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1 **KETONE MONOESTER INGESTION ALTERS METABOLISM AND SIMULATED**

2 **RUGBY PERFORMANCE IN PROFESSIONAL PLAYERS**

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25 **ABSTRACT**

26 Ketone ingestion can alter metabolism but effects on exercise performance are unclear,
27 particularly with regard to the impact on intermittent-intensity exercise and team-sport
28 performance. Nine professional male rugby union players each completed two trials in a
29 double-blind, randomized, crossover design. Participants ingested either 90±9 g carbohydrate
30 (CHO; 9% solution) or an energy matched solution containing 20±2 g carbohydrate (3%
31 solution) and 590 mg/kg body mass β-hydroxybutyrate monoester (CHO+BHB-ME) before
32 and during a simulated rugby union-specific match-play protocol, including repeated high-
33 intensity, sprint and power-based performance tests. Mean time to complete the sustained
34 high-intensity performance tests was reduced by 0.33±0.41 s (2.1%) with CHO+BHB-ME
35 (15.53±0.52 s) compared to CHO (15.86 ± 0.80 s) placebo (*p*=0.04). Mean time to complete
36 the sprint and power-based performance tests were not different between trials. CHO+BHB-
37 ME resulted in blood BHB concentrations that remained >2 mmol·L⁻¹ during exercise
38 (*P*<0.001). Serum lactate and glycerol concentrations were lower after CHO+BHB-ME than
39 CHO (*P*<0.05). Co-ingestion of a β-hydroxybutyrate monoester with carbohydrate can alter
40 fuel metabolism (attenuate circulating lactate and glycerol concentrations) and may improve
41 high-intensity running performance during a simulated rugby match-play protocol, without
42 improving shorter-duration sprint and power-based efforts.

43

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

45 INTRODUCTION

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

55 One of the most effective, practical methods to increase systematic BHB availability
56 without compromising endogenous carbohydrate stores is the oral ingestion of the BHB
57 monoester (BHB-ME), (R)-3-Hydroxybutyl (R)-3-hydroxybutyrate (Cox et al. 2016). When
58 compared to carbohydrate ingestion alone, the co-ingestion of carbohydrate and BHB-ME
59 before and during exercise can potently alter whole-body and skeletal muscle metabolism.
60 BHB-ME-carbohydrate co-ingestion has been shown to increase plasma BHB availability and
61 net intramuscular triglyceride utilization, whilst suppressing plasma non-esterified fatty acid
62 availability, glycolysis and net intramuscular glycogen utilization during moderate-intensity
63 exercise (Cox et al. 2016). Although it should be noted that glycogen sparing has not been
64 reported in all studies (Poffe et al. 2020). Furthermore, an increase in resting skeletal muscle
65 carnitine content, and a reduction in blood pH during exercise have also been observed with
66 acute BHB-ME ingestion (Cox et al. 2016; Dearlove et al. 2019; Poffe et al. 2021a). It is unclear
67 what implications these metabolic responses have for exercise performance, as some of these
68 changes may be beneficial (e.g. increased free carnitine content with potential to buffer acetyl-
69 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-

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

97

98 **METHODS**

99 *Participants*

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

106 *Experimental Design*

107 The study adopted a double-blind, placebo-controlled, randomised crossover design
108 comprising a preliminary session and two experimental trials, each separated by at least 6 days.
109 Supplements were prepared by an individual unconnected to the study and provided in
110 unmarked containers to those who interacted with participants. Sessions took place during the
111 players' off-season and on an indoor athletics training facility. One week prior to main trials,
112 participants completed a preliminary session to familiarise with the protocol (described below).
113 Prior to trials, habitual training was standardised for one week. Over the 48-h preceding the
114 first trial, each participant recorded their diet (estimated record) and replicated this diet before
115 the second trial. Participants refrained from high intensity exercise, caffeine, and alcohol for
116 24-h before sessions. Main trials required participants to complete the match-play protocol
117 ingesting either carbohydrate (CHO) or an energy matched drink containing carbohydrate and
118 β-hydroxybutyrate monoester (CHO+BHB-ME) before and at the mid-point of exercise.

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

121 *Simulated Rugby Union Match-play Protocol*

122 The Bath University Rugby Shuttle Test (BURST) is a rugby union-specific match-
123 play protocol based on physical demands data for elite rugby union (Roberts et al. 2010;
124 Roberts et al. 2008). The exercise requirements have been described in detail elsewhere
125 (Roberts et al. 2010). In brief, the protocol involves 16 exercise periods (~5 min each) arranged
126 into 4 x 21-min blocks (**Figure 1**). Blocks 1 and 3 are followed by 4-min active recovery. A
127 10-min “half-time” break follows block 2, comprising 7 min rest and 3 min active recovery.
128 Within each exercise period, participants perform shuttles of walking, cruising and jogging
129 interspersed with two bouts of simulated scrummaging and rucking, and one bout of mauling.
130 Timing is maintained by verbal instruction from an audio file. A Performance Test (described
131 below) and 15-m sprint are also completed within each exercise block and on 16 occasions in
132 total. Power-based performance was assessed during bouts of scrummaging using an explosive
133 sled-push (described below) and on 32 occasions. Performance test data are expressed as the
134 mean performance across the BURST protocol. Distance travelled during the BURST (7078
135 m) has been validated against match-play demands derived from time-motion analysis (6418
136 m), and time spent performing contact events (9.9%) is consistent with actual match play
137 (Roberts et al. 2010).

138 *Performance Testing*

139 The high-intensity performance test was designed to replicate a sustained high-intensity
140 exercise bout specific to rugby union competition, and combined aspects of resistance work,
141 sprinting, and agility. Testing required participants to pass through an initial infrared timing
142 gate (Smartspeed, Fusion Sport, Australia) and carry one 20-kg tackle bag over 9 m, followed
143 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°, 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- μ L fingertip capillary blood sample was
166 obtained (Softelix 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

169 baseline performance tests. Participants consumed an initial bolus of test drinks 25 mins pre-
170 exercise and rated drink pleasantness and acceptability using a 100-mm scale (Bartoshuk et al.
171 2004). After a second blood sample, participants then began the BURST. Blood samples and
172 subjective ratings were obtained after each exercise block. Participants received a further half-
173 bolus of test drinks at the midpoint of exercise and provided further ratings. Water was
174 permitted *ad libitum* during participants' first trials and then matched in subsequent trials. Body
175 mass was recorded post-exercise once participants had removed excess moisture from the skin.
176 Ambient temperature ($20\pm 3^{\circ}\text{C}$) and humidity ($40\pm 7\%$) was not different between trials.

177 *Drink Composition*

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

183 Participants received either carbohydrate (CHO) or carbohydrate with a β -
184 hydroxybutyrate monoester (CHO+BHB-ME) in equal drink volumes for each trial (629 ± 60
185 mL in total) divided into an initial pre-exercise bolus (419 mL) and a smaller mid-exercise
186 bolus (210 mL). Drinks were isocaloric and both made an estimated 16 kJ/kgBM available for
187 metabolism (total energy intake 1528 ± 145 kJ). The ketone monoester was provided at a total
188 dose of 590 mg/kgBM based on pilot data showing that this dosing level induces a sustained
189 moderate ketosis (blood β -hydroxybutyrate of $\sim 2\text{-}3\text{mmol/L}$) that was generally well-tolerated
190 and within the physiological response observed during fasting in humans (Clarke et al. 2012).
191 The total amount of carbohydrate ingested was 90 ± 9 g in CHO trials (3% solution) and 20 ± 2
192 g in CHO+BHB-ME trials (14% solution) and equates to ingestion rates of ~ 1.1 and ~ 0.3 g/min,
193 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 4°C (Biofuge Primo R, Heraeus). The serum fraction was extracted into 1.5-mL tubes
212 (Eppendorf Ltd., UK) and frozen at -80°C. 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*

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

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

236

237 **RESULTS**

238 *Exercise Performance*

239 Mean overall time to complete the sustained high-intensity performance test was
240 0.33 ± 0.41 s (2.1%) faster with CHO+BHB-ME (15.53 ± 0.52 s) compared to the energy-
241 matched CHO (15.86 ± 0.80 s) placebo ($p=0.04$, $d_z = 0.80$, **Figure 2A**). Subsequent 15-m sprint
242 performance was not different between trials (CHO = 2.57 ± 0.15 , CHO+BHB-ME = 2.56 ± 0.11
243 s, $p=0.80$). No differences were detected in mean time to complete the power-based sled push

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

247 *Blood β -hydroxybutyrate*

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

256 *Serum Variables*

257 Glucose concentrations increased from baseline to the end of the warm-up before
258 decreasing over the ensuing 20 min to near pre-supplementation values. Concentrations then
259 increased over the subsequent 25 min before gradually decreasing (time: $F=11$, $p<0.001$).
260 Glucose concentrations were higher after CHO than CHO+BHB-ME (treatment: $F=8$, $p<0.05$)
261 and were significantly different between trials pre-exercise ($p=0.02$; **Figure 3B**). Lactate
262 concentrations increased markedly from the onset of exercise and remained relatively stable
263 thereafter (time: $F=62$, $p<0.001$), with significantly lower concentrations after CHO+BHB-ME
264 than CHO (treatment: $F=10$, $p<0.05$; **Figure 3C**). Serum non-esterified fatty acid
265 concentrations remained near basal levels up to 20 min into exercise and gradually increased
266 thereafter with CHO but remained at or below pre-exercise values with CHO+BHB-ME
267 (**Figure 3D**). Neither the time course nor the magnitude of the response was significantly
268 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 $\sim 1\%$ dehydration in both trials.

291

292 **DISCUSSION**

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

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

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

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

342 shown that BHB-ME potently suppresses muscle glycogenolysis at 70% VO₂max (Cox et al.
343 2016), albeit with some inconsistency (Poffe et al. 2020). It is currently unclear whether BHB-
344 ME impairs the ability to access glycogen at very high exercise intensities. The intensity of
345 exercise in the present study is very likely to have exceeded 70% VO₂max based on the blood
346 lactate concentrations indicative of being well above lactate threshold.

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

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

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

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

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

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

403

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490

491 **FIGURE CAPTIONS**

492 **Figure 1.** A schematic representation of the study protocol.

493

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

499

500 **Figure 3.** Blood β -hydroxybutyrate (**A**), serum glucose (**B**), serum lactate (**C**), serum NEFA
501 (**D**), serum glycerol (**E**), and serum myoglobin (**F**) concentrations before and after acute
502 supplementation with carbohydrate alone (CHO ONLY) or isocaloric co-ingestion
503 carbohydrate with β -hydroxybutyrate monoester (CHO+BHB-ME) during a simulated rugby
504 union match-play protocol. Values are means \pm 95%CI. *Significant difference between
505 treatments ($p<0.05$). ***Significant difference between treatments ($p<0.001$).

506 ****Significant difference between treatments ($p<0.0001$). Asterisks next to figure labels
507 indicate a main effect of treatment.

508

509 **Figure 4.** Rating of perceived exertion (**A**), gastrointestinal discomfort ratings (**B**), thirst
510 ratings (**C**) and hunger ratings (**D**) during a simulated rugby union match-play protocol
511 following acute ingestion of carbohydrate alone (CHO ONLY) or isocaloric co-ingestion of
512 carbohydrate with β -hydroxybutyrate monoester (CHO+BHB-ME). Values are means \pm
513 95%CI. *Significant difference between treatments ($p<0.05$).