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Post-exercise dietary macronutrient composition modulates components of energy balance in young, physically active adults

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ABSTRACT

The effectiveness of exercise to reduce body mass is typically modest, partially due to energy compensation responses which may be linked to energy substrate availability around exercise. The present study aimed to investigate the effect of manipulating post-exercise energy substrate availability (high carbohydrate/low fat [HCLF] or low carbohydrate/high fat [LCHF] energy replacement) on energy balance components in the short-term (i.e., appetite, energy intake (EI) and energy expenditure (EE)).

Methods: Appetite, EI, activity- and total- EE were measured in twelve healthy, young (21.0 ± 2.3 years) physically active participants (10 men, 2 women) on two occasions across 4 days after a 75-min run and an isocaloric energy replacement drink (HCLF and LCHF). Appetite was measured daily by visual analogue scales, EI was calculated by subtracting the energy content of food leftovers from a provided food package, activity- and total- EE determined by heart-rate accelerometery.

Results: Composite appetite ratings between days were lower in HCLF (62.4 ± 12) compared to LCHF (68.3 ± 8.9 mm; $p = 0.048$). No differences between conditions were detected for EI. Cumulative activity-EE (HCLF= 20.9 ± 3.7, LCHF= 16.9 ± 3.1 MJ; $p = 0.037$), but not total-EE (HCLF= 44.6 ± 7.7, LCHF= 39.9 ± 4.7 MJ; $p = 0.060$), was higher for the HCLF condition than the LCHF across the measurement period.

Conclusion: Compared with low carbohydrate/high fat, immediate post-exercise energy replacement with a high carbohydrate/low fat drink resulted in higher short-term activity energy expenditure and lower appetite ratings.

1. Introduction

The prevalence of overweight and obesity has risen in the last few decades, impacting the health of around 2 billion people worldwide [1,2]. Overweight and obesity are commonly treated with lifestyle interventions, including exercise and diet. However, the efficacy of exercise and/or diet interventions on body-mass regulation varies amongst individuals [3] and such interventions often do not lead to the predicted body mass changes [4]. While this could be partially explained by a lack of compliance, physiological factors triggering energy compensation responses (ECRs) (i.e., increases in energy intake and/or reductions in energy expenditure components) could also play an important role [5]. A better understanding of ECRs could therefore lead to more effective strategies for controlling body mass.

ECRs are commonly described in long-term energy restriction, where reductions in fat- and fat-free mass lead to more than predicted decreases in energy expenditure, concomitant with increases in hunger and food intake, changes in the hormonal milieu and substrate utilisation [5,6]. On the other hand, ECRs could also be partially driven by short-term changes in substrate balances (e.g., carbohydrates and proteins) [7–9]. Indeed, changes in energy intake and/or energy expenditure following hours to days of exercise- and nutrition- interventions have been described even without body mass changes [10–12]. However, these phenomena remain to be fully understood, and more research is warranted.

Regarding carbohydrate metabolism, the gluco- and glycogeno-static theories [7,9,13] posit that blood glucose and liver and/or muscle glycogen levels regulate food intake. These carbohydrate stores are

Abbreviations: ANOVA, Analysis of variance; AEE, Activity energy expenditure; BF%, Body fat percentage; BM, Body mass; BMI, Body mass index; DEBQ, Dutch eating behaviour questionnaire; ECRs, Energy compensation responses; EDTA, Ethylenediamine tetra-acetic acid; EI_{diary}, Energy intake from food diaries; $EI_{leftovers}$, Energy intake from food leftovers; HCLF, High carbohydrate low fat; HR, Heart rate; LCHF, Low carbohydrate high fat; RMR, Resting metabolic rate; RER, Respiratory exchange ratio; SD, Standard deviation; TEE, Total energy expenditure; VAS, Visual analogue scale; VO_2peak, Peak oxygen uptake.

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relatively limited, essential for controlling glycaemia, and can be substantially affected by exercise and nutrition in the short term. As a result, reducing carbohydrate stores could increase food intake and lower physical activity levels. While this makes intuitive sense, evidence from studies manipulating nutrition only have not fully supported this notion [14–18]. For instance, a metabolic ward study by Stubbs and colleagues [16] showed a mild negative relationship between energy balance and carbohydrate balance during seven days of eating diets (ad libitum) with different levels of carbohydrates. Carbohydrate balance explained 5.5% of the variance in the subsequent day's energy balance. However, against a background of physical exercise, which has the potential to reduce carbohydrate availability markedly, the data suggest carbohydrates may exert a stronger regulatory role on ECRs [19–21].

In the context of exercise interventions, multiple lines of evidence have linked carbohydrates and ECRs [19–21]. Tremblay and colleagues investigated combining a 60-min run with three ad libitum diets differing in the fat-to-carbohydrate distribution and found that only the diets with high carbohydrate content led to a negative energy balance within the next 48 h [20]. However, the design does not allow for distinguishing between the effects of the exercise-induced energy deficit and substrate utilisation on energy balance. In this sense, stronger associations between carbohydrate metabolism and energy intake have been observed in situations where carbohydrate availability is even more limited (e.g., overnight-fasted exercise protocols). For instance, Hopkins and colleagues showed that in women with obesity, 60 min after the cessation of exercise, 37% of the variance in food intake during an ad libitum meal was explained by carbohydrate oxidation during exercise [22]. Furthermore, Edinburgh and colleagues, showed that in lean participants, blood glucose used during exercise was correlated with energy intake during a meal test performed two hours after finishing exercising [23]. On the other hand, carbohydrate availability has been linked to some extent with changes in energy expenditure, particularly in the physical activity domain. For example, during prolonged fasting periods, where carbohydrate availability is limited, physical activity levels tend to decrease spontaneously [24–28]. However, whether physical activity can be affected by higher carbohydrate demands and lower carbohydrate availability (e.g., with exercise and diet interventions) remains to be directly tested.

At present, no studies have expanded on the impact of overnight-fasted exercise and carbohydrate metabolism on short-term energy intake and physical activity, even though energy balance changes can have a lag time of days [29,30]. Therefore, the present study aimed to investigate the effects of an overnight-fasted aerobic exercise session followed by high carbohydrate/low fat or low carbohydrate/high fat energy replacement on short-term (i.e., across 4 days) energy balance components (i.e., appetite, energy intake and energy expenditure) in physically active normal-weight participants. It was hypothesised that compared with low carbohydrate/high fat (LCHF), high carbohydrate/low fat (HCLF) energy replacement after exercise would: (a) reduce appetite and energy intake and (b) increase physical activity and total energy expenditure during free-living conditions.

2. Materials and methods

2.1. Participants

Twelve healthy, physically active participants (10 men and two women) provided written informed consent and completed the study, which was approved by the local ethics committee of the University of Birmingham, UK (code: ERN_20–1826). Inclusion criteria were men or women, self-reported healthy, low food restraint levels (< 3.0 in the DEBQ) [31] VO_2peak > 50 and 45 ml·kg·min^{-1} for men and women respectively, 18–45 years old, BMI < 27.5 kg $\cdot m^{-2}$. Participants who were dieting, smoking, taking medications, pregnant, irregularly menstruating, or lactating were excluded. The final participants' characteristics were age - 21.0 ± 2.3 years (mean ± standard deviation); body mass (BM) - 72.8 ± 7.9 kg; body mass index (BMI) 22.9 ± 1.5 kg · m^{-2}; body fat percentage (BF%) - 14.3 ± 5.6%; peak oxygen uptake (VO_2peak) - 56.0 ± 6.4 ml · kg · min^{-1}; resting metabolic rate (RMR) – 7.94 ± 1.48 MJ · day^{-1}; Dutch eating behaviour questionnaire (DEBQ-R) score [32]: 1.9 ± 0.4.

2.2. Experimental design

Following the preliminary screening, every volunteer participated in a double-blinded randomised crossover design. Participants were randomised and counterbalanced to each condition by a third-party utilising www.randomizer.com. A familiarisation session and two trials were completed separated by one week of washout for men and four weeks for women (for the two women included, the trials were performed to match the same phase of their menstrual cycle). The experimental sessions were performed on the same weekdays to decrease the effect of daily life routine changes. To standardise nutrition before the experimental days, participants recorded their food intake and were asked to replicate it for the second experimental condition. Caffeine and alcohol intake was avoided 24 h before the trials, and strenuous physical activity for 48 h.

For every trial, the participants arrived at the laboratory ~ 8 a.m in an overnight-fasted state (10–12 h since last meal). Following the baseline measurements (i.e., appetite visual analogue scale (VAS), anthropometry, RMR, and a blood sample), a 75-min run was completed, followed by a LCHF or HCLF drink and a 4-h recovery within the laboratory. Subsequently, the participants left the facilities with a food package and returned on the morning of the fourth day, where the baseline measurements were repeated.

During the free-living time between the baseline- and follow-up visit, participants were requested to only eat from the food provided, to precisely complete a food diary record, and return all food leftovers. Participants wore an Actiheart device (Camntech, Cambridge, UK) to assess total- and activity-energy expenditure, only taking it off during water-related activities. To determine changes in daily appetite levels, participants received an email with a link to answer an appetite VAS before having breakfast.

After the washout period, the participants underwent the identical experimental condition using a different recovery drink. The drinks were designed to replace the energy expended during the run, differing only in the macronutrient distribution, but were carefully matched in flavour and texture.

2.3. Preliminary testing and familiarisation visit

Participants attended the laboratory, and after providing written informed consent and answering a general health screening questionnaire and the DEBQ, the RMR, anthropometry, and VO_2peak were assessed**.** Next, a 20-min familiarisation run was performed on a treadmill at the selected exercise intensity. At the end of this visit, the participants had to wear an Actiheart for at least 24 h to estimate energy needs based on energy expenditure and develop an individualised food menu.

2.3.1. Resting metabolic rate

The RMR was estimated by indirect calorimetry by a 30-min protocol (Cranlea Douglas bags system, Birmingham, UK). Participants laid down for a 15-min stabilisation period, followed by a 15-min breath sample collection period. Then, the VO_2 and VCO_2 concentration levels were measured with gas analysers (MOXAR Respirometry System, AEI technologies, USA), previously calibrated with two known concentration gases. Gas volume and temperature were measured by a dry gas metre (Harvard Bioscience, inc., Germany) and a gas thermometer (electronic temperature instruments, ltd., UK). Then, corrections for standard temperature and barometric pressure were performed. Based on these values, substrate utilisation and energy expenditure were estimated

utilising established equations [33].

2.3.2. Anthropometry

Body mass (Ohoaus Champ II scale, Cole-Parmer, UK) and height (SECA 213 stadiometer, UK) were measured to the closest 0.05 kg and 0.01 m, respectively.

2.3.3. Submaximal and maximal exercise testing

A submaximal running test was performed to establish the relationship between exercise intensity and oxygen uptake, determining the speed necessary to elicit 70% of the VO_2peak. After 3–5 min of warm-up, participants completed four 4-min stages commencing typically between 7 and 8 km/hr and with stepped increases of 2 km/hr. VO_2 and VCO_2 production were measured by a metabolic cart (Vyntus, Vyaire Medical, IL, US) previously calibrated for volume and gas concentrations following manufacturer recommendations. Heart rate was constantly monitored during the test (Polar H10 heart rate monitor, Finland).

After resting for 20 min, a maximal test was performed to determine participants' VO_2peak. The initial speed was 2 km/hr less than the last stage on the submaximal test, with an initial slope of 1%. Every minute the slope increased by one degree until reaching volitional fatigue. Participants were verbally encouraged during the test. The test finished once participants could not continue or if the maximal criteria variables were achieved for VO_2, heart rate (HR), and respiratory exchange ratio (RER) (i.e., no further increase in VO_2 (< 2 ml $\cdot$ kg $\cdot$ min^{-1}), HR $\pm$ 10 beats of the age estimated HR max, and RER > 1.1).

2.4. Experimental trials (Fig. 1)

2.4.1. Baseline visit

Participants arrived at the laboratory ~ at 8:00 am and were asked to void their bladder. Then they completed the first appetite VAS and body mass was measured while wearing light clothes. RMR was assessed by a 30-min protocol utilising Douglas bags and indirect calorimetry. Body composition was then estimated by bioelectrical impedance (Bodystat Quadscan 4000, Isle of Man) and a blood sample was obtained by venepuncture.

For the treadmill run, participants started with a 5-min warm-up followed by 75 mins at a running speed designed to elicit 70% VO_2peak. Every 15 min, a three-minute breath sample was collected and analysed to ensure that the targeted intensity was achieved (LCHF: 72.5 $\pm$ 3.8 & HCLF: 72.3 $\pm$ 3.2% VO_2peak; $p = 0.829$) (Vyntus, Vyaire Medical, IL, US). The heart rate and 6–20 Borg perceived exertions were measured throughout the protocol.

Once participants finished running, body mass and appetite were assessed again, a blood sample was taken by inserting a cannula into the antecubital vein, and the Actiheart was placed on the chest. Afterwards, participants had 10 min to change before staying in a research kitchen for the next four hours. Within the kitchen, they drank two bottles of the designated drink, separated by an hour. After 15 min of finishing the first bottle, a third appetite VAS was completed. During the recovery, a blood sample and appetite VAS were obtained hourly.

At the end of the four hours, participants left the building with a food package to cover the rest of that day and the next two days. Participants were requested to complete an appetite VAS before breakfast and a food record daily, wear the Actiheart for the whole-living period and bring back food leftovers on the morning of the fourth day.

2.4.2. Follow-up visit

After finishing the free-living period, participants returned to the lab on the morning of the fourth day (~ 8 a.m) in an overnight-fasted state. Here, the last appetite VAS was completed, followed by measuring body mass, RMR and body composition. Finally, a blood sample was taken, the food leftovers were collected, and Actiheart data was downloaded.

2.5. Nutritional manipulation

Participants were given one of the two isocaloric drinks (4.45 $\pm$ 0.6 MJ) after exercising. One was LCHF (5.3%, 87.7%, 7.0% of protein, fats, and carbohydrate, respectively), and the other was HCLF (7.3%, 0.5%, 92.2% of protein, fats, and carbohydrates, respectively). The drinks matched the energy expended during the running bout and were prepared by a trained third person who was not otherwise involved in the research. The LCHF drink was composed of semi-skimmed milk, double cream, and sucralose. The HCLF drink was made from semi-skimmed milk, sucrose and maltodextrin. To avoid visual recognition, the drinks were provided in opaque bottles.

For the free-living period, participants were instructed to eat "as much or as little as they want" but only from the food provided, which covered 125% of their total estimated energy needs (i.e., 1.25 x total energy needs —obtained by combining the RMR and the physical activity energy expenditure obtained by the Actiheart). Participants had the option to request additional food from the investigators if necessary. The average macronutrient distribution was 45%, 18%, and 37% of energy from carbohydrates, proteins, and fats. Two breakfasts, three lunches, three dinners and three snack bags were provided. The food provided and the ingredients utilised for the preparation of the drinks were obtained from commercially available products (Tesco, UK). The main meals (i.e., lunch and dinner) were "ready-to-eat food", and the labelled nutritional information was utilised to calculate energy intake.

2.6. Energy intake: from diaries and leftovers

Participants were instructed to complete food diary records during the free-living period. For this purpose, the investigator taught participants how to complete the paper-based form accurately. This approach was combined with the provision of food by the investigators to increase the reliability of dietary food records [34]. The mean reported energy intake was obtained directly from the food diaries (EI_{diary}). Furthermore, to calculate the energy intake more objectively, the investigators subtracted the food leftovers from the total caloric content of the food provided ($EI_{leftovers}$). For comparison purposes, EI_{diary} and $EI_{leftovers}$ outcomes are taken as cumulative values on each trial (i.e., the sum of food's caloric content across the days). For the analysis, the drinks' energy content is not considered within the cumulative EI.

2.7. Appetite VAS assessment

In the present study, the questions included were: How hungry do you feel now? How strong is your desire to eat? And how full do you feel now? These questions were answered by marking a 100 mm line using anchor statements as previously validated [35] in the laboratory and during free-living conditions. The investigator configurated an online questionnaire to allow the participants to answer the scales on their computers/mobiles/tablets. The data were collected and managed using REDcap electronic data capture tools hosted at the University of Birmingham, UK [36]. For free-living conditions, REDcap was programmed to email the questionnaires daily to the participants on a pre-specified schedule. The three questions were analysed individually and as a composite.

$$appetite\ composite = \frac{H.H\ +\ H.SD\ +\ (100 - H.F)}{3}$$

H.H= how hungry do you feel?; H.SD= How strong is your desire to eat?; H.F= how full do you feel? All the values are expressed in mm

2.8. Exercise energy expenditure within the lab

For estimating energy expenditure and substrate utilisation during the 75-min run at 70% VO_2peak (energy expenditure: 4.58$\pm$ 0.64 MJ; fat

oxidation: 48 ± 15 g; carbohydrate oxidation: 153 ± 31 g), a 3-minute breath sample was obtained every 15 min by indirect calorimetry utilising a metabolic cart (Vyntus, Vyaire Medical, IL, US). The values were calculated utilising the equations of Jeukendrup & Wallis [37]. For the analysis, exercise energy expenditure is not considered within the cumulative energy expenditure data.

2.9. Total- and activity-energy expenditure in free-living conditions (TEE & AEE)

The total- and activity-energy expenditure during free-living conditions was obtained from the data collected by the Actiheart [38]. Participants were instructed to wear the device all the time, from finishing the run until the morning they returned to the laboratory. They were only allowed to take it off during aquatic activities. The Actiheart utilised a previously validated algorithm based on a branched model [39] that combines heart rate and accelerometry to estimate the total- and activity-energy expenditure. The device was configurated to collect data every 15 s.

The participant's anthropometric information (i.e., body mass and height), date of birth, gender, sleeping- and maximum-heart rates were programmed into the software for data processing. For improved accuracy, individualised calibration curves were built based on the values of heart rate and VO_2 obtained in the submaximal and maximal exercise tests. Also, the measured RMR was included to improve further energy expenditure estimation on every trial. For interpretation, as done with energy intake, TEE and AEE outcomes are reported as cumulative values on each trial (i.e., the sum of TEE and AEE values across the days). Only participants without episodes of "non-wearing time", as detected by the Actiheart 5 software (i.e., no periods of more than 2 h of non-wearing), were included in the analysis.

2.10. Blood sampling & biochemical analyses

Venous blood samples were obtained and collected into pre-chilled 6 ml EDTA tubes. Blood samples were taken by venepuncture from the antecubital vein at baseline; and by cannulation after finishing the exercise, at 1, 2, 3, and 4 h. A protease inhibitor (Pefabloc® SC, Merck, Germany) was utilised to preserve the integrity of the samples for the subsequent measurement of appetite-related hormone concentrations. Tubes were kept on ice until they were centrifugated at 4 °C, 1500 g, for 10 min. Plasma aliquots were stored at – 70°C until they were analysed.

Plasma samples were analysed in using an automated clinical analyser (RX Daytona + Randox, London, UK) to determine glucose (Glucose hexokinase, Randox, UK) and lactate (Lactate Dehydrogenase, Randox, UK) concentrations. For insulin, acylated ghrelin, and leptin, ELISA assays were used (EMD Millipore Corporation, Missouri, USA). All biochemical analysis was performed in duplicate.

2.11. Statistics

The sample size was selected ($n = 12$) to be comparable with previous research investigating the impact of short-term exercise and nutrition manipulation on energy balance [22,23]. Unless otherwise stated, the statistical analyses included 12 participants. Data are described as mean ± standard deviation (SD). For cumulative values of energy intake, activity- and total- energy expenditure, a paired *t*-test was performed to compare mean values. The energy expenditure analyses account for all the data collected after the exercise bout, whereas the energy intake was calculated from the free-living period only. A two-way repeated-measures ANOVA was utilised for daily- appetite, activity- and total-energy expenditure, blood hormones and metabolites. For time x condition interactions, a Holm-Bonferroni *post hoc* was performed. Data were tested for sphericity with Mauchly's test. For the variables where the sphericity assumption was violated, a Greenhouse–Geisser correction was performed. Change in insulin was calculated for each trial and compared by paired *t*-test between conditions due to significant baseline differences. Statistical significance was set at $p < 0.05$. Statistics were performed using JASP 0.16.3 software and graphics with Graphpad PRISM 9.4.1.

3. Results

3.1. Energy and macronutrient intake

The mean cumulative EI_{diary} for the LCHF and HCLF conditions were 37.5 ± 7.6 and 38.7 ± 7.3 MJ ($p = 0.425$). The mean $EI_{leftovers}$ for the LCHF and HCLF conditions were 40.2 ± 9.7 and 40.3 ± 8.7 MJ ($p = 0.925$), respectively (see Fig. 2). The mean cumulative data for carbohydrates, proteins, fats, and fibre intake did not show significant differences between conditions (Table 1)

3.2. Cumulative total-and activity-energy expenditure

Data on $n = 8$ is presented as four participants were excluded from the analysis, as they had not worn the Actiheart for sufficient periods during the trials. The mean cumulative TEE for the LCHF and HCLF conditions were 39.9 ± 4.7 & 44.6 ± 7.7 MJ ($p = 0.060$). The corresponding values for AEE were 16.9 ± 3.1 & 20.9 ± 3.7 MJ ($p = 0.037$) for the LCHF and HCLF conditions (See Fig. 2). The breakdown of these data is shown in Table 2.

3.3. Appetite composite

Up to five participants were excluded from these analyses because they did not fully complete the appetite-VAS. During the recovery within the lab, there were no time ($p = 0.201$), condition ($p = 0.181$), or time x condition effects ($p = 0.775$). For the comparison between days, the LCHF condition presented higher levels of fasted appetite composite than the HCLF (LCHF= 68.3 ± 8.9, HCLF= 62.4 ± 12 mm, $p = 0.048$; Fig. 3.A). When the questions were analysed separately, statistically significant differences were only found for "How strong is your desire to eat" with lower values for the HCLF condition (LCHF= 67.6 ± 8.8, HCLF= 63.4 ± 12.4 mm; $p = 0.023$; Fig. 3.D).

3.4. Blood metabolites and hormones

3.4.1. Glucose

The effects of the intervention on blood glucose concentrations during the lab recovery were affected by time ($P = < 0.001$) but not by condition ($P = 0.335$), and there was a time x condition interaction ($P = < 0.001$) (Fig. 4.A). *Post hoc* analyses showed that within the lab, the HCLF condition presented lower glucose levels compared with LCHF 4 h after the exercise (5.07 ± 0.28 LCHF vs 3.72 ± 0.96 HCLF mmol · L^{-1} $P < 0.001$). For the comparison between day 1 and day 4, no differences were detected (Fig. 4.B).

3.4.2. Insulin

For the values obtained during the lab recovery period, there were differences by time ($p = < 0.001$), condition ($p = < 0.001$), and time x condition ($p = < 0.001$). *Post hoc* analyses showed significant differences between conditions at hour 1 (10.8 ± 8.8 LCHF vs 56.1 ± 29.4 HCLF μU·ml^{-1}; $p = < 0.001$), hour 2 (9.5 ± 6.5 LCHF vs 58.9 ± 33.5 HCLF μU·ml^{-1}; $p = < 0.001$), and hour 3 (6.3 ± 3.6 LCHF ± 40.7 ± 26.4 HCLF μU ml^{-1}; $p = 0.02$) (Fig. 4.C). For the comparison between day 1 and day 4, there were no differences between conditions ($p = 0.489$) (Fig. 4.D).

3.4.3. Lactate

For blood lactate concentration during the lab recovery, there were differences by condition ($P = < 0.001$), and time x condition ($P = < 0.001$), but not by time ($P = 0.063$). *Post hoc* analyses showed significant differences between conditions at hour 1 (0.89 ± 0.13 LCHF vs 2.09 ±

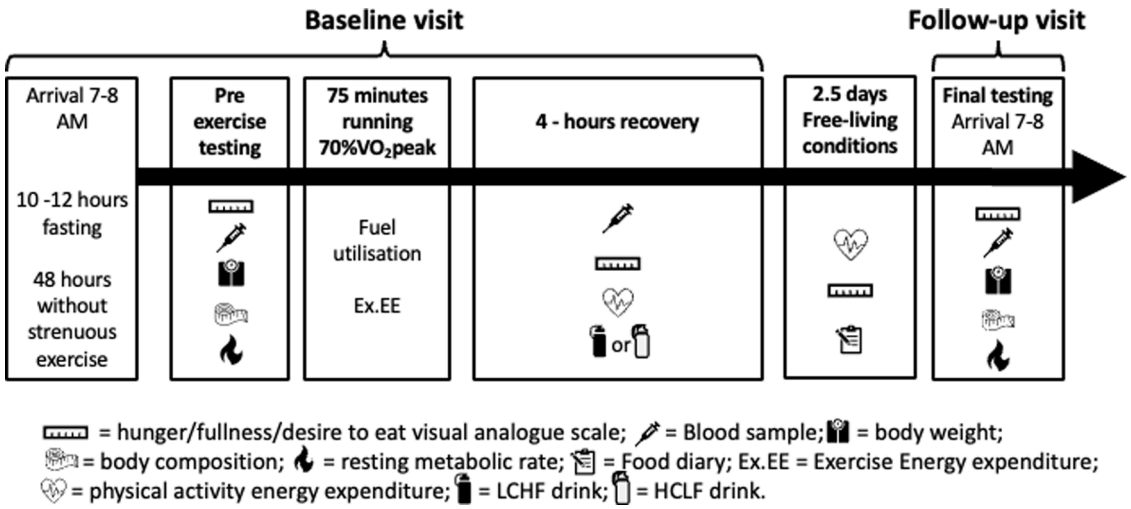

Fig. 1. Schematic overview of the experimental days.

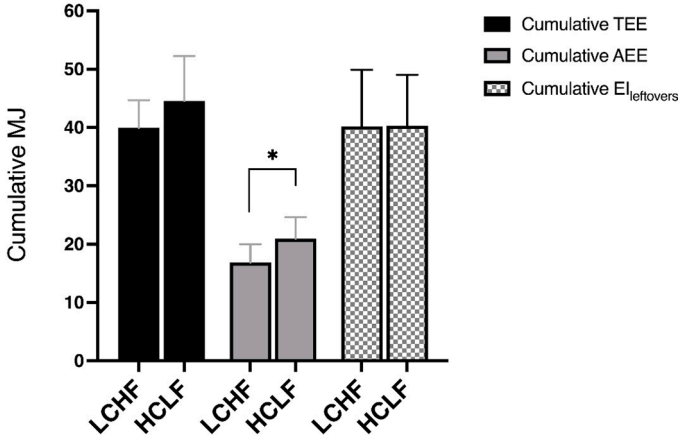

Fig. 2. Cumulative TEE, AEE, and $EI_{leftovers}$. Values are presented as mean ± SD. TEE= total energy expenditure ($n = 8$); AEE= activity energy expenditure ($n = 8$); $EI_{leftovers}$=Calculated energy intake from leftovers ($n = 12$). * $p = 0.037$.

Table 1
Cumulative macronutrient intake for each condition.

	LCHF	HCLF
Carbohydrates (g)	1124 ± 275 (47 ± 12% EI)	1129 ± 251 (47 ± 11% EI)
Proteins (g)	395 ± 63 (16 ± 3% EI)	398 ± 51 (14 ± 2% EI)
Fats (g)	352 ± 103 (33 ± 10% EI)	352 ± 94 (33 ± 9% EI)
Fibre (g)	126 ± 34 (4 ± 1% EI)	126 ± 28 (4 ± 1% EI)

Values are presented as mean ± SD in grams and as percentage of EI (% EI). No statistically significant differences between conditions were detected ($p > 0.05$). The data were calculated based on the information obtained after accounting for food leftovers.

0.68 HCLF mmol · L^{-1}; $p = < 0.001$), hour 2 (0.78 ± 0.09 LCHF vs 1.91 ± 0.53 HCLF mmol · L^{-1}; $p = < 0.001$), and hour 3 (0.75 ± 0.06 LCHF vs 1.52 ± 0.37 HCLF mmol · L^{-1}; $p = 0.04$) (Fig. 4.E). There were no differences in the comparisons between day 1 and day 4 (Fig. 4.F).

3.4.4. Acylated- ghrelin

The acylated ghrelin concentrations were measured on days 1 and 4. Here, significant differences were observed by condition ($p = 0.013$) and time x condition interaction ($p = 0.039$), where the HCLF condition showed higher concentrations than the LCHF condition. *Post hoc* analyses showed differences by condition on day 4 (329 ± 164 LCHF vs 518 ± 267 HCLF pg · mL^{-1}; $p = 0.008$) (Fig. 5).

Table 2
Total daily energy expenditure & daily activity energy expenditure corresponding to each condition.

	AEE (MJ)[a]		TDEE (MJ)[a]	
	LCHF	HCLF[b]	LCHF	HCLF
Day 1	4.6 ± 1.4	5.1 ± 1.6	9.7 ± 2.0	10.3 ± 2.5
Day 2	5.8 ± 2.4	6.6 ± 2.4	14.7 ± 2.7	15.6 ± 3.2
Day 3	6.5 ± 2.1	9.3 ± 2.5	15.5 ± 2.7	18.7 ± 4.0

Data are presented as mean ± SD; $N = 8$. A repeated measured ANOVA was performed for AEE= Activity energy expenditure and TDEE= Total daily energy expenditure.
[a] Time effect.
[b] Condition effect. Day 1 is considered from after the run until midnight. Days 2 and 3 run from midnight until midnight.

3.4.5. Leptin

For leptin concentration, there were no differences by time ($p = 0.223$), condition ($p = 0.583$) and time x condition interaction ($p = 0.335$) (Fig. 5).

4. Discussion

The present study aimed to investigate the effects of an aerobic exercise session followed by HCLF or LCHF energy replacement on short-term (i.e., across 4 days) energy balance components (i.e., appetite, energy intake and energy expenditure) in physically active normal-weight participants. Here, the HCLF condition led to higher AEE and fasted acylated ghrelin levels and reduced fasted-appetite composite rating compared with the LCHF condition, although no differences in energy intake were observed between conditions.

As above, a key finding in the present study was higher levels of AEE for the HCLF condition. TEE was not significantly increased in HCLF, which could suggest compensatory adaptations as implied by the constrained energy hypothesis [40]. However, this seems unlikely given TEE alterations were directionally consistent with AEE (i.e., increased in HCLF). Regardless, the present results agree with one of the study hypotheses and provide evidence that post-exercise dietary macronutrient composition can modulate AEE. Based on the glycogenostatic theory, the lower AEE in the LCHF condition could be explained by the reduced carbohydrate availability, as has been observed with other interventions combining overnight-fasting plus moderate to vigorous exercise [41].

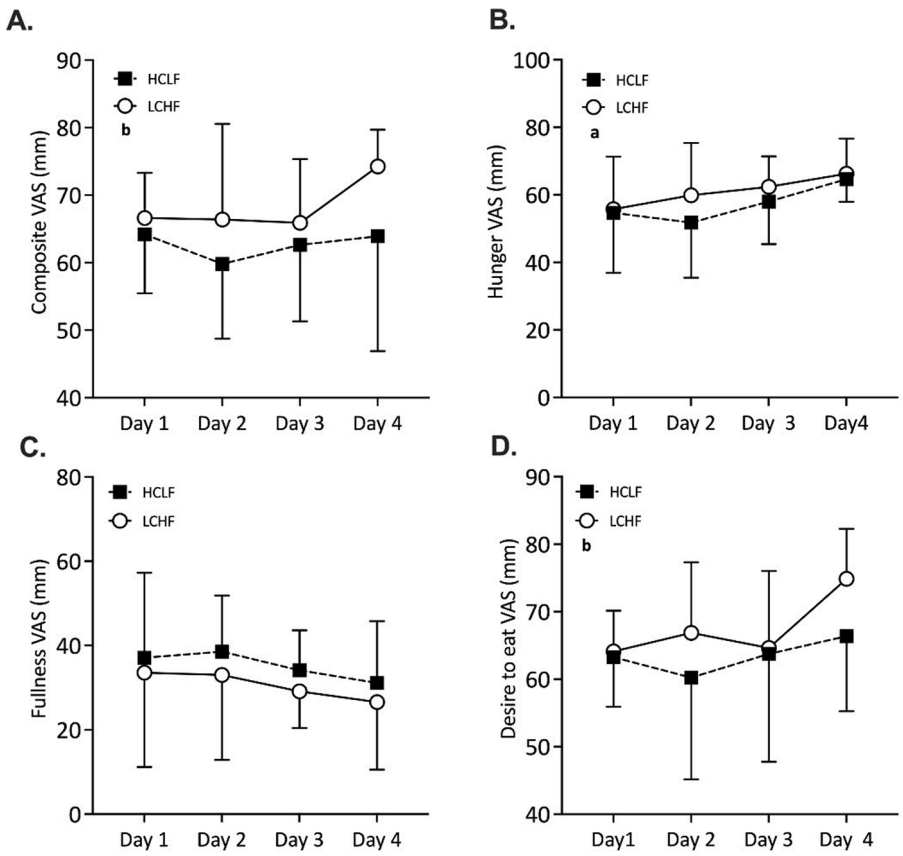

Fig. 3. Appetite VAS. A) appetite composite $n = 7$; B) How hungry do you feel? $n = 8$; C) How full do you feel? $n = 9$; D) How strong is your desire to eat? $n = 8$. Data are presented in mean ± SD. A two-way repeated measure ANOVA was performed. [a] time effect; [b] condition effect.

Although studies testing these effects on AEE directly are lacking, indirect evidence pointing to low carbohydrate availability and reduced AEE can be found in the context of fasting studies [24–28]. In this sense, it has been reported that overnight-fasted exercise can lead to higher glycaemic variability [42] and lower counterregulatory response to hypoglycaemia [43]. Consequently, if carbohydrate stores are not replenished after exercise, endogenous glucose production can be affected [44], triggering strategies to regulate glycaemia, such as reduced muscle glucose uptake [45,46] and increased hunger. Altogether, it could lead to decreased physical activity until glycaemic control is achieved again. On the other hand, it cannot be discarded that the observed LCHF results are driven by acute fat intake, as it could also modify glycaemic control and peripheral glucose metabolism [47–49]. Indeed, the type of fat intake by itself (saturated v. monounsaturated) can have different effects on physical activity and resting energy expenditure levels in healthy people, where a higher proportion of monounsaturated fat has led to higher energy expenditure [50]. If others corroborate that post-exercise macronutrient intake modulates AEE without modifying EI, it could open opportunities to develop nutritional-based approaches to minimise ECR in the context of exercise training and weight management programmes.

The appetite data collected in the present study showed that participants in the HCLF condition presented lower fasted-appetite levels in the days after the intervention. As no appetite-VAS differences between conditions were seen during the early recovery period within the laboratory, the direct satiating effect of the nutrients should not have influenced the results. Whether this points to a further influence of carbohydrate availability on short-medium-term appetite regulation is unclear. However, indirect evidence linking higher carbohydrate availability after exercising and reduced energy intake between days suggest a modulatory role [19–21]. Notwithstanding, even if there was a reduction in appetite ratings, the mean ~ 6 mm differences were modest and did not modify food intake. This agrees with the literature, which reports that a minimum 15 mm change in appetite VAS would be required to increase food intake [51]. Within the appetite-related hormones, the levels of acylated ghrelin were higher for the HCLF compared to the LCHF condition. This finding contradicts what was found for appetite, as it is well-known that acylated ghrelin is an orexigenic hormone. Nonetheless, it should be recognised that ghrelin is only one hormone from an array that influence appetite, and it is not uncommon to find uncoupled responses between endocrine signalling, appetite, and energy intake [52]. Even though the energy intake was not different between groups, the present data could help inform nutritional strategies to modulate appetite during high physical activity periods while eating ad libitum.

Despite the investigators' efforts, the present study is not free of limitations. It is important to mention that valuable information regarding physical activity and appetite during the free-living period could not be obtained. Four participants did not wear the device as requested and did not reply to the appetite-VAS, despite frequent reminders. Another limitation is the relatively homogenous population group, which limits the generalisation of the results. On the other hand, it is also possible to point out strengths. The design of the present study, combining lab and free-living conditions, gives control for the main manipulation (corroborated by the blood markers) and adds ecological validity, allowing capture of spontaneous behavioural changes after a controlled intervention. A 2.5 days free-living period allowed us to capture changes in physical activity that were not captured by shorter studies, suggesting the need to monitor longer after lifestyle interventions. Moreover, participants completed the trials matching the same days of the week between conditions, reducing the influence of routine changes on our results. Furthermore, limiting food variety

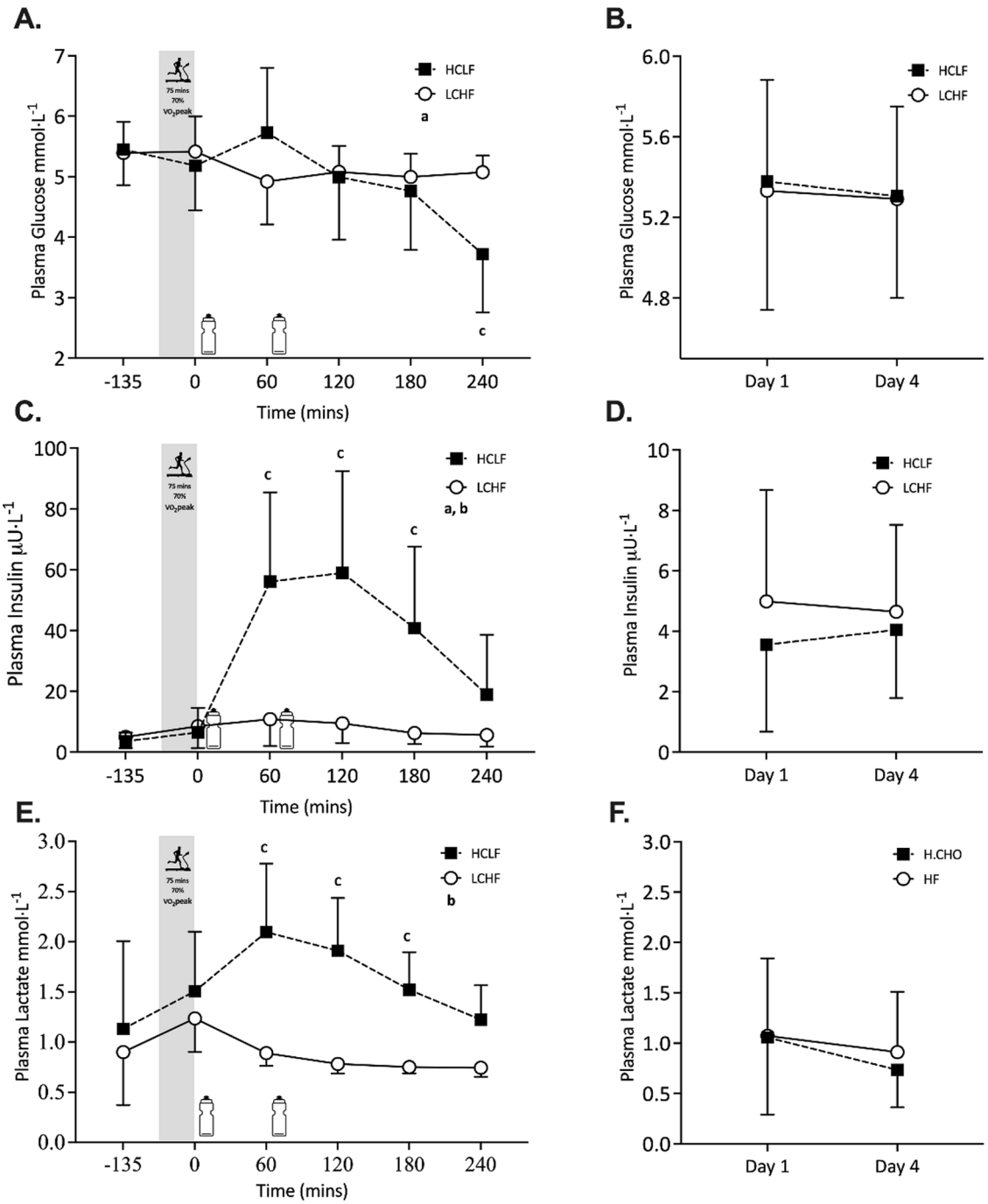

Fig. 4. Glucose A-B, insulin C-D, and lactate E-F concentrations values for the comparisons within the laboratory and between days. Values are presented as mean ± SD. A two-way repeated measure ANOVA was performed. [a] time effect; [b] condition effect; [c] Time x condition interaction.

helped avoid nutrient-different influences on appetite and food over-consumption. Indeed, there were no differences in macronutrients (Table 1) or caloric intake between conditions, isolating the energy replacement protocols' effects to explain the results.

In summary, this study shows that when the energy expended during exercise is replaced with different macronutrients, an HCLF composition favours higher levels of AEE and lower appetite ratings in the short term. However, as the present study did not include direct measurements of substrate availability (e.g., liver/muscle glycogen, continuous glucose monitoring, etc.), it is not feasible to offer stronger mechanistic insights. Future research should focus on studying the relationship between carbohydrate pools (blood glucose, liver and muscle glycogen) and their direct/indirect effects on energy balance components, with the potential to inform better nutritional strategies for physically active people where energy balance is a concern (e.g., weight loss programs, weight-categorised sports, etc.)

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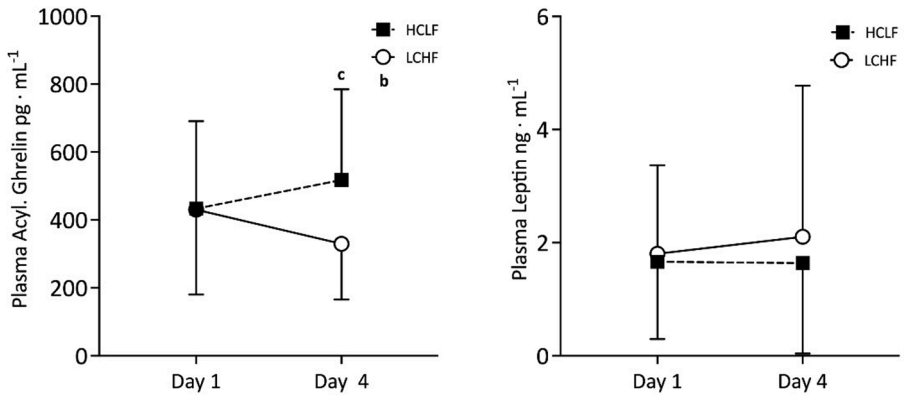

Fig. 5. Acylated ghrelin and leptin plasma concentration values for the comparisons between days. Values are presented as mean ± SD. [b] condition effect; [c] Time x condition interaction.

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Ethics approval

This study was performed following the principles of the Declaration of Helsinki and approved by the local ethics committee of the University of Birmingham, UK (code: ERNE_20–1826). All participants provided individually written consent before taking part in this study.

CRediT authorship contribution statement

I. Podestá D: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **A.K. Blannin:** Conceptualization, Supervision, Writing – review & editing. **G.A. Wallis:** Conceptualization, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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