Title: Appetite and energy intake responses to breakfast consumption and carbohydrate supplementation in hypoxia

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Abstract

Purpose: The purpose of experiment one was to determine the appetite, acylated ghrelin and energy intake response to breakfast consumption and omission in hypoxia and normoxia. Experiment two aimed to determine the appetite, acylated ghrelin and energy intake response to carbohydrate supplementation after both breakfast consumption and omission in hypoxia. Methods: In experiment one, 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. In experiment two, eleven participants rested and exercised in normobaric hypoxia (4300 m: F_iO_2 ~11.7%), twice after consuming a high carbohydrate breakfast and twice after breakfast omission. Participants consumed both a carbohydrate (1.2g·min-1 glucose) and a placebo beverage after breakfast consumption and omission. Measures of appetite perceptions and acylated ghrelin were taken at regular intervals throughout both experiments and an *ad-libitum* meal was provided post-exercise to quantify energy intake. Results: Breakfast consumption had no significant effect on post exercise energy intake or acylated ghrelin concentrations, despite reductions in appetite perceptions. As such, breakfast consumption increased total trial energy intake compared with breakfast omission in hypoxia (7136 ± 2047 kJ vs. 5412 ± 1652 kJ; $p = 0.02$) and normoxia (9276 ± 3058 vs. 6654 ± 2091 kJ; $p < 0.01$). Carbohydrate supplementation had no effect on appetite perceptions or acylated ghrelin concentrations after breakfast consumption or omission. As such, carbohydrate supplementation increased total energy intake after breakfast consumption (10222 ± 2831 kJ vs. 7695 ± 1970 kJ $p < 0.01$) and omission (8058 ± 2574 kJ vs. 6174 ± 2222 kJ $p = 0.02$). Conclusion: Both breakfast consumption and carbohydrate supplementation provide beneficial dietary interventions for increasing energy intake in hypoxic conditions.

Key words: altitude, ghrelin, feeding, nutrition

Abbreviations

AUC = area under the curve

B-CHO = breakfast consumption and carbohydrate supplementation

B-PLA = breakfast consumption and placebo

F-CHO = breakfast omission and carbohydrate supplementation

F_iO_2 = fraction of inspired oxygen

F-PLA = breakfast omission and placebo

GOAT = Ghrelin-O-Acyl-Transferase

MCFA = medium chain fatty acid

P_iO_2 = partial pressure of inspired oxygen

RPE = rating of perceived exertion

SD = standard deviation

SE = standard error

SpO_2 = peripheral oxygen saturation

VAS = visual analogue scale

CAS = composite analogue scale

$\dot{V}CO_2$ = volume of expired carbon dioxide

$\dot{V}O_2$ = volume of inspired oxygen

$\dot{V}O_{2max}$ = maximal oxygen uptake

1.0 Introduction

Hypoxic exposure has been demonstrated to supress appetite perceptions and subsequently attenuate energy intake in comparison with normoxia (Matu, et al., 2017a; Wasse, et al., 2012). In addition, resting energy expenditure has been suggested in be elevated in hypoxia, compared with normoxia (Matu, et al., 2017a; Westerterp, et al., 1994). As such, chronic hypoxic exposure is often associated with a negative energy balance and concomitant weight loss (Matu, et al., 2017c; Westerterp, et al., 1992), thus contributing to the deleterious effects of high altitude on physical capability (Sergi, et al., 2010). Logically, breakfast consumption provides an opportunity to increase caloric intake prior to exercise and abate body mass loss at high altitude. However, several studies in normoxia have demonstrated that the transitory reduction in appetite after exercise may be shorter in the fasted state (Cheng, et al., 2009; Deighton, et al., 2012; Gonzalez, et al., 2013; McIver, et al., 2018) compared with the fed state. In addition, studies in normoxia have previously demonstrated an increase in compensatory feeding after breakfast omission, *albeit* without an exercise intervention (Astbury, et al., 2011; Clayton & James, 2015). Whilst this overeating effect does not typically demonstrate a greater daily energy balance in normoxia, the magnitude of this acute response remains to be elucidated in hypoxia. In addition, alternative nutritional strategies to avoid glycogen depletion (Brouns, 1992) require investigation in hypoxia after both breakfast consumption and omission.

The prolonged hunger suppression after breakfast consumption compared with omission in the post-exercise period has often been demonstrated as transient, and has not resulted in any subsequent changes in post-exercise energy intake (Deighton, et al., 2012; Gonzalez, et al., 2013; McIver, et al., 2018). However, due to the suppressive effects of hypoxia on the orexigenic hormone, acylated ghrelin (Matu, et al., 2017a; Wasse, et al., 2012), it seems plausible that appetite perceptions following exercise after breakfast consumption may be further suppressed in hypoxia, compared with normoxia. In addition, it has also been suggested

that pre-exercise nutritional status and the resultant effects on substrate metabolism may determine post-exercise appetite perception and energy intake (Hopkins, et al., 2011). In this regard, there is growing evidence to suggest that whole body carbohydrate availability, and the rate of tissue specific utilisation (influenced by exercise and/or diet) may contribute to the regulation of energy balance (Edinburgh, et al., 2018; Gonzalez, et al., 2019; Hopkins, et al., 2014). The rate of carbohydrate oxidation during a bout of exercise has been positively associated with post-exercise energy intake, thus inducing post-exercise dietary compensation (Hopkins, et al., 2014). As substrate metabolism has been shown to differ in hypoxia compared with normoxia in varying states of energy balance (Griffiths, et al., 2019a; Griffiths, et al., 2019b; O'Hara, et al., 2019; O'Hara, et al., 2017; Péronnet, et al., 2006; Young, et al., 2018), these responses may induce differing effects on appetite and energy intake in hypoxia.

A number of studies have aimed to abate the loss of body mass experienced at high altitude (Matu, et al., 2017c; Westerterp, et al., 1992) via the use of nutritional interventions to augment energy intake in hypoxia (Berryman, et al., 2018; Butterfield, et al., 1992; Kayser, et al., 1993; Matu, et al., 2017b). However, the efficacy, practicality, adherence and palatability of these interventions at high altitude are somewhat limited. Previous research regarding the use of carbohydrate supplementation during exercise as a strategy to increase energy intake in hypoxia demonstrates an efficacious, practical and palatable alternative to the aforementioned interventions (Askew, et al., 1987; Macdonald, et al., 2009). Considering the suppressive effects of glucose load on the orexigenic hormone, acylated ghrelin (Shiiya, et al., 2002) it is necessary to determine the response to carbohydrate supplementation after breakfast consumption and omission in order to identify the optimum nutritional strategy. In addition, whilst the use of participants in a negative energy balance (as per previous research) is warranted for ecological validity, this response should also be determined in fully fed participants, to determine the applicability of this nutritional intervention for wider

populations, such as athletes or military personnel and mountaineers able to maintain energy balance (i.e. start of an expedition or rapid military deployment to high altitude).

This study aimed to determine the influence of breakfast consumption and omission on subsequent energy intake, as well as the efficacy of carbohydrate supplementation on increasing energy intake in hypoxia in the fasted and fed state. Findings from this study may be used to identify the optimum nutritional strategy (or strategies) for increasing energy intake during high altitude sojourns in mountaineers and military personnel. Within this manuscript we report the findings from two experimental studies. The purpose of experiment one was to determine the appetite, acylated ghrelin and energy intake response to breakfast consumption and omission in acute normobaric hypoxia (fraction of inspired oxygen (F_iO_2) ~11.7%, 4300 m) and normoxia. The purpose of experiment two was to determine the appetite, acylated ghrelin and energy intake response to carbohydrate supplementation after both breakfast consumption and omission in hypoxia.

2.0 Methods

2.1 Experiment one

Twelve (23 ± 3 years, 181.1 ± 6.4 cm, 79.8 ± 13.1 kg) physically active, healthy males provided written, informed consent prior to participation. The study received institutional ethical approval and was conducted in accordance with the Declaration of Helsinki (Leeds Beckett research ethics committee, application reference 32098). All participants were non-smokers, normotensive, and were free from diabetes, thyroid disorder, and sickle cell trait. In addition, participants did not possess any 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 of <500 m. Participants recorded their food intake for the 24 hours before each experimental trial and were instructed to replicate this for each subsequent trial. During this time participants were asked not to perform strenuous activity or consume caffeine or alcohol.

2.1.2 Experimental design

Experiment one was part of a larger study investigating substrate oxidation in hypoxia (Griffiths, et al., 2019a) but the methods included in this manuscript specifically relate to appetite and energy intake responses, which have not been published previously. participants completed sub-maximal and maximal exercise tests in both normobaric hypoxia (F_iO_2 ~11.7%, 4300 m) and normoxia (F_iO_2 ~ 20.93%) to calculate walking speeds required to elicit 40%, 50% and 60% maximal oxygen uptake ($\dot{V}O_{2max}$) relative to each environmental condition for the experimental trials. These two preliminary trials were separated by ≥48 hours and conducted in a single-blind randomised fashion. Participants then completed four 4-hour, 5-minute experimental trials, which included a 2-hour 15 minutes rest period, followed by a 1-hour incremental walking protocol (20 minutes at each intensity), a 30-minute post exercise rest period followed by an *ad-libitum* meal (Figure 1). All trials were conducted in an environmental chamber (TISS, Alton, UK and Sporting Edge, Sheffield on London, UK). Two of the trials were performed in 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), and two were performed in normoxia. One trial within each environmental condition involved breakfast consumption (see experimental trials), and one involved breakfast omission. These visits were separated by ≥7 days and were randomised independent of the preliminary trials, using a Latin Square design.

2.1.3 Preliminary trials

In experiment one, participants completed a preliminary trial involving a sub-maximal and maximal exercise test in both hypoxia and normoxia, to determine walking speeds for experimental trials in each environmental condition. In hypoxia, the sub-maximal phase involved four, 3-minute stages walking at 1.5 km/h, 2.5km/h, 3.5 km/h and 4.5 km/h at a 10% gradient throughout. In normoxia, participants walked at 3 km/h, 4 km/h, 4.5 km/h and 5.5 km/h. The initial two walking speeds were performed at a 10% gradient and the second two at a 15% gradient. Participants walked carrying a 10 kg backpack in both conditions. Lower speeds and gradients were used in normobaric hypoxia based on the reduced $\dot{V}O_{2max}$ elicited in hypoxia (Dill, et al., 1931), and the need for all participants to achieve 40 - 60% $\dot{V}O_{2max}$ during the 12-minute trial. The higher gradient utilised in normoxia was employed to ensure participants achieved 60% $\dot{V}O_{2max}$ with a walking gait.

Following completion of the sub-maximal phase, participants then rested for approximately 5 minutes, after which the maximal phase commenced. Participants ran without a rucksack, at a 1% gradient (Jones & Doust, 1996) at a constant speed dependant on fitness, aiming for a perceived exertion of 12. The gradient was increased by 1% every minute until volitional exhaustion. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measurements were made throughout both phases of the test using an online gas analysis system (Metalyser, Cortex, Germany), which was calibrated following the manufacturer's instructions. All participants were deemed to reach a 'true' $\dot{V}O_{2max}$ by fulfilment of $\geq$2 of the following criteria: a plateau in $\dot{V}O_2$ in the final exercise stage (Taylor, et al., 1955), respiratory exchange ratio $\geq$1.15 (Issekutz, et al., 1962), heart rate within 10 $b \cdot min^{-1}$ of age predicted maximum (220-age), rating of perceived exertion (RPE) $\geq$19 and/or blood lactate $\geq$8 mM (Midgley, et al., 2007; Shannon, et al., 2016).

2.1.4 Experimental trials

The evening prior to each experimental trial, participants consumed a standardised evening meal between 7pm and 8pm that included fusilli pasta, pasta sauce, cheddar cheese, milk and jelly beans (1037 kcal, 57% carbohydrate, 28% fat, 15% protein). Participants entered the environmental chamber at 8am, following an overnight fast. Participants then rested for an hour. At 1 hour, in both the normobaric hypoxia and normoxia breakfast consumption trials, participants were allowed 15 minutes to consume a standardised porridge 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 in the UK (Reeves, et al., 2013). At 1 hour in the normobaric hypoxia and normoxia breakfast omission trials, participants continued resting for 15 minutes, without the consumption of breakfast. At 1 hour 15 minutes, participants in all trials rested for a further hour. At 2 hours 15 minutes, participants completed a 1-hour walking test (20 minutes at 40%, 50% and 60% $\dot{V}O_{2max}$) at a 10% gradient, carrying a 10kg backpack, to mimic the demands of high-altitude trekking (Mellor, et al., 2017). Participants then rested for 30 minutes after exercise. Participants were then given 20 minutes to consume an *ad-libitum* meal (see *ad-libitum* meals section).

2.2 Experiment two

2.2.1 Participants

Eleven (23 ± 3 years, 178.0 ± 7.0 cm, 76.6 ± 7.0 kg) physically active, healthy males provided written, informed consent prior to participation. The study received institutional ethical approval and was conducted in accordance with the Declaration of Helsinki (Leeds Beckett research ethics committee, application reference 46180. Inclusion and exclusion criteria was replicated from experiment one, except participants in study two were all required to have a normal baseline 12 lead electrocardiogram, given emerging evidence indicating a

proarrhythmic effect of exposure to altitudes >4000m (Boos, et al., 2017). Pre-trial controls were also the same as experiment one.

2.2.2 Experimental design

Participants completed a sub-maximal and maximal exercise test to calculate walking speed required to elicit 50% $\dot{V}O_{2max}$ in normobaric hypoxia (F_iO_2 11.7%, 4300 m). Participants then completed a 4 hour 50 minutes experimental trial. This included a 1-hour 45 minute rest period, followed by a 1-hour 30 minute sub-maximal walking test (50% $\dot{V}O_{2max}$), 3 km time trial and a 30-minute post exercise rest period (Figure 2). All four trials were performed in normobaric hypoxia equivalent to 4300 m. Two trials involved pre-exercise breakfast consumption followed by ingestion of a carbohydrate or placebo beverage (B-CHO and B-PLA) throughout the 1-hour 30-minute sub-maximal walking test. The other two trials involved pre-exercise breakfast omission followed by ingestion of a carbohydrate or placebo beverage (F-CHO and F-PLA) throughout the walking test. The carbohydrate beverage trials involved ingestion of 1.2 $g \cdot min^{-1}$ (108 g) of glucose (D-glucose, Thornton and Ross LTD, Huddersfield, UK). Each beverage contained 25.7 mmol/L sodium chloride (2.25 g). These visits were separated by $\geq$ 7 days and pre-exercise nutritional status (breakfast consumption or omission) was randomised in a single blind fashion. The order of beverage ingestion was randomised in a double-blind fashion by a researcher independent to the study.

2.2.3 Preliminary trials

In experiment two, participants completed the previously described (see section 2.1.3) sub-maximal and maximal exercise test in hypoxia only, as there were no normoxic experimental trials. All participants fulfilled >2 of the criteria for $\dot{V}O_{2max}$ measurements as detailed in section 2.1.3.

2.2.4 Experimental trials

The evening prior to each experimental trial, participants consumed a standardised evening meal, *as per* experiment 1. Participants entered the environmental chamber (F_iO_2: ~11.7%, 4300 m) at 7:30am, following an overnight fast. Participants then rested for 30 minutes. At 30 minutes in the B-CHO and B-PLA trials, participants were allowed 15 minutes to consume a standardised breakfast (as per experiment one). At 30 minutes in the F-CHO and F-PLA trials, participants continued resting for 15 minutes, without the consumption of breakfast. At 45 minutes, participants in all trials rested for a further hour. At 1-hour 45 minutes participants completed a 1-hour 30 minute sub-maximal (50% $\dot{V}O_{2max}$) walking test at a 10% gradient, carrying a 10kg rucksack. Within each nutritional sub-group, one trial consumed a carbohydrate and one trial consumed a placebo beverage. Each beverage was consumed pre-exercise (600 ml) and every 15 minutes during exercise (150 ml). A total of 1.5 L of carbohydrate or placebo solution was consumed over the course of the trial. Participants then completed a self-paced 3 km time trial, however this was not the focus of this study and results are not presented herein. Following the 3 km time trial, participants rested for a further 30 minutes and then consumed an *ad-libitum* meal (see *ad-libitum* meals section).

2.3 Measurements

2.3.1 Ratings of perceived appetite

In experiment one, ratings of perceived appetite scores were recorded at baseline, pre-prandial (30 minutes and 1 hour), post-prandial (1 hour 15 minutes, 1 hour 45 minutes, 2 hour 15 minutes), exercise (2 hours 45 minutes), post-exercise (3 hours 15 minutes and 3 hours 45 minutes) and post *ad-libitum* meal (4 hours 5 minutes). In experiment two, ratings of perceived appetite scores were recorded at baseline, pre-prandial (30 minutes), post-prandial (45 minutes, 1 hour 15 minutes and 1 hour 45 minutes), exercise (2 hours 45 minutes), post-exercise (3 hours 15 minutes and 4 hours 30 minutes) and post *ad-libitum* meal (4 hours 50 minutes).

Appetite perceptions were measured using validated 100 mm visual analogue scales (VAS) (Flint, et al., 2000). Using these scales composite appetite score (CAS) was calculated using the following formula: CAS = ([hunger + prospective food consumption + (100 – fullness) + (100 – satisfaction)] / 4 (Stubbs, et al., 2000). A higher value is associated with a greater appetite sensation and subsequently a stronger motivation to eat.

2.3.2 Ad-libitum meals

An *ad-libitum* pasta meal was administered at 3-hours 45 minutes and 4-hours 30 minutes in experiment one and two respectively. The macronutrient content of the meal was designed to closely align with the UK dietary guidelines for macronutrient proportions (51% carbohydrate, 34 % fat and 15% protein). The meal consisted of penne pasta, cheddar cheese, tomato pasta sauce and olive oil (Deighton, et al., 2016).

Participants consumed the *ad-libitum* meal in isolation to prevent any social influence affecting food intake. Participants were provided with a bowl of the respective meal and this was replaced by an investigator before the participant had emptied it and with minimal interaction. No time limit was set for eating (although this did not exceed 20 minutes) and participants were instructed to eat until comfortably full before meal termination. Energy intake was determined as the weighted difference in food before and after eating, and with reference to the manufacturer's table of nutritional information. Participants were permitted to drink water *ad-libitum*.

2.3.3 Heart rate, SpO_2 and RPE

In experiment one and two, heart rate and peripheral oxygen saturation (SpO_2) were measured using a fingertip pulse oximeter (Nellcor PM10N, United States) every 15 minutes during rest. Measurements were taken for at least 20 seconds, until values had stabilised. Heart rate, SpO_2 and RPE were measured every 10 minutes throughout exercise.

2.3.4 Blood sampling

Venous blood samples were drawn from a 20-gauge cannula (Introcan Safety; B Braun, Sheffield, UK) which was inserted into an antecubital vein upon arrival. In experiment one, samples for the analysis of acylated ghrelin were drawn at baseline, 1-hour (pre-prandial), 2-hours 15 minutes (post-prandial), 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). In experiment two, samples for the analysis of acylated ghrelin were drawn at baseline, 30 minutes (pre-prandial), 1-hour 45 minutes (post-prandial), 2-hours 45 minutes (exercise (60 minutes)), 3-hours 15 minutes (exercise (90 minutes)) and post-exercise (4-hours 30 minutes). Samples were collected into a pre-cooled EDTA tube (Sarstedt, Leicester, UK). The tubes were treated on the morning of testing to minimise the degradation of acylated ghrelin, with 50 µl of a solution containing p-hydroxymercuribenzoic acid, potassium phosphate buffer and sodium hydroxide (Hosoda, et al., 2004). Tubes were spun at 1500 x g for 10 minutes in a centrifuge (CompactStar, CS4, VWR) immediately after being filled with venous blood. The supernatant was then transferred into separate Eppendorf tubes to be frozen immediately at -20 °C before being transferred to -80 °C until analysis.

2.4 Blood analysis

Commercially available enzyme-linked immunosorbent assay kits were used to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France). To eliminate interassay variation, all samples from each participant were analysed on the same plate. The within batch CV was 8.2%.

2.5 Statistical analysis

Data are expressed as mean ± standard deviation (SD) in text and mean ± standard error (SE) in figures to avoid distortion of the graphs. 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 appetite perceptions and acylated ghrelin concentration. Two-way repeated measure ANOVAs (time x trial) were used to determine differences between appetite perceptions and hormone concentrations between AUC periods in experiment one and two. One-way repeated measures ANOVAs were used to determine differences between energy intake, heart rate, SpO_2 and RPE. Where significant main effects of trial were found, further post-hoc analysis was performed using Bonferroni correction for multiple comparisons. A paired sample t test was used to determine the difference between $\dot{V}O_{2max}$ in hypoxia and normoxia in experiment one. Effect sizes were calculated as Cohen's *d* and subsequently corrected to Hedges *g* (Cumming, 2013), as a result of the small sample size in the present study ($n < 20$). As Hedges *g* is a variation of Cohen's *d* (Hedges & Olkin, 1985), values were interpreted as $\leq$ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large, as detailed by Hopkins (2004). The sample size was deemed sufficient to determine differences in CAS, acylated ghrelin and energy intake in experiment one and two. Regarding experiment one, based on previous studies in our laboratory (Matu, et al., 2017a) and an alpha value of 5%, a sample size of 12 would generate a power >80% for these variables. Regarding experiment two, based on previous studies in our laboratory (Matu, et al., 2017a) and similar previous literature (Askew, et al., 1987) a sample size of 11 would generate a power >80% for these variables.

3.0 Results

3.1 Experiment one

3.1.1 Exercise responses

$\dot{V}O_{2max}$ was significantly reduced in hypoxia compared with normoxia (38.3 ± 6.0 $mL \cdot kg^{-1} \cdot min^{-1}$ vs. 53.0 ± 8.6 $mL \cdot kg^{-1} \cdot min^{-1}$; $p < 0.001$, $g = 1.93$). Data regarding walking speeds and

relative percentage of $\dot{V}O_{2max}$ induced during the sub-maximal walking test in the present study have been reported previously (Griffiths, et al., 2019a). 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% $\dot{V}O_{2max}$ ($p = 0.06$). SpO_2, heart rate and RPE scores for the duration of the experimental trial are presented in Table 1. There were no significant differences between trials for heart rate ($p \geq 0.14$, $g \leq 0.93$) or RPE ($p \geq 0.86$, $g \leq 0.20$). SpO_2 was significantly lower in hypoxia compared with normoxia in both the breakfast consumption ($p < 0.01$, $g = 7.56$) and omission trials ($p < 0.01$, $g = 9.30$) (Table 1).

[Insert Table 1]

3.1.2 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; $p = 0.02$, $g = 0.92$) and breakfast omission trials (1349 ± 250 kJ vs. 1053 ± 140 kJ; $p = 0.001$, $g = 1.47$). Energy expenditure at rest was not significantly different between breakfast consumption and omission in hypoxia ($p = 0.66$, $g = 0.37$) or normoxia ($p = 0.49$, $g = 0.45$).

Energy expenditure during exercise was significantly reduced in hypoxia compared with normoxia after both breakfast consumption (1809 ± 218 kJ vs. 2477 ± 205 kJ, $p < 0.001$, $g = 3.05$) and omission (1734 ± 223 kJ vs. 2425 ± 262 kJ, $p < 0.001$, $g = 2.73$). Energy expenditure during exercise was not significantly different between breakfast consumption and omission in hypoxia ($p = 0.34$, $g = 0.21$) and normoxia ($p = 0.99$, $g = 0.32$).

3.1.3 Appetite perceptions

No significant differences in CAS (Figure 3A) were observed between any trial at baseline (all $p = 0.99$, $g \leq 0.30$) or in the pre-prandial period (all $p = 0.99$, $g \leq 0.12$). In the post-prandial,

exercise and post-exercise periods, CAS was significantly lower after breakfast consumption compared with omission in hypoxia (post-prandial: $p < 0.01$, $g = 2.90$, exercise: $p < 0.01$, $g = 1.93$, post-exercise: $p = 0.02$, $g = 0.54$) and normoxia (post-prandial: $p < 0.01$, $g = 2.95$, exercise: $p < 0.01$, $g = 2.37$, post-exercise: $p = 0.08$, $g = 0.62$). In the same periods, there were no significant differences in CAS between hypoxia and normoxia after breakfast consumption (trend for reduced CAS in hypoxia observed during exercise) (post-prandial: $p = 0.18$, $g = 0.45$, exercise: $p = 0.07$, $g = 0.60$, post-exercise: $p = 0.38$, $g = 0.44$) or omission (post-prandial: $p = 0.99$, $g = 0.42$, exercise: $p = 0.58$, $g = 0.51$, post-exercise: $p = 0.99$, $g = 0.26$). Immediately post meal, no significant differences in CAS were observed between any trial ($p \geq 0.40$, $g \leq 0.57$).

[Insert Figure 3]

3.1.4 Acylated ghrelin

No significant differences in acylated ghrelin concentration (Figure 4A) were observed between any trial at baseline (all $p = 0.99$, $g \leq 0.10$), or in the pre-prandial period (all $p = 0.99$, $g \leq 0.13$). In the post-prandial period, acylated ghrelin concentration tended to be lower in the breakfast consumption compared with omission trials in hypoxia ($p = 0.08$, $g = 0.51$) and normoxia ($p = 0.06$, $g = 0.58$). In the same period, no significant difference was observed in acylated ghrelin concentration between hypoxia and normoxia after breakfast consumption ($p = 0.99$, $g = 0.16$) or omission ($p = 0.48$, $g = 0.25$). During exercise, there was no significant difference in acylated ghrelin concentration between breakfast consumption and omission in hypoxia ($p = 0.10$, $g = 0.67$) or normoxia ($p = 0.85$, $g = 0.29$). In the same period, acylated ghrelin concentration was lower in hypoxia compared with normoxia after breakfast consumption ($p = 0.04$, $g = 0.52$) but not omission ($p = 0.99$, $g = 0.10$). In the post-exercise period, acylated ghrelin concentration was not significantly different between breakfast consumption and omission in hypoxia ($p = 0.57$, $g = 0.37$), or normoxia ($p = 0.58$, $g = 0.10$).

In the same period, acylated ghrelin concentration was significantly lower in hypoxia compared with normoxia after breakfast consumption (p = 0.01, g = 0.48) but not omission (p = 0.99, g = 0.04).

[Insert Figure 4]

3.1.5 Energy intake

Ad-libitum energy intake during the post-exercise meal was significantly lower in hypoxia compared with normoxia after breakfast consumption (4897 ± 2047 kJ vs. 7007 ± 3048 kJ; p = 0.04, g = 0.80) and omission (5412 ± 1653 kJ vs. 6654 ± 2091 kJ; p = 0.03, g = 0.64). No significant differences in *ad-libitum* energy intake during the post-exercise meal were observed between breakfast consumption and omission in hypoxia (p = 0.40, g = 0.27) or normoxia (p = 0.99, g = 0.14).

Total trial energy intake (including breakfast) was significantly higher after breakfast consumption compared with omission in hypoxia (7136 ± 2047 kJ vs. 5412 ± 1652 kJ; p = 0.02, g = 0.90) and normoxia (9276 ± 3058 vs. 6654 ± 2091 kJ; $p < 0.01$, g = 0.98).

3.2 Experiment two

3.2.1 Exercise responses

In hypoxia $\dot{V}O_{2max}$ was 40.6 ± 4.3 ml·kg·min^{-1} and this elicited a walking speed of 2.9 ± 0.5 km·h^{-1} in the experimental trials (B-CHO: 50.0 ± 8.4% $\dot{V}O_{2max}$; B-PLA: 49.0 ± 8.1 $\dot{V}O_{2max}$; F-CHO: 49.3 ± 8.3 $\dot{V}O_{2max}$; F-PLA: 49.0 ± 8.1 $\dot{V}O_{2max}$). Relative exercise intensity was not significantly different between any trial (p = 0.93). There were no significant differences between trials for mean SpO_2 (p = 0.08, $g \leq 0.49$), heart rate (p = 0.53, $g \leq 0.35$) and RPE (p = 0.62, $g \leq 0.35$) for the duration of the experimental trial (Table 2). There were no significant

differences in the walking speeds during the 3 km time trial (B-CHO: 2.54 km/h; B-PLA: 2.58 km/h, F-CHO: 2.56 km/h, F-PLA: 2.65, $p = 0.99$).

[Insert Table 2]

3.2.2 Energy expenditure

Total trial energy expenditure was not significantly different between any trial (B-CHO: 4003 ± 671 kJ; B-PLA: 3648 ± 726 kJ; F-CHO: 3768 ± 598 kJ; F-PLA: 3563 ± 621 kJ; $p = 0.16$, $g \leq 0.31$).

3.2.3 Appetite perceptions

No significant differences in CAS (Figure 3B) were observed between any trial at baseline ($p \geq 0.12$, $g \leq 0.51$) or in the pre-prandial period ($p \geq 0.37$, $g \leq 0.56$). In the post-prandial period, during exercise, post exercise and post meal, CAS was significantly lower after breakfast consumption compared with omission in the carbohydrate ($p \leq 0.03$, $g \geq 1.10$) and placebo trials ($p \leq 0.04$, $g \geq 1.47$). No significant difference in CAS was observed between the carbohydrate and placebo trials after breakfast consumption (all $p = 0.99$, $g \leq 0.20$ or omission ($p \geq 0.67$, $g \leq 0.41$) at any time point.

3.2.4 Acylated Ghrelin

No significant differences in acylated ghrelin concentration (Figure 4B) were observed between any trial at baseline (all $p = 0.99$, $g \leq 0.14$) or in the pre-prandial period (all $p = 0.99$, $g \leq 0.12$). In the post-prandial period, acylated ghrelin concentrations tended to be lower after breakfast consumption compared with omission in the carbohydrate ($p = 0.09$, $g = 0.75$) and placebo trials ($p = 0.08$, $g = 0.57$). In the same period, no significant differences in acylated ghrelin concentrations were observed between the carbohydrate and placebo trials after breakfast consumption ($p = 0.99$, $g = 0.12$) or omission ($p = 0.99$, $g = 0.03$). There were no

significant differences in acylated ghrelin concentration between trials during exercise ($p \geq 0.36$, $g \leq 0.91$) or in the post-exercise period (all $p = 0.99$, $g \leq 0.27$).

3.2.5 Energy intake

Ad-libitum energy intake during the post-exercise meal was not significantly different between trials (B-CHO: 6191 ± 2831 kJ, B-PLA: 5457 ± 1970 kJ, F-CHO: 6264 ± 2574 kJ, F-PLA: 6174 ± 2222 kJ; $p = 0.26$, $g \leq 0.33$).

Total trial energy intake (including breakfast and carbohydrate supplementation) was significantly higher in the carbohydrate compared with placebo trials after breakfast consumption (10222 ± 2831 kJ vs. 7695 ± 1970 kJ $p < 0.01$, $g = 1.01$) and omission (8058 ± 2574 kJ vs. 6174 ± 2222 kJ $p = 0.02$, $g = 0.76$). Total trial energy intake was also significantly higher after breakfast consumption compared with omission in the carbohydrate ($p < 0.01$, $g = 0.70$) and placebo trials ($p = 0.02$, $g = 0.77$).

4.0 Discussion

The purpose of this study was twofold. Firstly, experiment one aimed to determine the appetite, acylated ghrelin and energy intake response to breakfast consumption and omission in acute normobaric hypoxia and normoxia. Secondly, experiment two aimed to determine the appetite, acylated ghrelin and energy intake response to carbohydrate supplementation after both breakfast consumption and omission in hypoxia. In experiment one, we observed no significant effect of breakfast consumption on subsequent post-exercise energy intake in hypoxia or normoxia, despite a reduction in CAS after breakfast consumption compared with omission (trend observed post-exercise in normoxia). As such, pre-exercise breakfast consumption significantly increased total energy intake in hypoxia and normoxia. Secondary to this, we also observed a hypoxic induced suppression of appetite and acylated ghrelin after breakfast

consumption, but not omission. In experiment two, carbohydrate supplementation had no effect on CAS, acylated ghrelin concentration or post exercise *ad-libitum* energy intake in hypoxia. As such, carbohydrate supplementation increased total trial energy intake in both the breakfast consumption and omission trials. Pre-exercise breakfast consumption combined with carbohydrate supplementation induced the greatest total trial energy intake. It is important to note that the aforementioned findings were observed in acute normobaric hypoxia equivalent to 4300 m. Whilst we cannot be sure that these findings translate wholly to terrestrial altitude over chronic durations (as per high altitude sojourns), these acute exposures provide an alternative, to allow the quantification of these physiological responses prior to real world applications.

The discovery that breakfast consumption did not affect subsequent energy intake with hypoxia or normoxia, despite the associated reduction in CAS and acylated ghrelin is a novel finding. As such, breakfast consumption increased total trial energy intake and had no effect on energy expenditure at rest or during exercise. These findings concord with the normoxic literature in demonstrating that post-prandial exercise elicits a greater suppression of appetite than fasted exercise (Cheng, et al., 2009; Deighton, et al., 2012; Gonzalez, et al., 2013; McIver, et al., 2018). This is the first study to demonstrate that this effect does not differ in hypoxic conditions. The finding that energy intake did not differ in the breakfast consumption or omission trials post-exercise in hypoxia and normoxia may be explained by the rate of muscle glycogen utilisation during exercise. In this regard, muscle glycogen depletion as a result of exercise has demonstrated a positive relationship with post-exercise reductions in RER previously (Henderson, et al., 2007) and has also been suggested to promote muscle glycogen replenishment post exercise (Hopkins, et al., 2011). Previously published data from the same cohort as the present study (Griffiths, et al., 2019a) refutes this hypothesis and demonstrates that substrate oxidation was not different in the post-exercise period between fasted and fed

participants, suggesting that muscle glycogen utilisation was also not different during exercise between fasted and fed participant. This is in accordance with previous literature demonstrating no difference in post-exercise substrate oxidation (Deighton, et al., 2012; Gonzalez, et al., 2013; McIver, et al., 2018). Whilst speculative, these findings may explain the null effect of pre-exercise nutritional status on subsequent energy intake in the present study

In experiment one, CAS tended to be lower during exercise in hypoxia compared with normoxia after breakfast consumption but not omission. This finding is in accordance with findings from a meta-analysis demonstrating a reduction in post-prandial, but not fasted hunger scores in hypoxia compared with normoxia (Matu, et al., 2018). The effect of hypoxia on CAS after breakfast consumption was transient however, and was not sustained post-exercise. In addition, acylated ghrelin concentrations were significantly lower during exercise and post-exercise in hypoxia compared with normoxia after breakfast consumption but not omission. This is also in agreement with Matu, et al. (2017a) who found that the reduction in hunger scores after breakfast consumption was associated with reduced acylated ghrelin concentrations, thus implicating the orexigenic effect of acylated ghrelin as a moderator of appetite regulation in hypoxia.

The suppressive effect of normobaric hypoxia on appetite and acylated ghrelin concentration has been observed in randomised control trials previously (Bailey, et al., 2015; Matu, et al., 2017a; Wasse, et al., 2012) but a within participant comparison of fasted and fed participants has not been conducted. In addition, the aforementioned literature investigating appetite and acylated ghrelin responses to exercise in normobaric hypoxia have been conducted following breakfast consumption. To the author's knowledge, there are currently no studies investigating these variables in fasted participants during exercise in normobaric hypoxia. The appetite stimulating effects of ghrelin are induced via acylation of ghrelin with a medium chain fatty acid (MCFA) (Kojima, et al., 1999), catalysed by the enzyme Ghrelin-O-Acyl-Transferase

(GOAT) (Kojima, et al., 2016; Yang, et al., 2008). The condensation reaction involved in this acylation of ghrelin is not directly dependent upon molecular oxygen and is therefore not directly affected by hypoxia. As such, it has been suggested that hypoxia may elicit differing effects on the availability of MCFAs as a substrate (Matu, et al., 2017a; Matu, et al., 2017b), and as a hypoxia-related suppression of acylated ghrelin was only observed after breakfast consumption in the present study, this effect may be moderated by pre-exercise nutritional status in hypoxia. The present study suggests the discrepancy in findings between hypoxia and normoxia may be derived from the inability of acylated ghrelin to regenerate after both feeding and exercise in hypoxia. Further research is required to determine the physiological mechanisms associated with this effect. Interestingly, these differences in appetite and acylated ghrelin concentrations between pre-exercise nutritional status were not reflected in post-exercise *ad-libitum* energy intake, with hypoxia inducing a reduction in energy intake regardless of breakfast consumption or omission. These findings identify the need for novel nutritional interventions to augment energy intake in hypoxic environments.

This is the first study to determine the effect of a carbohydrate supplement on appetite, acylated ghrelin concentration and energy intake in hypoxia after both breakfast consumption and omission. In experiment two, carbohydrate supplementation had no effect on appetite perceptions or acylated ghrelin concentrations. This is somewhat surprising, as given the suppressive effect of meal ingestion on acylated ghrelin and subsequent increase in satiety (Drazen, et al., 2006), it may be expected that this effect is replicated following caloric intake via carbohydrate supplementation. In addition, the combined effect of breakfast consumption or omission and carbohydrate supplementation is of particular interest given the relationship between glucose load and lower plasma ghrelin concentrations observed in normoxia (Shiiya, et al., 2002). Further, carbohydrate supplementation typically induces an increase in insulin concentrations during exercise (Jeukendrup, et al., 1999), and these elevations in insulin

secretion have been suggested to be an inhibitor of ghrelin secretion (Erdmann, et al., 2004). However, this did not have any effect on acylated ghrelin concentration or appetite perception in hypoxia. These findings may be explained by the dominant, suppressive effect of hypoxia subsequently limiting any further effect of carbohydrate supplementation on acylated ghrelin concentrations. As such, future research is required to determine if the acylated ghrelin and appetite response to carbohydrate supplementation differs in acclimatised individuals or at lower altitudes.

As a result of the negligible effects of carbohydrate supplementation on appetite and acylated ghrelin concentration, total energy intake increased compared with placebo by ~33% and ~31% after breakfast consumption and omission respectively. Matu, et al. (2017b) demonstrated that pre-exercise high fat breakfast resulted in significantly higher appetite perceptions and acylated ghrelin concentrations during exercise than a high carbohydrate breakfast. However, in contrast to the present study, this did not result in an increase in energy intake. Further, high carbohydrate, rather that high fat diets provide a greater energy yield per litre of oxygen, which is likely an important consideration in environments of low oxygen availability (Hochachka, 1985). The increase in energy intake in the present study is in accordance with previous literature conducted in a field setting using *ad-libitum* carbohydrate supplementation (Askew, et al., 1987; Macdonald, et al., 2009). In this regard, Askew, et al. (1987) observed an increase in energy intake with *ad-libitum* carbohydrate supplementation compared with placebo (2325 kcal vs. 1787 kcal). In addition, Macdonald, et al. (2009) also observed a 15058 kcal increase in energy intake in the carbohydrate supplementation group during a 21-day incremental sojourn up to 5400 m. Specifically, an overall increase of 15058 kcal was observed and the expected energy deficit in hypoxia was completed abated in > 50% participants. The increase in energy intake following carbohydrate supplementation after both breakfast consumption and omission in the present study suggests that this nutritional strategy may be a useful tool in

potentiating energy intake in fully fed populations. Alternatively, this strategy may also provide a palatable, compensatory nutritional strategy for those in energy deficit. The largest energy intake observed was with a combination of breakfast consumption and carbohydrate supplementation, therefore identifying this strategy as the most efficient in augmenting energy intake in hypoxic conditions.

The present study provides novel insight regarding the effects of breakfast consumption and carbohydrate supplementation on appetite and energy intake. Nevertheless, some notable limitations must be acknowledged. First, whilst the measure of subsequent energy intake provides a better understanding of short-term appetite response, it is also necessary to determine the effect of breakfast consumption/omission and carbohydrate supplementation on total daily energy intake, as compensatory feeding may occur later in the day. Second, the placebo trial utilised in experiment two involved ingestion of a fluid solution containing electrolytes and flavouring. The effect of this fluid ingestion on acylated ghrelin concentration and appetite perceptions are unknown and it may be beneficial to include a no-fluid condition in future studies. Third, the hypoxic exposures used in the present study were acute in duration therefore measurement of body mass was not suitable for this experimental design and the efficacy of these nutritional interventions could not be confirmed. In addition, the physiological responses to chronic hypoxic exposure have been demonstrated to differ to acute exposure (Mazzeo, et al., 1991), therefore it is not definitive that these findings translate to chronic hypoxic exposure. As such, future research should determine the effects of breakfast consumption and carbohydrate supplementation on body mass during chronic terrestrial altitude exposures.

4.1 Conclusions

In conclusion, breakfast consumption suppressed appetite perceptions and acylated ghrelin concentrations in hypoxia and normoxia, however this effect was transient and resulted in no significant difference in subsequent post-exercise energy intake. As such, breakfast consumption increased total trial energy intake compared with breakfast omission in both hypoxia and normoxia. In addition, carbohydrate supplementation also increased energy intake in hypoxia regardless of pre-exercise nutritional status. Breakfast consumption and carbohydrate supplementation combined provided the largest increases in energy intake and should be considered as feeding strategies in hypoxic environments.

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List of Figures

Tables

Table 1. Mean SpO_2, heart rate and RPE across the full duration of all trials (experiment one)

	SpO_2	Heart rate	RPE
H breakfast	79±3	86±9	12±2
H fasted	80±4	88±21	12±2
N breakfast	97±3	86±9	12±2
N fasted			

H = hypoxia, N = normoxia, SpO_2 = peripheral oxygen saturation, RPE = rating of perceived exertion.

Table 2. Mean SpO_2, heart rate and RPE across the full duration of all trials (experiment two)

B-CHO			
B-PLA			
F-CHO			
F-PLA			

B-CHO = breakfast consumption and carbohydrate supplementation, B-PLA = breakfast consumption and placebo, F-CHO = breakfast omission and carbohydrate supplementation, F-PLA = breakfast omission and placebo, SpO_2 = peripheral oxygen saturation, RPE = rating of perceived exertion

Fig.1

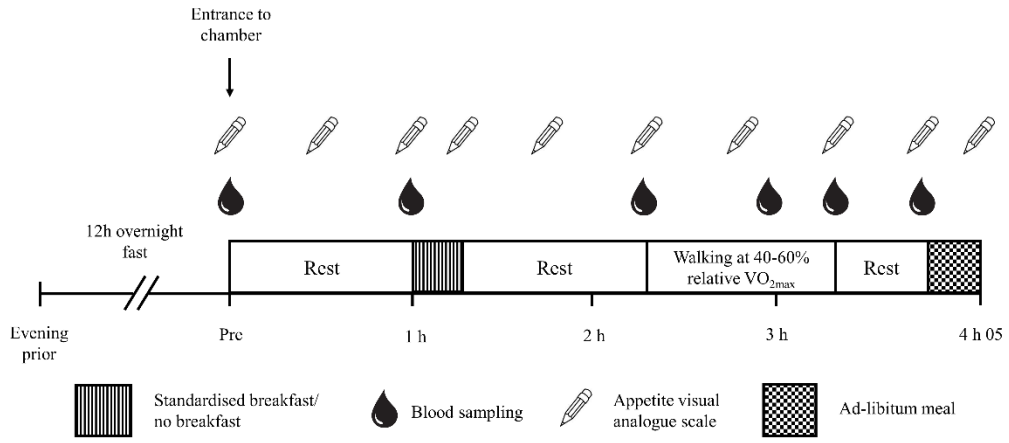

Entrance to chamber
12h overnight fast
Rest
Rest
Walking at 40-60% relative VO_{2max}
Rest
Evening prior
Pre
1 h
2 h
3 h
4 h 05
Standardised breakfast/ no breakfast
Blood sampling
Appetite visual analogue scale
Ad-libitum meal

Fig 2

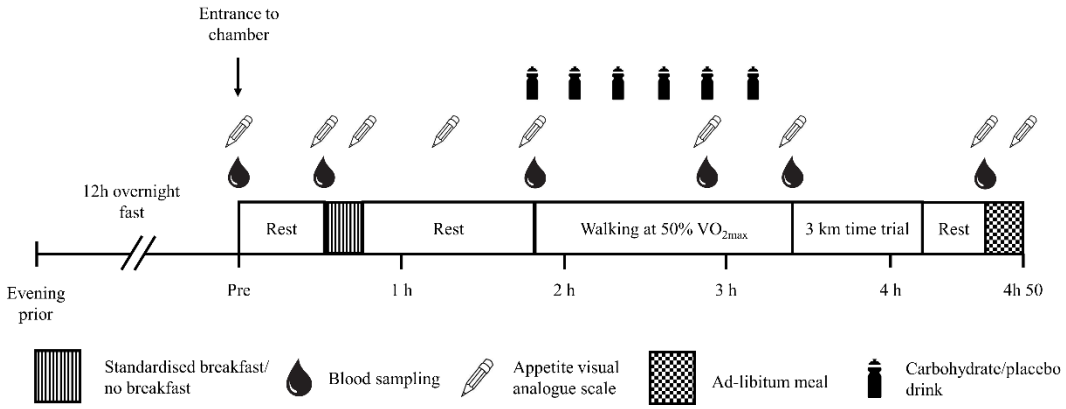

Entrance to chamber
12h overnight fast
Rest
Rest
Walking at 50% VO_{2max}
3 km time trial
Rest
Evening prior
Pre
1 h
2 h
3 h
4 h
4h 50
Standardised breakfast/ no breakfast
Blood sampling
Appetite visual analogue scale
Ad-libitum meal
Carbohydrate/placebo drink

Fig 3

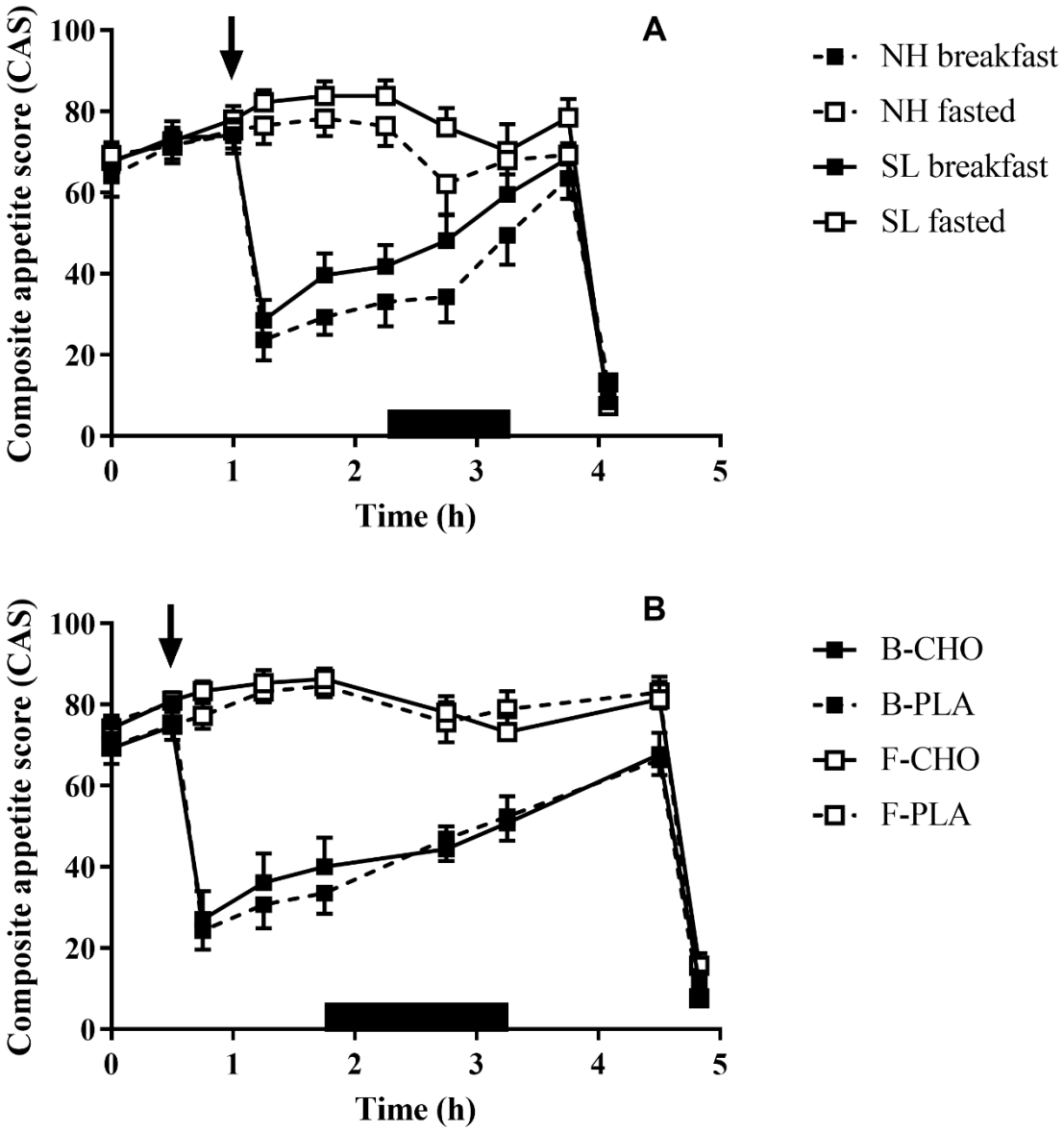
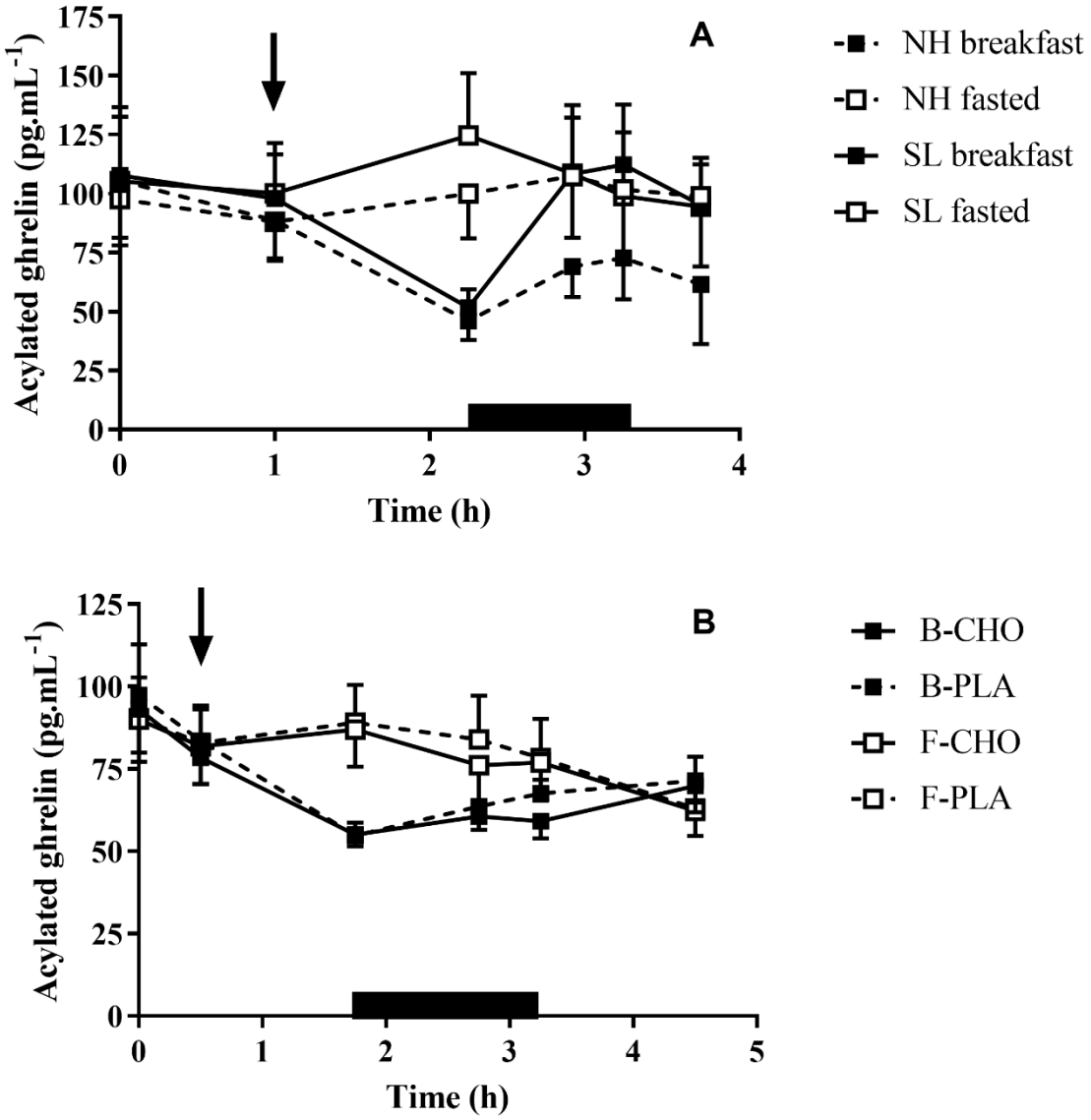

A
Composite appetite score (CAS)
100
80
60
40
20
0
0
1
2
3
4
5
Time (h)
NH breakfast
NH fasted
SL breakfast
SL fasted
B
Composite appetite score (CAS)
100
80
60
40
20
0
0
1
2
3
4
5
Time (h)
B-CHO
B-PLA
F-CHO
F-PLA

Fig 4


A
Acylated ghrelin (pg.mL-1)
175
150
125
100
75
50
25
0
0
1
2
3
4
Time (h)
NH breakfast
NH fasted
SL breakfast
SL fasted
B
Acylated ghrelin (pg.mL-1)
125
100
75
50
25
0
0
1
2
3
4
5
Time (h)
B-CHO
B-PLA
F-CHO
F-PLA