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Human Kinetics
ORIGINAL RESEARCH

The Effect of 1,3-Butanediol on Cycling Time-Trial Performance

David M. Shaw and Fabrice Merien
Auckland University of Technology

Andrea Braakhuis
The University of Auckland

Daniel Plews, Paul Laursen, and Deborah K. Dulson
Auckland University of Technology

This study investigated the effect of the racemic β-hydroxybutyrate (βHB) precursor, R,S-1,3-butanediol (BD), on time-trial (TT) performance and tolerability. A repeated-measures, randomized, crossover study was conducted in nine trained male cyclists (age, 26.7 ± 5.2 years; body mass, 69.6 ± 8.4 kg; height, 1.82 ± 0.09 m; body mass index, 21.2 ± 1.5 kg/m^2; VO_2peak, 63.9 ± 2.5 ml·kg^{-1}·min^{-1}; W_{max}, 389.3 ± 50.4 W). Participants ingested 0.35 g/kg of BD or placebo 30 min before and 60 min during 85 min of steady-state exercise, which preceded a ~25- to 35-min TT (i.e., 7 kJ/kg). The ingestion of BD increased blood D-βHB concentration throughout exercise (0.44–0.79 mmol/L) compared with placebo (0.11–0.16 mmol/L; all $p < .001$), which peaked 1 hr following the TT (1.38 ± 0.35 vs. 0.34 ± 0.24 mmol/L; $p < .001$). Serum glucose and blood lactate concentrations were not different between trials (all $p > .05$). BD ingestion increased oxygen consumption and carbon dioxide production after 20 min of steady-state exercise ($p = .002$ and $p = .032$, respectively); however, no further effects on cardiorespiratory parameters were observed. Within the BD trial, moderate to severe gastrointestinal symptoms were reported in five participants, and low levels of dizziness, nausea, and euphoria were reported in two participants. However, this had no effect on TT duration (placebo, 28.5 ± 3.6 min; BD, 28.7 ± 3.2 min; $p = .62$) and average power output (placebo, 290.1 ± 53.7 W; BD, 286.4 ± 45.9 W; $p = .50$). These results suggest that BD has no benefit for endurance performance.

Keywords: endurance, exogenous ketones, ketone supplements, ketosis

The interaction of energetic substrates during exercise has been investigated for over 100 years (Hawley et al., 2015). Endurance performance up to ~3–4 hr appears to be carbohydrate dependent (skeletal muscle and hepatic glycogen, blood glucose, lactate and exogenous sources; Hawley & Leckey, 2015), with the contribution of fat-derived fuel largely influenced by exercise intensity, training status, and diet (Maunder et al., 2018). These events require acute dietary interventions to sustain endogenous fuel supply, with the most common and effective approaches including carbohydrate loading and supplementation (Burke et al., 2011). However, recent scientific inquiry has focused on the metabolic and performance effects of an additional energetic substrate, ketone bodies (KB; Evans et al., 2016; Pinckaers et al., 2016).

Ketone bodies predominantly consist of the D-enantiomer of β-hydroxybutyrate (D-βHB), which is the major circulatory KB, and acetoacetate (Robinson & Williamson, 1980). KBs are synthesized primarily from the hepatic conversion of fatty acids during physiological states of low carbohydrate availability, such as starvation, ingestion of a very low carbohydrate, high-fat diet, and prolonged exercise to provide an alternative fuel source for extrahepatic tissues (e.g., skeletal muscle, brain, and heart). Postprandial blood KB concentration is ~0.1–0.2 mmol/L, whereas hyperketonemia is defined as concentrations above 0.5 mmol/L (Robinson & Williamson, 1980). The oral ingestion of ketone supplements, specifically salts and esters, have shown to induce hyperketonemia within 30 min (Stubbs et al., 2017), thus allowing the effect of KBs to be delineated from the interference to changes in substrate availability and metabolism resulting from alternative ketogenic strategies.

The ergogenic potential of KBs is linked to their direct contribution to the production of adenosine triphosphate (ATP) and ability to regulate substrate metabolism. Following entry into the tricarboxylic acid cycle, D-βHB liberates 13.0 ATP/mole per C_2 unit, compared with 12.67 and 10 ATP/mole per C_2 unit for glucose and pyruvate, respectively (Burgess et al., 2008), while increasing mitochondrial efficiency and the Gibbs free energy of ATP hydrolysis (Veech, 2004). This suggests that KBs are a more efficient substrate than carbohydrate. Although their energy yield is less than palmitate (i.e., 16.13 ATP/mole per C_2 unit), fat utilization declines from moderate- to high-intensity exercise (Achten et al., 2002), whereas absolute KB utilization increases (Cox et al., 2016). The ingestion of ketone esters has also been shown to attenuate carbohydrate utilization as demonstrated by reduced glycolytic rates (Cox et al., 2016) and reduced lactate accumulation during

Shaw, Plews, Laursen, and Dulson are with the Sports Performance Research Institute New Zealand (SPRINZ), Auckland University of Technology, Auckland, New Zealand. Merien is with AUT Roche Diagnostics Laboratory, School of Science, Auckland University of Technology, Auckland, New Zealand. Braakhuis is with the Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand. Shaw (nutrition@daveshaw.co.nz) is corresponding author.

moderate- to high-intensity exercise (Cox et al., 2016; Evans & Egan, 2018; Leckey et al., 2017). Concomitantly, there is a decline in adipose tissue lipolysis (Cox et al., 2016; Leckey et al., 2017), whereas intramuscular triacylglycerol lipolysis appears to increase (Cox et al., 2016).

The impact of acute hyperketonemia on endurance performance remains equivocal, with beneficial (Cox et al., 2016), detrimental (Leckey et al., 2017; O'Malley et al., 2017), and trivial (Evans & Egan, 2018; Rodger et al., 2017) effects reported. This is likely due to differences in methodology, particularly the use of varying forms of ketone supplements and the manipulation of carbohydrate availability before and during the performance test. In addition, the previously mentioned studies were unlikely to be of sufficient duration and intensity to deplete skeletal muscle glycogen stores, which may be required to elucidate an ergogenic effect of acute hyperketonemia. For example, the longest trial consisted of a 4-min cycling time trial (TT) preceded by 90 min of cycling at 80% of the power-eliciting secondary ventilatory threshold (VT_2; Rodger et al., 2017). Therefore, further investigations are warranted to elucidate the effect of different ketone supplements without the interference of concomitant carbohydrate supplementation on endurance performance during prolonged, high-intensity protocols.

To explore the effect of acute hyperketonemia on a ~30-min cycling TT performed following a glycogen-depletion phase, we used the racemic βHB precursor, R,S-1,3-butanediol (BD). R,S-1,3-butanediol is a widely available nontoxic dialcohol and component part of the R,S-1,3-butanediol acetoacetate diester (Leckey et al., 2017). Following ingestion, BD is passively absorbed in the gut (Shivva et al., 2016) and subsequently increases blood R- and S-BD, before rapidly undergoing hepatic conversion to the isotopic enantiomers, D-βHB and L-βHB (Desrochers et al., 1992). The ingestion of BD has shown to increase blood D-βHB concentrations to ~1.0 mmol/L within 30 min in rats (Kesl et al., 2016); however, there is currently no published literature on the dose response of BD in humans or its effect on endurance performance.

Methods

Participants

Nine trained male cyclists, consuming a mixed diet for at least 12 months and without a history of recurrent gastrointestinal symptoms volunteered to participate in the study (age, 26.7 ± 5.2 years; body mass, 69.6 ± 8.4 kg; height, 1.82 ± 0.09 m; body mass index, 21.2 ± 1.5 kg/m^2; VO_2peak, 63.9 ± 2.5 $ml \cdot kg^{-1} \cdot min^{-1}$; W_{max}, 389.3 ± 50.4 W; hours training per week, 12.3 ± 2.3 hr; weekly alcohol intake, 1.4 ± 1.2 standard units). All participants were informed of the rationale of the study, experimental procedures, and possible risks before providing their written consent. The study was approved by the health and disability ethics committee (Wellington, New Zealand; reference number 17/NTB/39/AM02) and prospectively registered with the Australian New Zealand clinical trials registry (ACTRN12617001347358).

Preliminary Testing and Familiarization

Participants presented to the laboratory on two occasions prior to the experimental trials. On the first visit, participants arrived at a time of convenience, having fasted for a minimum of 4 hr and refraining from caffeine, alcohol, and strenuous exercise for the preceding 24 hr. Participants' body mass (shorts only) and height were measured. To determine VO_2peak, VT_2, and maximal power output (W_{max}), participants performed a continuous incremental cycling protocol on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) commencing cycling at 95 W with increments of 35 W every 3 min until either (a) volitional exhaustion or (b) cadence could not be maintained above 60 rpm. Ratings of perceived exertion (RPE; Borg 6–20 scale; Borg, 1982) and heart rate (HR) using short-range telemetry (Garmin Fenix 3; Garmin Ltd., Olathe, KS) were noted during the final 30 s of each stage. The expired gas was collected and analyzed continuously using a computerized metabolic system with mixing chamber (TrueOne 2400; Parvo Medics, Salt Lake City, UT), and W_{max} was calculated according to the formula: $W_{max} = W_{final} + (t/T) \times W_{inc}$, where W_{final} is the power output (W) of the final completed stage, t is the time achieved in the final uncompleted stage (s), T is the duration of each stage (180 s), and W_{inc} is the workload increment (35 W). The power-eliciting VT_2 was determined using the V-slope method (Beaver et al., 1986) by two researchers in a pro-rata manner and VO_2peak was determined by the highest averaged 30 s. To familiarize participants with the TT, participants remounted the cycle ergometer after 15–20 min of rest, which was switched from hyperbolic to linear mode and commenced a TT equivalent to 7 kJ/kg (~25–35 min). The power output in linear mode is cadence (rpm) dependent, with power (W) calculated according to the formula: $W = L \times (\text{rpm})^2$. The linear factor (L) was calculated to elicit a power output of 70% W_{max} at an rpm of 90. For all cycling tests, bike dimensions were set to the participants' preferences and were repeated for subsequent trials. The participants returned after 3–7 days to perform a familiarization protocol, which involved completing the requirements of the experimental trial without the ingestion of BD, fluid restriction, or blood collection (described below).

Pilot Test

To investigate the dose response of BD on blood D-βHB concentration and tolerability, a pilot test was conducted prior to the experimental trials. Six healthy, recreationally active males undertook four different dosing protocols, which included the provision of BD within two 200-ml boluses of an artificially sweetened, flavored drink separated by 1.5 hr. These doses were a placebo of 0 + 0 g/kg (PLA), medium dose of 0.5 + 0 g/kg (M-BD), high dose of 0.7 + 0 g/kg (H-BD), and a split dose of 0.35 + 0.35 g/kg (Spl-BD) of BD. All tests commenced between 06:00 and 08:00 hr with participants in a fasted state, and D-βHB was measured across 3.5 hr following the ingestion of the initial bolus to align with time points of the experimental trial (Figure 1). We concluded that BD split into two 0.35 g/kg boluses elicited maximal D-βHB concentration and minimal side effects compared with BD given as a single, larger bolus of 0.5 or 0.7 g/kg, which tended to result in nausea, euphoria, and dizziness.

Experimental Trial

Using a repeated-measures, randomized, crossover design, participants were randomly (www.randomizer.org) assigned to either PLA or BD (proportional distribution of optical isomers unknown; product code 02-59620; Penta Manufacturing Ltd., Livingston, MT). Participants were not informed of their trial allocation. However, due to the difficulty masking the bitter taste of BD, achieving successful blinding was deemed unlikely. For the day prior to each experimental trial, participants were prescribed a diet

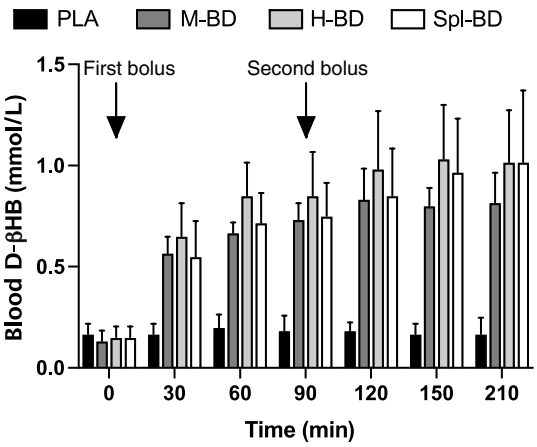

Figure 1 — Pilot test capillary blood D-βHB concentrations for each BD dosing protocol. Values are presented as mean ± *SD*. PLA = 0 + 0 g/kg, M-BD = 0.5 + 0 g/kg, H-BD = 0.7 + 0 g/kg, and Spl-BD = 0.35 g/kg + 0.35 g/kg of BD. D-βHB = D-β-hydroxybutyrate; BD = R,S-1,3-butanediol; PLA = placebo; Spl-BD = split dose; M-BD = medium dose; H-BD, high dose.

consisting of 6 g/kg of carbohydrate based on their dietary preferences by an experienced registered dietitian, which was to be consumed by 23:00 hr, and were asked to avoid caffeine and alcohol for the day prior to each experimental trial. Compliance was confirmed using an image-assisted weighed dietary record reported remotely in real time through a mobile phone application (WhatsApp, Mountain View, CA; Facebook, Menlo Park, CA). In addition, participants were asked to refrain from strenuous exercise for the preceding 48 hr and to consume 500 ml of water prior to arrival. The purpose of standardizing carbohydrate intake and exercise before each trial was to normalize carbohydrate availability to promote similar rates of muscle glycogen utilization prior to the TT. This was estimated at ~50% based on our participant characteristics and exercise protocol (Areta & Hopkins, 2018). Participants arrived at the laboratory between 07:00 and 08:00 hr having fasted from 23:00 hr the previous day. An indwelling intravenous teflon catheter (18G; Terumo Corp., Tokyo, Japan) was inserted into the antecubital vein for serial blood sampling. This was followed by the ingestion of a bolus of 0 or 0.35 g/kg BD within a 2 ml/kg artificially sweetened, orange-flavored drink (Thriftee; Hansells Food Group Ltd., Auckland, New Zealand), that is, presupplement. After 30 min (i.e., pre-exercise), participants commenced steady-state (SS) cycling at the power output eliciting 85% of their VT_2 (240.8 ± 28.3 W; 62.0% ± 2.4% W_{max}; 73.0% ± 5.2% VO_2peak) for 85 min. Every 20 min, HR and expired gas were collected, with participants providing their RPE. After 60 min of cycling (i.e., 60-min exercise), participants ingested a second bolus of PLA or 0.35 g/kg BD according to their trial allocation. Following completion (i.e., post-SS), participants rested for 5 min, then were instructed to complete the TT (as previously mentioned) as fast as possible while remaining in a seated position. Participants were blinded to their power output and elapsed time; however, they were notified at each quarter of completion and were counted down from 100 kJ in 10 kJ decrements. No respiratory or blood samples were collected during the TT; however, HR was collected at each quarter of completion. All trials were conducted by the same researcher and standardized encouragement was provided. Fluid intake was restricted to 2 ml/kg of water every 15 min during the SS and TT phases. Following the TT (i.e., post-TT), participants removed wet clothing and towel dried themselves prior to having their body mass measured. Participants then completed a customized questionnaire adapted from published sources (Clarke et al., 2012; Pfeiffer et al., 2009), including 27 items pertaining to systemic (13), upper abdominal (six), and lower abdominal (eight) symptoms, and prior tested by two experienced registered dietitians for understanding and literacy. Participants were prompted for additional symptoms not stated on the questionnaire and were asked to identify their trial allocation. They were then provided with 5 ml/kg of water and rested for 60 min prior to departing. All exercise trials throughout the study were performed in standard laboratory conditions of 18.3 ± 0.6°C and 67.6% ± 7.4% relative humidity and were separated by 7–10 days.

Blood Sampling and Analysis

Capillary blood D-βHB concentration was measured (FreeStyle Optium Neo; Abbott Diabetes Care, Victoria, Australia), and venous blood samples were collected at presupplement, pre-exercise, 30-min exercise, 60-min exercise, post-SS, post-TT, and 1-hr post-TT into 8-ml serum vacutainers (Becton Dickinson and Co., Franklin Lakes, NJ) with the participants seated in an upright position. Serum vacutainers were left to clot for 30 min prior to centrifugation at 1,500*g* for 10 min at 4°C and separation into two 1.5-ml aliquots to be stored at −80°C prior to the analysis of glucose concentration (Cobas Modular P800 Analyser; Roche Diagnostics, Auckland, New Zealand). Capillary blood lactate concentration was measured (Lactate 2 Pro; Arkray, Kyoto, Japan) at pre-exercise, post-SS, and post-TT. All capillary blood samples were collected from participants' finger tips using standardized techniques.

Data Analysis

All data are expressed as mean ± *SD* unless otherwise stated. Data were checked for normality as indicated by the Shapiro–Wilk score, and where appropriate, statistical analysis was performed on the logarithmic transformation of the data. Paired *t* tests were used to compare TT duration, average TT power output and HR, and change in body mass between trials. A two-way (Trial × Time) repeated-measures analysis of variance was performed for glucose, D-βHB, lactate, cardiorespiratory, and RPE data (IBM SPSS Statistics software, version 21; IBM Corp., Chicago, IL). If Mauchly's test of sphericity was violated, adjustments to the degrees of freedom were made for the analysis of variance using Greenhouse–Geisser ϵ. Where a significant effect was observed, post hoc analysis was conducted using Student's paired *t* tests with Holm–Bonferroni adjustments for multiple comparisons applied to the unadjusted *p* value to locate specific differences. Significance level was accepted at an alpha of $p < .05$. To interpret the magnitude of effect and identify trends within nonsignificant data, Cohen's *d* effect sizes (±90% confidence limits) were estimated using a purpose-built spreadsheet (Hopkins, 2006), with effect size thresholds set at <0.2, >0.2, >0.6, >1.2, >2.0, and >4.0 for *trivial*, *small*, *moderate*, *large*, *very large*, and *extremely large* effects, respectively (Hopkins et al., 2009). However, as Cohen's *d* may overestimate the effect of BD on D-βHB concentration compared with ketone supplements with higher bioavailability, we used a novel approach to determine the magnitude of effect whereby the possible range of change was transformed into a full scale of deflection (FSD; Hopkins, 2010). The deflection (±90% confidence limits) was estimated by the difference in D-βHB concentration between the PLA and BD trials for each time point and the range was calculated by subtracting D-βHB concentration of the PLA trial for each time

point from 3.5 mmol/L, which is approximately the highest D-βHB concentration reported during exercise of a similar intensity following the ingestion of a ketone supplement (Cox et al., 2016). Each range was set at 0–100%, and the magnitude thresholds were defined as >10%, >30%, >50%, >70%, and >90% for *small, moderate, large, very large*, and *extremely large* effects, respectively. If the 90% confidence limits overlapped 0 for either effect size statistics, the magnitude of effect was deemed *unclear*.

Results

D-βHB, Glucose, and Lactate Concentration

Presupplement blood D-βHB concentration (PLA, 0.12 ± 0.04; BD, 0.14 ± 0.05 mmol/L) and serum glucose concentration (PLA, 4.33 ± 0.30; BD, 4.49 ± 0.26 mmol/L) did not differ between trials (all $p > .05$). A significant Trial × Time interaction was observed for blood D-βHB concentration ($p < .001$), with increased concentrations from pre-exercise to 1-hr post-TT in the BD compared with PLA trial (all $p < .001$; Figure 2a). However, the *small* increase in blood D-βHB concentration at pre-exercise (FSD = 12.57 [10.48, 14.64]) declined to a *trivial* effect at 30-min exercise (FSD = 9.84 [8.68, 11.01]) and 60-min exercise (FSD = 9.52 [7.93, 11.10]). The second bolus of BD at 60-min exercise led to a *small* increase in blood D-βHB concentration at post-SS (FSD = 15.38 [11.73, 19.03]) and post-TT (FSD = 19.41 [16.06, 22.76]), which elevated to a *moderate* increase at 1-hr post-TT (FSD = 32.45 [26.04, 38.85]) compared with PLA, coinciding with peak concentrations of 1.38 ± 0.35 mmol/L. Despite a significant Trial × Time interaction being observed for serum glucose concentration ($p = .027$), post hoc analysis could not locate specific differences. However, there was a trend for a *small* reduction in serum glucose concentration in the BD compared with PLA trial at post-TT ($p = .60$, $d = -0.48$ [−0.93, −0.02]). A significant effect for time was also observed for serum glucose concentration ($p < .001$), with higher concentrations from 30-min exercise to post-TT compared with pre-exercise and at post-TT compared with post-SS (all $p < .001$; Figure 2b). Pre-exercise blood lactate concentration did not differ between trials (PLA, 2.33 ± 0.62; BD, 2.02 ± 0.52 mmol/L, $p = .37$), and BD had no effect on blood lactate concentration

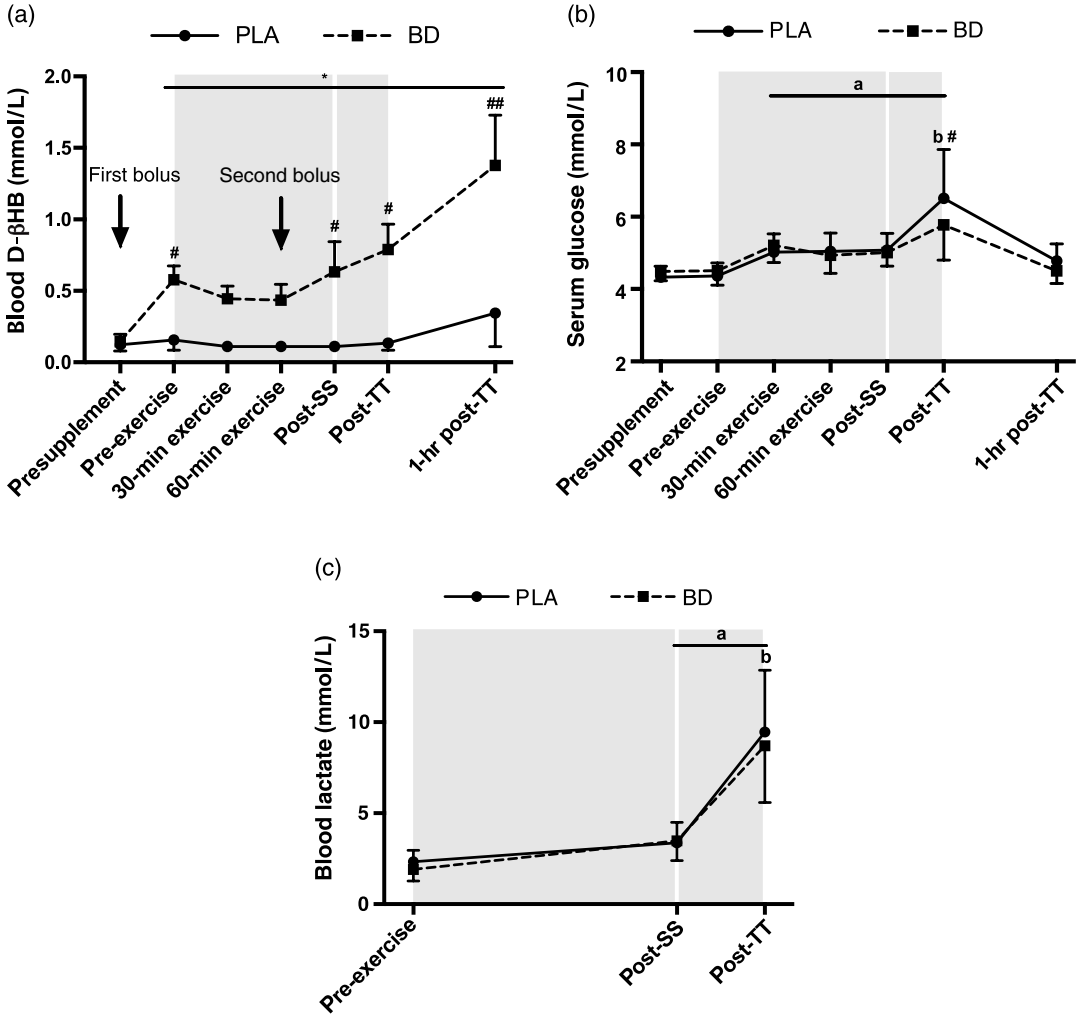

Figure 2 — (a) Capillary blood D-βHB, (b) serum glucose, and (c) capillary blood lactate concentrations. Values are presented as mean ± *SD*. The gray area denotes the SS and TT phases. Effect size (*d* or FSD); [#]*small* and [##]*moderate* effects for BD compared with PLA at time point. [a]Main effect for time; significantly higher to pre-exercise ($p < .001$). [b]Main effect for time; significantly higher to post-SS ($p < .001$). D-βHB = D-β-hydroxybutyrate; BD = R,S-1, 3-butanediol; ex = exercise; TT = time trial; PLA = placebo; SS = steady state; FSD = full scale of deflection. *Significantly higher in BD compared with PLA at time point ($p < .001$).

during exercise. However, there was a significant effect for time ($p < .001$), with blood lactate concentration elevated at post-SS compared with pre-exercise and at post-TT compared with post-SS (Figure 2c).

SS Exercise Cardiorespiratory Variables and Perceived Exertion

There was a significant Trial × Time interaction for VO_2 and VCO_2 ($p = .021$ and $p = .032$, respectively). Post hoc analysis revealed *small* increases in VO_2 and VCO_2 in the BD trial compared with PLA at 20-min exercise ($p = .002$, $d = 0.32$ [0.22, 0.42] and $p = .032$, $d = 0.29$ [0.10, 0.49], respectively); however, no other differences were located (Table 1). A Trial × Time interaction was also observed for relative intensity ($p = .03$), with a *small* increase occurring at 20 min in the BD compared with PLA trial ($p = .001$, $d = 0.45$ [0.32, 0.59]). There were no significant effects of BD on respiratory exchange ratio (RER), HR, or RPE (all $p > .05$). However, there was a trend for *moderately* higher RPE in the BD trial compared with PLA at 80-min exercise ($d = 0.65$ [0.09, 1.21]; Table 1). Significant effects for time were observed for HR ($p < .001$), which increased from 20-min exercise to 40-min exercise, then from 60-min exercise to 80-min exercise (all $p < .05$), and RPE ($p = .004$), which increased between subsequent time points (all $p < .05$; Table 1).

Time-Trial Performance

BD had no effect on TT duration (PLA, 28.49 ± 3.59; BD, 28.72 ± 3.23 min; $p = .62$, $d = 0.06$ [–0.15, 0.26]; Figure 3a), average power output (PLA, 290.10 ± 53.70; BD, 286.42 ± 45.88 W; $p = .50$, $d = –0.06$ [–0.22, 0.10]; Figure 3b), or average HR (PLA, 174.9 ±10.6; BD, 173.3 ± 7.4 beats/min; $p = .38$, $d = –0.13$ [–0.39, 0.13]). Nor was there a carryover effect between trials ($p > .05$). Five participants believed that they performed better during the BD trial compared with PLA; however, only three were correct.

Tolerability

There was no difference in the change of body mass (corrected for fluid intake) between trials (PLA, –2.14 ± 0.48 kg; BD, –2.07 ± 0.42 kg; $p = .15$, $d = 0.13$ [–0.02, 0.28]). Within the BD trial, two participants experienced transient symptoms of low levels of nausea, euphoria, and dizziness, which they related to a state of alcohol intoxication. Five participants reported low to moderate

Table 1 Cardiorespiratory Variables and Perceived Exertion Values During Steady-State Cycling for PLA and BD Trials

	Pre-exercise	20-min exercise	40-min exercise	60-min exercise	80-min exercise
VO_2 (L/min)					
PLA		3.15 ± 0.30	3.22 ± 0.32	3.24 ± 0.26	3.28 ± 0.32
BD		3.26 ± 0.32**,#	3.25 ± 0.25	3.21 ± 0.21	3.23 ± 0.30
ES (±90% CL)		0.32 (0.22, 0.42)	0.07 (–0.14, 0.27)	–0.11 (–0.32, 0.09)	–0.14 (–0.37, 0.10)
VCO_2 (L/min)					
PLA		2.91 ± 0.26	2.95 ± 0.27	2.93 ± 0.26	2.95 ± 0.27
BD		2.99 ± 0.27*,#	2.97 ± 0.23	2.90 ± 0.25	2.91 ± 0.24
ES (±90% CL)		0.29 (0.14, 0.45)	0.07 (–0.16, 0.29)	–0.09 (–0.26, 0.09)	–0.12 (–0.44, 0.19)
RER					
PLA		0.92 ± 0.03	0.92 ± 0.03	0.90 ± 0.03	0.90 ± 0.03
BD		0.92 ± 0.04	0.91 ± 0.03	0.91 ± 0.04	0.90 ± 0.02
ES (±90% CL)		–0.16 (–0.46, 0.15)	–0.07 (–0.51, 0.37)	0.02 (–0.31, 0.36)	0.05 (–0.60, 0.69)
%VO_2peak					
PLA		71.21 ± 4.69	72.75 ± 5.47	73.29 ± 6.16	73.99 ± 5.64
BD		73.57 ± 4.42*,#	73.39 ± 5.11	72.59 ± 5.95	72. 95 ± 5.66
ES (±90% CL)		0.45 (0.32, 0.59)	0.11 (–0.18, 0.39)	–0.10 (–0.30, 0.09)	–0.17 (–0.48, 0.15)
HR (beats/min)[a,b]					
PLA	59.0 ± 10.5	154.4 ± 15.5	158.1 ± 14.3	158.1 ± 14.3	159.7 ± 14.1
BD	59.3 ± 5.6	157.2 ± 13.5	159.3 ± 14.1	158.3 ± 13.2	159.8 ± 13.1
ES (±90% CL)	0.03 (–0.65, 0.71)	0.16 (0.01, 0.32)	0.08 (–0.11, 0.26)	0.01 (–0.14, 0.17)	0.01 (–0.20, 0.21)
RPE[a,c,d]					
PLA		12.9 ± 0.9	13.6 ± 1.1	13.9 ± 0.9	14.3 ± 0.9
BD		12.9 ± 1.6	13.7 ± 1.6	14.0 ± 1.6	15.0 ± 1.4##
ES (±90% CL)		–0.06 (–0.80, 0.67)	0.05 (–0.65, 0.76)	0.05 (–0.82, 0.92)	0.65 (0.09, 1.21)

Note. Values are presented as mean ± *SD*. ES = Cohen's *d* effect size as interpreted according to <0.2, >0.2, >0.6, >1.2, >2.0, and >4.0 for *trivial*, *small*, *moderate*, *large*, *very large*, and *extremely large*, respectively; VO_2 = volume of oxygen consumes; BD = R,S-1,3-butanediol; RPE = ratings of perceived exertion; HR = heart rate; PLA = placebo; CL = confidence limits; RER = respiratory exchange ratio; VCO_2 = volume of carbon dioxide produced; VO_2peak = peak oxygen uptake.

*Significantly higher in BD compared with PLA ($p < .05$). **Significantly higher in BD compared with PLA ($p < .01$). ES (*d*); #*small* and ##*moderate* effect for BD compared with PLA at time point.

Main effect for time: [a]significantly higher at 40-min exercise compared with 20-min exercise ($p < .001$); [b]significantly higher at 80-min exercise compared with 60-min exercise ($p = .02$); [c]significantly higher at 60-min exercise compared with 40-min exercise ($p = .01$); and [d]significantly higher at 80-min exercise compared with 60-min exercise ($p < .001$).

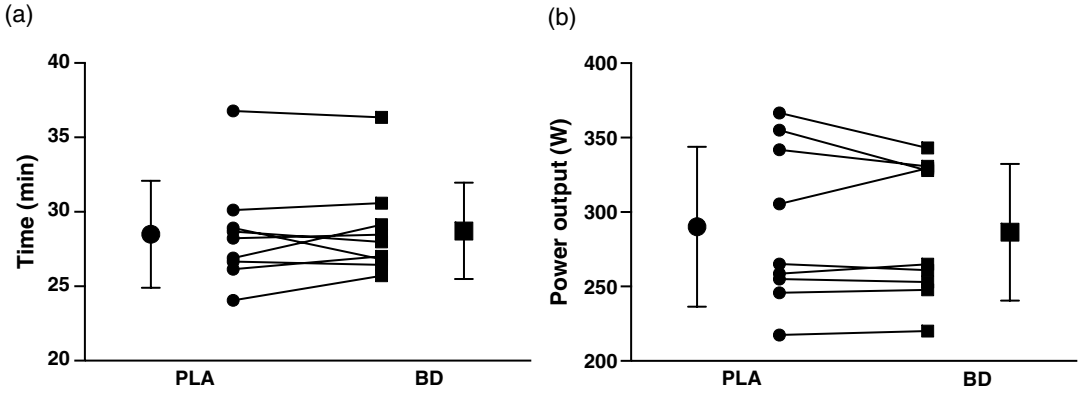

Figure 3 — Time-trial performance presented as (a) mean ± *SD* and individual time and (b) mean ± *SD* and individual power output. BD = R,S-1,3-butanediol; PLA = placebo.

levels of belching and burping, and one participant reported severe abdominal pain. No participants reported similar symptoms during the PLA trial. Everyone disliked the taste of BD, which resulted in retching in four participants. Everyone correctly identified their trial allocation, which was likely due to the difficulty masking the taste of BD and subsequent gastrointestinal effects.

Discussion

To our knowledge, this is the first study investigating the effects of BD ingestion on performance. Despite BD increasing blood D-βHB concentration, no other significant differences in metabolic, cardiorespiratory, or performance variables were observed. These findings support previous work suggesting ketone supplements eliciting blood D-βHB concentrations up to ~1 mmol/L do not benefit endurance performance. Furthermore, we found BD to elicit gastrointestinal distress, in particular belching and burping, as well as symptoms of nausea, euphoria, and dizziness in some participants.

In this study, BD paralleled the bioavailability of other racemic ketone supplements. The use of 2 × 0.35 g/kg of BD increased blood D-βHB concentration to levels during exercise (0.44–0.79 mmol/L) similar to the ingestion of 2 × ~0.4 g/kg of a racemic βHB salt solution (total of 24–37 g D,L-βHB; Evans et al., 2018; Rodger et al., 2017) and 2 × 0.25 g/kg of a R,S-1,3-butanediol acetoacetate diester (Leckey et al., 2017). It is likely these racemic ketone supplements also increased blood L-βHB; however, as L-βHB largely resides intracellularly in very low concentrations and does not directly contribute to energy production, it is not considered an important substrate in this context (Desrochers et al., 1992; Stubbs et al., 2017). Notably, our peak D-βHB concentrations occurred at 1-hr post-TT, which was substantially higher than values observed in our pilot test; however, post-exercise ketogenesis was a likely contributor (Koeslag et al., 1980). Nevertheless, our blood D-βHB concentrations were markedly lower compared with the ingestion of the nonracemic D-βHB R-1,3-butanediol monoester, which can increase D-βHB to ~2.5–3.5 mmol/L during exercise (Cox et al., 2016). This highlights the low bioavailability and differences in componentry of BD and other racemic ketone supplements compared with the D-βHB R-1,3-butanediol monoester.

Following the onset of exercise, blood D-βHB concentration declined and plateaued until after the second bolus of BD ingestion at 60-min exercise. Compared with our pilot data in a separate resting population, blood D-βHB concentration was ~0.2–0.3 mmol/L lower from 30-min exercise to post-TT. Collectively, this suggests D-βHB was continuously released into the circulation, while being taken up by muscle cells through monocarboxylate transporters (Halestrap & Wilson, 2012). Although there was not a shift in the RER toward βHB and acetoacetate's respiratory quotient of 0.89 and 1.0, respectively, this effect appears to be abrogated at exercise intensities above 60% VO_2max (Evans et al., 2018). An earlier study estimated D-βHB oxidation rates by comparing area under the curve for blood D-βHB concentrations between resting and exercising conditions following the ingestion of D-βHB R-1,3-butanediol monoester (Cox et al., 2016); however, as we did not measure resting D-βHB concentrations for our cyclists, we could not compare D-βHB oxidation rates. Importantly, the validity of these calculations have not been confirmed using direct calorimetry and may be compromised due to the inclusion of unclear D-βHB volume distribution values, which could also change following exercise or ketone supplement ingestion (Frayn, 1983).

Exercise efficiency did not change following BD ingestion. There was no sustained difference in oxygen utilization during the SS exercise, reciprocating studies using racemic βHB salts (Evans et al., 2018; O'Malley et al., 2017; Rodger et al., 2017) and ketone esters (Cox et al., 2016; Leckey et al., 2017), suggesting the enhanced metabolic efficiency of KBs often referred to in an isolated perfused heart (Sato et al., 1995) may not translate to whole-body exercise. Nor was there an effect of BD on blood lactate accumulation, corroborating studies using racemic βHB salts (Evans et al., 2018; O'Malley et al., 2017; Rodger et al., 2017), but not ketone esters (Cox et al., 2016; Leckey et al., 2017). We could not locate significant differences in serum glucose concentration between trials, which is in contrast to other studies (Cox et al., 2016; Evans et al., 2018; Leckey et al., 2017; O'Malley et al., 2017; Rodger et al., 2017). However, there was a trend toward a *small* reduction in serum glucose concentration immediately following the TT in the BD trial, which may be due to an attenuation of hepatic glycogenolysis. Nonetheless, this did not appear to affect performance.

Clearly, the physiological and nutritional conditions suitable for ketone supplements to augment performance remain difficult to identify. Considering the potential for hyperketonemia to downregulate glycolysis and suppress adipocyte lipolysis, the optimal range of KB concentration to enhance substrate provision and energy production remains obscure. This is exacerbated by differences in the bioavailability of various ketone supplements and measurement discrepancy of point-of-care versus laboratory-based

methods (Guimont et al., 2015; Leckey et al., 2017), thus making comparisons between studies difficult. For example, following the ingestion of the D-βHB R-1,3-butanediol monoester within a carbohydrate drink, plasma D-βHB was maintained above ~2.0–3.0 mmol/L and improved performance during a preloaded 30-min cycling TT by ~2% compared with the ingestion of an isoenergetic carbohydrate-only drink (Cox et al., 2016). However, these performance benefits have not been replicated and may be abrogated in exercise trials 90 min or less proceeding the ingestion of recommended carbohydrate intakes (Cox et al., 2016; Evans & Egan, 2018; Leckey et al., 2017). It is possible that the interaction between KB concentration and carbohydrate availability has a mediating role on KB metabolism (Chari & Wertheimer, 1954), with high rates of KB oxidation requiring high-carbohydrate availability to maintain the anaplerotic flux in the tricarboxylic acid cycle (Russell & Taegtmeyer, 1991a, 1991b). However, how this translates to real-world performance is yet to be elucidated.

The ingestion of BD also led to symptoms synonymous with low levels of alcohol intoxication within two participants. Typically, BD is rapidly converted to βHB by hepatic alcohol and aldehyde dehydrogenases (Desrochers et al., 1992). However, these steps are rate limiting and may be influenced by previous ethanol exposure (Münst et al., 1981). In this study, participants habitually consumed low levels of alcohol (~1.5 standard drinks per week); therefore, their maximal capacity to metabolize BD into βHB could have been below the dose ingested, resulting in the accumulation of BD in the circulation. Furthermore, five participants reported moderate to severe gastrointestinal symptoms and despite it not effecting TT performance, it is a deterrent to the use of some ketone supplements (Evans et al., 2018; Leckey et al., 2017). Arguably, these effects may have been exacerbated by ingesting BD in a fasted state, which is not reflective of real-world conditions. We also did not observe significant elevations in RPE as in the case of R,S-1,3-butanediol acetoacetate diester, which were likely due to the resultant gastrointestinal distress (Leckey et al., 2017). However, there was a trend toward a *moderate* increase in RPE nearing the end of SS cycling in the BD trial, which may have been due to approaching an upper limit of BD ingestion. Collectively, this would limit the application of some ketone supplements to endurance events where exogenous substrate provision and management of gastrointestinal distress are critical factors for performance.

Conclusions

Similar to other ketone supplements eliciting blood D-βHB concentrations up to ~1 mmol/L, BD does not benefit endurance performance. However, it is uncertain whether this absence of effect persists in events for >2–3 hr. BD may also induce symptoms related to a low level of alcohol intoxication, including nausea, euphoria, and dizziness, as well as moderate to severe gastrointestinal symptoms, suggesting that ingestion should be avoided in higher doses. Further attempts to identify the ergogenic properties of ketone supplements need to focus on products with higher bioavailability and their interaction with varying levels of carbohydrate availability.

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