Title Page

Title: The effect of 1,3-butanediol and carbohydrate supplementation on running performance.

Preferred running head: Run performance with 1,3-BD and CHO

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Abstract

Objectives: Ingested ketogenic agents offer the potential to enhance endurance performance via the provision of an alternative exogenous, metabolically efficient, glycogen-sparing fuel (i.e. ketone bodies). This study aimed to assess the impact of combined carbohydrate and 1,3-butanediol (CHO-BD) supplementation on endurance performance, blood beta-hydroxybutyrate (βHB) concentration and glycolytic activity, in comparison to carbohydrate supplementation alone (CHO). **Design:** Eleven male runners (age 38 ± 12 years, mass 67.3 ± 6.5 kg, height 174.5 ± 5.0 cm, $\dot{V}O_{2peak}$ 64.2 ± 5.0 ml·kg^{-1}·min^{-1}) performed two experimental trials in a randomised crossover design. **Methods:** Each trial consisted of 60 min of submaximal running, followed by a 5 km running time-trial (TT), and was performed following the ingestion of an energy matched ~650 ml drink (CHO-BD or CHO). **Results:** There was no difference in TT completion time between the trials (CHO: 1265 ± 93, CHO-BD: 1261 ± 96 s; p=0.723). However, blood βHB concentration in the CHO-BD trial was at least double that of the CHO trial at all time points following supplementation ($p<0.05$). While blood lactate concentration was lower in the CHO-BD versus CHO trial after 30 min submaximal exercise (CHO-BD: 1.46 ± 0.67 mmol·L^{-1}, CHO: 1.77 ± 0.46 mmol·L^{-1}, p=0.040), it was similar at other time points. Blood glucose concentrations were higher post-TT in the CHO-BD trial (CHO-BD: 5.83 ± 1.02 mmol·L^{-1}, CHO: 5.26 ± 0.95 mmol·L^{-1}, p=0.015). **Conclusions:** An energy matched CHO-BD supplementation drink raised βHB concentration and acutely lowered blood lactate concentration, without enhancing 5km TT running performance.

Keywords: ketone bodies, substrate, exercise performance, dietary supplements, sports nutrition

Introduction

Dietary manipulation and supplementation strategies can impact substrate utilisation[1], a well-known determinant of endurance exercise performance. While less investigated, supplementation of ketone bodies (i.e. acetoacetate, beta-hydroxybutyrate (βHB) and acetone) can be used to enhance endurance performance[2]. When present in elevated concentrations, ketone bodies have been shown to be preferentially metabolised over other fuels[3,4]. By acting as an alternative substrate and as a signalling molecule, ketone bodies appear to modulate the mobilisation and metabolism of substrates during exercise, suppressing glycolysis, even in conditions that typically favour carbohydrate oxidation[5]. Hypothetically, elevated levels of ketone bodies could prove advantageous for long-duration endurance exercise, by sparing precious glucose and gluconeogenic reserves, thus allowing greater performance capacity later in exercise.

Adherence to a ketogenic diet increases ketone bodies[6], however its performance effects remain unproven[7,8]. For elite athletes, there are practical limitations to the ketogenic diet. First, moderate levels of ketosis can take several days to achieve, and long-term adherence is considered notoriously difficult, due to reasons related to adherence, palatability and gastrointestinal comfort[9]. Second, switching from a high carbohydrate diet to a ketogenic diet can, temporarily at least, reduce glycogen stores and the ability to oxidise carbohydrate[6,10,11], which may in turn negatively impact performance[12].

Alternatively, ingestion of exogenous ketogenic agents (i.e. a supplement that when ingested increases levels of circulating ketone bodies) can allow ketone bodies to be absorbed directly through the gut epithelium, elevating blood ketone body concentrations without the need to maintain a strict diet, and without compromising glycogen stores. Indeed, exogenous ketogenic agents can act synergistically with glucose[13]. Ingestion of ketone esters (i.e. compounds that link an alcohol body to a ketone body) in combination with carbohydrate has improved 30 min time trial performance in highly trained cyclists following a 1 h fixed workload period at 75% W_{max} [5]. However, ketone ester supplements are currently expensive and have been reported to cause gastrointestinal side effects[14].

1,3-butanediol (1,3-BD), a component part of the ketone ester used by Cox et al.[5], could itself be a cost-effective alternative fuel source capable of inducing ketosis without gastrointestinal side-effects. When consumed, 1,3-BD undergoes a series of oxidation steps in the liver to produce βHB. Despite showing prolonged elevations of blood βHB concentration within 30 min of consumption in rodent models[15], 1,3-BD alone, rather than as a ketone ester, is yet to be examined as a potential ergogenic supplement in humans.

The purpose of the present study therefore was to investigate the effects of 1,3-BD supplementation in combination with carbohydrate (CHO-BD), in comparison to an energy matched carbohydrate only supplementation (CHO), on blood βHB concentration, lactate concentration (proxy of glycolytic activity) and endurance running performance. It was hypothesised that CHO+BD supplementation would increase blood βHB concentrations and also enhance running performance.

73 **Methods**

74 Eleven healthy male runners (age 38 ± 12 years, mass 67.3 ± 6.5 kg, height 174.5 ± 5.0 cm, $\dot{V}O_{2peak}$
75 64.2 ± 5.0 ml·kg^{-1}·min^{-1}) provided written informed consent to participate in this study approved by
76 the Loughborough University Ethics Approvals (Human Participants) Sub-committee.
77
78 Participants visited the laboratory four times, for preliminary testing, a familiarisation session and two
79 main experimental trials. Experimental trials assessed endurance running performance using a double-
80 blind, randomised crossover design. Participants performed a self-paced, pre-fatigued 5 km time trial
81 (TT) following two experimental conditions: ingestion of a drink containing 1,3-butanediol and
82 carbohydrates (CHO-BD) or carbohydrates only (CHO). All exercise took place in a temperature-
83 controlled chamber (20.5 ± 0.4°C, 52.5 ± 4.0% relative humidity; Weiss Gallenkamp, Loughborough,
84 United Kingdom).
85
86 Participants performed a discontinuous incremental protocol to lactate threshold (4 min stages at
87 progressive speeds of 1 km·h^{-1}), followed by a maximal continuous fixed-speed incremental ramp
88 protocol (13.5 ± 0.9 km·h^{-1}), with treadmill gradient increasing by 1% every min until volitional
89 exhaustion. Peak oxygen uptake ($\dot{V}O_{2peak}$) was calculated as the highest sample of oxygen consumed
90 ($\dot{V}O_2$) averaged over 60 s. Data from this test were used to extrapolate running speeds for subsequent
91 trials.
92
93 The familiarisation trial followed the same procedures as the experimental trials. All trials were
94 performed in the morning at the same time of day to minimise circadian variations.
95
96 Participants also followed several pre-trial standardisation practices that were replicated for each trial:
97 1) completing a 24h food diary before the first trial and replicating before the second trial and also
98 refraining from strenuous exercise and alcohol consumption in the 24 h prior to testing (light exercise
99 was recorded and replicated), 2) consuming 500 mL of water 1 h before arriving, and 3) arriving
100 following an overnight fast. All running was performed on a motorised treadmill (pulsar® 3p;

h/p/cosmos, Nussdorf-Traunstein, Germany), at a 1% gradient. Trials were identical apart from the drink ingested.

For each trial, upon entering the laboratory, participants provided a urine sample for assessment of osmolality (Osmocheck; Vitech Scientific, Horsham, United Kingdom) and had nude body mass measured (CFW-150; Adam Equipment Ltd, Milton Keynes, United Kingdom). After 5 min of seated rest, baseline measures of heart rate (HR) and gastrointestinal (GI) comfort were taken (0 = very comfortable to 10 = extremely uncomfortable), along with fingertip capillary blood samples using 20 µl end-to-end glass tubes (EKF, Cardiff, UK) and 300 µl microvettes® (300 CB K2E; Sarsedt, Nümbrecht, Germany) containing potassium ethylenediaminetetraacetic acid anticoagulant (K+ EDTA), for later analysis of blood lactate, blood glucose and blood βHB concentrations. Participants then ingested 50% of the volume of an ~650 mL drink (exact volume dependent on composition of drink based on participant body mass), either CHO-BD or CHO. Following a further 30 min of seated rest, baseline measures were repeated and a further 25% of the drink was ingested. Participants then completed 60 min of submaximal running at a fixed speed equivalent to 75% $\dot{V}O_{2peak}$ (12.6 ± 0.9 $km \cdot h^{-1}$). Blood sampling was repeated after 30 min (participants stopped running for 30 s) and 60 min of submaximal running. Rating of perceived exertion (RPE), GI comfort and HR were measured every 15 min.

Upon completion of the submaximal exercise, participants were given a 10 min rest period, during which they ingested the remaining 25% of the drink, before they completed the TT. Participants were told to complete the TT as fast as possible. To account for inconsistencies that can occur in accelerating from a standstill up to desired speed, participants started running from a rolling start at a speed equivalent to 80% $\dot{V}O_{2peak}$ (13.5 ± 0.9 $km \cdot h^{-1}$) before self-selecting their running speed using a control panel mounted to the side of the treadmill. Verbal progress updates of distance completed were provided every 500 m for the first 4 km and every 250 m thereafter; HR and time were recorded at these time points. Participants were not verbally encouraged and were not made aware of time

128 elapsed. Upon completion of the TT, final measures of RPE, GI, blood lactate, glucose and βHB
129 concentrations were taken.
130
131 Participants ingested one of two isocaloric drinks. Both drinks contained 7 g.kg body mass^{-1} of water,
132 1 g.kg body mass^{-1} of orange squash (Robinson's, Brtivic PLC, Hertfordshire, UK) and 0.015 g.kg
133 body mass^{-1} of artificial sweetener (Canderel, Merisant Company, Chicago, US) and either
134 carbohydrate (CHO) or carbohydrate plus food grade 1,3-butanediol (CHO+BD). Each drink
135 contained 60 g of carbohydrate (Maltodextrin; MyProtein, Northwich, UK). The CHO-BD drink also
136 contained 0.5 g.kg body mass^{-1} 1,3-butanediol (Product Number 02-59620; Penta Manufacturing Ltd,
137 Livingston, USA), whilst the CHO drink contained additional carbohydrate (total carbohydrate in
138 CHO drink: 110 ± 5 g) to create the matched calorie content. Participants were told they would be
139 consuming a novel sports drink, but were not informed of the specific contents (i.e. containing 1,3-
140 butanediol and carbohydrate or carbohydrate only) until after completing the study.
141
142 The 20 µl blood samples were analysed for glucose and lactate concentrations using a calibrated
143 Biosen C-Line analyser (EKF, Cardiff, UK). The 300 µl samples were centrifuged at 2000 g for 5 min
144 (accuSpin™ Micro 17 centrifuge; Fisher Scientific™, Pittsburgh, USA), from which plasma was
145 aliquoted and analysed in duplicate for βHB concentration using an enzymatic assay (RANBUT;
146 Randox, Crumlin, United Kingdom) and spectrophotometric measurement of absorbance (Varioskan
147 Flash™; ThermoScientific™, Waltham, USA).
148
149 Before each test, electrochemical (O_2) and infrared (CO_2) gas analyser calibrations were carried out
150 using gases of known concentrations; the digital volume transducer was calibrated using a 3 L syringe
151 (Carefusion, San Diego, USA). Participants wore a low-dead space face mask (Hans-Rudolph, Kansas
152 City, USA), and breath-by-breath gas exchange data were collected continuously throughout the
153 preliminary testing and submaximal component of the trials, using an automated open circuit
154 metabolic cart (Jeager™ Vytnus™ CPX; Carefusion, San Diego, USA). Experimental trial breath-by-
155 breath data were pooled into 5 min segments for analysis: 10-15, 25-30, 40-45 and 55-60 min.

156

157 Height was measured using a wall-mounted stadiometer (Seca, Hamburg, Germany). HR was
158 recorded using a Polar M400 heart rate monitor and Polar H7 transmitter (Polar, Kempele, Finland).
159

160 Data are reported as mean ± standard deviation (SD) unless otherwise stated. Data were checked for
161 normality of distribution using a Shapiro-Wilks test. A two-way repeated measures ANOVA was used
162 to analyse data sets containing two factors. A Greenhouse-Geisser estimate was used to correct the
163 degrees of freedom if the assumption of sphericity was violated. A one-way repeated measures
164 ANOVA was used to analyse data sets containing one factor. If a significant main effect was found, a
165 post hoc Holm-Bonferroni corrected t-test was applied. Statistical significance was accepted when
166 $p<0.05$, with analyses performed using SPSS Statistics (Version 21; IBM©, Chicago, USA).
167 Magnitude-based inferences (MBI) were also used to identify practically substantial differences in
168 performance using a modified statistical spreadsheet[16]. Effect sizes (ES), calculated from standardised
169 change in mean (Std. Δ Mean) from CHO-BD to CHO, were defined using Cohen's d, with an ES
170 <0.2 considered trivial, >0.2 small, >0.6 moderate, >1.2 large and >2.0 very large. Uncertainty is
171 expressed as ±90% confidence limits (CL), which define the likely range of true values. Qualitative
172 descriptors representing likelihood of effects being negative, trivial or positive are provided[17]; an
173 effect was deemed unclear if it had >5% likelihood for more than one of these descriptors.

Results

Urine osmolality (446 ± 290 mOsm·kg^{-1}; 479 ± 246 mOsm·kg^{-1}) and body mass (68.4 ± 6.8 kg; 67.3 ± 6.7 kg) on arrival at the laboratory were similar between CHO-BD and CHO trials, respectively ($p>0.05$), indicating similar hydration status between trials[18].

TT performances were similar in CHO-BD (1261 ± 96 s) and CHO trials (1265 ± 93 s) ($p=0.723$) ($p>0.05$; Figure 1A). Comparison using MBI revealed an *unclear* difference (-0.04 ± 90% CL 0.19). Pacing, defined by 1 km split times, was similar between trials ($p=0.829$) (Figure 1B). MBI comparison revealed *very likely trivial* differences 0-3 km (0-1km: 257 ± 22 vs 258 ± 23 s; 1-2km: 254 ± 20 vs 253 ± 21 s; 2-3km: 254 ± 19 vs 254 ± 19 s), and *unclear* differences between 3-5 km (3-4km: 252 ± 20 vs 253 ± 17 s; 4-5km: 245 ± 20 vs 247 ± 16 s).

Blood βHB concentrations were similar between CHO-BD (0.36 ± 0.13 mmol·L^{-1}) and CHO (0.36 ± 0.06 mmol·L^{-1}) trials at baseline ($p=0.384$), but blood βHB concentrations in CHO-BD trial was at least double that of the CHO trial thereafter ($p<0.05$). βHB concentration remained stable in CHO trial ($p>0.05$), but at least two-fold higher than baseline from 30 min onwards in the CHO-BD trial ($p<0.05$), without any additional increase thereafter ($p>0.05$) (Figure 2A).

Blood glucose concentrations were similar across trials at baseline ($p>0.05$), rising ~70% after 30 min seated rest in both trials ($p<0.0001$). Glucose concentrations then returned to baseline after 30 min submaximal exercise ($p<0.0001$) in both trials. Glucose concentration was higher for the CHO-BD trial compared to the CHO trial post-TT ($p=0.015$) (Figure 2B).

In both trials, blood lactate concentrations rose from baseline after 30 min submaximal exercise ($p<0.05$), when lactate concentrations were lower in the CHO-BD trial versus the CHO trial ($p=0.040$). Blood lactate concentrations were similar between trials at all other time points ($p>0.05$), peaking post-TT in both trials ($p<0.0001$) (Figure 2C).

Heart rate, $\dot{V}O_2$, respiratory exchange ratio (RER), RPE and GI comfort (Table 1) were similar across trials at all time points measured ($p>0.05$). Furthermore, GI comfort was similar to baseline at all time points ($p>0.05$).

205 **Discussion**

206 The present study demonstrated that CHO-BD supplementation did not improve 5 km TT
207 performance compared to an energy matched carbohydrate drink. However, CHO-BD did acutely
208 increase blood βHB concentrations higher than those observed when supplementing with medium-
209 chain triacylglycerol[19] and ketone salts[20], comparable with results attained after 1-3 days of fasting[21],
210 and lower than those produced through combined ketone ester and carbohydrate supplementation[5].
211
212 The performance findings of this study are consistent with others that have induced small to moderate
213 elevations in blood βHB concentrations[19,20,22]. In contrast, ketone esters capable of inducing greater
214 levels of ketosis (>2 mmol·L^{-1}) have been shown to improve the performance of elite cyclists when
215 ingested in combination with carbohydrate[5]. Therefore, the lack of a clear performance effect in this
216 study may be due to insufficient ketosis and consequently a reduced ketone body utilisation. In
217 addition, both drinks contained a minimum of 60 g of a single source of carbohydrate and therefore
218 would likely have saturated transporters responsible for intestinal absorption[23], rendering the
219 additional carbohydrate in the CHO trial ineffective. The fact that 1,3-BD did not enhance
220 performance by providing an additional substrate provides further evidence to suggest that the state of
221 ketosis in this study was insufficient. However, further investigation may be warranted into the effect
222 of CHO-BD when the carbohydrate dose is lower than 60 g·h^{-1}.
223
224 Specific comparisons of the changes in blood βHB concentration following CHO-BD seen in this
225 study are also limited, as many of the studies showing elevations in blood βHB concentrations
226 following the ingestion or infusion of 1,3-BD have used animal models[15,24]. Human studies have
227 previously shown 1,3-BD to be a source of energy supply for humans, but failed to raise blood βHB
228 levels with the small doses used[25,26]. Therefore, the finding of increased blood βHB concentration
229 following human supplementation with 1,3-BD in the current study is novel.
230
231 Blood lactate concentrations were lower after 30 min of submaximal exercise during the CHO-BD
232 trial compared with CHO trial, while post-TT glucose concentrations in CHO-BD trials were higher

than in the CHO trial. These findings align with differences in exercising blood lactate and glucose concentrations that have been observed, to a greater magnitude, in studies comparing combined ketone ester and carbohydrate supplementation with carbohydrate only supplementation[5]. Taken together these results suggest that 1,3-BD may reduce lactate formation or increase lactate clearance during submaximal exercise, which might spare glucose reserves. That said, this difference had disappeared by 60 min, suggesting any effects of 1,3-BD supplementation in this regard may be short-lived.

GI comfort was similar between CHO-BD and CHO trials at all time points. This is contrary to previous research investigating the effects of ketone esters where sensations of bloating, nausea, belching, intestinal cramps and general gastrointestinal discomfort have been reported[5,19]. In order to elicit a greater state of ketosis future work is likely to involve increasing the amount of 1,3-BD supplementation, which may increase the susceptibility to GI discomfort.

The absence of any between-trial differences in RER contrasts with previous research where similar ketosis induced using the ketogenic diet reduced RER[27]. This discrepancy may be due to the different way endogenous and exogenous ketone bodies are formed or perhaps differences in endogenous glycogen availability. Endogenous ketone body production occurs in the liver from the adipolysis of high levels of circulating free fatty acids. In contrast, ketone bodies provided exogenously are absorbed through the gut epithelium and monocarboxylate transporters, without effecting fat oxidation[28]. Indeed, βHB is anti-lipolytic, part of a negative homeostatic feedback system to prevent ketoacidosis[29], meaning that supplementation with exogenous ketone agents can reduce fat oxidation. Although elevated ketone body concentrations may signal to lower glucose oxidation, the impact on RER may also be masked by the stoichiometry of ketone body oxidation, producing RER values more similar to carbohydrates compared to lipids (βHB = 0.89; Acetoacetate = 1.00). Therefore, without knowledge of total ketone body storage, utilisation and excretion, it is impossible to draw definite conclusions from the reported RER values in this study.

Blood βHB concentrations in this study did not reach therapeutic levels described by Hashim and VanItallie[4] of >2 mmol·L^{-1}. It is possible that higher levels of circulating ketone bodies, achieved through larger 1,3-BD dosing or combining 1,3-BD with other ketogenic agents, would have increased utilisation by metabolically demanding extra-hepatic tissues, and thus produced a performance effect[3,21], as well as greater effect on blood βHB, glucose and lactate concentrations, as seen with studies using ketone esters[5].

βHB is a chiral compound with two optical isoforms: D-3HB and L-3HB; research in rodents has suggested the effect of this molecule on metabolism to be stereo-selective, meaning that D-3HB:L-3HB ratio could play an important role in fuel selection. Relative glucose utilisation decreases as concentration of D-3HB increases while L-3HB has been shown to reverse the glycolytic inhibition caused by elevated D-3HB[30]. Within the present study, subjects consumed a formulation of 1,3-BD, which will have been broken down into either the D-3HB or L-3HB isomer (or both). Although the relative contributions of D-3HB and L-3HB were not quantified in this study, it is possible that the 1-3-BD elevated both forms in relatively equal proportions; this is in contrast to esters that are >99% D-3HB.

With the techniques used in this study, the precise assessment of ketone body, glucose, and fatty acid metabolism cannot be determined. Although it was shown that 1,3-BD increases circulating βHB concentrations, these concentrations are not a true reflection of ketone body production, but rather a snapshot of metabolic flux, the balance between the absorption and production, and utilisation and clearance of βHB.

Conclusion

Despite elevating blood βHB concentrations supplementation with CHO-BD did not improve 5 km running performance following a 60 min submaximal running phase and did not alter substrate utilisation, compared with CHO only. Further research is needed to establish the ergogenic effects of ketone esters using different ketone ester type and dosing strategies.

Practical Implications

- 1,3-BD + CHO supplementation maintained 5km TT performance compared to CHO ingestion
- 1,3-BD supplementation produced mild levels of ketosis
- Ingestion of 1,3-BD with CHO did not cause symptoms of gastrointestinal discomfort

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Tables

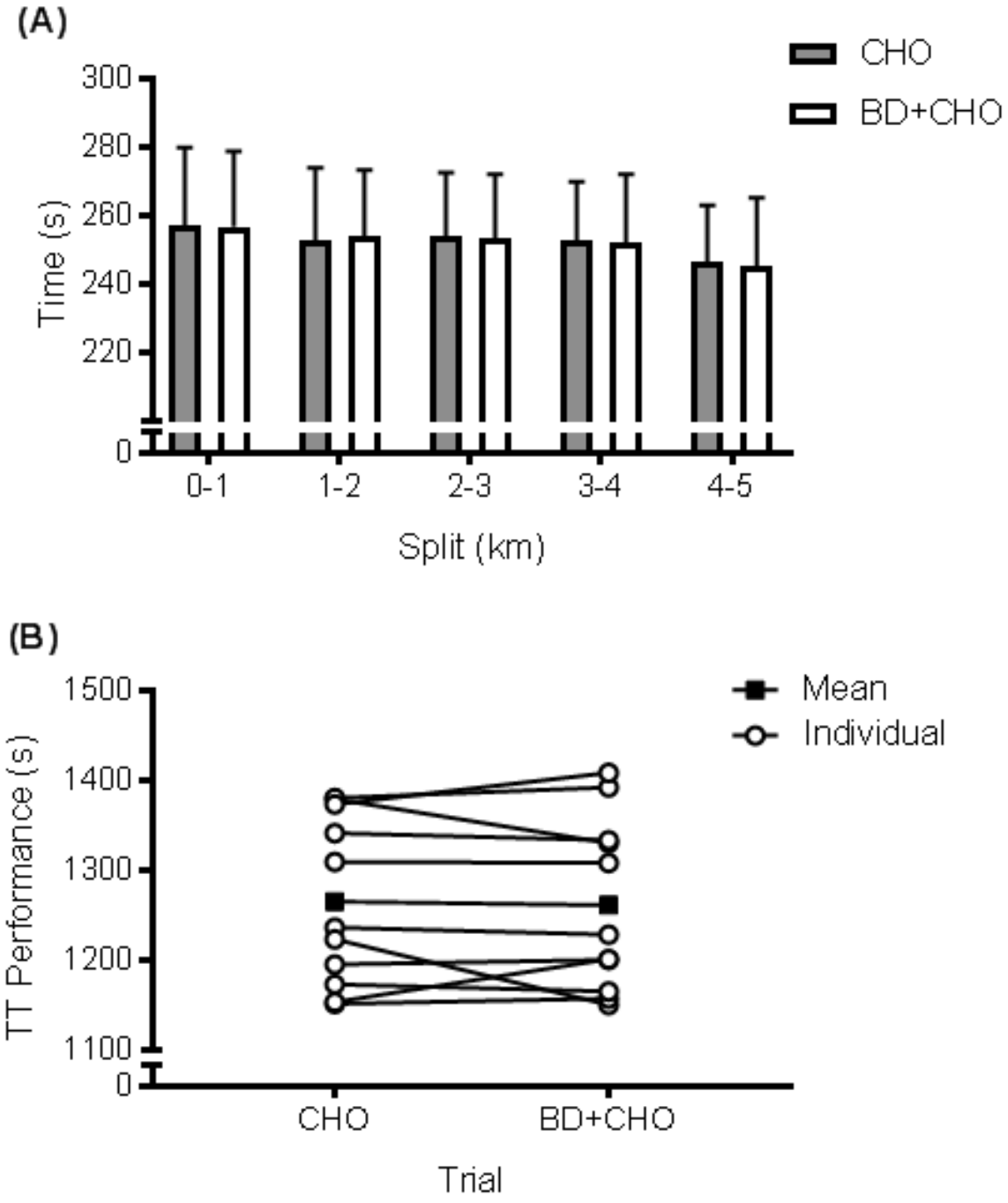
Table 1 Heart rate, respiratory and perceptual scale results. Mean ± SD

	CHO	CHO-BD
HR (b·min at TT end)	182 ± 10	183 ± 13
VO_2 during steady state ($L \cdot min^{-1}$)	46.9 ± 3.6	46.8 ± 3.1
RER	0.89 ± 0.05	0.89 ± 0.04
RPE (steady state mean)	12 ± 1	12 ± 1
RPE (TT)	18 ± 1	18 ± 1
GI comfort (mean all timepoints)	2 ± 2	2 ± 2

372 **Figure Captions**

373 **Figure 1. (A)** Pre-fatigued 5 km time-trial performance for each trial. Individual and mean results
374 presented. **(B)** 1 kilometre split times for the pre-fatigued 5km time-trial. Mean ± SD.

375 **Figure 2.** Blood beta-hydroxybutyrate **(A)**, blood glucose **(B)**, and blood lactate **(C)** concentrations
376 over the duration of each trial * denotes difference between trials, 1,2,3,4,5 denotes difference from
377 baseline and subsequent time-points a denotes difference in CHO trial only, b denotes difference in
378 BC+C trial only from baseline in CHO-BD trials only ($p<0.05$). Mean ± SD.



379

Figure 1. (A) Pre-fatigued 5 km time-trial performance for each trial. Individual and mean results presented. **(B)** 1 kilometre split times for the pre-fatigued 5km

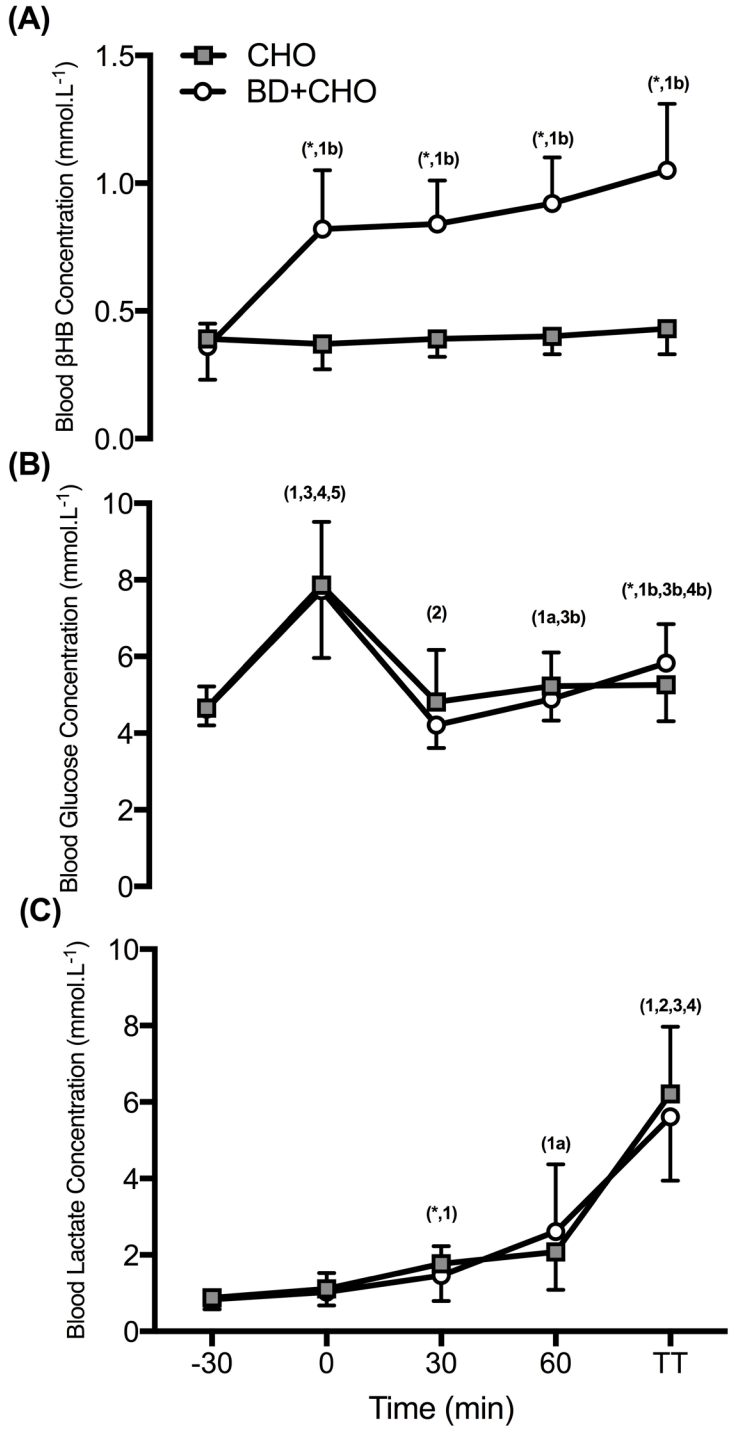


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Figure 2. Blood beta-hydroxybutyrate **(A)**, blood glucose **(B)**, and blood lactate **(C)** concentrations over the duration of each trial * denotes difference between trials, **1,2,3,4,5** denotes difference from baseline and subsequent time-points **a** denotes difference in CHO trial only, **b** denotes difference in BC+C trial only from baseline in CHO-BD trials only ($p<0.05$). Mean ± SD.

381