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1 **Muscle glycogen utilization during exercise following ingestion of alcohol**

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15

16 **Keywords:** Ethanol, Fat, Carbohydrate, Lactate, Metabolism

17

18 **Abstract**

19 **PURPOSE:** Ingested ethanol (EtOH) is metabolized gastrically and hepatically,

20 which may influence resting and exercise metabolism. Previous exercise studies

21 have provided EtOH via intravenous infusion rather than oral ingestion, which

22 alters the metabolic effects of EtOH. No studies to date have investigated the

23 effects of EtOH *ingestion* on systemic and peripheral (e.g. skeletal muscle)

24 exercise metabolism.

25 **METHODS:** Eight men (Mean \pm SD, Age: 24 ± 5 y; Body Mass: 76.7 ± 5.6 kg;

26 Height: 1.80 ± 0.04 m; $\dot{V}O_{2peak}$: 4.1 ± 0.2 L.min⁻¹) performed two bouts of fasted

27 cycling exercise at 55% $\dot{V}O_{2peak}$ for 2-h, with (EtOH) and without (Control) prior

28 ingestion of EtOH 1-h and immediately before exercise (total dose: 0.1 g·kg lean

29 body mass⁻¹·h⁻¹; 30.2 ± 1.1 g 40% ABV Vodka; fed in 2 equal boluses) in a

30 randomized order, separated by 7-10 days.

31 **RESULTS:** Muscle glycogen breakdown during exercise was not different
32 between conditions (Control: -257.7 [-330.8, 184.6] vs EtOH: -221.4 [-287.6, 141.4]
33 mmol·kg dm⁻¹; means with normalized 95% confident intervals). Mean plasma
34 glucose concentrations during exercise were similar (Control: 5.26 [5.17,
35 5.34] vs EtOH: 5.26 [5.18, 5.34] mmol·L⁻¹; *p* = 0.04). EtOH ingestion resulted in
36 similar plasma non-esterified fatty acid (NEFA) concentrations compared to rest
37 (Control: 0.43 [0.31,0.55] vs EtOH: 0.30 [0.21,0.40] mmol·L⁻¹) and during exercise.
38 Mean plasma lactate concentration was higher during the first 30-min of rest
39 following EtOH consumption (mean concentration: Control: 0.83 [0.77, 0.90]
40 vs EtOH 1.00 [0.93, 1.07] mmol·L⁻¹) but the response during exercise was similar
41 between conditions.

42 **CONCLUSIONS:** Ingesting a small dose of EtOH transiently altered resting
43 concentrations of systemic lactate, but not during exercise. Muscle glycogen
44 utilization was similar during exercise with or without prior alcohol ingestion,
45 reflected in similar total whole-body carbohydrate oxidation rates observed.

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55 **Introduction**

56 Ethanol (EtOH) is the relatively energy-dense ($\sim 7.1 \text{ kcal}\cdot\text{g}^{-1}$) ingestible form of
57 alcohol and can be preferentially oxidized over other nutrients [1-3]. Low doses
58 are primarily metabolized through the action of alcohol dehydrogenase (ADH)
59 and aldehyde dehydrogenase (ALDH) in the gut and the liver. The resultant
60 reduction in the cellular $\text{NAD}^+:\text{NADH}$ redox ratio disturbs metabolic pathways in
61 the liver that either require NAD^+ or are inhibited by NADH [4]. Specifically, this
62 includes pathways vital for energy turnover (i.e. glycolysis, citric acid cycle,
63 pyruvate dehydrogenase, fatty acid oxidation, and gluconeogenesis). At the
64 whole-body level, maximal rates of EtOH oxidation ($0.1 \text{ g}\cdot\text{kg}\cdot\text{h}^{-1}$ lean body mass)
65 have been suggested to transiently spare the oxidation of other substrates (i.e.
66 carbohydrate and fat) up to a maximum level of half an individual's resting
67 metabolic rate [5, 6]. However, it remains unclear whether substrate sparing
68 manifests during times where the requirement for energy turnover is high (i.e.
69 during exercise).

70 Necessarily, any alterations in circulating concentrations of metabolic
71 substrates as a result of EtOH ingestion could influence metabolic fuel selection
72 during exercise [7]. Specifically, as muscle glycogen depletion is a largely
73 dictated by the total amount of carbohydrate available to the system [8],
74 preferential oxidation of EtOH over carbohydrate may spare carbohydrate at the
75 systemic level during moderate intensity exercise; resulting in sparing of muscle
76 glycogen concentrations during a bout of exercise. However, the influence of
77 EtOH on circulating glucose and non-esterified fatty acids (NEFA) during exercise
78 remains indistinct [9-12]. Discrepancy in findings between studies may be

79 explained by inconsistencies in dose of EtOH, with a greater effect being
80 observed following larger relative doses (~20-40 g) [9, 12]. Similarly, EtOH blunts
81 the typical blood glucose response to exercise during moderate (>50% $\dot{V}O_{2max}$)
82 [9, 13], but not lower intensity exercise (30% $\dot{V}O_{2max}$) [10].

83 Importantly, the majority of exercise studies to date have investigated the
84 influence of EtOH on carbohydrate and fat metabolism following infusion, rather
85 than ingestion, which could alter the extent of displacement of hepatic, and
86 therefore skeletal muscle carbohydrate and fat metabolism during exercise [14-
87 18]. Furthermore, whilst it has been generally accepted that exercise *per se* will
88 not increase the rate of EtOH metabolism [19], the 2-fold elevation in hepatic $\dot{V}O_2$
89 during exercise (~60 mL.min⁻¹ at rest vs ~135 mL.min⁻¹ during exercise),
90 suggests the increase in liver metabolic rate could augment EtOH metabolism
91 especially when fed a dose within the liver's capacity to oxidize EtOH [20-22]. No
92 studies to date have investigated the effects of EtOH *ingestion* on systemic and
93 peripheral (e.g. skeletal muscle) metabolism during exercise.

94 Based on previous work it was expected that a low dose of orally ingested
95 EtOH would alter circulating metabolites thereby meaningfully displacing
96 carbohydrate and fat oxidation at rest and during exercise, sparing skeletal
97 muscle glycogen utilization. Therefore, the objective of the current study was to
98 investigate the whole body metabolic and skeletal muscle glycogen responses to
99 acute ingestion of a dose of EtOH estimated to maximally stimulate hepatic
100 oxidation, prior to a bout of prolonged, moderate-intensity exercise in young,
101 healthy men.

102

103 **Materials and Methods**

104 ***Experimental design***

105 Participants performed two bouts of cycle ergometry in a randomized
106 cross-over design, interspersed by an interval of 7-10 days. A dietary record was
107 collected during the 48-hour period prior to the first experimental trial and was
108 subsequently replicated with exact types and amounts of foods before the final
109 trial (2726 ± 490 kcal·day⁻¹, 47 ± 3 % carbohydrate, 33 ± 8 % fat, 21 ± 9 % protein).
110 Furthermore, participants were asked not to perform vigorous physical activity,
111 consume alcohol, or caffeine 24-h prior to testing, confirmed by questionnaire
112 upon entering the laboratory. Main trials involved ingestion of EtOH beverages or
113 volume-matched water as a control, followed by 1-hour of rest and then 2-hours
114 cycling at 55% of individual $\dot{V}O_{2peak}$. The study randomisation plan was created
115 using <https://www.random.org/>.

116 ***Participants***

117 Seven healthy recreationally active men (**Table 1**) were recruited to
118 participate in the study. Participants were informed of potential risks and
119 discomfort involved in the study prior to providing written informed consent. The
120 study was approved by the National Health Service Research Ethics Committee:
121 Bristol (17/SW/0219), the Research Ethics Approval Committee for Health at the
122 University of Bath (EP 17/18 090) and was registered at clinicaltrials.gov
123 (NCT03404947). All procedures were performed in accordance with the
124 Declaration of Helsinki.

125 ***Preliminary measurements***

126 Prior to experimental sessions participants visited the human performance
127 laboratory at the University of Bath for fitness and body composition analysis.
128 Body mass was assessed to the nearest 0.1 kg using electronic weighing scales
129 (BC543 Monitor; Tanita, Netherlands) and height was measured to the nearest
130 0.1 cm using a stadiometer (Seca Ltd, Germany), before lean and fat mass were
131 determined using dual-energy X-ray absorptiometry (DEXA; Discovery, Hologic,
132 Bedford, UK). An incremental cycling test was then completed on an electronically
133 braked ergometer, at a self-selected cadence, to assess maximum oxygen
134 uptake ($\dot{V}O_{2peak}$) (Excalibur Sport; Lode[®], Netherlands). Participants were
135 permitted to adjust the handlebar and saddle heights to their preference. Power
136 output was initially set at 50 Watts (W), increasing in 50 W increments every 4
137 minutes for four stages. Thereafter, power output increased in 20-W increments
138 every 1-minute until volitional exhaustion was achieved. Heart rate was monitored
139 throughout (Polar H7; Polar Electro, Finland) and breath-by-breath assessment
140 of $\dot{V}O_2$ was made using an online gas analysis system (TrueOne2400;
141 Parvomedics, USA). Volume and gas analysers were calibrated with a 3-litre
142 calibration syringe (Hans Rudolph, USA) and known concentrations of a
143 calibration gas (15.99% O₂; 5.08% CO₂). Peak oxygen uptake ($\dot{V}O_{2peak}$) was
144 recorded as the highest average $\dot{V}O_2$ (L.min⁻¹) over a consecutive 30-s period.
145 Peak power output (W_{peak}) was calculated using the following equation [23].

146
$$W_{peak} = W_{final} + \left(\frac{t}{T} \cdot W_{increment}\right)$$

147 Where W_{peak} is peak power output (Watts), W_{final} is the power output of the final
148 completed stage (Watts), t is time completed of the final stage (s), T is total stage

149 time (s), and $W_{increment}$ is the power increment between stages at exhaustion
150 (Watts).

151 ***Experimental protocol***

152 Participants arrived at the laboratory in an overnight fasted state (~10 h).
153 Body mass was assessed (Sliding Beam Column Scale, Weylux, UK) before
154 participants were instructed to rest in a semi-supine position (~60°), prior to the
155 assessment of resting metabolic rate (RMR) via the Douglas bag technique.
156 Collection of expired gas through the Douglas bag technique allows for
157 calculation of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$),
158 thereby allowing for calculation of whole-body carbohydrate and fat oxidation
159 [24]. An intravenous cannula was placed into an antecubital vein and a baseline
160 sample of 5 mL venous blood was collected (BD Venflon Pro; BD, Switzerland).
161 Cannulae were kept patent by flushing with 0.9% sodium chloride infusion (B.
162 Braun; UK). Participants then ingested 50% of the total EtOH (Absolut Raspberri,
163 40% ABV; Absolut, Sweden) or volume matched water before a 60-minute rest
164 period during which venous blood samples were collected at 15, 30, 45 and 60
165 minutes, alongside a 10-min gas sample between 50-60 minutes. Participants
166 then remained in a semi-supine position while muscle was sampled from the
167 *vastus lateralis* under local anaesthetic (1% lidocaine; Hameln Pharmaceuticals
168 Ltd., Brockworth, UK). Samples were taken immediately prior to the second bolus
169 of EtOH, from a 3-5 mm incision in the anterior aspect of the thigh using a
170 Bergstrom needle adapted for suction and were snap-frozen in liquid nitrogen for
171 subsequent storage at -80°C [25]. A second incision was also made and
172 temporarily dressed for immediate post-exercise muscle sampling. Thereafter,

173 participants were asked to ingest the remaining 50% of the EtOH or control
174 beverage, immediately prior to the initiation of exercise. Participants were then
175 asked to cycle for 2-hours at 55% of their pre-determined $\dot{V}O_{2peak}$ on a cycle
176 ergometer (Monark 894E; Monark, Vansbro, Sweden). One-minute expired
177 breath, and 5-ml venous blood samples were collected, alongside heart rate
178 (Polar H1; Polar Electro, Kempele, Finland), every 15-minutes for the first hour
179 and every 30-minutes during the second hour (Figure 1). In both trials, throughout
180 exercise participants ingested water at a rate of $0.5 \text{ mL}\cdot\text{kg}^{-1}$ every 10-minutes
181 (Total: $376 \pm 26 \text{ ml}$). Immediately post exercise, participants were transferred from
182 the ergometer to the bed, where the post-exercise muscle sample was collected.

183 ***Test beverage composition***

184 The rate of EtOH ingestion in the EtOH trial was $0.1 \text{ g}\cdot\text{kg LBM}^{-1}\cdot\text{h}^{-1}$ [6],
185 which aimed to provide sufficient EtOH to contribute meaningfully as a metabolic
186 substrate but without intending to exceed the maximum rate of EtOH metabolism
187 and unnecessarily overspill into systemic circulation [6]. As such, total EtOH
188 provided was $12.1 \pm 0.4 \text{ g}$ ingested as a 15% solution in water ($30.2 \pm 1.1 \text{ g}$ 40%
189 ABV Vodka; $\sim 67 \text{ kcal}$). In the control trial, participants ingested a volume matched
190 water beverage.

191 ***Blood analysis***

192 Blood samples were immediately transferred into tubes treated with
193 ethylenediaminetetraacetic acid (EDTA) prior to being centrifuged at 3466 g
194 (5000 rpm) for 10 minutes at 4°C (Heraeus Primo R; Thermo Fisher Scientific,
195 UK) and frozen on dry ice for storage. All samples were later analyzed for plasma
196 glucose (colormetric), non-esterified fatty acids (colormetric), lactate

197 (colormetric), and a subset of samples for EtOH (colormetric) using a
198 spectrophotometric analyser (RX, Daytona, Randox Laboratories Ltd., UK). Inter-
199 assay CV was < 3% for glucose, < 7% for NEFA, and < 3% for lactate. Intra-
200 assay CV was < 2% for glucose, < 5% for NEFA, and < 3% for lactate.

201 ***Muscle analysis***

202 Frozen muscle samples were placed in a freeze dryer (Mechatech
203 Systems, UK) for ~16 hours at -55°C. Following removal of visible connective
204 tissue, freeze-dried muscle samples were reduced to a fine powder using a pestle
205 and mortar and then used to determine muscle glycogen concentrations. Briefly,
206 the muscle powder was digested in 0.1 mM NaOH and neutralized with HCl-
207 citrate buffer, pH = 5.0. The glycogen present in the supernatant was hydrolyzed
208 with α -amylglucosidase and analyzed for glucosyl units in duplicate by an
209 enzymatic method [26]. Relative concentrations of muscle glycogen were
210 assessed in duplicate using a spectrophotometric plate reader (SpectraMax 190,
211 Molecular Devices, USA). To account for possible measurement error associated
212 with fluid shift during exercise, glycogen concentrations are reported as mmol
213 glucosyl units per kilogram of dry mass ($\text{mmol}\cdot\text{kg}^{-1}\text{dm}^{-1}$). Total rates of muscle
214 glycogen utilization in relation to whole-body carbohydrate oxidation were then
215 calculated as the change in glycogen content of the *vastus lateralis* ($\text{g}\cdot\text{kg}^{-1}$ dry
216 mass $\cdot\text{min}^{-1}$) multiplied by the estimated active muscle mass during exercise,
217 which is almost exclusively the thigh muscles for seated cycling and can be
218 estimated as ~10 kg wet mass in young men [27].

219

220

221 ***Expired gas analysis***

222 Inspired air concentrations were assessed during Douglas bag collection
223 to correct for changes in atmospheric O₂ and CO₂ concentrations [28]. Expired
224 gas concentrations of O₂ and CO₂ were analysed in a known volume of sample,
225 using paramagnetic and infrared analysers, respectively (Mini HF 5200;
226 Servomex Group Ltd, Crowborough, UK). Total volumes of expired gas were
227 determined using a dry gas meter (Harvard Apparatus, Holliston, USA) and
228 temperature measured using a digital thermometer (Edale Instruments,
229 Longstanton, UK). Substrate utilization during exercise was determined using the
230 equations of Jeukendrup and Wallis (2005) [29] (where $\dot{V}O_2$ and $\dot{V}CO_2$ are
231 expressed in L·min⁻¹). EtOH oxidation was assumed to be complete, based on
232 feeding at the maximal hepatic oxidation rate reported in Schutz [6]. Measured
233 RER was adjusted for EtOH oxidation to give Non-EtOH RER. This was
234 calculated by subtracting the $\dot{V}O_2$ and $\dot{V}CO_2$ associated with complete oxidation
235 of ingested EtOH (i.e. 3O₂ & 2CO₂ per mol EtOH respectively) from measured
236 values of $\dot{V}O_2$ and $\dot{V}CO_2$.

237 ***Statistical analysis***

238 All data in the text are reported as means [normalized 95% confidence
239 interval] unless otherwise stated. Normality of data was assessed using the
240 Shapiro-Wilk test, with a paired t test or Wilcoxon's test employed to analyse
241 parametric data and non-parametric data respectively. A mixed model ANOVA
242 (condition, time, and condition x time) was used to examine differences in plasma
243 metabolite in data, with post-hoc Bonferroni corrections applied in GraphPad
244 Prism (GraphPad Software Inc., California, USA). Effect sizes (Cohen's *d*) were

245 calculated and interpreted in accordance with [30]. Error bars shown on figures
246 are confidence intervals (CI) corrected for inter-individual variation using the
247 specific error term from the pairwise contrast at each time-point [31]. Rather than
248 describing the variability of individual values around the mean in each condition,
249 the magnitude of these confidence intervals provides a visual representation of
250 the contrast between means such that, in general, plotted means whose
251 confidence intervals overlap by no more than half one side of an interval would
252 typically generate a p -value less than 0.05 if using a paired t-test at that time-
253 point [32]. There was no evidence of trial order effects for any variable, which was
254 verified using 2-way ANOVA of Sequence x Condition interactions (all $p = 0.2$ -
255 0.9). Based on differences in arterial glucose concentrations observed in Juhlin-
256 Dannfelt *et al* [11] a sample size of 10 participants was deemed sufficient to
257 provide an 80% chance of detecting such a difference at an alpha level of 0.05
258 (G Power 3.1). Statistical analyses were performed using SPSS Statistics v.24
259 (IBM Corp., Armonk, NY, USA) and figures were created using GraphPad Prism
260 v.7 (GraphPad Software, San Diego, CA, USA).

261

262 **Results**

263 ***Muscle glycogen and substrate metabolism***

264 Pre-exercise muscle glycogen content was not different between EtOH
265 and Control conditions (471 [387, 555] vs 469 [385, 553] mmol·kg dm⁻¹
266 ¹ respectively; $p = 0.86$) and muscle glycogen used during exercise was also not
267 clearly different between trials (EtOH: 229 [156, 302] vs CONTROL: 258 [185,
268 331] mmol·kg dm⁻¹; $p = 0.67$) (Figure 2A).

269 ANOVA revealed main effects for time (both $p < 0.01$) but not condition (p
270 = 0.85 & 0.35), or time x condition ($p = 0.30$ & 0.30) for $\dot{V}O_2$ and $\dot{V}CO_2$
271 respectively (Figure 3A & 3B). No effect was seen for time ($p = 0.30$), condition
272 ($p = 0.94$), or time x condition ($p = 0.24$) for raw respiratory exchange ratio (RER)
273 (Figure 3C). When adjusted for complete EtOH oxidation, resting RER increased
274 following EtOH consumption but was not different from the control condition (1.03
275 [0.92, 1.15] vs 0.91 [0.79, 1.02]), with no effects of time ($p = 0.22$), condition ($p =$
276 0.21), or time x condition ($p = 0.24$) (Figure 3D).

277 Extra-muscular carbohydrate, and therefore total whole-body
278 carbohydrate oxidation was similar following EtOH ingestion when compared to
279 the Control condition ($P = 0.56$) (Figure 2B). Total fat oxidation during exercise
280 was also similar following EtOH ingestion compared to the control trial (1.42 [1.12,
281 1.72] vs 1.71 [1.41, 2.01] mJ, respectively; $p = 0.33$).

282 **Systemic metabolites**

283 *Plasma Glucose Concentrations* - Pre-ingestion plasma glucose
284 concentrations were similar between EtOH and Control conditions (5.38 [5.29,
285 5.46] mmol·L⁻¹ and 5.31 [5.22, 5.39] mmol·L⁻¹, respectively). No effect of time ($p =$
286 0.13), condition ($p = 0.47$), or time x condition ($p = 0.63$) for plasma glucose
287 response was revealed.

288 *Plasma NEFA Concentrations* - Baseline (resting) plasma NEFA
289 concentrations were similar between conditions (Figure 4). ANOVA revealed
290 main effects for time ($p < 0.01$), but not condition ($p = 0.40$), or time x condition
291 ($p = 0.34$) for plasma NEFA responses.

292 *Plasma lactate Concentrations* – Main effects of time ($p < 0.01$), but not
293 condition ($p = 0.15$), or time x condition ($p = 0.40$) were revealed for plasma
294 lactate responses (Figure 4). However, across the first 30-mins of the rest period
295 plasma lactate was higher following EtOH ingestion relative to the control
296 condition (mean concentration: Control: 0.83 [0.77, 0.90] vs EtOH 1.00 [0.93, 1.07]
297 $\text{mmol}\cdot\text{L}^{-1}$; $p = 0.04$, $d = 0.77$).

298 *Plasma EtOH Concentrations* - Plasma EtOH remained below the lower
299 detectable limit of the assay ($0.72 \text{ mmol}\cdot\text{L}^{-1}$; EtOH, Randox Laboratories Ltd., UK)
300 throughout the entire protocol.

301 ***Exercise intensity***

302 Average power output (Mean \pm SD) for the cycling exercise was 156 ± 14
303 W at a self-selected cadence of 80 ± 9 rpm. Average heart rate during exercise
304 was 154 [150, 157] bpm in the EtOH trial and 157 [154, 161] bpm in the control
305 trial.

306

307 **Discussion**

308 This is the first study to investigate exercise metabolism following oral
309 alcohol ingestion, with measurements made at the level of skeletal muscle,
310 systemic metabolites and whole-body substrate oxidation. Accordingly, we report
311 the novel observations that prior alcohol ingestion does not alter the utilization of
312 either muscle glycogen or extra-muscular carbohydrate sources during exercise.
313 However, at rest the ingestion of alcohol did increase plasma lactate
314 concentrations. During the subsequent exercise, whole-body fat oxidation was
315 then lower following EtOH ingestion. These metabolic effects were apparent with

316 a relatively low dose of vodka within the capacity for alcohol metabolism and thus
317 without any measurable appearance of EtOH in the systemic circulation.

318 Interestingly, ingestion of $0.1 \text{ g EtOH}\cdot\text{kg LBM}^{-1}\cdot\text{h}^{-1}$ ($12.1 \pm 0.4 \text{ g}$) did not
319 result in differing muscle glycogen use relative to water ingestion in the current
320 study. This is consistent with the one previous study to have quantified muscle
321 glycogen use during exercise under conditions where EtOH is present in the
322 circulation [11]. Despite demonstrating a reduction in muscle glycogen at rest
323 following EtOH infusion, the latter study by Juhlin-Dannfelt and colleagues [11]
324 found infusion of EtOH did not result in differing muscle glycogen utilisation during
325 exercise. The relatively low dose of EtOH ingested in the current study (~30 mL
326 of 40% ABV vodka) was intended not to exceed the capacity for gastric/hepatic
327 metabolism such that EtOH would not appear systemically, so it remains a
328 possibility that a higher dose may stimulate hepatic lipogenesis and/or suppress
329 hepatic glucose production sufficient to modify the balance of skeletal muscle
330 fatty acid, glucose and thus glycogen utilization [6]. Notably, one participant's
331 glycogen use was near zero, so caution must be taken in interpreting absolute
332 values which depend on assumed constants, albeit the relative pattern would not
333 be systematically altered between treatments.

334 Notably, the current study was the first to assess substrate metabolism
335 using indirect calorimetry during both rest and exercise following EtOH ingestion.
336 Previous studies propose that acute ingestion of EtOH at rest transiently spares
337 the oxidation of carbohydrate and fat up to a maximum level of half an individual's
338 resting metabolic rate (~15-43% sparing of carbohydrate and ~30% sparing of fat
339 oxidation) [5]. RER adjusted for EtOH oxidation was similar between conditions

340 with initial decreases over rest likely reflective of a shift towards the RER for EtOH
341 (~0.66) in the EtOH trial, and greater lipid oxidation in the Control condition [33].
342 Likewise, when adjusted for assumed complete EtOH oxidation, resting RER was
343 not different between conditions during rest or exercise, reflected in similar whole
344 body extra-muscular carbohydrate, and fat oxidation (Figure 2A).

345 Following an approximate 10-hour overnight fast, plasma glucose
346 concentrations were similar at rest between conditions in the current study.
347 Previous research has demonstrated that at rest, EtOH results in either a
348 reduction, or no change in blood glucose concentration, with conflicting results
349 primarily explained by the nutritional status of participants. The current study
350 therefore agrees with previous literature demonstrating no influence of EtOH on
351 resting blood glucose concentrations in humans following an overnight fast of
352 ~12-hours [34-36]. However, ingestion of EtOH lead to higher plasma lactate
353 concentrations during the first 30-mins of the rest period relative to the control
354 group, consistent with the possibility that EtOH can inhibit the conversion of
355 lactate to glucose, and further research should seek to measure this [34, 37].
356 Whilst blood glucose homeostasis may have been maintained through a
357 compensatory rise in hepatic glycogenolysis in this scenario [34], such a rise in
358 lactate is still well within the normal physiological range, such that any
359 compensatory increase in hepatic glycogenolysis is likely to be minimal.

360 Plasma concentrations of NEFA were also similar between conditions in the
361 current study, which is likely due to the relatively small dose of EtOH ingested by
362 participants [38, 39]. Notably, the area under the curve response of acetate to
363 EtOH is dose-dependent and whilst the current study did not assess the plasma

364 acetate responses, it is likely that EtOH ingestion at the dose fed in the current
365 study was not sufficient to elevate plasma acetate concentration and therefore
366 inhibit adipose tissue lipolysis [39, 40].

367 During bouts of exercise performed in the fasted state, elevation in hepatic
368 gluconeogenesis maintains delivery of glucose to the working muscles [7].
369 Following ingestion of a small dose of EtOH fed in two boli, blood glucose
370 concentration during exercise did not differ between conditions in the current
371 study. Moreover, both plasma lactate and NEFA concentrations did not differ
372 between conditions during the 2-h exercise protocol. Interestingly, the responses
373 of circulating metabolites during exercise were remarkably similar, suggesting
374 that any observed effect of acute EtOH ingestion at rest is not present during
375 exercise. Bearing in mind that hepatic $\dot{V}O_2$ increases over two-fold during exercise
376 ($\sim 60 \text{ mL}\cdot\text{min}^{-1} \rightarrow \sim 135 \text{ mL}\cdot\text{min}^{-1}$), and that disposition and first pass metabolism
377 of EtOH is affected by liver function, the increase in liver metabolic rate *may* have
378 augmented EtOH metabolism in the current study [20, 21].

379 **Conclusion**

380 A low pre-exercise dose of alcohol within the capacity for complete
381 oxidation does not meaningfully alter oxidation of fat or carbohydrate during
382 subsequent exercise.

383

384 **Acknowledgements**

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386 Further thanks to Rob Edinburgh for helping to get the study up and running.

387

388 **Conflict of Interest**

389 The authors have no conflicts of interest to declare. The results of the present
390 study results of the study are presented clearly, honestly, and without fabrication,
391 falsification, or inappropriate data manipulation and do not constitute
392 endorsement by the American College of Sports Medicine (ACSM).

393

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509 **Figure 1.** Schematic representation of the experimental protocol. EtOH =
510 Ethanol. $\dot{V}O_{2max}$ = maximal oxygen uptake. n=7.

511

512 **Figure 2.** Mean \pm normalised 95% confidence interval A. Substrate contribution
513 to energy expenditure (left) and individual muscle glycogen responses (right).
514 EtOH was assumed fully oxidised, lipid oxidation was calculated from non-EtOH
515 RER, Extra-muscular carbohydrate oxidation was calculated as the difference
516 between whole-body carbohydrate oxidation and measured muscle glycogen
517 utilisation. B. Pre and post exercise muscle glycogen content following EtOH
518 ingestion and volume matched, non-caloric Control. n=6.

519

520 **Figure 3.** Mean \pm normalised 95% confidence interval **A.** Raw $\dot{V}O_2$ **B.** Raw $\dot{V}CO_2$
521 **C.** Raw respiratory exchange ratio **D.** Non-EtOH adjusted respiratory exchange
522 ratio at rest and during exercise following EtOH ingestion and volume matched,
523 non-caloric Control. n=7.

524

525 **Figure 4.** Mean \pm normalised 95% confidence interval Plasma Glucose, Lactate,
526 and Non-esterified fatty acid (NEFA) responses to EtOH ingestion at rest and
527 during exercise compared to a volume matched, non-caloric Control. * $p < 0.05$.
528 n=7.







