Muscle glycogen utilization during exercise following ingestion of alcohol

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Abstract

PURPOSE: Ingested ethanol (EtOH) is metabolized gastrically and hepatically, which may influence resting and exercise metabolism. Previous exercise studies have provided EtOH via intravenous infusion rather than oral ingestion, which alters the metabolic effects of EtOH. No studies to date have investigated the effects of EtOH *ingestion* on systemic and peripheral (e.g. skeletal muscle) exercise metabolism.

METHODS: Eight men (Mean ± SD, Age: 24 ± 5 y; Body Mass: 76.7 ± 5.6 kg; Height: 1.80 ± 0.04 m; $\dot{V}O_{2peak}$: 4.1 ± 0.2 L.min^{-1}) performed two bouts of fasted cycling exercise at 55% $\dot{V}O_{2peak}$ for 2-h, with (EtOH) and without (Control) prior ingestion of EtOH 1-h and immediately before exercise (total dose: 0.1 g·kg lean body mass^{-1}·h^{-1}; 30.2 ± 1.1 g 40% ABV Vodka; fed in 2 equal boluses) in a randomized order, separated by 7-10 days.

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

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

Introduction

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

Necessarily, any alterations in circulating concentrations of metabolic substrates as a result of EtOH ingestion could influence metabolic fuel selection during exercise [7]. Specifically, as muscle glycogen depletion is a largely dictated by the total amount of carbohydrate available to the system [8], preferential oxidation of EtOH over carbohydrate may spare carbohydrate at the systemic level during moderate intensity exercise; resulting in sparing of muscle glycogen concentrations during a bout of exercise. However, the influence of EtOH on circulating glucose and non-esterified fatty acids (NEFA) during exercise 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

Materials and Methods

Experimental design

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

Participants

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

Preliminary measurements

Prior to experimental sessions participants visited the human performance laboratory at the University of Bath for fitness and body composition analysis. Body mass was assessed to the nearest 0.1 kg using electronic weighing scales (BC543 Monitor; Tanita, Netherlands) and height was measured to the nearest 0.1 cm using a stadiometer (Seca Ltd, Germany), before lean and fat mass were determined using dual-energy X-ray absorptiometry (DEXA; Discovery, Hologic, Bedford, UK). An incremental cycling test was then completed on an electronically braked ergometer, at a self-selected cadence, to assess maximum oxygen uptake ($\dot{V}O_{2peak}$) (Excalibur Sport; Lode®, Netherlands). Participants were permitted to adjust the handlebar and saddle heights to their preference. Power output was initially set at 50 Watts (W), increasing in 50 W increments every 4 minutes for four stages. Thereafter, power output increased in 20-W increments every 1-minute until volitional exhaustion was achieved. Heart rate was monitored throughout (Polar H7; Polar Electro, Finland) and breath-by-breath assessment of $\dot{V}O_2$ was made using an online gas analysis system (TrueOne2400; Parvomedics, USA). Volume and gas analysers were calibrated with a 3-litre calibration syringe (Hans Rudolph, USA) and known concentrations of a calibration gas (15.99% O_2; 5.08% CO_2). Peak oxygen uptake ($\dot{V}O_{2peak}$) was recorded as the highest average $\dot{V}O_2$ ($L.min^{-1}$) over a consecutive 30-s period. Peak power output (W_{peak}) was calculated using the following equation [23].

$$W_{peak} = W_{final} + (\frac{t}{T} . W_{increment})$$

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

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

Experimental protocol

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

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

Test beverage composition

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

Blood analysis

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

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

Muscle analysis

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

221 ***Expired gas analysis***

222 Inspired air concentrations were assessed during Douglas bag collection
223 to correct for changes in atmospheric O_2 and CO_2 concentrations [28]. Expired
224 gas concentrations of O_2 and CO_2 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 $L \cdot min^{-1}$). 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. $3O_2$ & $2CO_2$ 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

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

Results

Muscle glycogen and substrate metabolism

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

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

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

Systemic metabolites

Plasma Glucose Concentrations - Pre-ingestion plasma glucose concentrations were similar between EtOH and Control conditions (5.38 [5.29, 5.46] $mmol \cdot L^{-1}$ and 5.31 [5.22, 5.39] $mmol \cdot L^{-1}$, respectively). No effect of time ($p = 0.13$), condition ($p = 0.47$), or time x condition ($p = 0.63$) for plasma glucose response was revealed.

Plasma NEFA Concentrations - Baseline (resting) plasma NEFA concentrations were similar between conditions (Figure 4). ANOVA revealed main effects for time ($p < 0.01$), but not condition ($p = 0.40$), or time x condition ($p = 0.34$) 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 mmol·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 mmol·L^{-1}; EtOH, Randox Laboratories Ltd., UK)
300 throughout the entire protocol.
301 ***Exercise intensity***
302 Average power output (Mean ± 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 g EtOH·kg LBM^{-1}·h^{-1} (12.1 ± 0.4 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

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

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

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

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

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

Conclusion

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

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Conflict of Interest

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

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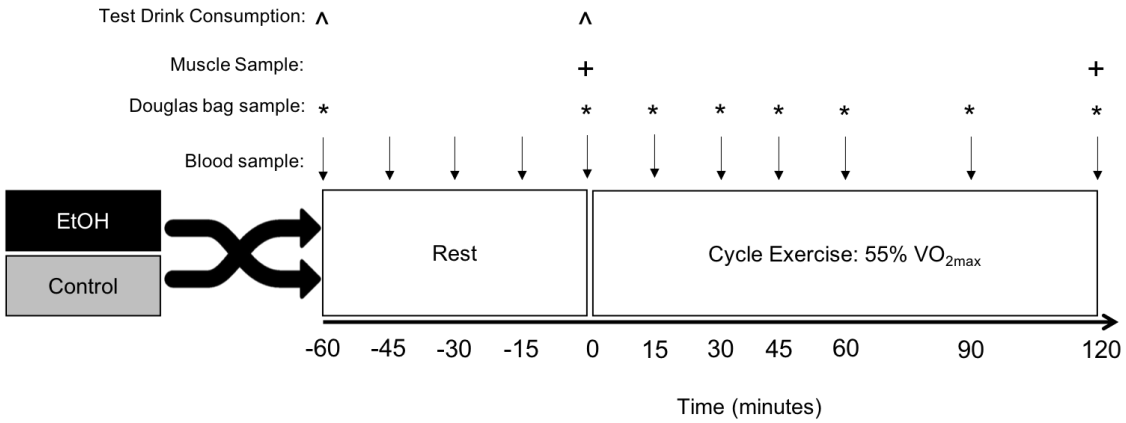
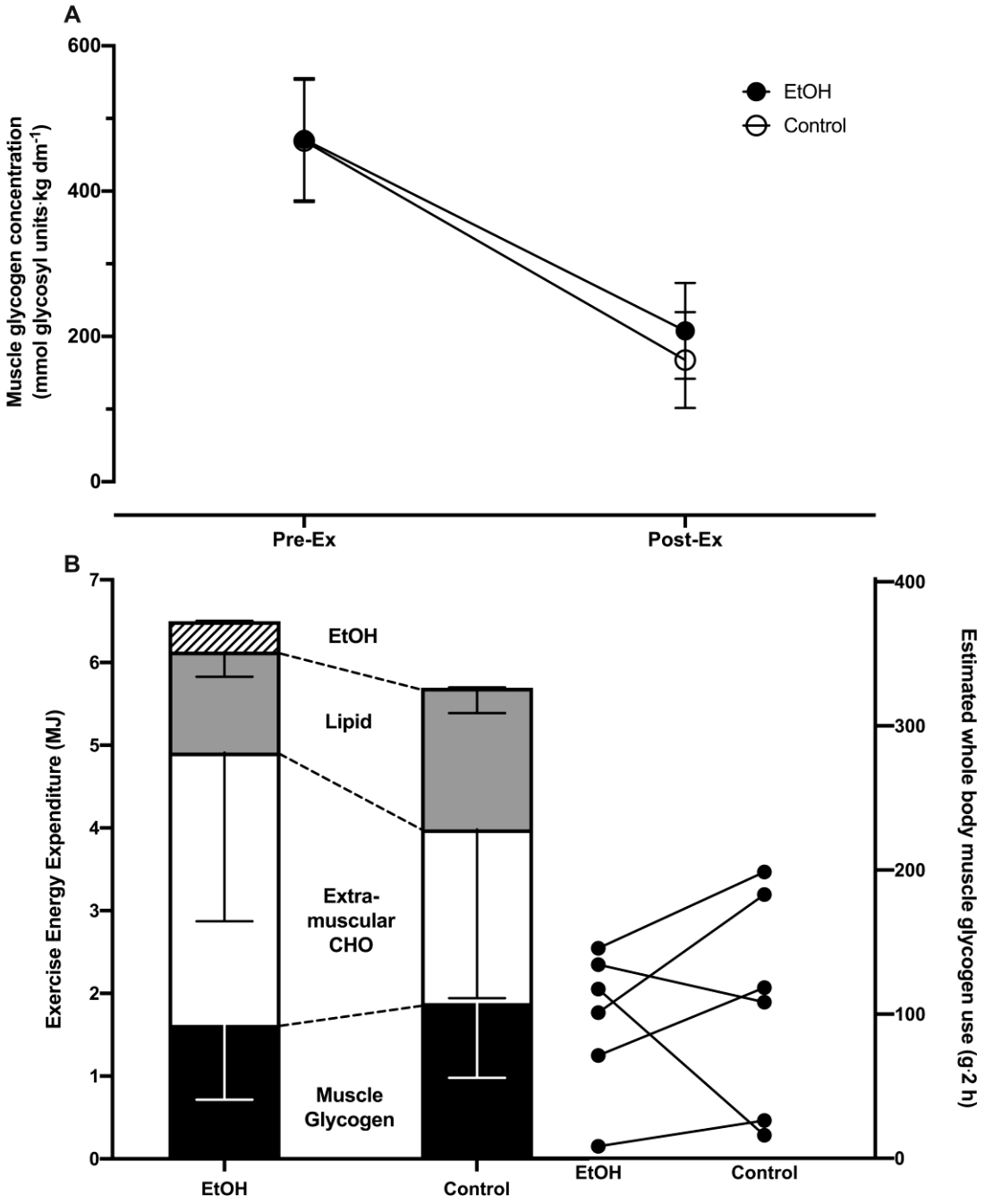
Figure 1. Schematic representation of the experimental protocol. EtOH = Ethanol. $\dot{V}O_{2max}$ = maximal oxygen uptake. n=7.

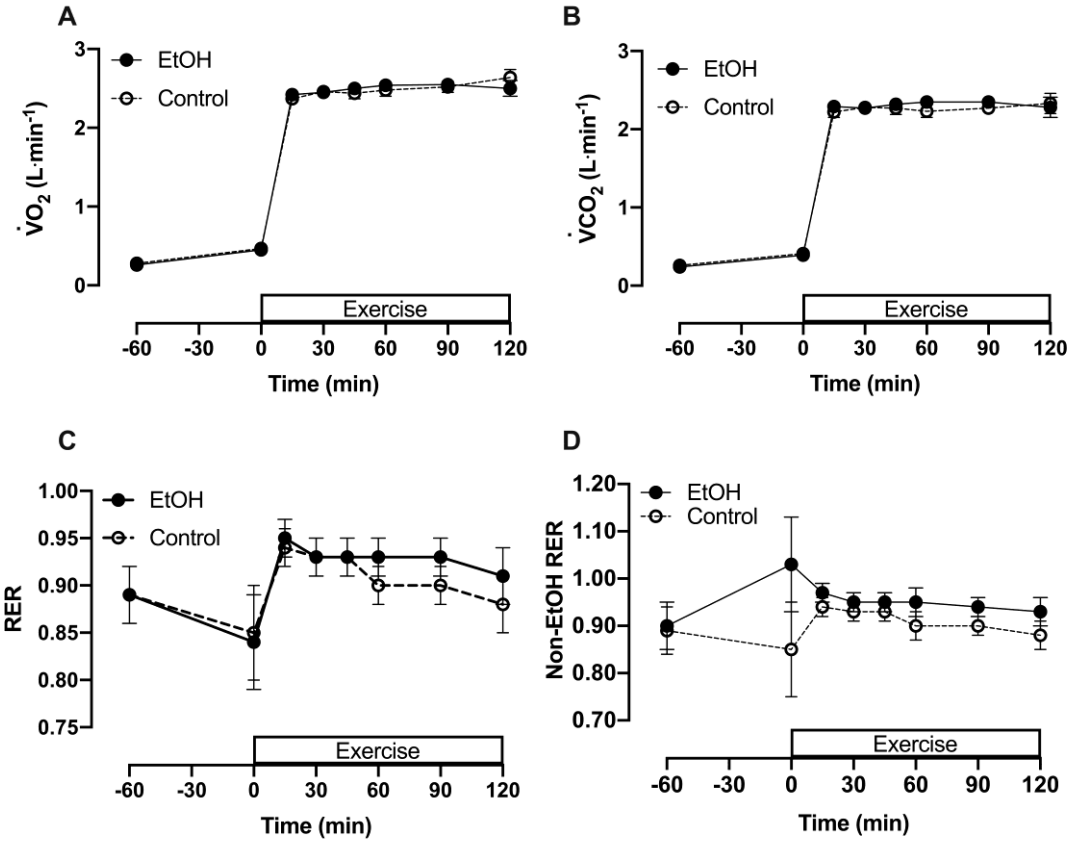
Figure 2. Mean ± normalised 95% confidence interval A. Substrate contribution to energy expenditure (left) and individual muscle glycogen responses (right). EtOH was assumed fully oxidised, lipid oxidation was calculated from non-EtOH RER, Extra-muscular carbohydrate oxidation was calculated as the difference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

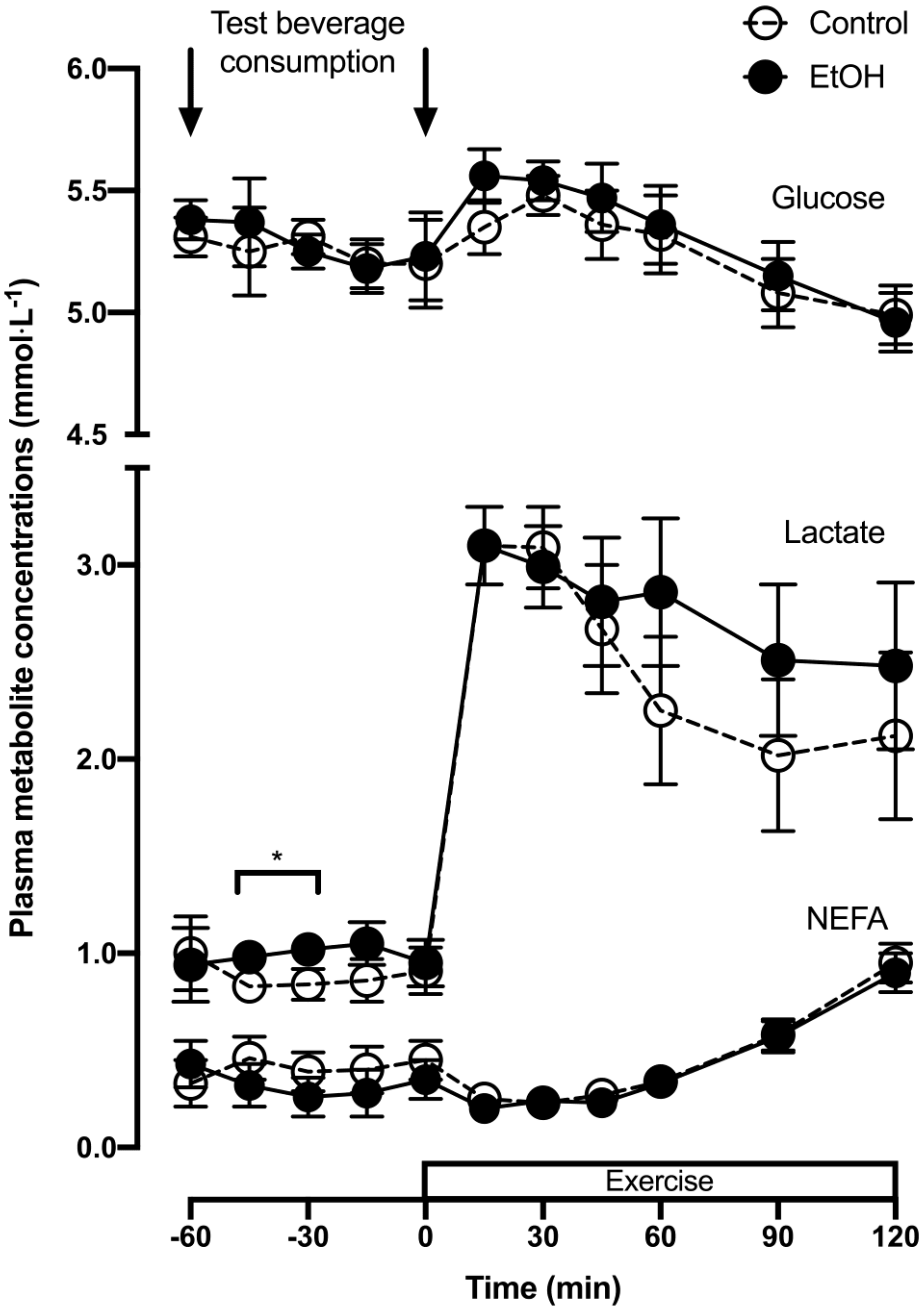
Figure 3. Mean ± normalised 95% confidence interval **A**. Raw $\dot{V}O_2$ **B**. Raw $\dot{V}CO_2$ **C**. Raw respiratory exchange ratio **D.** Non-EtOH adjusted respiratory exchange ratio at rest and during exercise following EtOH ingestion and volume matched, non-caloric Control. n=7.

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


Test Drink Consumption:
Muscle Sample:
Douglas bag sample:
Blood sample:
EtOH
Control
Rest
Cycle Exercise: 55% VO2max
-60
-45
-30
-15
0
15
30
45
60
90
120
Time (minutes)


A
600
400
200
0
Muscle glycogen concentration (mmol glycosyl units·kg dm-1)
EtOH
Control
Pre-Ex
Post-Ex
B
7
6
5
4
3
2
1
0
Exercise Energy Expenditure (MJ)
EtOH
Lipid
Extra-muscular CHO
Muscle Glycogen
400
300
200
100
0
Estimated whole body muscle glycogen use (g·2 h)
EtOH
Control
EtOH
Control


A
EtOH
Control
$\dot{V}O_2$ (L·min^{-1})
Exercise
Time (min)
B
EtOH
Control
$\dot{V}CO_2$ (L·min^{-1})
Exercise
Time (min)
C
EtOH
Control
RER
Exercise
Time (min)
D
EtOH
Control
Non-EtOH RER
Exercise
Time (min)


Test beverage consumption
Control
EtOH
Glucose
Lactate
NEFA
Plasma metabolite concentrations (mmol·L-1)
6.0
5.5
5.0
4.5
3.0
2.0
1.0
0.0
*
Exercise
-60
-30
0
30
60
90
120
Time (min)