KETONE MONOESTER INGESTION ALTERS METABOLISM AND SIMULATED RUGBY PERFORMANCE IN PROFESSIONAL PLAYERS

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ABSTRACT

Ketone ingestion can alter metabolism but effects on exercise performance are unclear, particularly with regard to the impact on intermittent-intensity exercise and team-sport performance. Nine professional male rugby union players each completed two trials in a double-blind, randomized, crossover design. Participants ingested either 90±9 g carbohydrate (CHO; 9% solution) or an energy matched solution containing 20±2 g carbohydrate (3% solution) and 590 mg/kg body mass β-hydroxybutyrate monoester (CHO+BHB-ME) before and during a simulated rugby union-specific match-play protocol, including repeated high-intensity, sprint and power-based performance tests. Mean time to complete the sustained high-intensity performance tests was reduced by 0.33±0.41 s (2.1%) with CHO+BHB-ME (15.53±0.52 s) compared to CHO (15.86 ± 0.80 s) placebo (p=0.04). Mean time to complete the sprint and power-based performance tests were not different between trials. CHO+BHB-ME resulted in blood BHB concentrations that remained >2 mmol·L^{-1} during exercise (P<0.001). Serum lactate and glycerol concentrations were lower after CHO+BHB-ME than CHO (P<0.05). Co-ingestion of a β-hydroxybutyrate monoester with carbohydrate can alter fuel metabolism (attenuate circulating lactate and glycerol concentrations) and may improve high-intensity running performance during a simulated rugby match-play protocol, without improving shorter-duration sprint and power-based efforts.

Keywords: β-hydroxybutyrate, lactate, glycerol, athletes, team sport, exercise performance

INTRODUCTION

Ketone bodies comprise acetyl-CoA-derived compounds produced by the liver during low carbohydrate availability (Cahill 2006). The principal ketone body is β-hydroxybutyrate (BHB), which displays the highest systemic concentrations and utilization by the brain and skeletal muscle (Cahill 2006; Mikkelsen et al. 2015). High systemic BHB availability suppresses hepatic glucose production and whole-body glycerol release (Mikkelsen et al. 2015) suggesting sparing of liver glycogen and a suppression of lipolysis. The suppression of lipolysis may act *via* both direct and indirect (e.g. insulin) mechanisms (Mikkelsen et al. 2015). The metabolic properties of BHB has led to growing interest in methods of raising systemic BHB availability with the putative potential to influence human health and performance.

One of the most effective, practical methods to increase systematic BHB availability without compromising endogenous carbohydrate stores is the oral ingestion of the BHB monoester (BHB-ME), (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate (Cox et al. 2016). When compared to carbohydrate ingestion alone, the co-ingestion of carbohydrate and BHB-ME before and during exercise can potently alter whole-body and skeletal muscle metabolism. BHB-ME-carbohydrate co-ingestion has been shown to increase plasma BHB availability and net intramuscular triglyceride utilization, whilst suppressing plasma non-esterified fatty acid availability, glycolysis and net intramuscular glycogen utilization during moderate-intensity exercise (Cox et al. 2016). Although it should be noted that glycogen sparing has not been reported in all studies (Poffe et al. 2020). Furthermore, an increase in resting skeletal muscle carnitine content, and a reduction in blood pH during exercise have also been observed with acute BHB-ME ingestion (Cox et al. 2016; Dearlove et al. 2019; Poffe et al. 2021a). It is unclear what implications these metabolic responses have for exercise performance, as some of these changes may be beneficial (e.g. increased free carnitine content with potential to buffer acetyl-CoA during high-intensity exercise (Wall et al. 2011)), but others detrimental (e.g. reduced

70 blood pH and glycogenolysis potentially impairing high-intensity performance (Poffe et al.
71 2020, 2021a; Poffe et al. 2021b; Stellingwerff et al. 2006)). BHB-ME ingestion, therefore,
72 produces a relatively unique metabolic milieu, with the potential effects on exercise
73 performance currently unclear.

74 The evidence to date of ketone body ester ingestion on exercise performance
75 demonstrates positive (Cox et al. 2016), neutral (Evans et al. 2019; Poffe et al. 2020, 2021a),
76 and negative (Leckey et al. 2017; Poffe et al. 2021b) effects during continuous, endurance-type
77 exercise. Only one study to date has examined the effect of BHB-ME ingestion on intermittent
78 running capacity, using a test originally designed to test soccer performance (Evans and Egan
79 2018). The addition of ketone ester to carbohydrate ingestion did not influence time-to-
80 exhaustion or 15-m sprint times. However, any potential performance advantage due to the
81 metabolic effects of ketone ester co-ingestion could have been counteracted by a higher
82 prevalence of gastrointestinal issues with the addition of ketone compared with carbohydrate
83 alone (Evans and Egan 2018). Rugby Union is a team sport characterized by periods of low-
84 speed running interspersed with bouts of high-intensity activity, some of which can be
85 prolonged in duration and comprise physical contact and sprinting elements (Austin et al. 2011;
86 Read et al. 2018). The distinct physical demands and characteristics of rugby limit the ability
87 to translate evidence from previous work involving endurance-type exercise, with some short
88 sprints, to nutritional practices in rugby. It remains unknown whether isocaloric ingestion of
89 ketone ester and carbohydrate influences intermittent exercise performance relevant to team
90 sports, and no study has examined the effects of BHB-ME ingestion in professional rugby
91 players during simulated match play.

92 Accordingly, the aim of this study was to assess the effect of ketone body ester plus
93 carbohydrate co-ingestion on simulated rugby union match play performance, compared to
94 isocaloric carbohydrate ingestion alone. It was hypothesised that ketone ester-carbohydrate co-

ingestion would augment simulated rugby union performance, when compared to isocaloric carbohydrate ingestion.

METHODS

Participants

Seventeen professional male players (age, 20±1 years; body mass, 97.8±5.3 kg; playing experience 11±4 years) recruited from an English Premiership rugby union club consented to participate. Of the players originally recruited, eight dropped out of the study due to illness or injury and a total of nine participants (5 forwards and 4 backs) completed the study. Experimental procedures were approved by the University of Bath Research Ethics Approval Committee for Health and conducted according to the Declaration of Helsinki.

Experimental Design

The study adopted a double-blind, placebo-controlled, randomised crossover design comprising a preliminary session and two experimental trials, each separated by at least 6 days. Supplements were prepared by an individual unconnected to the study and provided in unmarked containers to those who interacted with participants. Sessions took place during the players' off-season and on an indoor athletics training facility. One week prior to main trials, participants completed a preliminary session to familiarise with the protocol (described below). Prior to trials, habitual training was standardised for one week. Over the 48-h preceding the first trial, each participant recorded their diet (estimated record) and replicated this diet before the second trial. Participants refrained from high intensity exercise, caffeine, and alcohol for 24-h before sessions. Main trials required participants to complete the match-play protocol ingesting either carbohydrate (CHO) or an energy matched drink containing carbohydrate and β-hydroxybutyrate monoester (CHO+BHB-ME) before and at the mid-point of exercise.

Performance testing was integrated as part of the validated match-play protocol and included repeat assessment of high-intensity running, sprint and power-based performance.

Simulated Rugby Union Match-play Protocol

The Bath University Rugby Shuttle Test (BURST) is a rugby union-specific match-play protocol based on physical demands data for elite rugby union (Roberts et al. 2010; Roberts et al. 2008). The exercise requirements have been described in detail elsewhere (Roberts et al. 2010). In brief, the protocol involves 16 exercise periods (~5 min each) arranged into 4 x 21-min blocks (**Figure 1**). Blocks 1 and 3 are followed by 4-min active recovery. A 10-min "half-time" break follows block 2, comprising 7 min rest and 3 min active recovery. Within each exercise period, participants perform shuttles of walking, cruising and jogging interspersed with two bouts of simulated scrummaging and rucking, and one bout of mauling. Timing is maintained by verbal instruction from an audio file. A Performance Test (described below) and 15-m sprint are also completed within each exercise block and on 16 occasions in total. Power-based performance was assessed during bouts of scrummaging using an explosive sled-push (described below) and on 32 occasions. Performance test data are expressed as the mean performance across the BURST protocol. Distance travelled during the BURST (7078 m) has been validated against match-play demands derived from time-motion analysis (6418 m), and time spent performing contact events (9.9%) is consistent with actual match play (Roberts et al. 2010).

Performance Testing

The high-intensity performance test was designed to replicate a sustained high-intensity exercise bout specific to rugby union competition, and combined aspects of resistance work, sprinting, and agility. Testing required participants to pass through an initial infrared timing gate (Smartspeed, Fusion Sport, Australia) and carry one 20-kg tackle bag over 9 m, followed by a second bag over the same distance, pick up a ball and sprint 14 m before completing an

144 unanticipated rapid change in direction (prompted by a flashing beacon), and then sprint
145 through a final timing gate. Time taken between the timing gates was recorded as the
146 performance test time. Participants had 25 s to return to the start and then perform a single 15-
147 m sprint between two timing gates. There were no significant differences in the time taken to
148 complete the high-intensity performance test and 15-m sprint between two preliminary tests,
149 with a mean coefficient of variation (CV) of 1.4% and 1.8%, respectively.

150 The power-based sled push was performed on a custom-built machine designed to
151 simulate the demands of rugby scrummaging. The machine (RJF Design, Surrey) incorporates
152 a steel frame with a horizontal sled that runs along the frame. Participants adopt a semi-
153 crouched position with flexion at the knees and hips and with their shoulders resting against
154 the sled. Participants used an explosive leg drive to push the sled with maximal force through
155 a distance of ~3.5 m. Timing gates (Brower Speed Trap 2, USA) assess sled movement times
156 with the first set placed 10 cm in front of the sled and then 3 m from the first set. Based on pilot
157 testing, the runner angle was set at an incline of 3^{o}, and additional resistance was provided by
158 loading 100-kg onto the 80-kg sled. There was no significant difference in the time to complete
159 the sled push between two preliminary tests, with a CV of 0.6%.

160 *Experimental Trials*

161 Each participant began trials in the morning following a 10-h overnight fast and having
162 consumed their normal high-carbohydrate breakfast 3 h before exercise (providing a mean total
163 of 165 g of carbohydrate) and at least 500 mL of water. On arrival, participants provided a
164 urine sample, and were weighed in underwear and shorts using a digital balance scale accurate
165 to 0.05 kg (Avery Ltd., UK). Thereafter, a resting 500-µL fingertip capillary blood sample was
166 obtained (Softclix Pro, Roche, Switzerland) and subjective ratings of thirst, hunger and overall
167 gastrointestinal symptoms recorded (scale 1-15). Participants then began a standardized 10-
168 min warm-up that consisted of stretching, jogging, sprinting, one period of the BURST and

baseline performance tests. Participants consumed an initial bolus of test drinks 25 mins pre-exercise and rated drink pleasantness and acceptability using a 100-mm scale (Bartoshuk et al. 2004). After a second blood sample, participants then began the BURST. Blood samples and subjective ratings were obtained after each exercise block. Participants received a further half-bolus of test drinks at the midpoint of exercise and provided further ratings. Water was permitted *ad libitum* during participants' first trials and then matched in subsequent trials. Body mass was recorded post-exercise once participants had removed excess moisture from the skin. Ambient temperature (20±3^{o}C) and humidity (40±7%) was not different between trials.

Drink Composition

(R)-3-Hydroxybutyl (R)-3-hydroxybutyrate was synthesized at the University of Oxford as a colourless oil comprising ethyl-(R)-3-hydroxybutyrate (~1%) and (R)-1,3-butanediol (~1%), which were the starting materials, (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (94%), 3-betahydroxybutryl 1,3-butanediol monoester (~1%), and di-b-hydroxybutyrate 1,3-butanediol diester (~3%).

Participants received either carbohydrate (CHO) or carbohydrate with a β-hydroxybutyrate monoester (CHO+BHB-ME) in equal drink volumes for each trial (629±60 mL in total) divided into an initial pre-exercise bolus (419 mL) and a smaller mid-exercise bolus (210 mL). Drinks were isocaloric and both made an estimated 16 kJ/kgBM available for metabolism (total energy intake 1528±145 kJ). The ketone monoester was provided at a total dose of 590 mg/kgBM based on pilot data showing that this dosing level induces a sustained moderate ketosis (blood β-hydroxybutyrate of ~2-3mmol/L) that was generally well-tolerated and within the physiological response observed during fasting in humans (Clarke et al. 2012). The total amount of carbohydrate ingested was 90±9 g in CHO trials (3% solution) and 20±2 g in CHO+BHB-ME trials (14% solution) and equates to ingestion rates of ~1.1 and ~0.3 g/min, respectively. These intake rates ensured that the CHO trial matched guidance for supporting

194 exercise performance i.e. equal to or above 1 g/min (Jeukendrup 2004). Some carbohydrate
195 was added to the ketone monoester to enhance drink palatability and given that carbohydrate
196 in the mouth may have a central effect on performance (Carter et al. 2004; Chambers et al.
197 2009). The carbohydrate content of CHO+BHB-ME was 100% glucose and was achieved by
198 adding a commercially available sports drink (Glacueau, Vitamin Water). The CHO solution
199 included exactly the same volume of this 'base drink', while additional carbohydrate in the
200 form of 35% sucrose and 65% maltodextrin was added as liquid gel (MaxiNutrition,
201 ViperActive) with the primary intention of matching solutions for consistency, texture and
202 mouthfeel. Given that the raw ketone monoester is bitter in taste, pre-testing was conducted to
203 ensure the best possible matching of drinks. To make the taste comparable, CHO was flavoured
204 by adding 10 mL/L bitters and CHO+BHB-ME by adding 100 ng/L sweetener (Symrise, UK).
205 Four out of 9 participants (44%) correctly guessed the order in which they received test drinks.
206 *Sampling and Analysis*
207 Capillary fingertip blood samples were assessed for blood levels of BHB using a
208 portable analyser (Abbott Medisense Precision Xtra Advanced Diabetes Monitoring System,
209 Abbott). Blood was collected into serum Microvette 500 collection tubes (Sarstedt Ltd., UK)
210 and allowed to clot for 15 min at room temperature before being centrifuged at 3000 g for 10
211 min at 4ºC (Biofuge Primo R, Heraeus). The serum fraction was extracted into 1.5-mL tubes
212 (Eppendorf Ltd., UK) and frozen at -80ºC. Immunoassays were used to measure serum lactate,
213 glucose, myoglobin, glycerol (Randox, Ireland) and free fatty acids (Wako Chemical GmbH,
214 Germany) in duplicate using an automated spectrophotometer (Cobas Mira, Roche
215 Diagnostics, Burgess Hill, UK). The CV was less than 5% for all parameters. Urine samples
216 were measured for urine specific gravity using a handheld refractometer (Atago, Model A300,
217 USA).
218 *Statistical Analysis*

A sample size estimation was performed based on data from (Cox et al. 2016), whereby CHO+BHB-ME improved time trial performance by 411±458 s compared with CHO. Using this effect size (d=0.90), 12 participants should provide greater than 80% power to detect such a difference with a two-tailed t-test and an alpha-level of 0.05.

Data that required a single comparison of two means was tested for normality of distribution using the Shapiro-Wilk test. A paired two-tailed t-test was used to identify differences between means. A two-way repeated-measures analysis of variance (ANOVA) was used to identify differences over time. Where significant interactions were observed, multiple t-tests were applied to determine the location of variance both between treatments at each time point and between time points within each treatment relative to baseline, with both methods subject to a Holm-Bonferroni correction (Atkinson 2001). Statistical tests were conducted using GraphPad Prism version 8.2.1 (San Diego, CA). The p value was converted into 95% confidence intervals to derive a mechanistic inference about the true value of the effect statistic (Hopkins 2007). Effects sizes were calculated for performance data using Cohen's d_z. Data are expressed as means ± *SD* in text and means ±95% confidence interval (CI) in figures. Data for performance tests are presented as the mean overall difference between trials. For all comparisons, α was set at .05.

RESULTS

Exercise Performance

Mean overall time to complete the sustained high-intensity performance test was 0.33±0.41 s (2.1%) faster with CHO+BHB-ME (15.53±0.52 s) compared to the energy-matched CHO (15.86±0.80 s) placebo (p=0.04, d_z = 0.80, **Figure 2A**). Subsequent 15-m sprint performance was not different between trials (CHO = 2.57±0.15, CHO+BHB-ME= 2.56±0.11 s, p=0.80). No differences were detected in mean time to complete the power-based sled push

between trials (p=0.12, d_z = 0.58, **Figure 2B**). No trial order effects were observed for any of the performance tests (p≥0.11) and no baseline differences were identified for any performance measure (p=0.26 – 0.76).

Blood β-*hydroxybutyrate*

Pre-supplementation concentrations of blood β-hydroxybutyrate were similar between trials (**Figure 3A**). There was a marked increase in β-hydroxybutyrate concentrations 20 min after CHO+BHB-ME (2.53±0.85 mmol·L^{-1}) compared with CHO (0.01±0.03 mmol·L^{-1}). Blood β-hydroxybutyrate concentrations following CHO+BHB-ME remained elevated above CHO throughout the entire trial (treatment: F=570, p<0.001), with significant differences observed between trials pre-exercise and at all successive time-points (treatment x time: F=44, p<0.001). A second slight increase in β-hydroxybutyrate concentrations was observed from 40 to 80 min from ~2 to ~2.6 mmol·L^{-1} (**Figure 3A**).

Serum Variables

Glucose concentrations increased from baseline to the end of the warm-up before decreasing over the ensuing 20 min to near pre-supplementation values. Concentrations then increased over the subsequent 25 min before gradually decreasing (time: F=11, p<0.001). Glucose concentrations were higher after CHO than CHO+BHB-ME (treatment: F=8, p<0.05) and were significantly different between trials pre-exercise (p=0.02; **Figure 3B**). Lactate concentrations increased markedly from the onset of exercise and remained relatively stable thereafter (time: F=62, p<0.001), with significantly lower concentrations after CHO+BHB-ME than CHO (treatment: F=10, p<0.05; **Figure 3C**). Serum non-esterified fatty acid concentrations remained near basal levels up to 20 min into exercise and gradually increased thereafter with CHO but remained at or below pre-exercise values with CHO+BHB-ME (**Figure 3D**). Neither the time course nor the magnitude of the response was significantly different between trials, although there was a trend for an interaction (treatment x time: F=4,

269 p=0.07), whereby concentrations were lower 45 min into exercise with CHO+BHB-ME than
270 CHO (p<0.01). A similar pattern of response was identified for serum glycerol (time: F=20,
271 p<0.001), although concentrations were significantly lower after CHO+BHB-ME than CHO
272 (treatment: F=8, p<0.05; **Figure 3E**). There was a progressive rise in serum myoglobin
273 concentrations throughout the exercise irrespective of trial (time: F=19, p<0.05; **Figure 3F**).
274 *Subjective Ratings*
275 Ratings of perceived exertion increased throughout the exercise protocol irrespective
276 of trial (**Figure 4A**), from initial values of 13±1 ("fairly hard") to 15±2 ("hard"). As the time-
277 course of response was not different for ratings of gastrointestinal distress as well as drink
278 pleasantness and acceptability, data were combined across these time periods. Results showed
279 a trend for gastrointestinal upset to be greater after CHO+BHB-ME (10±2) than CHO (8±1)
280 trials (T=2, p=0.08; **Figure 4B**), while ratings of drink palatability were higher for CHO (47±7)
281 compared with CHO+BHB-ME (21±9) trials (T=2, p<0.001).
282 *Initial hydration Status and Fluid Balance*
283 Adequate hydration status was shown by similar pre-testing values for urine specific
284 gravity in both trials (CHO = 1.018±0.009, CHO+BHB-ME = 1.021±0.005). Total fluid intake
285 (i.e. prescribed and that consumed *ad libitum*) was not different between trials (CHO =
286 1675±728 mL, CHO+BHB-ME = 1777±743 mL). There was no significant difference between
287 trials in estimated fluid losses through sweat (CHO = 2462±563 mL, CHO+BHB-ME =
288 2573±642 mL) or for total urine production (CHO = 198±135 mL, CHO+BHB-ME = 226±220
289 mL). Body mass loss was apparent post-exercise (CHO = 984±482 g, CHO+BHB-ME =
290 1022±420 g) and equivalent to ~1% dehydration in both trials.
291

DISCUSSION

The present study demonstrates that co-ingestion of ketone body ester with carbohydrate suppresses circulating glycerol and lactate concentrations during exercise, and may improve certain aspects of rugby union performance, when compared to isocaloric carbohydrate ingestion. The data suggest that ingestion of ketone monoester improved simulated rugby union match play performance, without improving shorter duration sprint and power-based performance. These performance effects were observed in the presence of marginally higher ratings of gastrointestinal distress.

Co-ingestion of CHO+BHB-ME resulted in blood BHB concentrations remaining above a mean of 2 $mmol \cdot L^{-1}$ throughout exercise, compared to negligible BHB concentrations with isocaloric carbohydrate ingestion. β-hydroxybutyrate concentrations above ~1.5 $mmol \cdot L^{-1}$ can suppress hepatic glucose output and muscle glycogenolysis (Cox et al. 2016; Mikkelsen et al. 2015). Although it should be noted that one recent study reported no difference in plasma lactate concentrations or muscle glycogen breakdown with substantial and sustained increases in β-hydroxybutyrate concentrations during continuous cycling (Dearlove et al. 2021; Poffe et al. 2021a). It is therefore possible that circulating β-hydroxybutyrate concentrations above 1.5 $mmol \cdot L^{-1}$ combined with intermittent high-intensity exercise is required to detect meaningful metabolic effects. The present study reports marked metabolic effects with BHB-ME plus carbohydrate co-ingestion, as serum lactate, non-esterified fatty acid, and glycerol concentrations were lower with co-ingestion of CHO+BHB-ME, compared to isocaloric carbohydrate ingestion alone. Lower glycerol concentrations suggest a suppression of adipose tissue lipolysis. Whilst lower lactate concentrations could theoretically be due to an increase in lactate clearance rates, a reduction in lactate appearance rate is more likely, as this would be consistent with previous reports (Cox et al. 2016) of a suppression of glycolysis and/or better matching of glycolytic to pyruvate dehydrogenase flux.

The present study demonstrates that co-ingestion of CHO+BHB-ME may enhance high-intensity intermittent performance, without affecting sprint or power-type performance. It is noteworthy that lactate concentrations were higher in the present study than in many of the other endurance-type studies on ketone supplementation and thus the potential metabolic effects of ketones could be accentuated within our protocol i.e., during high-intensity rugby-related performance. While there was no improvement in very short duration maximal intensity sprints or power-based performance throughout the BURST protocol, there was also no negative impact, and therefore ketone ester-carbohydrate co-ingestion may represent an effective nutritional strategy for actual match play in professional Rugby Union players.

If lower lactate concentrations reflect a lower rate of glycogenolysis, as has been reported during steady-state exercise (Cox et al. 2016), then the implications of glycogen sparing as a mechanism for performance enhancement in high-intensity exercise is unclear. Whilst the strong association between liver and muscle glycogen depletion with fatigue has led to speculation that sparing of glycogen stores may enhance endurance performance (Bergström et al. 1967; Gonzalez et al. 2016), it is also possible that the inability to access glycogen could impair the capacity for high-intensity exercise (Stellingwerff et al. 2006). Muscle glycogen is the most rapid fuel for ATP re-synthesis (Walter et al. 1999), and therefore is the most appropriate fuel for very high-intensity exercise (Gonzalez et al. 2017). Some nutritional strategies that spare glycogen by suppressing glycogenolysis and PDH activity, such as low-carbohydrate, high-fat diets, have been shown to impair sprint performance (Stellingwerff et al. 2006). However, other nutritional strategies (e.g. L-carnitine supplementation), have been shown to spare muscle glycogen utilisation at moderate exercise intensities (50% Wmax), but still allow for high rates of glycogenolysis at higher exercise intensities (80% Wmax) (Wall et al. 2011). Whether these responses are translatable to the exercise intensity in the present study, remains to be assessed. Glycogen data are not available in the present study, but prior work has

shown that BHB-ME potently suppresses muscle glycogenolysis at 70% VO_2max (Cox et al. 2016), albeit with some inconsistency (Poffe et al. 2020). It is currently unclear whether BHB-ME impairs the ability to access glycogen at very high exercise intensities. The intensity of exercise in the present study is very likely to have exceeded 70% VO2max based on the blood lactate concentrations indicative of being well above lactate threshold.

An additional explanation for the lower lactate concentrations observed in the present study is a better matching of glycolytic to PDH flux (Stephens et al. 2007). If this is the case, it is currently the most likely explanation for a potential performance enhancement with co-ingestion of BHB-ME. During high-intensity exercise, the production of acetyl groups from high PDH flux can exceed utilisation by the TCA cycle, resulting in acetyl-CoA accumulation, depletion of the free CoA pool and, ultimately, inhibition of PDH and TCA flux (Stephens et al. 2007). Skeletal muscle carnitine can act as an acetyl group buffer by forming acetylcarnitine and thereby facilitate better matching of glycolytic, PDH and TCA flux during high-intensity exercise (Stephens et al. 2007; Wall et al. 2011). Acute CHO+BHB-ME co-ingestion has previously been shown to increase skeletal muscle carnitine content by up to ~50% within 25 mins of ingestion (Cox et al. 2016). Whilst this marked increase in muscle carnitine needs confirming, if acute BHB-ME ingestion is able to raise skeletal muscle free carnitine content, this could explain the improvement in performance observed in the present study. Future work will be required to establish whether this is indeed a plausible mechanism by which BHB-ME alters performance.

Any potentially advantageous metabolic effects of BHB-ME ingestion for performance could be negated by other metabolic or non-metabolic effects, such as a reduction in blood pH and/or an increase in gastrointestinal distress. The precise dose of BHB-ME to produce sufficient circulating BHB concentrations to influence metabolism, whilst limiting gastrointestinal distress and acidosis requires clarification. In the present study where there was

a suggestion of a ~2% (0.3 s) improvement in overall performance, the absolute dose of BHB-ME was ~58 g (590 mg/kgBM), which was co-ingested with 0.3 g/min of carbohydrate (as a glucose-fructose mixture), compared to 1.1 g/min of carbohydrate alone in the control group. The only previous study to have assessed the effects of ketone body ester ingestion in intermittent running found a neutral effect on time-to-exhaustion when ketone body ester (750 mg/kgBM; 59 g) was ingested in addition to glucose (~1.2 g/min) (Evans and Egan 2018). It was noted in that study that there was a greater prevalence of gastrointestinal distress with addition of ketone body ester to glucose ingestion, compared to glucose ingestion alone. This is consistent with other observations of gastrointestinal distress and impaired performance with the addition of acetoacetate diester to carbohydrate ingestion. Therefore, a consistent picture appears to be emerging, whereby the addition of ketone ester ingestion to relatively large carbohydrate intakes may result in sufficient gastrointestinal distress to impair or negate any potentially beneficial performance effects. However, the substitution of ketone ester for carbohydrate intake to result in isocaloric comparisons may explain the lesser prevalence of gastrointestinal complaints in the present study compared to prior work. It should still be noted that the difference in mean gastrointestinal symptoms score in the present study was 2 points higher (15-point scale) with CHO+BHB-ME compared to carbohydrate ingestion alone – with two participants reporting more severe gastrointestinal symptoms (scores >12) only with CHO+BHB-ME. Thus, the potential for gastrointestinal distress should be carefully considered if translating these findings into practice.

Some limitations with the present study are worthy of acknowledgment. First, there is potential for the study to be underpowered based on the dropout rate, and having not achieved the desired sample size. This could both inflate any effect size observed or increase the chance of a type II error. Furthermore, due to the nature of the protocol and the athletes recruited, there is a lack of mechanistic insight and therefore the proposed mechanisms remain speculative.

Future work should aim to establish the mechanisms underpinning any potential alterations in metabolism or performance with β-hydroxybutyrate monoester ingestion during high-intensity intermittent activity.

In summary, the present study demonstrates that co-ingestion of a β-hydroxybutyrate monoester with carbohydrate can alter metabolism (reduce circulating lactate and glycerol concentrations) and may improve high-intensity intermittent performance during a rugby simulation protocol in professional players, without altering shorter-duration sprint and power-type efforts. Some evidence of gastrointestinal distress was also prevalent. Therefore, Rugby Union players may consider consuming β-hydroxybutyrate monoester with carbohydrate before and during competition, although individual tolerance should first be tested in training prior to competition due to the potential for gastrointestinal problems.

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FIGURE CAPTIONS

Figure 1. A schematic representation of the study protocol.

Figure 2. Time to complete the simulated match-play performance (**A**) and 3-m sled push (**B**), following acute supplementation with carbohydrate alone (CHO ONLY) or co-ingestion of carbohydrate with β-hydroxybutyrate monoester (CHO+BHB-ME). Forwards are solid circles and backs are open circles. Values are means ± 95%CI. *Significant difference between treatments (p=0.04).

Figure 3. Blood β-hydroxybutyrate (**A**), serum glucose (**B**), serum lactate (**C**), serum NEFA (**D**), serum glycerol (**E**), and serum myoglobin (**F**) concentrations before and after acute supplementation with carbohydrate alone (CHO ONLY) or isocaloric co-ingestion carbohydrate with β-hydroxybutyrate monoester (CHO+BHB-ME) during a simulated rugby union match-play protocol. Values are means ± 95%CI. *Significant difference between treatments (p<0.05). ***Significant difference between treatments (p<0.001). ****Significant difference between treatments (p<0.0001). Asterisks next to figure labels indicate a main effect of treatment.

Figure 4. Rating of perceived exertion (**A**), gastrointestinal discomfort ratings (**B**), thirst ratings (**C**) and hunger ratings (**D**) during a simulated rugby union match-play protocol following acute ingestion of carbohydrate alone (CHO ONLY) or isocaloric co-ingestion of carbohydrate with β-hydroxybutyrate monoester (CHO+BHB-ME). Values are means ± 95%CI. *Significant difference between treatments (p<0.05).