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1 **Substituting carbohydrate at lunch for added protein increases fat oxidation during**
2 **subsequent exercise in healthy males**

3 Tommy Slater¹, William J A Mode¹, Louise C Bonnard¹, Cian Sweeney¹, Mark P Funnell²,
4 Harry A Smith³, John Hough¹, Ruth M James¹, Ian Varley¹, Craig Sale⁴, James A Betts³, Lewis J
5 James², and David J Clayton^{1*}

6 **Affiliations:**

7 ¹*Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement*
8 *Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham,*
9 *NG11 8NS, UK.*

10 ²*National Centre for Sport and Exercise Medicine, School of Sport, Exercise and Health*
11 *Sciences, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK.*

12 ³*Centre for Nutrition Exercise and Metabolism, Department for Health, University of Bath, Bath,*
13 *BA2 7AY, UK.*

14 ⁴*Department of Sport and Exercise Sciences, Manchester Metropolitan University Institute of*
15 *Sport, Manchester, M1 7EL, UK.*

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17 ***Corresponding author:** Dr David J Clayton

18 Senior Lecturer in Nutrition and Exercise Physiology

19 Sport, Health and Performance Enhancement Research Centre, School of Science and
20 Technology; Clifton Campus; Nottingham Trent University; Nottingham; Nottinghamshire;
21 NG11 8NS; UK

22 Email: David.Clayton@ntu.ac.uk

23 Tel: +44 (0) 115 84 85514

24 ORCID ID: 0000-0001-5481-0891

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27

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2 energy intake.
3
4 **Clinical Trial Registration:** <https://clinicaltrials.gov/ct2/show/NCT05107583>

ACCEPTED MANUSCRIPT

1 Abstract

2 **Context:** How pre-exercise meal composition influences metabolic and health responses to
3 exercise later in the day is currently unclear. **Objective:** Examine the effects of substituting
4 carbohydrate for protein at lunch on subsequent exercise metabolism, appetite, and energy
5 intake. **Methods:** Twelve healthy males completed three trials in randomized, counterbalanced
6 order. Following a standardized breakfast (779 ± 66 kcal; $\sim 08:15$), participants consumed a lunch
7 (1186 ± 140 kcal; $\sim 13:15$) containing either $0.2 \text{ g}\cdot\text{kg}^{-1}$ carbohydrate and $\sim 2 \text{ g}\cdot\text{kg}^{-1}$ protein (LO-
8 CARB), $2 \text{ g}\cdot\text{kg}^{-1}$ carbohydrate and $\sim 0.4 \text{ g}\cdot\text{kg}^{-1}$ protein (HI-CARB), or fasted (FAST).
9 Participants later cycled at $\sim 60\% \dot{V}O_{2\text{peak}}$ for 1 h ($\sim 16:15$) and post-exercise *ad-libitum* energy
10 intake was measured ($\sim 18:30$). Substrate oxidation, subjective appetite, and plasma
11 concentrations of glucose, insulin, non-esterified fatty acids (NEFA), peptide YY (PYY),
12 glucagon-like peptide-1 (GLP-1), and acylated ghrelin (AG) were measured for 5 h post-lunch.
13 **Results:** Fat oxidation was greater during FAST ($+11.66 \pm 6.63$ g) and LO-CARB ($+8.00 \pm 3.83$
14 g) than HI-CARB ($p < 0.001$), with FAST greater than LO-CARB ($+3.67 \pm 5.07$ g; $p < 0.05$).
15 NEFA were lowest in HI-CARB and highest in FAST, with insulin demonstrating the inverse
16 response (all $p < 0.01$). PYY and GLP-1 demonstrated a stepwise pattern, with LO-CARB greatest
17 and FAST lowest (all $p < 0.01$). AG was lower during HI-CARB and LO-CARB *versus* FAST
18 ($p < 0.01$). Energy intake in LO-CARB was lower than FAST (-383 ± 233 kcal; $p < 0.001$) and HI-
19 CARB (-313 ± 284 kcal; $p < 0.001$). **Conclusion:** Substituting carbohydrate for protein in a pre-
20 exercise lunch increased fat oxidation, suppressed subjective and hormonal appetite, and reduced
21 post-exercise energy intake.

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1 **Abbreviations:** DTE: Desire to eat, EER: Estimated energy requirements, FAST: No lunch
2 (extended fasting) experimental trial, LO-CARB: Low-carbohydrate lunch experimental trial,
3 HI-CARB: High-carbohydrate lunch experimental trial.
4

5 **Introduction**

6 Regular physical exercise is associated with numerous well-established health benefits (1) and
7 can aid weight management (2). Interestingly, pre-exercise nutritional state can mediate the
8 benefits of exercise, as performing a single bout of exercise after a prolonged fast (>12 h) has
9 been shown to increase fat oxidation (3, 4) which, if performed regularly over a 6-week period,
10 can improve fat oxidative capacity (5, 6). This is associated with improved markers of metabolic
11 health (7), meaning regular overnight-fasted exercise training may augment improvements in
12 insulin sensitivity (6, 8). Compared to fed exercise, overnight-fasted exercise may also aid in
13 regulating energy balance. For example, compared to consuming breakfast before exercise, an
14 acute bout of fasted exercise has no effect (4, 9, 10), or only slightly increases (3) lunch energy
15 intake. This leads to a reduced cumulative energy intake which appears to persist over 24 h (9),
16 without any compensatory effects on 24 h energy expenditure (3).

17 Most fasted exercise studies have been conducted in the morning, but morning exercise may not
18 be possible or desirable for many. Macronutrient metabolism and appetite demonstrate circadian
19 variation (11), so findings from overnight-fasted morning exercise may not translate to other
20 times of day. One study showed that fasted exercise later in the day upregulates fat oxidation
21 (12), but prolonged daytime fasting also elevates appetite, increases energy intake, and
22 ultimately reduces motivation to exercise, exercise enjoyment, and exercise performance (13).

1 The metabolic benefits of fasted exercise may be driven by carbohydrate restriction, rather than
2 fasting *per se*, with studies demonstrating that low-carbohydrate, high-protein feeding before
3 morning exercise does not blunt fat oxidation compared to fasted exercise (14–16). Moreover,
4 markers of training adaptation with implications for improved insulin sensitivity, such as AMPK
5 signaling, as well as CD36 and PGC-1 α mRNA expression, are upregulated following protein-
6 only feeding (16–18). A high-protein meal also reduces appetite and energy intake to a greater
7 extent than high-carbohydrate or high-fat meals (19), which may aid weight management efforts.
8 This provides a practical rationale for overcoming some of the difficulties associated with
9 conducting fasted exercise later in the day, but the metabolic and appetite-related effects of a
10 low-carbohydrate, high-protein pre-exercise meal, relative to a more typical high-carbohydrate
11 pre-exercise meal and fasting, are not well understood.

12 Exercise often takes place later in the day, meaning the composition of meals consumed earlier
13 in the day likely influence metabolic and health outcomes. However, the physiological and
14 subjective responses to different pre-exercise meal compositions are not well understood.
15 Therefore, the aim of this study was to examine the effects of consuming a low-carbohydrate,
16 high-protein lunch prior to late-afternoon/early-evening (*i.e.*, 16:15) cycling exercise on substrate
17 oxidation, compared to a high-carbohydrate, lower-protein lunch, and fasting. Secondary aims
18 were to assess the effects of pre-exercise meal composition on exercise metabolism, appetite, and
19 subsequent energy intake.

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1 **Methods**

2 *Participants*

3 After ethical approval (Nottingham Trent University Ethical Advisory Committee: application
4 number 704; ClinicalTrials registration no: NCT05107583), 12 healthy males completed the
5 study (age 25 ± 2 y; height 1.81 ± 0.08 m; body mass 81.4 ± 10.2 kg; body fat $17 \pm 6\%$; $\dot{V}O_{2\text{peak}}$
6 45 ± 7 mL·kg⁻¹·min⁻¹). Participants were required to be aged 18–40 years, recreationally active
7 (1–10 h·wk⁻¹), and self-reported to have been weight stable for the 6 months before commencing
8 the study to be included. Participants were excluded if they currently smoked, were classified as
9 clinically restrained, disinhibited, or hungry eaters (20), a severe dislike or intolerance of any
10 study foods or drinks, were currently undergoing a structured diet and/or exercise intervention
11 aiming to achieve weight loss, reported a history of gastric, digestive, cardiovascular, or renal
12 disease, were taking medication or undergoing treatment known to affect glucose/lipid
13 metabolism or appetite, or were consuming >14 units of alcohol per week. Participants provided
14 written informed consent and completed a health screening questionnaire before commencing the
15 study.

16 Sample size was estimated using G*Power software (v3.1), an α of 0.05, and β of 0.90. Using fat
17 oxidation data from a similar study which observed an effect size of 0.54 when comparing
18 carbohydrate- versus protein-fed exercise (15), it was estimated that 11 participants would be
19 required to detect a 15% difference in fat oxidation during exercise. For energy intake, an energy
20 deficit of 100 kcal·day⁻¹ is recommended to prevent excess weight gain in 90% of the US
21 population (21). Mean energy intake based on our previous work using an identical meal was
22 ~1070 kcal for males (13). Therefore, we deemed 10% a clinically meaningful difference

1 between experimental conditions. Based on these values and an effect size of 0.61 from our
2 previous study (13), it was estimated that 8 participants would be required to detect a 10%
3 difference in post-exercise energy intake. As such, we recruited 12 participants to adequately
4 power both aims of the study and enable a counterbalanced study design.

6 ***Study design***

7 Participants completed two preliminary trials, and three experimental trials (completed between
8 November 2021 and March 2022 in Nottingham Trent University laboratories) in a randomized
9 (trial order drawn out of a bag), counterbalanced order, separated by ≥ 7 days (11 ± 7 days).
10 Experimental trials involved consuming a standardized carbohydrate-rich breakfast at home (779
11 ± 66 kcal; $\sim 08:15$), before a lunch (1186 ± 140 kcal; $\sim 13:15$) containing either 0.2 g·kg body
12 mass^{-1} carbohydrate and ~ 2 g·kg body mass^{-1} protein (LO-CARB), 2 g·kg body mass^{-1}
13 carbohydrate and ~ 0.4 g·kg body mass^{-1} protein (HI-CARB), or a water-only lunch (FAST) in
14 the laboratory. The energy content of the LO-CARB and HI-CARB meals was matched by
15 substituting carbohydrate for protein, whilst keeping fat content similar. Three hours later
16 ($\sim 16:15$), participants completed 1 h of cycling ($\sim 60\%$ $\dot{V}O_{2\text{peak}}$), before *ad-libitum* energy intake
17 was assessed at dinner and from a selection of snacks provided after participants left the
18 laboratory (Figure 1). Participants were blinded to the compositional differences between LO-
19 CARB and HI-CARB meals until completion of the study. Trials took place in a laboratory (20.6
20 ± 3.0 °C, $44.9 \pm 8.7\%$ relative humidity, 751 ± 8 mmHg barometric pressure).

21

22

1 ***Preliminary trials***

2 The first preliminary trial involved measuring participants' body mass, height, and skinfold
3 thickness (22), before $\dot{V}O_{2\text{peak}}$ was determined on an electronically braked cycle ergometer (Lode
4 Corival, Netherlands). The test involved 4-min incremental stages separated by ~5 min rest until
5 volitional exhaustion, with 1-min expired gas samples collected during the final minute of each
6 increment. The second preliminary trial involved familiarization with key aspects of the
7 experimental protocol (*i.e.*, cycling and *ad-libitum* eating procedures).

8 9 ***Pretrial standardization***

10 Participants recorded all dietary intake (including caffeine intake) and physical activity in the 24
11 h before the first experimental trial, replicating this before subsequent trials. Strenuous activities
12 and alcohol intake were prohibited during this period, with adherence confirmed verbally upon
13 arrival at the laboratory prior to each trial. On the evening before trials, participants ceased food
14 and caffeine intake at 20:00 and fasted overnight (other than plain water, which was standardized
15 between trials). Participants arrived at the laboratory via motorized transport.

16 17 ***Protocol***

18 At ~08:15 (-5 h), participants completed baseline measures of subjective appetite before
19 consuming a standardized breakfast. Participants arrived at the laboratory between 12:15–12:45
20 (consistent between trials within-participant). An indwelling cannula was inserted into an
21 antecubital vein, and after 30 min supine rest, a baseline blood sample, expired gas sample, and

1 subjective appetite measures, were collected. At ~13:15 (0 h), participants consumed an
2 experimental lunch meal (LO-CARB and HI-CARB), or volume of water equal to the water
3 content of LO-CARB and HI-CARB meals (FAST). After lunch (0.5 h), subjective perceptions
4 of the meal were collected (LO-CARB and HI-CARB only), and participants rested in the
5 laboratory, with blood and expired gas samples collected after 30 min of supine rest at 1, 1.75,
6 and 2.75 h. At 3 h (~16:15), subjective measures of appetite, mood, exercise readiness, and a pre-
7 exercise blood sample were collected before participants completed 1 h of cycling at an intensity
8 calculated to elicit ~60% $\dot{V}O_{2peak}$. During exercise, expired gas samples were collected between
9 28–30 and 58–60 min, venous blood samples collected at 30 and 60 min, and heart rate and
10 rating of perceived exertion (RPE) recorded every 15 min, with subjective appetite and exercise
11 enjoyment measured immediately post-exercise. Final expired gas and venous blood samples
12 were collected 1 h post-exercise after 30 min of supine rest. An *ad-libitum* meal was served 1.25
13 h post-exercise (~18:30), with participants permitted 20 min to eat. Participants then left the
14 laboratory, taking a selection of snacks which they could choose to consume *ad-libitum* at home
15 between 20:00–22:00 only. Outside of the snacking window, participants were instructed to
16 refrain from food and caffeine intake until after the final subjective appetite questionnaire was
17 completed at 08:15 the following day, although *ad-libitum* water intake was permitted (volume
18 recorded). Adherence to these instructions was confirmed via text messaging.

19 20 ***Standardized breakfast meal***

21 Meals were provided as a percentage of estimated energy requirements (EER; resting metabolic
22 rate (23) multiplied by a physical activity factor of 1.7 to account for the exercise component of

1 the trial). A standardized breakfast (25% EER; Table 1) consisting of porridge (Oatso Simple
2 Golden Syrup, Quaker, UK), cereal bars (Belvita, Mondelez, UK), yoghurt (Ski Strawberry,
3 Nestlé, UK), and strawberry milkshake (Yazoo, Campina Ltd., UK) was provided in all
4 experimental trials (Table S1) (24).

6 *Experimental lunch meals*

7 In HI-CARB and LO-CARB, lunch (35–40% EER) consisted of tuna and mayonnaise
8 sandwiches, crisps, and a blended drink. Meals provided either 0.2 g·kg body mass⁻¹
9 carbohydrate and ~2.0 g·kg body mass⁻¹ protein (LO-CARB) or 2 g·kg body mass⁻¹ carbohydrate
10 and ~0.4 g·kg body mass⁻¹ protein (HI-CARB) (Table 1). The energy content of meals was
11 matched primarily via the manipulation of carbohydrate and protein content. Fat content was
12 closely matched between trials to limit effects of dietary fat on substrate oxidation (25). The
13 bread (Hovis, UK) and crisps (Walkers, UK) provided in HI-CARB were substituted for low-
14 carbohydrate, high-protein bread (LivLife, UK) and crisps (MyProtein, UK) in LO-CARB. The
15 HI-CARB blended drink comprised of water, maltodextrin (MyProtein, UK), full-fat milk,
16 chocolate milkshake powder (Nesquik, Nestlé, UK), sucralose sweetener (ASDA, UK), and
17 thickening agent xanthan gum (Doves Farm, UK). The LO-CARB drink consisted of water,
18 chocolate-flavored soy protein isolate (MyProtein, UK), double cream (ASDA, UK), and
19 sucralose sweetener (ASDA, UK) (Table S1) (24). In FAST, participants consumed water equal
20 to the water content of LO-CARB and HI-CARB meals. To eliminate the possibility of water
21 intake influencing appetite, water intake was provided at 30 mL·kg body mass⁻¹ in all trials,

1 distributed into 7 equal volumes consumed: 08:15–10:30; 10:30–12:30; 14:15–15:15; 15:15–
2 16:15; first half of exercise (16:15–16:45); second half of exercise (16:45–17:15); 17:15–18:15.

3

4 ***Ad-libitum energy intake***

5 *Ad-libitum* dinner energy intake was determined by weighing food items before and after
6 consumption. Dinner consisted of pasta, tomato sauce, and extra virgin olive oil, containing 1.25
7 ± 0.01 kcal·g⁻¹ (69% carbohydrate, 11% protein, 18% fat, and 2% fiber), and was provided in
8 excess of expected consumption (Table S2) (24). Participants ate in isolation to eliminate
9 distractions until they felt “*comfortably full and satisfied*”, with water available *ad-libitum*.
10 Participants remained in the booth for the entire 20-min period and reported ceasing to eat within
11 this time in all trials. In all experimental trials, the selection of snacks which could be consumed
12 *ad-libitum* at home included 4 chocolate bars (Mars, UK), 2 cereal bars (Special K, Kellogg’s,
13 UK), 2 packets of ready salted crisps (Walkers, UK), 2 apples, and 2 satsumas (Table S2) (24).
14 All items consumed outside the laboratory were weighed before being provided and reweighed
15 the following day within the laboratory after collection from the participant by a researcher. The
16 energy densities used to derive total energy intake from each macronutrient were: 4 kcal·g⁻¹ for
17 carbohydrate, 4 kcal·g⁻¹ for protein, 9 kcal·g⁻¹ for fat, and 2 kcal·g⁻¹ for fiber.

18

19 ***Expired gas samples***

20 At rest, expired gas was sampled for 10 min after laying supine for 25 min. The first 5 min
21 served as a familiarization period, after which the sample was discarded. During the second 5

1 min, expired gas was collected into a Douglas bag for analysis. During exercise, expired gas was
2 sampled for 3 min, which included a 1 min familiarization period (sample discarded), with the
3 subsequent 2 min sample collected and analyzed. Expired gas was analyzed for oxygen and
4 carbon dioxide concentrations (MiniHF 5200, Servomex, UK), volume (Harvard Dry Gas Meter,
5 Harvard Ltd., UK), and temperature, and substrate oxidation rates calculated (26).

6 7 *Subjective responses*

8 Participants rated hunger, fullness, desire to eat (DTE), prospective food consumption (PFC),
9 and nausea on digital visual analogue scales (VAS) sent to their mobile telephone at -5, 0, 0.5, 1,
10 1.75, 3, 4, 5.25, 5.75, 6.75, 8.75, and 19 h. Additionally, motivation to exercise, readiness to
11 exercise, tiredness, and energy, were rated pre-exercise (3 h). VAS were administered using
12 SurveyMonkey.com, with 0–100 sliding scales including written anchors “not at all”/“no desire
13 at all”/“none at all” and “extremely”/“a lot” at 0 and 100, respectively. Participants also
14 completed a paper-based Positive and Negative Affect Schedule (PANAS) (27); pre-exercise.
15 Enjoyment of exercise was assessed immediately post-exercise using a paper-based, shortened
16 version of the Physical Activity Enjoyment Scale (PACES-8) (28).

17 Additional VAS relating to perceptions of the overall meal (how pleasant), the sandwich (how
18 pleasant, dry, moist, chewy), and the drink (how pleasant, bitter, sweet, creamy, thick, sticky,
19 salty) were completed by participants immediately after lunch in LO-CARB and HI-CARB.

20

21

1 ***Blood sampling and analyses***

2 Blood samples (~10 mL) were drawn from an antecubital vein. The first 2 mL was discarded,
3 before 4.9 mL blood was collected into an EDTA monovette (1.6 mg·mL⁻¹; Sarstedt AG & Co.,
4 Germany). A further 2.7 mL blood was collected into an EDTA monovette (1.6 mg·mL⁻¹)
5 containing 27 µL of a potassium phosphate buffer (PBS; 0.05 M), P-hydroxymercuribenzoic acid
6 (PHMB; 0.05 M), and sodium hydroxide (NaOH; 0.006 M) solution, to prevent degradation of
7 acylated ghrelin. Following collection, blood samples were centrifuged (1700 g, 15 min, 4°C),
8 the supernatant (1 mL) of the PHMB/PBS/NaOH-treated blood was mixed with 100 µL
9 hydrochloric acid (1 M), and plasma was stored at -80°C until analysis. Acylated ghrelin (intra-
10 assay CV 1.8–6.2%; LoD <5 pg·mL⁻¹; Bertin Technologies, France; Catalog #A05106, RRID:
11 AB_3083805), insulin (intra-assay CV 2.7–5.8%; LoD 6 pmol·L⁻¹; Merckodia, Sweden; Catalog
12 #10-1113-01, RRID: AB_2877672), total PYY (intra-assay CV 1.6–4.0%; LoD 6.5 pg·mL⁻¹;
13 Merck Millipore Ltd., UK; Catalog #EZHPYYT-66K, RRID: AB_2910201), and total GLP-1
14 (intra-assay CV 2.2–4.4%; LoD 1.5 pM·L⁻¹; Merck Millipore Ltd.; Catalog #EZGLP1T-36K,
15 RRID: AB_2813786) concentrations were determined by ELISA. Plasma glucose (intra-assay
16 CV 0.2–0.4%; LoD 0.1 mmol·L⁻¹; Horiba Ltd., UK), non-esterified fatty acid (NEFA; intra-assay
17 CV 1.0%; LoD 0.072 mmol·L⁻¹; Randox Laboratories Ltd., UK), and glycerol (intra-assay CV
18 7.5%; LoD 14.5 µmol·L⁻¹; Randox Laboratories Ltd.) concentrations were determined by
19 enzymatic colorimetric assay. To avoid inter-assay variation from influencing results, samples
20 from the same participant were analyzed within the same run/assay.

21

22

1 *Statistical analyses*

2 Data were analyzed using SPSS v26.0 (IBM, USA). Raw data were checked for normality using
3 a Shapiro-Wilk test. Incremental area under the curve (iAUC) or total area under the curve
4 (tAUC) were calculated with the trapezoid method using the Time Series Response Analyzer
5 tool (29). For appetite-related variables, AUC values were determined in response to breakfast
6 (08:15–13:15), lunch (13:15–16:15), exercise (16:15–18:30), and dinner/overnight (18:30–
7 08:15). Data containing one factor (*ad-libitum* energy intake, exercise subjective responses,
8 laboratory conditions, iAUC, and tAUC) were analyzed using one-way repeated-measures
9 ANOVA and data containing two factors (plasma substrate/hormone concentrations, energy
10 expenditure and substrate oxidation rates, and subjective appetite sensations) were analyzed
11 using two-way repeated-measures ANOVA. Assumptions of sphericity of the ANOVA were
12 checked and adjustments for the degrees of freedom were made using the Greenhouse-Geiser (ϵ
13 < 0.75) or Huynh-Feldt ($\epsilon > 0.75$) correction, where appropriate. Significant ANOVA effects
14 were explored with *post-hoc* paired samples *t*-tests (normally distributed data), or Wilcoxon
15 Signed-Rank tests (non-normally distributed data), with Holm-Bonferroni stepwise correction.
16 Data sets were considered statistically different when $p < 0.05$. Data are presented as mean \pm SD,
17 unless stated. Effect sizes (Cohen's *dz*) were calculated, with 0.2, 0.5, and 0.8 representing small,
18 medium, and large effect sizes (30). A one-way repeated-measures ANOVA was conducted on
19 the primary outcome of fat oxidation during exercise to show no systematic effect of trial order
20 ($p \leq 0.677$).

21

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1 Results

2 *Energy expenditure and substrate oxidation*

3 There were trial-by-time interaction effects for fat and carbohydrate oxidation rates (both $p <$
4 0.001), where total fat oxidation across the trial period was higher and carbohydrate oxidation
5 lower in LO-CARB and FAST versus HI-CARB, as well as in FAST versus LO-CARB ($dz =$
6 0.98–2.03; all $p < 0.01$; see Figure 2 for specific time points). There was a main effect of trial (p
7 < 0.001), but no trial-by-time interaction effect for energy expenditure ($p = 0.119$). Total energy
8 expenditure across the trial period was greater in LO-CARB ($dz = 2.49$; $p < 0.001$) and HI-
9 CARB ($dz = 0.87$; $p = 0.012$) versus FAST, and in LO-CARB versus HI-CARB ($dz = 1.00$; $p <$
10 0.01; Figure 2).

11 During exercise, fat oxidation was 8.00 ± 3.83 g greater in LO-CARB ($dz = 2.10$; $p < 0.001$) and
12 11.66 ± 6.63 g greater in FAST ($dz = 1.75$; $p < 0.001$) versus HI-CARB and was also 3.67 ± 5.07
13 g greater in FAST versus LO-CARB ($dz = 0.73$; $p = 0.029$). Carbohydrate oxidation was $17.21 \pm$
14 10.16 g lower in LO-CARB ($dz = 1.71$; $p < 0.01$) and 30.25 ± 17.39 g lower in FAST ($dz = 1.75$;
15 $p < 0.001$) versus HI-CARB and was also 13.04 ± 13.55 g lower in FAST versus LO-CARB (dz
16 $= 0.98$; $p < 0.01$). Exercise energy expenditure was 17 ± 16 kcal greater in LO-CARB versus
17 FAST ($dz = 1.12$; $p < 0.01$; Figure 3).

18 19 *Energy intake*

20 *Ad-libitum* dinner energy intake in LO-CARB was 262 ± 174 kcal lower than FAST ($dz = 1.52$; p
21 < 0.001) and 215 ± 135 kcal lower than HI-CARB ($dz = 1.58$; $p < 0.001$) but was not different
22 between FAST and HI-CARB ($dz = 0.41$; $p = 0.194$; Table 1). Snack energy intake (LO-CARB:

1 575 ± 272 kcal, FAST: 696 ± 246 kcal, HI-CARB: 673 ± 245 kcal; $dz = 0.09$ – 0.50 ; $p = 0.274$)
2 and macronutrient intake ($dz = 0.05$ – 0.84 ; all $p \geq 0.055$) were not different between trials.
3 Cumulative energy intake across the day was 803 ± 279 kcal greater in LO-CARB ($dz = 2.86$; p
4 < 0.001) and 1116 ± 315 kcal greater in HI-CARB ($dz = 3.56$; $p < 0.001$) versus FAST but was
5 also 313 ± 284 kcal greater during HI-CARB than LO-CARB ($dz = 1.10$; $p < 0.01$).

7 ***Blood parameters***

8 There were trial-by-time interaction effects for plasma PYY and GLP-1 concentrations (both $p <$
9 0.001). For plasma acylated ghrelin concentrations, there was a main effect of trial ($p < 0.001$),
10 but no trial-by-time interaction effect ($p = 0.067$). PYY and GLP-1 tAUC were greater in LO-
11 CARB ($dz = 2.66$ – 4.01 ; both $p < 0.001$) and HI-CARB ($dz = 1.16$ – 1.48 ; both $p < 0.01$) versus
12 FAST and in LO-CARB versus HI-CARB ($dz = 1.98$ – 3.22 ; both $p < 0.001$; see Figure 4 for
13 specific time points). Acylated ghrelin tAUC was lower in LO-CARB ($dz = 1.19$; $p < 0.01$) and
14 HI-CARB ($dz = 1.32$; $p < 0.01$) versus FAST (Figure 4).

15 There were trial-by-time interaction effects for plasma insulin, glucose, NEFA, and glycerol
16 concentrations (all $p < 0.001$). Insulin iAUC was lower in LO-CARB ($dz = 1.65$; $p < 0.001$) and
17 FAST ($dz = 1.87$; $p < 0.001$) versus HI-CARB and in FAST versus LO-CARB ($dz = 1.04$; $p <$
18 0.01; see Figure 5 for specific time points). Glucose iAUC was lower in both LO-CARB ($dz =$
19 1.17; $p < 0.01$) and FAST ($dz = 1.41$; $p < 0.01$) versus HI-CARB, although glucose was lower at
20 1 h ($dz = 1.16$ – 1.31 ; both $p \leq 0.036$), and higher at 3 h ($dz = 1.54$ – 1.68 ; both $p < 0.01$), in LO-
21 CARB and FAST versus HI-CARB. tAUC for NEFA was greater in LO-CARB ($dz = 0.93$; $p <$
22 0.01) and FAST ($dz = 2.61$; $p < 0.001$) versus HI-CARB, and in FAST versus LO-CARB ($dz =$

1 1.31; $p < 0.01$; see Figure 5 for specific time points). tAUC for glycerol was greater in FAST
2 versus LO-CARB ($d_z = 1.48$; $p < 0.01$) and HI-CARB ($d_z = 1.78$; $p < 0.001$; see Figure 5 for
3 specific time points).

5 ***Subjective appetite responses***

6 There were trial-by-time interaction effects for hunger, fullness, DTE, and PFC (all $p < 0.001$;
7 Figure 6), but not nausea ($p = 0.367$). Following lunch, hunger, DTE, and PFC were lower, and
8 fullness was higher until 3 h in LO-CARB and HI-CARB versus FAST ($d_z = 1.60$ – 3.66 ; all $p <$
9 0.001), and these differences were still apparent at 4 ($d_z = 1.04$ – 1.73 ; all $p < 0.001$) and 5.25 h
10 ($d_z = 1.43$ – 2.00 ; all $p < 0.001$) between LO-CARB and FAST. Hunger and DTE were lower in
11 LO-CARB versus HI-CARB at 4 and 5.25 h, with fullness also higher at 5.25 h in LO-CARB (d_z
12 $= 1.30$ – 1.56 ; all $p \leq 0.019$).

13 Hunger, DTE, and PFC tAUC were all lower, and fullness tAUC greater in response to lunch and
14 exercise in LO-CARB ($d_z = 1.75$ – 3.55 ; all $p < 0.001$) and HI-CARB ($d_z = 1.47$ – 2.87 ; all $p <$
15 0.01) versus FAST, and in LO-CARB versus HI-CARB ($d_z = 1.00$ – 2.09 ; all $p < 0.01$).

17 ***Subjective exercise responses***

18 Participants reported lower pre-exercise energy in FAST versus LO-CARB ($d_z = 0.78$; $p =$
19 0.044) and HI-CARB ($d_z = 0.91$; $p = 0.028$), although motivation, tiredness, and readiness to
20 exercise were not different between trials ($d_z = 0.00$ – 0.58 ; all $p \geq 0.121$). Pre-exercise positive
21 affect ($d_z = 0.32$ – 0.51 ; $p = 0.103$) and negative affect ($d_z = 0.28$ – 0.66 ; $p = 0.137$), enjoyment of

1 exercise sessions ($dz = 0.12-0.55$; $p = 0.186$), RPE ($dz = 0.00-1.47$; $p = 0.070$), and heart rate (dz
2 $= 0.26-0.39$; $p = 0.249$) were not different between trials.

4 **Meal perceptions**

5 Overall pleasantness of the lunch meal was lower in LO-CARB ($dz = 2.18$; $p = 0.011$). The drink
6 was rated as both creamier and thicker in LO-CARB ($dz = 0.80-2.49$; $p \leq 0.014$), with no further
7 differences in perceptions ($dz = 0.32-0.95$; $p \geq 0.058$). The sandwich was rated as less pleasant
8 and chewier in LO-CARB ($dz = 1.02-1.16$; $p \leq 0.047$), with no further perceptual differences (dz
9 $= 0.42-0.51$; $p \geq 0.143$).

11 **Discussion**

12 The novel aspects of the present study were that a low-carbohydrate, high-protein lunch
13 increased fat oxidation during late-afternoon/early-evening exercise compared to an energy-
14 matched high-carbohydrate, lower-protein lunch, whilst also increasing the secretion of
15 anorexigenic hormones PYY and GLP-1 from the gastro-intestinal tract. Accordingly, the LO-
16 CARB lunch suppressed appetite and reduced *ad-libitum* energy intake in the evening by ~315
17 kcal compared to HI-CARB, and by ~385 kcal compared to FAST. These findings suggest that
18 carbohydrate restriction via consuming a low-carbohydrate, high-protein lunch could be used to
19 achieve many of the positive metabolic responses achieved from fasted exercise and mitigate the
20 appetite-related challenges associated with fasted exercise via endocrine signaling.

1 Our findings showed that fat oxidation was increased by 8.00 ± 3.83 g during 1 h of exercise
2 performed 3 h after consuming a low-carbohydrate (0.2 g·kg body mass⁻¹ carbohydrate; 6% of
3 energy), high-protein (~ 2 g·kg body mass⁻¹ protein; $\sim 53\%$ of energy) lunch, compared to an
4 isocaloric high-carbohydrate (2 g·kg body mass⁻¹ carbohydrate; 55% of energy), lower-protein
5 (~ 0.4 g·kg body mass⁻¹ protein; 10% of energy) lunch, albeit to a lesser extent than after an 8 h
6 fast (11.66 ± 6.63 g). This builds upon previous observations that similar fat oxidation can be
7 achieved during morning exercise with low-carbohydrate, high-protein feeding or complete
8 fasting (14–16). The $\sim 65\%$ difference in fat oxidation between protein- and carbohydrate-fed
9 exercise observed in the present study is considerably greater than the $\sim 19\%$ difference reported
10 in previous work (15). As the inhibition of fat oxidation during fed exercise is governed
11 primarily by the insulinemic response to carbohydrate ingestion (31), this is likely due to the
12 larger carbohydrate content of the LO-CARB meal compared to the previous study. Protein
13 feeding does not appear to attenuate fat oxidation rates to the same extent ($\sim 20\%$ difference in fat
14 oxidation between LO-CARB and fasting), suggesting that substituting pre-exercise
15 carbohydrate for protein may achieve much of the metabolic response associated with increased
16 fat oxidation, without enduring extended fasting during the day.

17 The stepwise increase in fat oxidation between trials was mirrored by a stepwise reduction in
18 insulin concentrations. Consuming carbohydrate increases plasma glucose and insulin
19 concentrations (32), inhibiting hormone-sensitive lipase activity and lipolysis (33), and
20 stimulating fatty acid re-esterification in adipose tissue (32, 33). This ultimately reduces fatty
21 acid availability for oxidation during exercise after carbohydrate intake (29, 30). Accordingly,
22 plasma NEFA concentrations showed stepwise increases between trials in-line with differences
23 in fat oxidation. The fat content of the pre-exercise meals was closely matched (44 ± 5 g versus

1 50 ± 6 g, or 34% versus 38% of energy), so it is unlikely that the differences in NEFA
2 concentrations following the LO-CARB and HI-CARB meals were a product of dietary fat
3 appearance, but rather indicate increased mobilization of endogenous lipid stores in LO-CARB.

4 Plasma glycerol concentrations, which are often used as a surrogate marker of adipose tissue
5 lipolysis (36), were, however, only elevated during FAST. This suggests that different
6 mechanisms may explain the increased fat oxidation in LO-CARB and FAST, likely increased
7 intramuscular triglyceride utilization (30, 35). It should be noted, however, that our data reflect
8 single time-point measures of plasma glycerol concentrations, and so it cannot be distinguished
9 whether changes represent alterations in glycerol appearance (lipolytic rate) or glycerol uptake
10 (14, 36). Therefore, it remains possible that any subtle differences in lipolytic rate between LO-
11 CARB and HI-CARB trials may have been masked by changes in glycerol uptake. Studies have,
12 however, reported elevated NEFA and glycerol concentrations during exercise after smaller
13 doses of protein (14, 37, 38), suggesting the high protein dose and the resultant insulin
14 concentrations in LO-CARB might have reduced lipolysis and fat oxidation compared to FAST.
15 This is supported by observations that even small increases in plasma insulin concentrations can
16 suppress lipolysis (41).

17 Postprandial concentrations of GLP-1 and PYY were greater in LO-CARB compared to HI-
18 CARB and FAST. This is likely due to the increased protein content of the LO-CARB meal, as
19 evidence supports a dose-dependent relationship between protein intake and postprandial
20 concentrations of GLP-1 and PYY (40, 41). Both GLP-1 and PYY are secreted from intestinal L-
21 cells in response to nutrient ingestion and are associated with reduced appetite and food intake in
22 humans via central effects on the hypothalamus, as well as other mechanisms including the
23 slowing of gastric emptying (44). In addition to its effects on appetite, GLP-1 is an incretin,

1 enhancing glucose-dependent insulin secretion (45), indicating that elevated GLP-1 in LO-
2 CARB also has the potential to benefit postprandial glucose control at subsequent meals.
3 Acylated ghrelin is an orexigenic hormone secreted by the stomach and is often regarded as a
4 biological mechanism to promote hunger and food intake (46). Acylated ghrelin was suppressed
5 by both lunch meals compared to fasting but was not different between LO-CARB and HI-
6 CARB. This suggests that the increase in protein intake in LO-CARB did not alter the acylated
7 ghrelin response, agreeing with some (40, 45), but not all (48) previous studies. However,
8 acylated ghrelin concentrations typically fall rapidly after the onset of food intake, often reaching
9 nadir values within 1 h (49). Therefore, although our findings indicate the appetite-suppressing
10 effects of the LO-CARB lunch were likely mediated via GLP-1 and PYY, delaying the first
11 blood sample until 1 h post-lunch may have missed potential differences in acylated ghrelin
12 concentrations between LO-CARB and HI-CARB meals.

13 The lunch provided during LO-CARB reduced evening energy intake by 22% and 27%
14 compared to HI-CARB and FAST. These responses may have been mediated by increased
15 secretion of GLP-1 and PYY. Our findings corroborate those of Oliveira et al. (40), who
16 similarly reported lower post-exercise hunger and greater post-exercise concentrations of PYY
17 and GLP-1 when performed after a high-protein meal. As well as suppressing appetite in the
18 postprandial period, meals with increased protein content have been shown to reduce appetite
19 and promote satiety during the meal (50). It is plausible that the high-protein content of the lunch
20 provided in LO-CARB may have reduced the volume of food consumed at this eating occasion.

21 A smaller meal would generally induce a smaller insulinemic response, possibly allowing for
22 greater rates of fat oxidation during exercise, which may have resulted in closer mirroring of
23 fasted exercise. We chose to match pre-exercise energy intake within the two eating occasions in

1 this study, so it was not possible to assess the effect of meal composition on within-meal satiety,
2 but this does represent an interesting avenue for future research. It should be acknowledged that
3 the LO-CARB lunch contained an additional ~9 g fiber than the HI-CARB lunch. Indeed, dietary
4 fiber can suppress hunger and reduce subsequent energy intake via mechanisms including
5 prolonged oral processing and increased gastric distension (51), which, in addition to its protein
6 content, may have contributed to the superior appetite-suppressing effects of the LO-CARB
7 lunch.

8 One important factor to note is that the reduction in *ad-libitum* evening energy intake after the
9 LO-CARB meal only compensated for ~32% of the lunch, so energy intake over the day was still
10 803 ± 278 kcal lower in FAST. This is consistent with previous studies exploring overnight-
11 fasted morning exercise (3, 4, 9, 10), but it is important to acknowledge the additional challenges
12 associated with fasted exercise later in the day. Slater et al. (13) showed that fasting from 11:30
13 until exercise at 18:30 increased appetite and reduced motivation to exercise, exercise
14 enjoyment, and exercise performance, agreeing with previous findings showing that elevated
15 appetite can reduce resistance exercise performance (52). Similarly, the present study showed
16 that fasting before late afternoon/early-evening exercise increased appetite and reduced pre-
17 exercise energy levels compared to both LO-CARB and HI-CARB, although other subjective
18 markers including motivation, tiredness, and readiness to exercise were unaffected. In any case, a
19 low-carbohydrate, high-protein lunch may help achieve a better psychological state for engaging
20 in regular exercise, whilst still increasing fat oxidation.

21 Whilst this study provides novel insights into the metabolic, perceptual, and energy intake
22 responses to late-afternoon/early-evening exercise after a low-carbohydrate, high-protein lunch,
23 findings must be interpreted in light of the study design. First, rates of substrate oxidation and

1 energy expenditure were not corrected for rates of protein oxidation. Although rates of protein
2 oxidation during exercise are generally considered negligible (26), protein content of the low-
3 carbohydrate lunch was high, so it is possible that the contribution of protein to oxidative
4 metabolism was greater than previously assessed. Secondly, we aimed to compare meals closely
5 matched for taste/texture that contained ecologically-valid components typically consumed at
6 lunch in the real world. The resultant high protein content of the low-carbohydrate lunch may be
7 challenging to replicate within the real world, although previous studies have shown increased
8 fat oxidation (14–16) and reduced appetite (40) following smaller, more ecologically-valid
9 protein doses. This study also recruited lean, healthy, and active men, meaning results cannot be
10 directly extrapolated to other population groups, particularly individuals with overweight or
11 obesity, females, or older adults, who may respond differently to fasting-based interventions (13,
12 49). For example, females generally exhibit higher relative rates of fat oxidation during exercise
13 than males (54), potentially enhancing the metabolic benefits associated with increased fat
14 oxidation during fasted or carbohydrate-restricted exercise. In contrast, older-adults often have
15 reduced fat oxidative capacity (55), which may attenuate the potential benefits of such exercise
16 interventions. Finally, this was an acute study, so it is not known whether these findings would
17 persist chronically.

18

19 **Conclusion**

20 This study showed that the acute consumption of a low-carbohydrate, high-protein lunch before
21 late-afternoon/early-evening exercise (*i.e.*, 16:15) increased fat oxidation compared to a high-
22 carbohydrate, lower-protein lunch, although the increase was less than that following an 8 h fast.
23 The low-carbohydrate, high-protein lunch also increased satiety-related hormone concentrations,

1 and reduced subjective appetite and subsequent energy intake, meaning that this meal
2 composition could offer some of the metabolic benefits associated with fasted exercise without
3 the need to endure daytime fasting. Future studies are required to explore whether acute exercise
4 performed after a low-carbohydrate, high-protein meal can be implemented on a regular basis as
5 a method of managing body weight/composition and maintaining metabolic health.

6

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10

11 **Author Contributions**

12 T.S., J.H., R.M.J, C.Sa., L.J.J., and D.J.C. designed the research, T.S., W.J.A.M., L.C.B., C.Sw.,
13 J.H., R.M.J., and D.J.C. conducted the research; T.S., W.J.A.M., M.P.F., H.A.S., J.A.B., I.V.,
14 and D.J.C. analyzed the data; the first draft of the manuscript was written by T.S., and all authors
15 critically reviewed previous versions of the manuscript. T.S. and D.J.C. had primary
16 responsibility for final content. All authors have read and approved the final manuscript.

17

18 **Data Availability**

19 Some or all datasets generated during and/or analyzed during the current study are not publicly
20 available but are available from the corresponding author on reasonable request.

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1 **Figure Legends**

2 **Figure 1.** Schematic representation of the study protocol.

3
4 **Figure 2. (a)** Fat oxidation, **(b)** carbohydrate oxidation, and **(c)** energy expenditure during HI-CARB,
5 LO-CARB and FAST. Data are presented at each time point (left) and as total area under the curve
6 (tAUC) for each trial (right). Data are mean \pm SD. White rectangle represents standardized lunch;
7 diagonal striped rectangle represents exercise. * LO-CARB vs. HI-CARB ($p < 0.05$); † LO-CARB vs.
8 FAST ($p < 0.05$); # HI-CARB vs. FAST ($p < 0.05$).

9
10 **Figure 3. (a)** Total fat oxidation, **(b)** total carbohydrate oxidation, and **(c)** total energy expenditure during
11 the 1 h cycling exercise in HI-CARB, LO-CARB and FAST. The bars display mean values, with vertical
12 error bars representing SD. The lines display individual subjects' substrate oxidation and energy
13 expenditure for each experimental trial. * LO-CARB vs. HI-CARB ($p < 0.05$); † LO-CARB vs. FAST (p
14 < 0.05); # HI-CARB vs. FAST ($p < 0.05$).

15
16 **Figure 4.** Plasma concentrations of **(a)** GLP-1, **(b)** PYY, and **(c)** acylated ghrelin during HI-CARB, LO-
17 CARB and FAST. Data are presented at each time point (left) and as total area under the curve (tAUC)
18 for each trial (right). Data are mean \pm SD (GLP-1 and PYY) or mean \pm SEM (acylated ghrelin). White
19 rectangle represents standardized lunch; diagonal striped rectangle represents exercise. * LO-CARB vs.
20 HI-CARB ($p < 0.05$); † LO-CARB vs. FAST ($p < 0.05$); # HI-CARB vs. FAST ($p < 0.05$).

21
22 **Figure 5.** Plasma concentrations of **(a)** glucose, **(b)** insulin, **(c)** non-esterified fatty acids (NEFA), and **(d)**
23 glycerol during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as
24 incremental area under the curve (iAUC) or total area under the curve (tAUC) for each trial (right). Data
25 are mean \pm SD. White rectangle represents standardized lunch; diagonal striped rectangle represents
26 exercise. * LO-CARB vs. HI-CARB ($p < 0.05$); † LO-CARB vs. FAST ($p < 0.05$); # HI-CARB vs. FAST
27 ($p < 0.05$).

28
29 **Figure 6. (a)** Hunger, **(b)** fullness, **(c)** prospective food consumption (PFC), and **(d)** desire to eat (DTE)
30 during HI-CARB, LO-CARB and FAST. Data are presented at each time point (left) and as time-
31 averaged total area under the curve (tAUC) for each trial (right). Data are mean \pm SEM. White rectangle
32 represents standardized lunch; grey rectangle represents *ad-libitum* dinner and snacking; diagonal striped
33 rectangle represents exercise. * LO-CARB vs. HI-CARB ($p < 0.05$); † LO-CARB vs. FAST ($p < 0.05$); #
34 HI-CARB vs. FAST ($p < 0.05$).

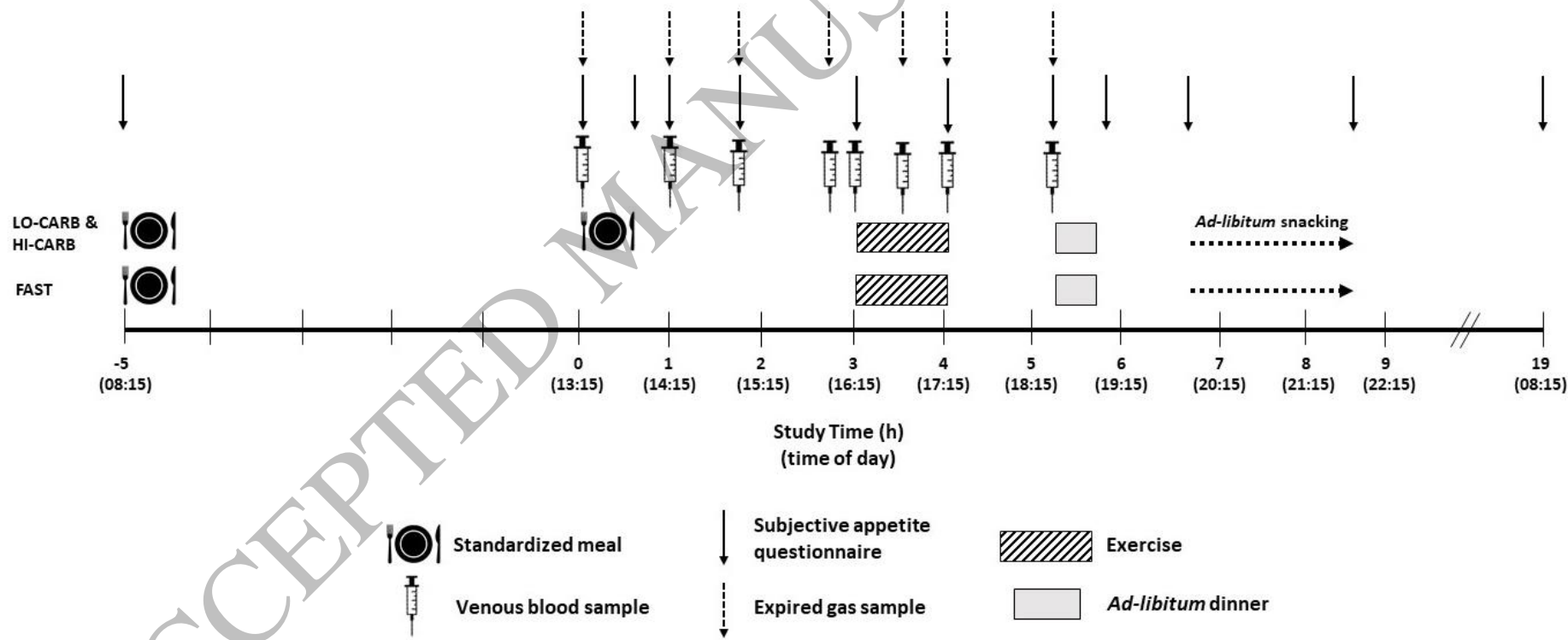
1 **Tables**2 **Table 1.** Macronutrient composition of each meal.

	Carbohydrate (g)	Protein (g)	Fat (g)	Fiber (g)	Energy (kcal)
Standardized Breakfast					
All	121.2 ± 9.2	24.1 ± 1.9	20.0 ± 1.7	9.4 ± 0.7	779 ± 66
Experimental Lunch					
LO-CARB	18.4 ± 2.5	157.7 ± 18.8	50.1 ± 5.9	15.3 ± 1.4	1186 ± 140 [†]
HI-CARB	163.2 ± 19.3	30.6 ± 3.9	44.3 ± 5.3	6.0 ± 0.5	1186 ± 140 [#]
FAST	0	0	0	0	0 [#]
Ad-Libitum Dinner					
LO-CARB	149.8 ± 38.0	23.6 ± 6.0	17.6 ± 4.5	8.2 ± 2.1	869 ± 220* [†]
HI-CARB	186.9 ± 43.1	29.4 ± 6.8	22.0 ± 5.1	10.3 ± 2.4	1084 ± 250*
FAST	195.0 ± 50.3	30.7 ± 7.9	22.9 ± 5.9	10.7 ± 2.8	1131 ± 292 [†]
Ad-Libitum Snack					
LO-CARB	83.1 ± 39.3	7.2 ± 3.5	22.9 ± 11.4	4.0 ± 2.9	575 ± 272
HI-CARB	98.8 ± 40.2	9.0 ± 4.4	25.5 ± 7.7	6.4 ± 3.9	673 ± 245
FAST	101.7 ± 37.7	9.6 ± 3.1	26.5 ± 9.6	6.2 ± 3.1	696 ± 246
Total					
LO-CARB	372.5 ± 60.4	212.6 ± 24.8	110.6 ± 16.0	36.8 ± 5.2	3409 ± 466* [†]
HI-CARB	570.1 ± 78.8	93.1 ± 11.4	111.7 ± 13.0	32.0 ± 5.6	3722 ± 478* [#]
FAST	417.8 ± 67.8	64.4 ± 9.8	69.4 ± 11.1	26.3 ± 4.9	2606 ± 403 ^{†#}

3 Data are mean ± SD

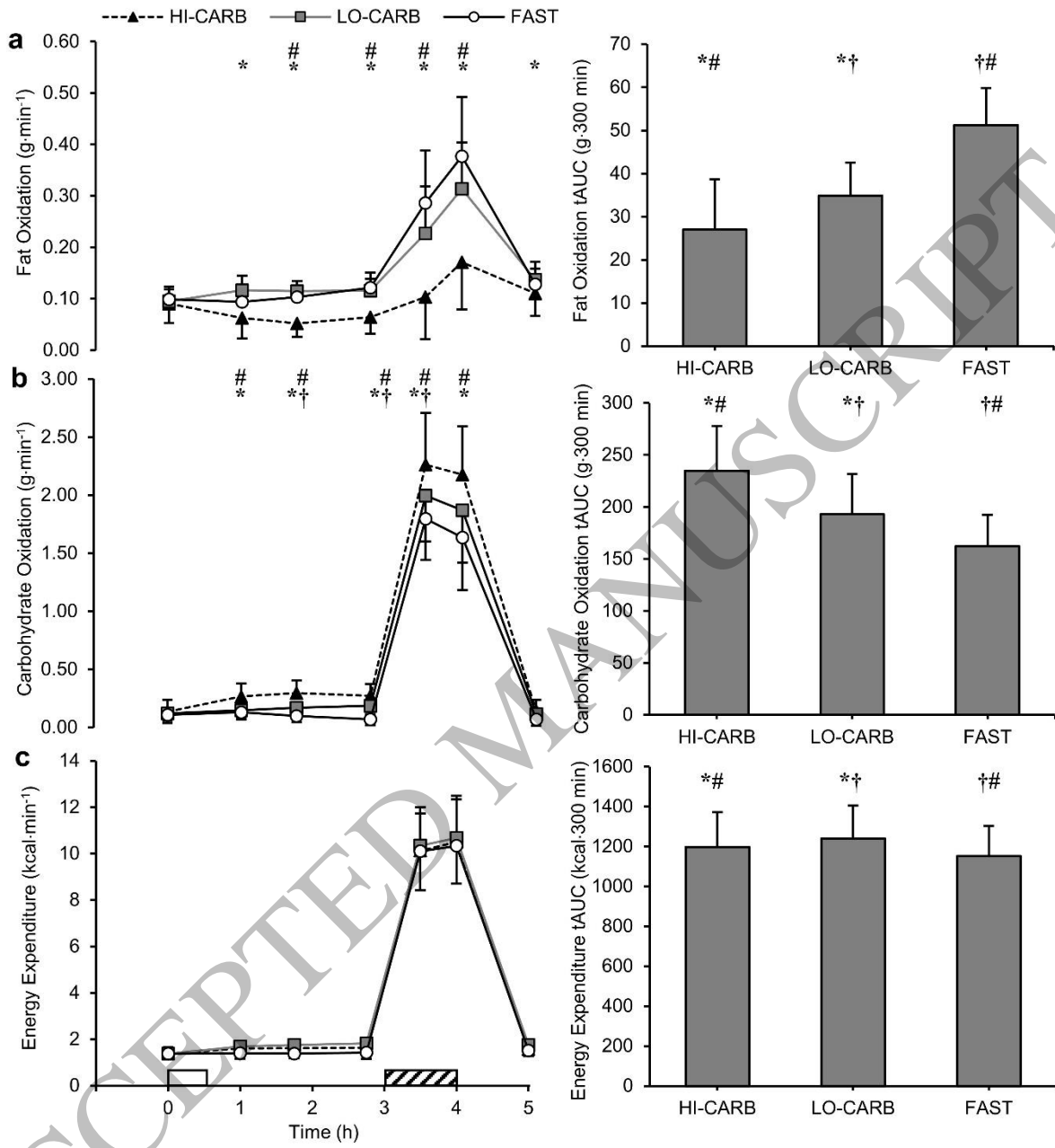
4 * LO-CARB vs. HI-CARB total energy intake ($p < 0.05$)5 † LO-CARB vs. FAST total energy intake ($p < 0.05$)6 # HI-CARB vs. FAST total energy intake ($p < 0.05$)

1 **Figures**



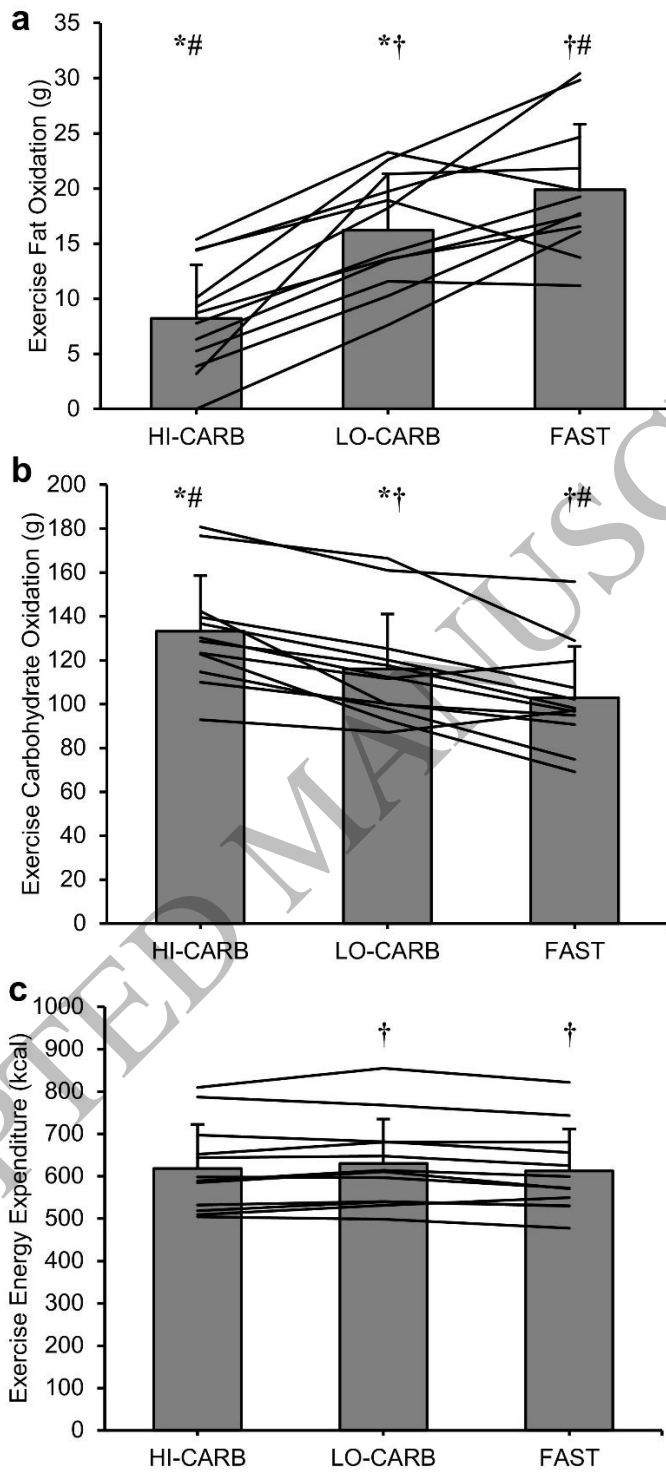
2 **Figure 1.**

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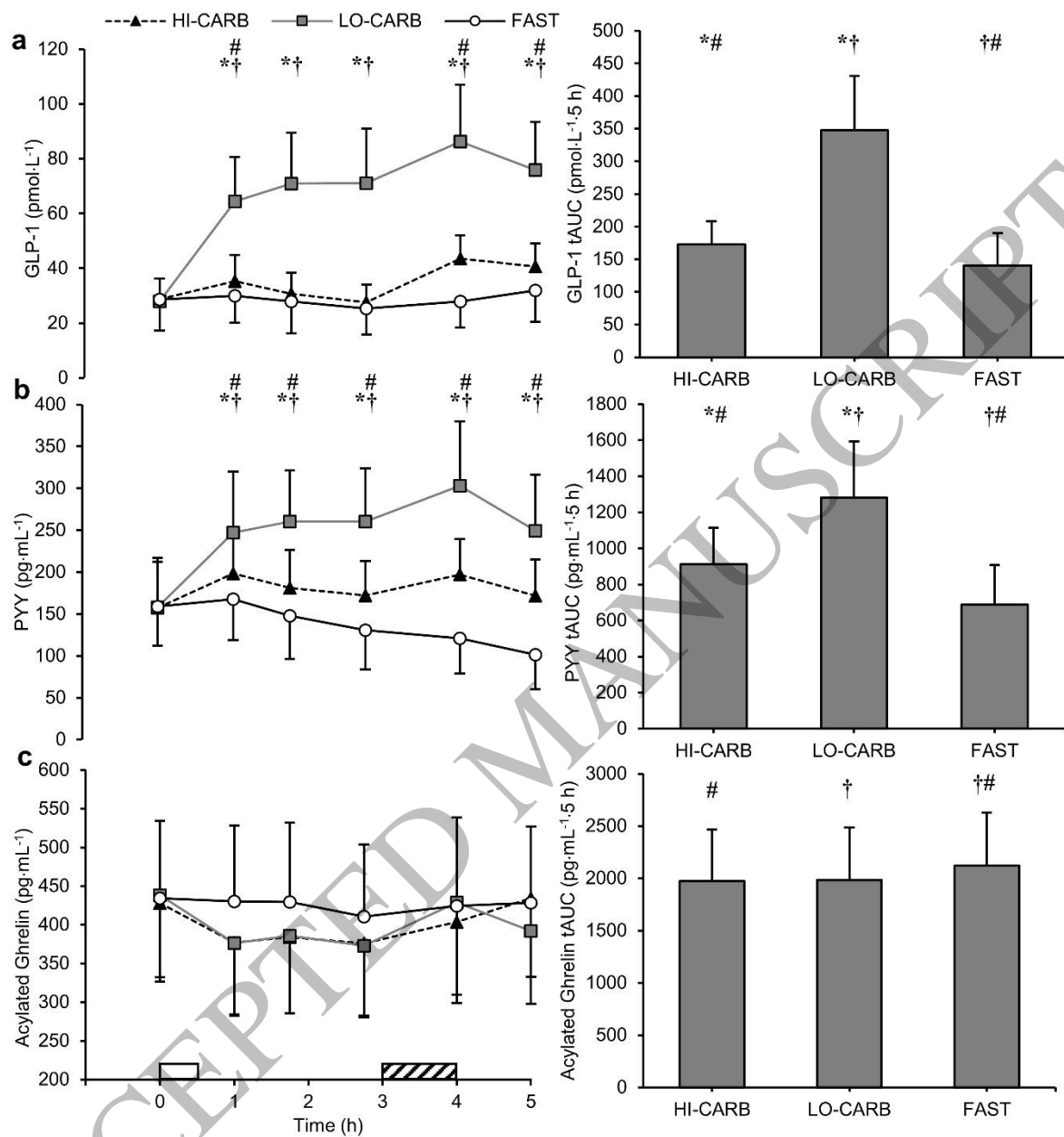
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2 **Figure 2.**



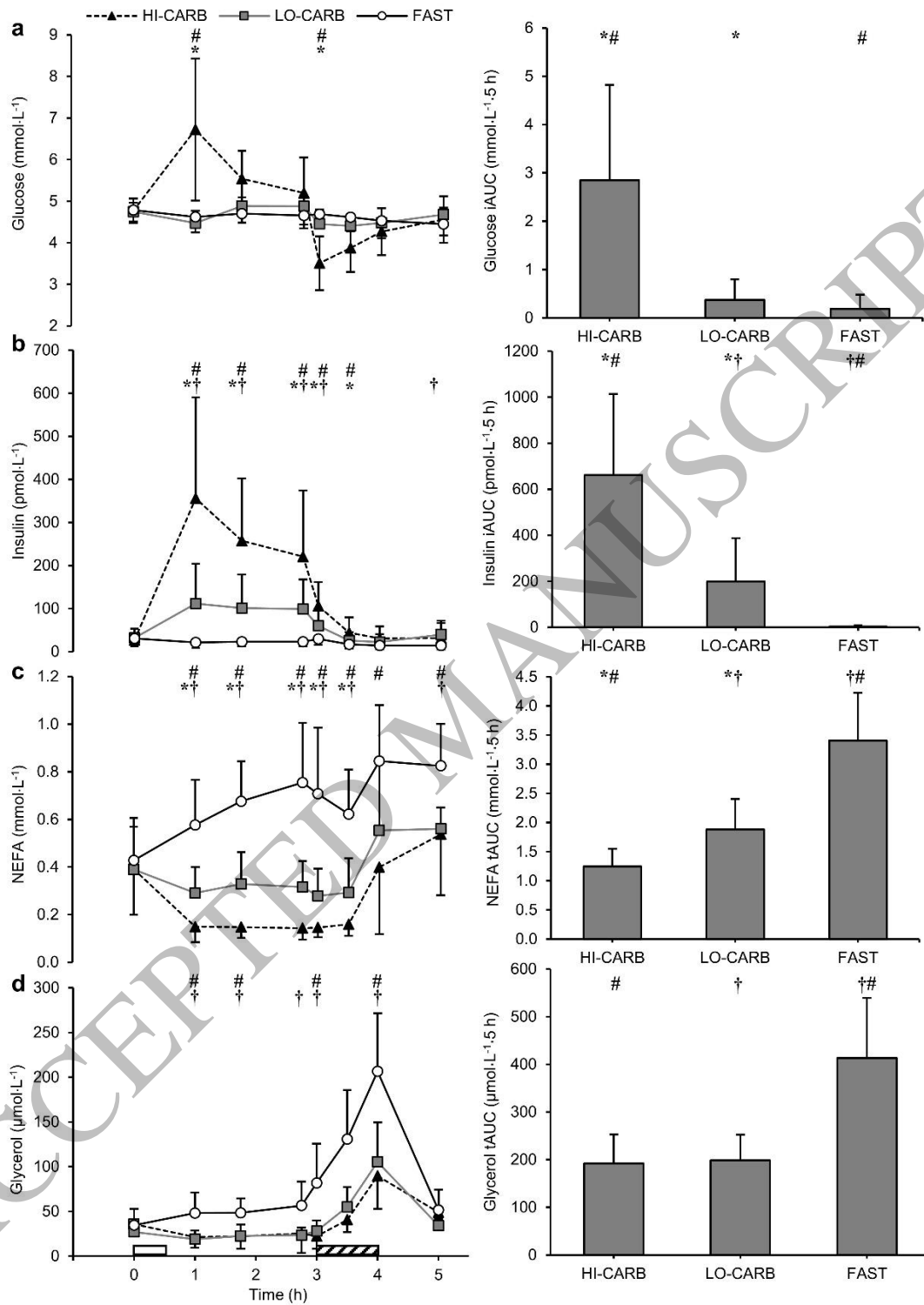
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Figure 3.



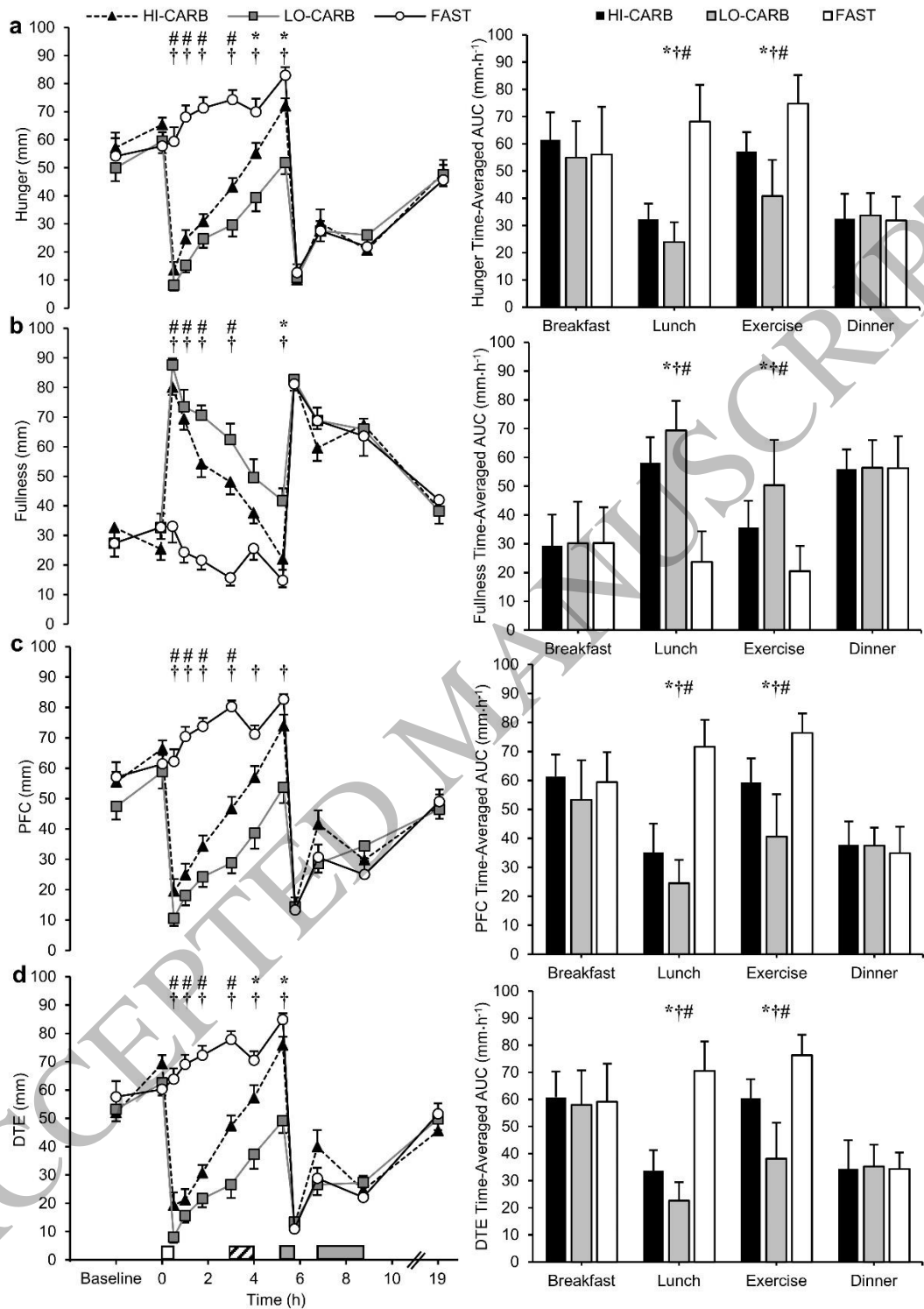
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2 **Figure 4.**



1

2 **Figure 5.**



1

2 **Figure 6.**