\pm$ 0.29	3.54 $\pm$ 0.41	3.54 $\pm$ 0.33	3.53 $\pm$ 0.31
RER	0.92 $\pm$ 0.03	0.93 $\pm$ 0.03	0.91 $\pm$ 0.03	0.97 $\pm$ 0.06	0.97 $\pm$ 0.02	0.97 $\pm$ 0.03	0.99 $\pm$ 0.06	0.99 $\pm$ 0.04	0.99 $\pm$ 0.03	1.00 $\pm$ 0.04	1.02 $\pm$ 0.01	1.02 $\pm$ 0.03

292 Time trial data

293 *Time*

294 The average CV across the 20 km time trials was 1.68%. Mean $\pm$ SD
 295 time for the 20 km was PYC-B: 1990.07 $\pm$ 93.18 s, PLA: 2030.30 $\pm$
 296 124.73 s and CHA: 2008.56 $\pm$ 97.50 s (Figure 1). Time taken to
 297 complete the 20 km TT was not significantly different between trials
 298 ($p = .115$, eta-squared = .24, power = .43). Effect size for the mean
 299 difference between the PYC-B and PLA trials was calculated at 0.46
 300 and 0.37 for the CHA and PLA trials.

301

302

303 Figure 1 Time taken (s) to complete 20 km for the 3 trials.

304

305

306

307

308 *Power output*

309 Mean $\pm$ SD power output over the 20 km was PYC-B: 250.58 ± 27.89
 310 W, CHA: 244.98 ± 29.29 W and PLA: 240.69 ± 35.31 W. There was
 311 no significant difference in 20 km power output between the trials (p
 312 = .171, eta-squared = .20, power = .35). However, there was a
 313 significant difference between trials in the final 5 km of the TT (p =
 314 .038, eta-squared = .34, power = .63) with a paired samples t-test
 315 revealing that power output was significantly greater during PYC-B
 316 compared to PLA (p = .022, ES = .45) (Figure 2). Mean $\pm$ SD in the
 317 final 5 km was PLA: 247.29 ± 36.98 W and PYC-B: 263.77 ± 25.1 W.

318

319

320 Figure 2

321

322

323

324

325

Mean power output (W) for the final 5 km of the 3 trials.*
 significantly greater power output compared to PLA.

326 *Blood lactate and Heart rate*

327 No significant differences were observed between trials for HR at 10,
 328 15, 18 and 20 km ($p = .160$, eta-squared = .16, power = .64). B_{lac} ,
 329 collected at 19.5 km, were not significantly different between trials (p
 330 = .859, eta-squared = .19, power = .07) (Table 2).

331
 332 Table 2. B_{lac} (mmol·l⁻¹) at 19.5 km into the 3 trials and average HR
 333 (b·min⁻¹) at 10, 15, 18 and 20 km of the TT (mean ± SD).

	PLA	CHA	PYC-B
B_{lac}	6.66 ± 2.58	6.77 ± 2.03	6.41 ± 2.23
HR ₁₀	163 ± 13.64	158.8 ± 11.42	161.77 ± 8.96
HR ₁₅	167 ± 14.86	161.44 ± 10.10	165.33 ± 8.39
HR ₁₈	167.44 ± 10.19	166.66 ± 10.23	168.44 ± 8.45
HR ₂₀	176.66 ± 12.19	175.22 ± 10.4	178.11 ± 10.82

334

335 **Discussion**

336 The primary aim of the study was to determine if supplementing with
 337 2 different polyphenol mixtures (PYC-B and CHA) for only 3 days
 338 could improve cycling TT performance. It was found that after PYC-B
 339 supplementation, participants were able to generate a significantly
 340 greater power output over the final 5 km by 6.2% (Figure 2).
 341 However, overall 20 km TT performance was not significantly
 342 different between trials. No performance benefits were observed at
 343 any time point following CHA supplementation. Furthermore, neither
 344 PYC-B nor CHA had any effect on GME, HR, RER, VO_2 or B_{lac} at 40,
 345 50, 60 and 70% of W_{max} when compared to the PLA. The only

346 physiological measures taken during the TT was HR and B_{lac} at 19.5
347 km, which were both not significantly different between the 3 trials.

348 In previous similar studies, acute PYC consumption extended time to
349 fatigue in cycling trials [17, 18] and enabled participants to run for
350 29.6% longer following 30 days of PYC (200 mg/day)
351 supplementation [16]. This however is the first study to assess the
352 effects of a PYC based supplement during a performance test and
353 supplementing for as little as 3 days. Although there was no
354 statistical difference between trials, a small effect size of .46 was
355 observed for 20 km time in favour of PYC-B. Participants completed
356 the 20 km 1.98% faster during the PYC-B trial compared to the PLA.
357 It is possible that any small effects resulting from the supplements
358 could have been better detected amongst a group of elite cyclists
359 since elite cyclists have demonstrated a CV of 1.1% for the 20 km TT
360 [28] compared to the 1.68% demonstrated in this study.

361 The significant improvement in the last 5 km of the TT in PYC-B
362 could be related to the greater intensity of exercise being performed.
363 This is demonstrated by the increase in HR over this time (see table
364 2). The increase in intensity would have decreased muscle pH and
365 increased VO_2 , causing the exercising muscles to produce more free
366 radicals [3, 11]. This is likely to result in more cell damage and
367 disruption to skeletal muscle contraction and an increased need for
368 exogenous AOs [3, 11]. It is therefore possible that the AO effects of
369 PYC-B were only significant at this time when skeletal muscle

370 contractions were most likely to be compromised by increased
371 oxidative stress [1, 3, 15].

372 Performance may also have been enhanced by PYC-B through
373 increased blood flow, since the release of the vasodilator NO and
374 reductions in endothelial damage and platelet formation are some of
375 the proposed benefits of PYC [4, 15, 29]. This increased blood flow
376 may help to overcome any potential circulatory limitations towards
377 the end of the TT, attenuating the diminishing O₂ supply to the
378 exercising muscles and maintaining force production during the final
379 5 km [6, 30]. Support for these mechanisms is provided by various
380 studies of the physiological effects of PYC and bioflavonoids [29, 31].

381 Both supplements had no effect on B_{lac} levels at 40, 50, 60 and 70%
382 of W_{max} and 19.5 km into the TT. These results are comparable to
383 previous studies that found consuming polyphenol supplements had
384 no effect on B_{lac} levels throughout and after HI cycling exercise [2,
385 17]. One study however, found that B_{lac} levels were reduced by
386 ingesting a pre-trial AO and PYC beverage when cycling at a fixed
387 output of 70% of W_{max} [18]. It was proposed that the polyphenols
388 within PYC encourage lactate removal, possibly via the vasodilatory-
389 induced increases in blood flow [18, 29]. Whether this effect occurs
390 has not been investigated and requires more research.

391 The supplements had no effect on RER when cycling at 40, 50, 60
392 and 70% of W_{max}. Another study also found that substrate use was
393 unaffected by AO-rich quercetin consumption during a 30 km cycling
394 TT [9]. In two other studies, no significant differences were found, but

395 there was a slight reduction in RER throughout running exercise after
396 ingesting a pre-trial dose of *Ecklonia cava* [4] and a cocoa
397 polyphenol supplement prior to cycling exercise [2]. Only studies
398 using polyphenol mixtures such as green tea, which contains
399 epigallocatechin gallate, have found significant increases in fat
400 oxidation during exercise [32, 33].

401 The CHA and PYC-B supplements also had no effect on GME and
402 VO_2 ($\text{L}\cdot\text{min}^{-1}$) (Table 2). Few studies investigating polyphenols and
403 exercise performance have measured efficiency. Although one study
404 found that VO_2 was lower throughout a 30 km TT following ingestion
405 of an AO and quercetin supplement GME was not calculated [9].
406 How polyphenols contribute to superior efficiency remains unclear,
407 other studies investigating nitrate consumption have implied that
408 boosting NO production could lower VO_2 , leading to improved
409 exercise efficiency [34, 35]. Based on this, it is possible that in this
410 study the polyphenol supplements effects on NO were insufficient to
411 make any major difference to efficiency, however NO was not
412 measured.

413 Further research to help explain the results should be undertaken as
414 no measures of FR damage or muscle blood flow were taken
415 throughout the TT and therefore it can only be speculated that this
416 was the case during the final 5 km. Furthermore, an optimal dosage
417 of PYC-B and CHA has not yet been determined and it is
418 conceivable that for an exercise protocol of this volume and intensity
419 a greater daily dose and/or longer supplementation period is required

420 for both supplements to have a significant impact on performance,
421 particularly during the CHA trial and should be the focus of future
422 research. It should also be considered that the low sample size may
423 have confounded the results, as VO_2 , RER and GME data was only
424 available for 6 participants, a recognized limitation of this study.
425 Future research should build upon these findings and employ
426 designs with greater participant numbers.

427 **Conclusion**

428 To conclude, neither the PYC-B nor CHA polyphenol supplement
429 significantly enhanced 20 km TT performance or had any
430 physiological effect during sub-maximal cycling. PYC-B enhanced
431 final 5 km power output suggesting that the supplement may provide
432 some benefit towards the end of an intense bout of exercise.
433 However as total 20 km time was not significantly different between
434 trials the dosing protocol employed does not appear to benefit 20 km
435 cycling time trial performance. Future studies should focus on using a
436 greater dosage of the supplements and also the use of longer
437 performance tests to see if further beneficial effects can be revealed
438 under these conditions.

439

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444

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447

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