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**Title**: Substrate oxidation and the influence of breakfast in normobaric hypoxia and normoxia

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## Abstract

**Purpose:** Previous research has reported inconsistent effects of hypoxia on substrate oxidation, which may be due to differences in methodological design, such as pre-exercise nutritional status and exercise intensity. This study investigated the effect of breakfast consumption on substrate oxidation at varying exercise intensities in normobaric hypoxia compared with normoxia.

**Methods:** Twelve participants rested and exercised once after breakfast consumption and once after omission in normobaric hypoxia (4300 m: $F_iO_2$ ~11.7%) and normoxia. Exercise consisted of walking for 20-minutes at 40%, 50% and 60% of altitude-specific $\dot{V}O_{2max}$ at 10-15% gradient with a 10kg backpack. Indirect calorimetry was used to calculate carbohydrate and fat oxidation.

**Results:** The relative contribution of carbohydrate oxidation to energy expenditure was significantly reduced in hypoxia compared with normoxia during exercise after breakfast omission at 40% (22.4 ± 17.5% vs. 38.5±15.5%, $p$ = 0.03) and 60% $\dot{V}O_{2max}$ (35.4±12.4 vs. 50.1±17.6%, $p$ = 0.03), with a trend observed at 50% $\dot{V}O_{2max}$ (23.6±17.9% vs. 38.1± 17.0%, $p$ = 0.07). The relative contribution of carbohydrate oxidation to energy expenditure was not significantly different in hypoxia compared with normoxia during exercise after breakfast consumption at 40% (42.4±15.7% vs. 48.5±13.3%, $p$ = 0.99), 50% (43.1±11.7% vs. 47.1±14.0%, $p$ = 0.99) and 60% $\dot{V}O_{2max}$ (54.6±17.8% vs. 55.1±15.0%, $p$ = 0.99).

**Conclusions:** Relative carbohydrate oxidation was significantly reduced in hypoxia compared with normoxia during exercise after breakfast omission but not during exercise after breakfast consumption. This response remained consistent with increasing exercise intensities. These findings may explain some of the disparity in the literature.



**Key words:** Carbohydrate, fat, utilisation, fasted, fed, altitude



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47 Abbreviations

48 AUC – area under the curve

49 FFA – free fatty acids

50 $F_IO_2$ – fraction of inspired oxygen

51 HIF-1α – hypoxia inducible factor 1 alpha

52 $PiO_2$ – partial pressure of inspired oxygen

53 PPARα – peroxisome proliferator-activated receptor alpha

54 RPE – rating of perceived exertion

55 SD – standard deviation

56 SE – standard error

57 $\dot{V}CO_2$ – carbon dioxide production

58 $\dot{V}O_2$ – oxygen uptake

59 $\dot{V}O_{2max}$ – maximal oxygen update

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## Introduction

Disparate metabolic responses have been observed during exercise matched for relative intensities in hypoxia compared with normoxia (Young et al. 1982; Braun et al. 2000; Beidleman et al. 2002; Lundby and Van Hall 2002; Friedmann et al. 2004; Péronnet et al. 2006; Katayama et al. 2010; Morishima et al. 2014; O'Hara et al. 2017; Matu et al. 2017). These contrasting findings within the literature appear to be due to differences in experimental design, specifically pre-exercise nutritional status and exercise intensity (Griffiths et al. 2019). Whilst the effect of pre-exercise breakfast consumption (Edinburgh et al. 2018) and exercise intensity (Van Loon et al. 2001) on metabolism are well documented in normoxic conditions, the metabolic response to these factors is yet to be quantified in hypoxia. In addition, due to the inconsistent use of pre-exercise breakfast consumption in the literature, a direct comparison of the two distinct states during exercise of varying intensities in normoxia and hypoxia may provide clarity on such equivocal findings. Further, whilst the use of studies utilising fasted participants to control for baseline metabolic status is warranted, knowledge of how this differs to fed participants is necessary to generate practical recommendations for relevant populations.

It has been proposed that during exercise matched for relative intensities, the relative contribution of carbohydrate oxidation to energy expenditure is higher in hypoxia compared with normoxia when performed after breakfast consumption, but lower in hypoxia than normoxia when exercise was performed after breakfast omission (Griffiths et al. 2019). A potential explanation of findings observed after breakfast consumption is that greater oxidation and mobilisation of endogenous carbohydrate stores may be stimulated via the combined effect of hypoxia (Katayama et al. 2010) and feeding (Tentolouris et al. 2003) on the sympathetic nervous system. Additionally, a similar effect of hypoxia (Matu et al. 2018) and feeding (Blom et al. 2005) may increase circulating insulin concentration and subsequently inhibit lipolysis and free fatty acid (FFA) mobilisation (Coyle et al. 1997). It also seems plausible that greater

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fat oxidation may be observed in hypoxia, compared with normoxia after breakfast omission. Increased expression of the transcription factor hypoxia inducible factor 1 alpha (HIF-1α) may upregulate the fatty acid-activated transcription factor peroxisome proliferator-activated receptor alpha (PPARα) as per the metabolic response to hypoxia (Aragones et al. 2008). This response may be further stimulated by the fasted state (König et al. 1999), subsequently inhibiting pyruvate dehydrogenase activity (Huang et al. 2002) and enabling greater mobilisation and oxidation of fat stores (Spriet and Watt 2003).

Exercise intensity was also identified as a significant moderator of substrate oxidation during exercise matched to relative intensities in hypoxia (Griffiths et al. 2019). Specifically, the relative contribution of carbohydrate oxidation to energy expenditure was higher in hypoxia compared with normoxia during exercise performed at higher intensities. This was attributed to the hypoxic effect of both altitude and high intensity exercise, augmenting skeletal muscle hypoxia. The subsequent change in substrate oxidation could therefore be explained as per the normoxic response to increased exercise intensity (i.e. reduction in adipose tissue blood flow and lipolysis and/or downregulation of carnitine palmitoyltransferase-1) (Sahlin 1990; Romijn et al. 1993; Van Loon et al. 2001). Alternatively, sympathetic nervous system activity may be potentiated by hypoxia and greater exercise intensities, augmenting glycogenolysis and therefore carbohydrate oxidation (Watt et al. 2001).

An investigation into the effects of pre-exercise nutritional status and exercise intensity in hypoxia compared with normoxia may provide clarity on the current literature and facilitate the development of nutritional strategies for high altitude mountaineers and military personnel alike. As such, the purpose of this study was to investigate the effect of breakfast consumption or omission on substrate oxidation during exercise matched for relative intensities in normobaric hypoxia and normoxia. These exercise intensities ranged from 40%-60% of altitude specific maximal oxygen uptake ($\dot{V}O_{2max}$). We hypothesised that the relative

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contribution of carbohydrate oxidation to energy expenditure would be increased during exercise matched to relative intensities in hypoxia compared with normoxia after breakfast consumption, but that this response would not occur after breakfast omission. We also hypothesised that the relative contribution of carbohydrate oxidation would be potentiated with increasing exercise intensities in hypoxia compared with normoxia after both breakfast consumption and omission. Due to the differing metabolic response to hypoxia in females compared with males (Braun et al. 2000), all participants in the present study were male. Inclusion of a female sub-group was beyond the scope of the present study but warrants investigation in future studies.

## Methods

### Participants

Twelve, physically active (structured exercise $\geq$ 3 times a week), healthy male volunteers (23 ± 3 years, 181.1 ± 6.4 cm, 79.8 ± 13.1 kg) provided written, informed consent to participate in this study. The study received institutional ethical approval (Leeds Beckett research ethics committee, application reference: 46180) and was conducted in accordance with the Declaration of Helsinki. All participants were non-smokers, normotensive, free from food allergies and were not taking any medication. None of the participants had travelled to an altitude of >1500 m within the previous three months and were all currently residing at an altitude <500 m.

### Experimental design

Participants were required to make a total of seven visits to the laboratory. The first visit involved pre-exercise screening, anthropometry, verbal familiarisation with testing procedures

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and a sickle cell trait test. Sickle cell trait was an exclusion criterion due to complications that may occur at altitude, for example splenic infarction (Goodman et al. 2014). Further exclusion criteria included diabetes and thyroid disorders. The second and third visit required participants to be acutely exposed to normobaric hypoxia (fraction of inspired oxygen ($F_iO_2$): ~11.7% when considering water vapour partial pressure (Conkin 2011; Fenn et al. 1946) and daily fluctuations in barometric pressure) equivalent to 4300 m (partial pressure of inspired oxygen ($P_iO_2$): 83 mmHg) in an environmental chamber (TISS, Alton, UK and Sporting Edge, Sheffield on London, UK) or normoxia (absolute altitude ~113 m). Ambient temperature was maintained at 20 °C and relative humidity at 50% for all trials. Participants completed sub-maximal and maximal exercise tests to calculate walking speeds matched for relative exercise intensity in each environmental condition for the experimental trials. These two preliminary trials were separated by ≥48 hours and conducted in a single-blind randomised fashion. On visits 4-7 the participants completed a 3-hour 45 minutes experimental trial, which included a 2-hour 15 minutes rest period, followed by a 1-hour incremental walking protocol and a 30-minute post exercise rest period (Figure 1). Two of the trials were performed in normobaric hypoxia equivalent to 4300 m (one trial with breakfast consumption (HB), and one with breakfast omission (HF)) and two were performed in normoxia (one trial with breakfast consumption (NB), and one with breakfast omission (NF)). These visits were separated by ≥ 7 days and were randomised independent of the preliminary trials, using a Latin Square design.

[Insert Figure 1]

Preliminary testing

Participants completed an exercise test on a motorised treadmill (Woodway PPS S5; Waukesha, WI), which comprised of a sub-maximal and maximal phase. In the normoxic condition, the incremental sub-maximal phase involved four, 3-minute stages walking at 3

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164 km/h, 4 km/h, 4.5 km/h and 5.5 km/h. Participants carried a 10 kg backpack to mimic the
165 physiological demands of the experimental trials. The initial two walking speeds in the
166 normoxic condition were performed at a 10% gradient and the second two at a 15% gradient.
167 In normobaric hypoxia, participants walked at 1.5 km/h, 2.5 km/h, 3.5 km/h and 4.5 km/h, at a
168 10% gradient throughout. Lower speeds and gradients were used in normobaric hypoxia based
169 on the reduced $\dot{V}O_{2max}$ elicited at altitude (Dill et al. 1931), and the need for all participants to
170 achieve 60% $\dot{V}O_{2max}$ within the 12-minute trial. The higher gradient utilised in normoxia was
171 employed to ensure participants achieved 60% $\dot{V}O_{2max}$ with a walking gait. Following
172 completion of the sub-maximal phase, participants then rested for approximately 5 minutes,
173 after which the maximal phase of the test commenced. Within this 5 minutes, participants were
174 permitted to rest seated, or complete some light stretching. Participants ran without a backpack,
175 at a 1% gradient (Jones and Doust 1996), at a constant speed dependant on fitness and
176 environmental condition, aiming for a perceived exertion of 12. The gradient was increased by
177 1% every minute until volitional exhaustion. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide
178 production ($\dot{V}CO_2$) measurements were made throughout both phases of the test using an online
179 gas analysis system (Metalyser, Cortex, Germany), which was calibrated following the
180 manufacturer's instructions. In this regard, the online gas analyser was calibrated with daily
181 barometric pressure, a 3 L syringe (volume), as well as ambient gases and two known
182 concentrations of gas (15% $O_2$ and 5% $CO_2$). These gases were subsequently checked before
183 use with an acceptance limit set at ± 0.02% for both $O_2$ and $CO_2$. All participants were deemed
184 to reach a 'true' $\dot{V}O_{2max}$ by fulfilment of >2 of the following criteria: a plateau in $\dot{V}O_2$ in the
185 final exercise stage, respiratory exchange ratio $\geq$1.15, heart rate within 10 $b \cdot min^{-1}$ of age
186 predicted maximum (220-age), rating of perceived exertion (RPE) $\geq$19 and/or blood lactate $\geq$8
187 mM (Howley et al. 1995). The sub-maximal and maximal data was used to establish walking

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speeds that would elicit 40%, 50% and 60% $\dot{V}O_{2max}$ relative to both normoxia and hypoxia whilst carrying a 10 kg backpack at a 10% gradient.

Diet and Physical Activity Before Testing

Participants recorded their food intake for the 24 hours before the first experimental trial and were instructed to repeat the quantity and timing of this intake for each subsequent visit. During these 24 hours, participants were asked not to perform strenuous activity or consume caffeine or alcohol. Participant adherence to these requirements was verbally confirmed before each trial. In addition, on the day before each experimental trial, participants were provided with, and consumed a standardised evening meal at home between 7pm and 8pm that included fusilli pasta, pasta sauce, cheddar cheese, milk and jelly beans (1037 kcal, 57% carbohydrate, 28% fat, 15% protein). This meal was consumed to minimise the possibility of a second meal effect confounding glycaemic control or any other measured variables (Wolever et al. 1988; Stevenson et al. 2005).

Experimental trials

Participants arrived at the research facilities following a 12 hour fast and entered the environmental chamber at 8am. Verbal confirmation that participants had fasted for the previous 12 hours was obtained prior to commencing each trial. Participants then rested for an hour. During rest periods, participants were seated upright and permitted to undertake personal activities such as reading. At 1 hour, in both the normobaric hypoxia and normoxia breakfast consumption trials, participants were allowed 15 minutes to consume a standardised breakfast (535 kcal, 58% carbohydrate, 24% fat, 18% protein). This meal included rolled oats, semi-skimmed milk and orange juice, and was designed to replicate typical breakfast consumption

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212 in the UK (Reeves et al. 2013). At 1 hour in the normobaric hypoxia and normoxia breakfast
213 omission trials, participants continued resting for 15 minutes, without the consumption of
214 breakfast. At 1 hour 15 minutes, participants in all trials rested for a further hour. At 2 hours
215 15 minutes, participants completed a 1-hour walking test (20 minutes at 40%, 50% and 60%
216 $\dot{V}O_{2max}$) at a 10% gradient, carrying a 10kg backpack, to mimic the demands of high altitude
217 trekking (Mellor et al. 2017). Participants then rested for 30 minutes after exercise. Water was
218 allowed ad-libitum throughout all trials. See Figure 1 for a schematic representation of the
219 experimental trials.

220 Measurements

221 Heart rate, capillary oxygen saturation and RPE

222 Heart rate and capillary oxygen saturation were measured using a fingertip pulse oximeter
223 (Nellcor PM10N, United States) every 15 minutes during rest. Heart rate, capillary oxygen
224 saturation and RPE were measured every 10 minutes throughout exercise.

225 Expired breath analysis

226 Expired gas breath samples were collected using an online gas analysis system (Metalyser,
227 Cortex, Germany) for two 10-minute resting periods in the first hour (pre-prandial) of exposure
228 (5 – 15 minutes and 35-45 minutes). In the hour following breakfast consumption or omission
229 (post-prandial), a further two 10-minute resting periods of expired gas breath samples were
230 collected (1 hour 20 minutes – 1 hour 30 minutes and 1 hour 50 minutes – 2 hours). In addition,
231 these measurements were made continuously throughout the 1-hour walking protocol, and for
232 the final 10 minutes in the 30 minutes post exercise period. Participants were fitted with a
233 facemask by researchers 5 minutes prior to the collection period whilst the participant was
234 seated. At the end of the collection period, participants were asked to remove the facemask to
235 minimise unnecessary opening and closing of the chamber door. This approach (5 minutes prior

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to collection and 10 minutes collection period) has demonstrated reliability previously (correlation coefficient: 0.8) (Spaeth et al. 2015). Coefficient of variation values from the pre-prandial period in the normoxia and normobaric hypoxia trials in the present study (repeated measurements under same conditions) were within 4-9% as recommended by Skinner et al. (1999) (normoxia: $VO_2$ = 6.2%, $VCO_2$ = 5.2%; normobaric hypoxia: $VO_2$ = 4.4%, $VCO_2$ = 5.2%). Substrate oxidation was calculated using relevant equations for both resting (Frayn 1983) and exercise periods (Jeukendrup and Wallis 2005).

Blood sampling

Researchers entered the chamber to draw venous blood samples from a 20-gauge cannula (Introcan Safety; B Braun, Sheffield, UK) which was inserted into an antecubital vein upon arrival. Samples were drawn at baseline (before entry to the chamber) for the analysis of plasma glucose, plasma lactate, serum FFA and serum insulin. Subsequent blood samples variables were drawn at 1-hour (pre-prandial), 2-hours 15 minutes (post-prandial), 2-hours 35 minutes (40% $\dot{V}O_{2max}$), 2-hours 55 minutes (50% $\dot{V}O_{2max}$), 3-hours 15 minutes (60% $\dot{V}O_{2max}$) and 3-hours 45 minutes (post exercise) (no insulin at this time point due to plate layout). For samples in close proximity (i.e. during exercise), researchers stayed in the chamber to avoid unnecessary opening and closing of chamber doors. Fluoride oxalate tubes used for plasma glucose and lactate, were spun at 1500 x g for 10 minutes in a centrifuge (CompactStar CS4, VWR) immediately after being filled with venous blood. The serum separator tubes used for FFA and insulin were spun at the same speed, 30 minutes after collection to allow for clotting. The supernatant was then transferred into separate Eppendorf tubes to be frozen immediately at -20 ˚C before being transferred to -80 ˚C until analysis.

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Blood analysis

Commercially available enzyme-linked immunosorbent assay kits were used to determine serum concentrations of insulin (IBL, Hamburg, Germany). To eliminate interassay variation, all samples from each participant were analysed on the same plate. Plasma glucose and lactate, and serum FFA were measured photometrically with reagents from Instrumentation Laboratories (Lexington, MA) and Randox Laboratories (Crumlin, UK). The within batch coefficients of variation were as follows: insulin 5.9%, glucose 1.8%, lactate 2.8% and FFA 3.7%.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) in text and mean ± standard error (SE) in figures. All data were analysed using IBM SPSS statistics (v24 for Windows; SPSS; Chicago, IL). The trapezoid method was used to calculate area under the curve (AUC) for substrate oxidation and hormone concentrations. The periods of AUC were defined as pre-prandial (0 – 1 hour), post-prandial (1-hour 15 minutes – 2-hour 15 minutes), 40% relative $\dot{V}O_{2max}$ (2-hour 15 minutes – 2-hour 35 minutes), 50% relative $\dot{V}O_{2max}$ (2-hour 35 minutes – 2-hour 55 minutes), 60% relative $\dot{V}O_{2max}$ (2-hour 55 minutes – 3-hour 15 minutes) and post exercise (3-hour 15 minutes – 3-hour 45 minutes). Normality of distribution was evaluated using histograms and Shapiro-Wilk test and approximated normal distribution. A paired sample t-test was used to determine differences between $\dot{V}O_{2max}$ in normoxic and hypoxic conditions. One-way repeated measures ANOVA was used to determine differences between trials for energy expenditure, heart rate, capillary oxygen saturation and RPE. Two-way repeated measures ANOVA (time x trial) was used to determine differences between absolute and relative carbohydrate and fat oxidation and hormone concentrations between AUC periods. Where significant main effects of trial were found, further post-hoc analysis was performed

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using Bonferonni correction for multiple comparisons. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > very large and > 4 extremely large (Hopkins 2004).

## Results

### Maximal oxygen uptake and walking speeds

$\dot{V}O_{2max}$ was significantly reduced in hypoxia compared with normoxia (38.3 ± 6.0 mL·kg$^{-1}$·min$^{-1}$ vs. 53.0 ± 8.6 mL·kg$^{-1}$·min$^{-1}$; $p < 0.001$, $d = 2.00$). In hypoxia, this elicited walking speeds of 1.8 ± 0.4 km·h$^{-1}$ (HB: 40.3 ± 4.1%; HF: 38.9 ± 3.1% $\dot{V}O_{2max}$), 2.7 ± 0.5 km·h$^{-1}$ (HB: 47.8 ± 3.3% $\dot{V}O_{2max}$; HF: 48.0 ± 4.4% $\dot{V}O_{2max}$;) and 3.5 km·h$^{-1}$ (HB: 59.6 ± 5.9% $\dot{V}O_{2max}$; HF: 59.1 ± 5.0% $\dot{V}O_{2max}$). In normoxia, this elicited walking speeds of 3.4 ± 0.3 km·h$^{-1}$ (NB: 38.4 ± 3.4% $\dot{V}O_{2max}$; NF: 38.1 ± 3.9% $\dot{V}O_{2max}$), 4.1 ± 0.4 km·h$^{-1}$ (NB: 45.8 ± 3.3% $\dot{V}O_{2max}$; NF: 45.1 ± 2.9% $\dot{V}O_{2max}$) and 4.6 ± 0.5 km·h$^{-1}$ (NB: 61.4 ± 2.5% $\dot{V}O_{2max}$; NF: 61.3 ± 3.3% $\dot{V}O_{2max}$) for each 20-minute exercise period. Relative exercise intensity was not significantly different between any trial at 40% ($p = 0.39$), or 60% $\dot{V}O_{2max}$ ($p = 0.18$) however, a trend for an increased relative exercise intensity in hypoxia compared with normoxia after breakfast omission was observed at 50% $\dot{V}O_{2max}$ ($p = 0.06$).

### Experimental trials

#### Energy expenditure

Energy expenditure at rest was significantly greater in hypoxia compared with normoxia in both the breakfast consumption (1252 ± 158 kJ vs. 1108 ± 145 kJ; $p = 0.02$, $d = 0.95$) and breakfast omission trials (1349 ± 250 kJ vs. 1053 ± 140 kJ; $p = 0.001$, $d = 1.52$). Energy

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expenditure at rest was not significantly different between breakfast consumption and omission in hypoxia ($p$ = 0.66, $d$ = 0.38) or normoxia ($p$ = 0.49, $d$ = 0.47).

Energy expenditure during exercise was significantly reduced in hypoxia compared with normoxia after both breakfast consumption (1809 ± 218 kJ vs. 2477 ± 205 kJ, $p < 0.001$, $d$ = 3.16) and omission (1734 ± 223 kJ vs. 2425 ± 262 kJ, $p < 0.001$, $d$ = 2.83). Energy expenditure during exercise was not significantly different between breakfast consumption and omission in hypoxia ($p$ = 0.34, $d$ = 0.22) and normoxia ($p$ = 0.99, $d$ = 0.33).

Pre-prandial Carbohydrate and Fat Oxidation

In the pre-prandial period, absolute (Table 1) carbohydrate oxidation ($p \geq 0.11$, $d \leq 0.86$) and its relative contribution to energy expenditure were not significantly different between trials (HB: 43.8 ± 16.8%, HF: 40.8 ± 24.0%, NB: 34.8 ± 16.2%, NF: 38.4 ± 15.5%, $p \geq 0.63$, $d \leq 0.54$). In the same period, absolute ($p$ = 0.99, $d \leq 0.42$, Table 1) and relative contributions of fat oxidation were not significantly different between trials (HB: 56.2 ± 16.8%, HF: 59.2 ± 24.0%, NB: 65.2 ± 16.2%, NF: 61.6 ± 15.5%, $p \geq 0.63$, $d \leq 0.54$).

Post-prandial Carbohydrate and Fat Oxidation

In the post-prandial period, absolute carbohydrate oxidation (Table 1) was significantly higher after breakfast consumption, compared with breakfast omission in hypoxia (absolute: $p < 0.001$, $d$ = 1.92) and normoxia ($p$ = 0.04, $d$ = 1.25). In addition, the relative contribution of carbohydrate oxidation was significantly higher after breakfast consumption, compared with breakfast omission in hypoxia (46.2 ± 14.1% vs. 19.6 ± 14.9%, $p < 0.001$; $d$ = 1.84), but not normoxia (45.5 ± 15.4% vs. 29.6 ± 16.9%, $p$ = 0.14, $d$ = 0.99). Absolute carbohydrate oxidation was not significantly different between hypoxia and normoxia after breakfast consumption ($p$ = 0.50, $d$ = 0.56) or omission ($p$ = 0.99, $d$ = 0.20). The relative contribution of carbohydrate

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oxidation was not significantly different between hypoxia and normoxia after breakfast consumption ($p$ = 0.99, $d$ = 0.05) or omission ($p$ = 0.56, $d$ = 0.63).

In the same period, absolute fat oxidation was significantly higher after breakfast omission, compared with consumption in hypoxia ($p$ < 0.01, $d$ = 1.72) but not normoxia ($p$ = 0.99, $d$ = 0.49). In addition, the relative contribution of fat oxidation was significantly higher after breakfast omission, compared with consumption in hypoxia (80.4 ± 14.9 vs. 53.8 ± 14.1, $p$ < 0.001, $d$ = 1.84) but not normoxia (70.4 ± 16.9 vs. 54.5 ± 15.4, $p$ = 0.14, $d$ = 0.99). Absolute fat oxidation was significantly higher in hypoxia compared with normoxia after breakfast omission ($p$ < 0.01, $d$ = 1.72) but not consumption ($p$ = 0.48, $d$ = 0.68). The relative contribution of fat oxidation was not significantly different between hypoxia and normoxia after breakfast omission ($p$ = 0.56, $d$ = 0.63), or consumption ($p$ = 0.99, $d$ = 0.05).

[Insert Table 1]

Exercise Carbohydrate and Fat Oxidation

During exercise at all intensities, absolute (Table 1) carbohydrate oxidation was significantly lower in hypoxia compared with normoxia after breakfast omission (40% $p$ < 0.01, $d$ = 1.49; 50% $p$ = 0.01, $d$ = 1.27; 60% $p$ = 0.001, $d$ = 1.96). Absolute carbohydrate oxidation was also significantly lower in hypoxia compared with normoxia after breakfast consumption at 60% $\dot{V}O_{2max}$ ($p$ = 0.02, $d$ = 1.42), and approached significance at 50% $\dot{V}O_{2max}$ ($p$ = 0.06, $d$ = 1.00), but not at 40% $\dot{V}O_{2max}$ ($p$ = 0.71, $d$ = 0.71). The relative contribution of carbohydrate (with the exclusion of 50% $\dot{V}O_{2max}$) was significantly lower in hypoxia, compared with normoxia after breakfast omission (40% $p$ = 0.03; $d$ = 0.98; 50% $p$ = 0.07, $d$ = 0.83; 60% $p$ = 0.03, $d$ = 0.98) but not breakfast consumption at any intensity (all $p$ = 0.99, $d \leq$ 0.42). Absolute carbohydrate oxidation was significantly higher after breakfast consumption compared with omission at all intensities in hypoxia (40%: $p$ = 0.02, $d$ = 1.14; 50%: $p$ = 0.001, $d$ = 1.30; 60%: $p$ < 0.01, $d$ =

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353 1.24) but not normoxia ($p \geq 0.09$, $d = 0.68$). The relative contribution of carbohydrate oxidation
354 was significantly higher after breakfast consumption, compared with omission at all intensities
355 in hypoxia (40%: $p < 0.01$, $d = 1.20$; 50%: $p < 0.01$, $d = 1.32$; 60% $p = 0.01$, $d = 1.28$), but not
356 in normoxia (all $p \geq 0.28$, $d \leq 0.69$).

357 During exercise, absolute fat oxidation (Table 1) was not significantly different between
358 hypoxia and normoxia at any exercise intensity after breakfast consumption ($p \geq 0.14$, $d \leq 1.12$)
359 or omission (all $p = 0.99$, $d \leq 0.43$). The relative contribution of fat oxidation (with the
360 exclusion of 50% $\dot{V}O_{2max}$) (Figure 2) was significantly higher in hypoxia compared with
361 normoxia after breakfast omission (40%: $p = 0.03$, $d = 0.98$; 50%: $p = 0.07$; $d = 0.83$; 60%: $p$
362 $= 0.03$, $d = 0.98$) but not at any intensity after breakfast consumption (all $p = 0.99$, $d \leq 0.42$).
363 In addition, absolute fat oxidation was significantly higher at all exercise intensities after
364 breakfast omission compared with consumption in hypoxia (40%: $p = 0.04$, $d = 0.93$; 50%: $p$
365 $= 0.02$, $d = 1.12$; 60%: $p = 0.02$, $d = 1.24$) but not normoxia ($p \geq 0.58$, $d \leq 0.68$). The relative
366 contribution of fat oxidation was significantly higher at all exercise intensities after breakfast
367 omission compared with consumption in hypoxia (40%: $p < 0.01$, $d = 1.20$; 50%: $p < 0.01$, $d =$
368 1.32; 60%: $p = 0.01$, $d = 1.28$), but not normoxia ($p \geq 0.28$, $d \leq 0.69$).

369 [Insert Figure 2]

370 Post-exercise Carbohydrate and Fat Oxidation

371 In the post-exercise period, absolute carbohydrate oxidation was not significantly different
372 between trials ($p \geq 0.12$, $d \leq 0.92$). The relative contribution of carbohydrate oxidation was
373 also not significant between trials (HB: 19.9 ± 19.8%, HF: 6.6 ± 10.0%, NB: 30.01 ± 21.2%,
374 NF: 19.7 ± 15.5%, $p \geq 0.10$, $d \leq 1.02$). In the same period, absolute fat oxidation was
375 significantly higher after breakfast omission compared with consumption in normoxia ($p =$
376 0.001, $d = 1.97$), but not hypoxia ($p = 0.85$, $d = 0.71$). Absolute fat oxidation was not

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significantly different between hypoxia and normoxia after breakfast consumption ($p = 0.10$, $d = 1.10$) or omission ($p = 0.99$, $d = 0.28$). The relative contribution of fat oxidation was not significantly different between any trial (HB: 80.1 ± 19.8%, HF: 93.4 ± 10.0%, NB: 69.9 ± 21.2%, NF: 80.3 ± 15.5%, $p \geq 0.10$, $d \leq 1.02$).

Blood biochemistry

A significant effect of time (all $p < 0.01$) and trial (all $p < 0.01$) was observed for all analytes. Further, a significant interaction effect of time x trial was also observed for all analytes (all $p \leq 0.03$). All significant pairwise statistical comparisons are presented in Figure 3.

[Insert Figure 3)

Heart rate, capillary oxygen saturation and RPE

Capillary oxygen saturation, heart rate and RPE scores for the duration of the experimental trial are presented in Table 2. There were no significant differences between trials for heart rate ($p \geq 0.14$, $d \leq 0.96$) and RPE ($p \geq 0.86$, $d \leq 0.21$). Capillary oxygen saturation was significantly lower in hypoxia compared with normoxia in both the breakfast consumption and omission trials ($p < 0.01$, d = 7.83) ($p < 0.01$, d= 9.63).

[Insert Table 2]

Discussion

This study investigated the effect of breakfast consumption and exercise intensity on substrate oxidation in hypoxia compared with normoxia during both rest and exercise. In this regard, the relative carbohydrate contributions to energy expenditure decrease, while relative fat contributions increase, during exercise matched for relative intensities in hypoxia compared with normoxia after breakfast omission, but *not* consumption. This effect of breakfast consumption in hypoxia compared with normoxia appears to be exclusive to exercise, with no

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differences in relative substrate oxidation between hypoxia and normoxia after breakfast consumption or omission at rest. Higher exercise intensities did not potentiate carbohydrate oxidation in hypoxia, compared with normoxia after either breakfast consumption or omission.

Absolute carbohydrate oxidation was significantly lower in hypoxia compared with normoxia during exercise at all intensities after breakfast omission. It has long been established that hypoxia induces a lower $\dot{V}O_{2max}$ than normoxia (Dill et al. 1931), which subsequently elicits lower absolute workloads during exercise matched to relative intensities in hypoxia, compared with normoxia (Lundby and Van Hall 2002). As such, the reduction in absolute carbohydrate oxidation in hypoxia, compared with normoxia after breakfast omission is likely due in part to the reduced energy expenditure during exercise in hypoxia. Interestingly, this effect was less pronounced during exercise after breakfast consumption in hypoxia compared with normoxia (significance at 60% $\dot{V}O_{2max}$ only and approaching significance at 50% $\dot{V}O_{2max}$). However, due to the confounding factor associated with utilising absolute substrate oxidation, the use of substrate oxidation data relative to total energy expenditure is warranted to determine an effect of hypoxia beyond a reduced absolute workload.

A novel finding of this study is the lower relative contribution of carbohydrate and higher relative contribution of fat oxidation observed during exercise matched for relative intensities in hypoxia, compared with normoxia after breakfast omission. This finding is in agreement with some existing literature utilising overnight fasted male participants in acute hypoxia (O'Hara et al. 2017) but not others (Katayama et al. 2010; Morishima et al. 2014). O'Hara et al. (2017) observed an increased relative contribution of fat to energy expenditure during exercise matched to relative intensities (74% $\dot{V}O_{2max}$) in hypoxia compared with normoxia. It was proposed that this may in part, be associated with augmented lipolysis and therefore FFA

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oxidation. The increased rates of lipolysis were supported by elevated concentrations of metanephrine and normetanephrine, as well as a subsequent increase in circulating FFA. This effect was observed despite consumption of a carbohydrate drink in both conditions (1.2 g·min$^{-1}$ glucose, 0.6 g·min$^{-1}$ fructose), demonstrating the potency of being fasted during exercise in hypoxia. In the present study, no significant differences in FFA concentrations were observed between hypoxia and normoxia after breakfast consumption or omission. This suggests that the higher relative contribution of fat oxidation observed in hypoxia, compared with normoxia after breakfast omission in the present study may not be associated with increased lipolysis of triglycerides stored in adipose tissue. As such, it seems plausible that an increased oxidation of intramuscular trigylcerides, which would not influence circulating FFA concentrations, may contribute to the increased relative contribution of fat oxidation during exercise after breakfast omission in hypoxia, compared with normoxia.

At the mitochondrial level, it has been proposed that the increased relative contribution of fat oxidation to energy expenditure in hypoxia may be a result of increased expression of the transcription factor HIF-1α and the upregulation of PPARα (Aragones et al. 2008). Specifically, PPARα has been demonstrated to deactivate pyruvate dehydrogenase (Huang et al. 2002), inhibiting the conversion of pyruvate to acetyl-coA and therefore enabling greater mobilisation and oxidation of fat stores (Spriet and Watt 2003). Subsequently, pyruvate may then be shunted towards lactate production and away from oxidative metabolism. Logically, the reduced relative carbohydrate oxidation observed after breakfast omission in hypoxia, compared with normoxia may be associated with a potentiated PPARα response induced by fasting (König et al. 1999). Therefore, as expected, increased lactate concentrations were observed after breakfast omission in hypoxia, compared with normoxia however, this effect was also evident after breakfast consumption. *Albeit*, lactate concentrations in the fed state may also be inflated by the metabolism of fructose, derived from the consumption of orange juice

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during breakfast. Specifically, fructose metabolism can occur without the rate limiting step of glycolysis (catalysed by phosphofructokinase) and is therefore rapidly phosphorylated leading to increased rates of glycolysis and elevated plasma lactate concentrations (Jentjens et al. 2004; Tappy 2018).

In contrast to findings from our recent meta-analysis (Griffiths et al. 2019), we observed no significant change in the relative contribution of carbohydrate or fat to energy expenditure during exercise matched for relative intensities in hypoxia, compared with normoxia after breakfast consumption. This finding is in accordance with a number of studies investigating substrate oxidation during exercise matched to relative intensities in hypoxia, compared with normoxia (Lundby and Van Hall 2002; Young et al. 1982). However, it is in contrast to those who observed an increased relative contribution of carbohydrate (Péronnet et al. 2006; Friedmann et al. 2004) and fat (Matu et al. 2017; Braun et al. 2000) to energy expenditure in the same conditions. The variance in the literature regarding the use of fed participants is difficult to explain but may be due to the numerous differing experimental characteristics such as carbohydrate supplementation and the sex of participants. The isolation of each of these characteristics in randomised control trials is required to further understand their influence on substrate oxidation in hypoxia.

Absolute carbohydrate oxidation was significantly higher in the post-prandial period after breakfast consumption compared with omission in both hypoxia and normoxia. Interestingly, the increased absolute carbohydrate oxidation observed after breakfast consumption in hypoxia and normoxia was associated with lower plasma glucose concentrations than breakfast omission. This effect remained significant at 40% and 50% $\dot{V}O_{2max}$ in normoxia and approached significance at 40% $\dot{V}O_{2max}$ in hypoxia. This is likely due to the synergistic effect of augmented insulin concentrations and skeletal muscle contraction during exercise on GLUT-4 trafficking, subsequently inducing an alteration from fat to carbohydrate metabolism (Geiger

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et al. 2006). Evidence from Edinburgh et al. (2018) suggests that the upregulation of carbohydrate metabolism induced by insulin secretion in the post-prandial state is not solely matched by the subsequent glucose delivery to the muscle, and that for a period before feeding-derived carbohydrate entering the blood stream, muscle glycogen stores are also utilised. Taylor et al. (1993) also observed a reduction in muscle glycogen concentrations one-hour post meal, before increasing again in the subsequent hours (1-7 hours). Whilst we cannot confirm this in the present study, this may have implications for the timing of breakfast before exercise, and also provide a consideration for an additional exogenous carbohydrate source during exercise if the primary goal is to spare muscle glycogen and improve performance.

Alterations in substrate oxidation between hypoxia and normoxia after both breakfast consumption and omission were consistent across varying exercise intensities, suggesting that increasing exercise intensities may not potentiate carbohydrate oxidation in hypoxia, as previously proposed (Griffiths et al. 2019). However, the range of exercise intensities used in the present study were low to moderate intensity (40-60% $\dot{V}O_{2max}$) and therefore may not have been sufficient to potentiate sympathetic nervous system activity and glycogenolysis, as plasma epinephrine concentrations have been demonstrated to increase exponentially with exercise intensities increasing up to 85% $\dot{V}O_{2max}$ (Romijn et al. 1993).

The finding that energy expenditure at rest was significantly higher in normobaric hypoxia compared with normoxia is consistent with numerous other studies (Butterfield et al. 1992, Matu et al. 2017). An increase in resting energy expenditure and/or a reduction in energy intake has been associated with a negative energy balance and therefore weight loss during chronic hypoxic exposure (Armellini et al. 1997, Sergi et al. 2010). The increase in resting energy expenditure has been attributed to the elevated cardiovascular and ventilatory responses experienced during hypoxic exposure (Butterfield 1999). The reduction in energy intake observed in hypoxia is likely explained by impaired appetite regulation, a result of the hypoxic-

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induced suppression of the orexigenic hormone, acylated ghrelin (Debevec 2017; Matu et al. 2017). Whilst a reduction in body mass may induce debilitating effects on performance for high altitude mountaineers and military personnel alike, this finding may have implications for weight loss strategies in obese populations (Kayser and Verges 2013). The finding that energy expenditure during exercise was reduced in normobaric hypoxia compared with normoxia substantiates previous research (Matu et al. 2017), and is likely due to the reduced absolute workload in hypoxia. However, this reduced workload in hypoxia has been suggested to induce a similar metabolic load to normoxia, therefore facilitating physical activity adherence while reducing the risk of musculoskeletal injury in obese individuals (Girard et al. 2017).

Despite the novel findings of this study, some notable limitations must be acknowledged. The use of muscle biopsies to investigate a key theory involving PPARα was beyond the scope of this study and as such, a physiological explanation for the changes in substrate oxidation in the fasted state could not be confirmed. Further, whilst we could calculate whole body substrate oxidation, muscle/liver glycogen oxidation could not be analysed without muscle biopsies or tracer derived methods. In addition, the present study only investigated changes in young men, therefore caution should be applied when applying the results to other populations. For example, women have been shown to elicit differing metabolic responses to hypoxia, when compared with men (Braun et al. 2000). Finally, the use of a normobaric hypoxic chamber in the present study should be acknowledged when considering the practical applicability of these findings to terrestrial altitude. Future research should investigate the effect of nutritional strategies (i.e. carbohydrate supplementation) in hypoxia after both breakfast consumption and omission to determine their effect on substrate oxidation and performance. In addition, it would be necessary to investigate substrate oxidation during exercise across a broader range of exercise intensities to determine if high intensity exercise induces different substrate oxidation responses than low intensity exercise when performed in hypoxia compared with normoxia.

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## Conclusions

In conclusion, we observed a reduced relative contribution of carbohydrate oxidation to energy expenditure during exercise matched for relative intensities in hypoxia, compared with normoxia after breakfast omission but no difference was observed during exercise after breakfast consumption. The effect of hypoxia on substrate oxidation was not altered with increasing exercise intensities when compared with normoxia. These data provide clarity on the current literature and may be useful in the design of nutritional strategies for high altitude mountaineers and military personnel.

**Contributions**: AG, JOH, KD and RK conceived and designed the study. AG, OS and JM collected the data. AG analysed the data and wrote the manuscript. All authors read and provided critical feedback on the manuscript before approving.

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**Conflict of interest**: The authors declare that they have no conflict of interest

**Ethical approval**: All procedures performed in studies involving human participants were in accordance with the ethical standards of Leeds Beckett University School of Sport research ethics committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent**: Informed consent was obtained from all individual participants included in the study

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547 Figures

548 **Fig. 1** Schematic of full experimental trial. Trials were completed twice in hypoxia (once
549 with breakfast consumption, once with breakfast omission) and twice in normoxia (once with
550 breakfast consumption, once with breakfast omission). Exercise involved 20 minutes at 40%,
551 50% and 60% relative $\dot{V}O_{2max}$.

552 **Fig. 2** The relative (% energy yield) contribution of carbohydrate and fat oxidation during
553 uphill walking at 40%, 50% and 60% $\dot{V}O_{2max}$ in normoxia and hypoxia after breakfast
554 consumption and omission.

555 **Fig. 3** Plasma glucose, plasma lactate, serum FFA and serum insulin concentrations over the
556 full experimental trial. Values are mean ± SE. The thin arrow represents the timing of breakfast
557 in the hypoxic and normoxic breakfast consumption trials. Breakfast was not consumed in the
558 hypoxic and normoxic fasted trials. The black rectangle represents the exercise period. (a)
559 indicates a significant difference between breakfast consumption and omission in normoxia.
560 (b) represents a significant difference between breakfast consumption and omission in hypoxia.
561 (c) indicates a significant difference between hypoxia and normoxia after breakfast
562 consumption. (d) indicates a significant difference between hypoxia and normoxia after
563 breakfast omission. Significance $p < 0.05$.

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Figure 1

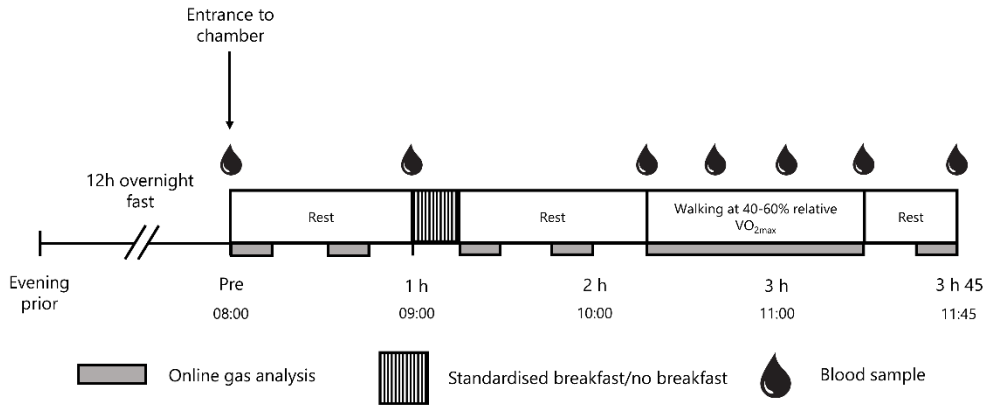


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Figure 2

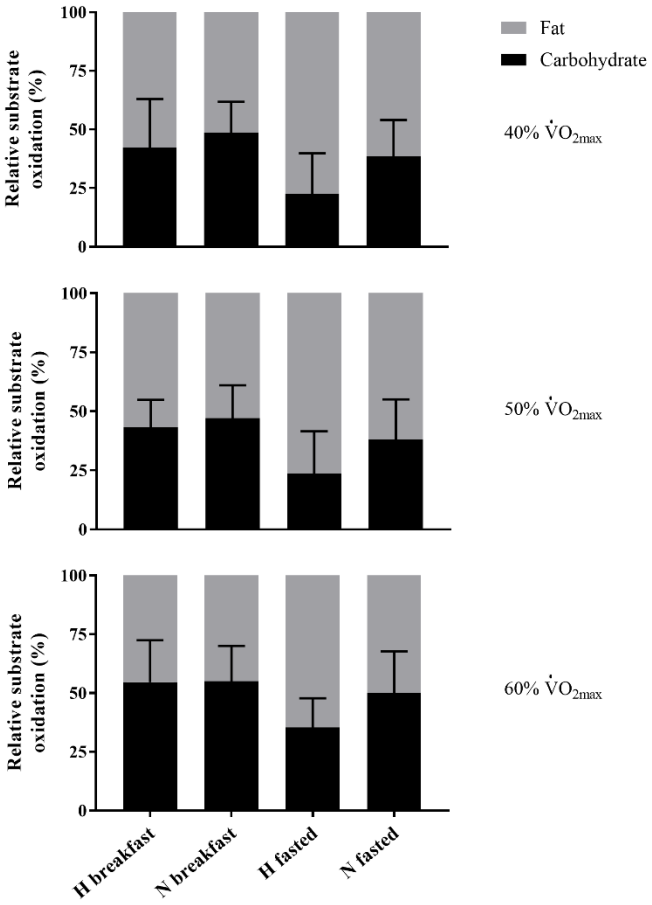


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Figure 3

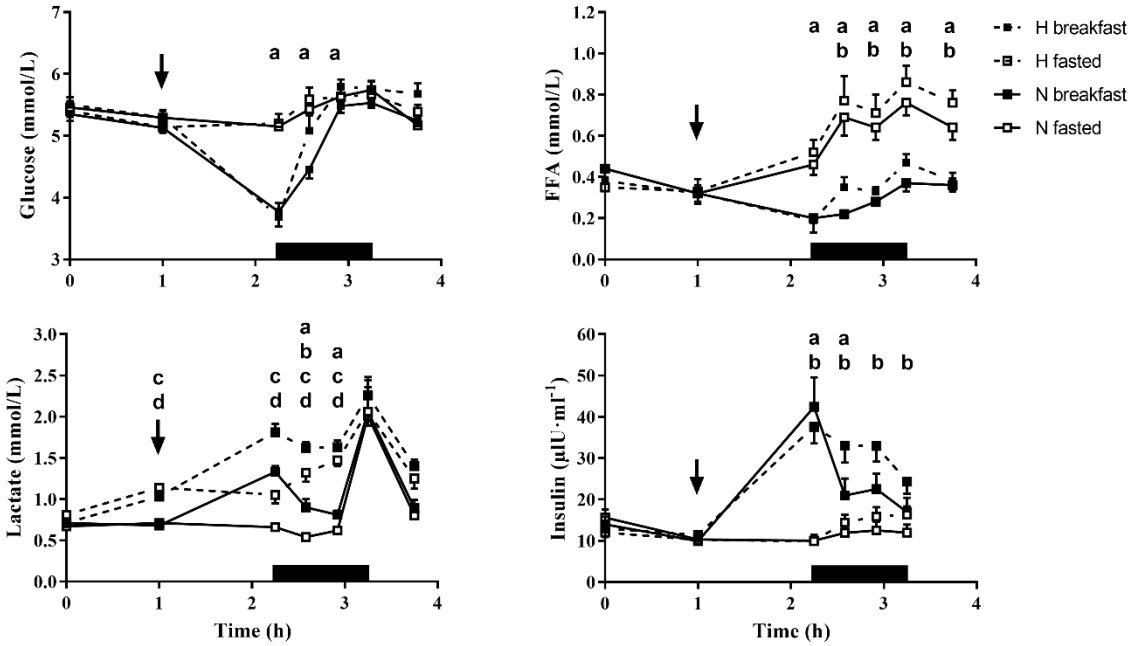


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**Table 1**. Area under the curve (AUC) values for absolute carbohydrate and fat oxidation in all trials. H = hypoxia, N = normoxia

| | Pre-prandial | | Post-prandial | | Exercise | | | | | | Post exercise | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 40% $\dot{V}O_{2max}$ | | 50% $\dot{V}O_{2max}$ | | 60% $\dot{V}O_{2max}$ | | | |
| | CHO oxidation (g) | Fat oxidation (g) | CHO oxidation (g) | Fat oxidation (g) | CHO oxidation (g) | Fat oxidation (g) | CHO oxidation (g) | Fat oxidation (g) | CHO oxidation (g) | Fat oxidation (g) | CHO oxidation (g) | Fat oxidation (g) |
| H breakfast | 12.83±5.90 | 7.20±2.16 | 16.05±5.23 | 7.63±1.55 | 13.54±8.22 | 7.33±1.54 | 15.33±5.05 | 8.53±1.43 | 21.77±6.42 | 8.90±1.81 | 3.40±3.87 | 3.68±0.94 |
| H fasted | 12.70±7.76 | 7.60±2.72 | 6.18±5.04 | 10.70±2.62 | 6.07±4.92 | 9.15±2.38 | 8.63±5.27 | 10.83±2.70 | 14.63±5.13 | 11.70±2.71 | 0.95±1.45 | 5.10±3.06 |
| N breakfast | 8.38±4.48 | 6.53±1.29 | 13.10±5.37 | 6.55±1.60 | 18.60±5.98 | 8.42±1.83 | 21.67±7.54 | 10.33±2.28 | 34.90±12.12 | 12.00±3.70 | 4.30±3.01 | 2.73±0.79 |
| N fasted | 9.40±3.64 | 6.63±1.92 | 7.10±4.20 | 7.48±2.15 | 14.47±6.33 | 9.98±2.78 | 16.62±7.28 | 11.90±3.76 | 30.72±11.27 | 13.35±4.99 | 2.53±1.94 | 4.53±1.03 |

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**Table 2.** Mean capillary oxygen saturation ($SpO_2$), heart rate and RPE in all trials. H = hypoxia and N = normoxia

| | SpO2 | Heart rate | RPE |
|---|---|---|---|
| H breakfast | 79±3 | 86±9 | 12±2 |
| H fasted | 80±4 | 88±21 | 12±2 |
| N breakfast | 79±3 | 86±9 | 12±2 |
| N fasted | 97±1* | 75±13 | 12±2 |

*Denotes significance in comparison with corresponding nutritional status in hypoxia.

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