



**Manchester
Metropolitan
University**

Slater, Tommy, Mode, William JA, Pinkney, Mollie G, Hough, John, James, Ruth M, Sale, Craig ORCID logoORCID: <https://orcid.org/0000-0002-5816-4169>, James, Lewis J and Clayton, David J (2023) Fasting before evening exercise reduces net energy intake and increases fat oxidation, but impairs performance in healthy males and females. *International Journal of Sport Nutrition and Exercise Metabolism*, 33 (1). pp. 11-22. ISSN 1526-484X

Downloaded from: <https://e-space.mmu.ac.uk/630590/>

Version: Accepted Version

Publisher: Human Kinetics

DOI: <https://doi.org/10.1123/ijsnem.2022-0132>

Please cite the published version

<https://e-space.mmu.ac.uk>

Fasting before evening exercise reduces net energy intake and increases fat oxidation, but impairs performance in healthy males and females

Authors: Tommy Slater¹, William J A Mode¹, Mollie G Pinkney¹, John Hough¹, Ruth M James¹, Craig Sale^{1,2}, Lewis J James³ and David J Clayton^{1*}

Affiliations: ¹*Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK.*

²*Institute of Sport, Manchester Metropolitan University, Manchester, M1 7EL, UK*

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

Running Head: Fasting, evening exercise and energy intake

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

Senior Lecturer in Nutrition and Exercise Physiology

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

Email: David.Clayton@ntu.ac.uk

Tel: +44 (0) 115 84 85514

Word count: 4222

1 ABSTRACT

2 Acute morning fasted exercise may create a greater negative 24-h energy balance than the
3 same exercise performed after a meal, but research exploring fasted evening exercise is
4 limited. This study assessed the effects of 7 h fasting before evening exercise on energy
5 intake, metabolism, and performance. Sixteen healthy males and females ($n=8$ each)
6 completed two randomised, counterbalanced trials. Participants consumed a standardised
7 breakfast (08:30) and lunch (11:30). Two hours before exercise (16:30), participants
8 consumed a meal (543 ± 86 kcal; FED) or remained fasted (FAST). Exercise involved 30 min
9 cycling ($60\% \text{VO}_{2\text{peak}}$) and a 15-min performance test ($\sim 85\% \text{VO}_{2\text{peak}}$; 18:30). *Ad-libitum*
10 energy intake was assessed 15 min post-exercise. Subjective appetite was measured
11 throughout. Energy intake was 99 ± 162 kcal greater post-exercise ($P<0.05$), but 443 ± 128
12 kcal lower over the day ($P<0.001$), in FAST. Appetite was elevated between the pre-exercise
13 meal and *ad-libitum* meal in FAST ($P<0.001$), with no further differences ($P\geq 0.458$). Fat
14 oxidation was greater ($+3.25 \pm 1.99$ g), and carbohydrate oxidation lower (-9.16 ± 5.80 g)
15 during exercise in FAST ($P<0.001$). Exercise performance was 3.8% lower in FAST ($153 \pm$
16 57 vs. 159 ± 58 kJ; $P<0.05$), with pre-exercise motivation, energy, readiness, and post-
17 exercise enjoyment also lower in FAST ($P<0.01$). Fasted evening exercise reduced net energy
18 intake and increased fat oxidation compared to exercise performed 2 h after a meal. However,
19 fasting also reduced voluntary performance, motivation, and exercise enjoyment. Future
20 studies are needed to examine the long-term effects of this intervention as a weight
21 management strategy.

22 **Keywords:** Exercise performance, appetite, energy intake, energy balance, fasting, fat
23 oxidation

24 INTRODUCTION

25 Weight gain occurs when energy intake exceeds energy expenditure for a sustained period
26 (Hill et al., 2012). Counter-regulatory changes to energy balance systems appear more
27 profound for weight loss than weight gain (Hill et al., 2012), meaning early intervention in
28 lean individuals to prevent weight gain might be a more efficacious approach than attempting
29 to reduce obesity once established (Monnier et al., 2021).

30 Physical activity/exercise can aid weight management by increasing energy expenditure
31 (Donnelly et al., 2009). Despite this, long-term exercise interventions for weight management
32 are often less effective than predicted (Martin et al., 2019), perhaps explained by
33 compensatory reductions in energy expenditure (Thompson et al., 2014), and/or increases in
34 energy intake (King et al., 2008). Manipulating the timing of exercise around meals has the
35 potential to optimise exercise as a strategy to sustain an energy deficit and/or improve
36 metabolic health.

37 Exercise after a prolonged fast (>12 h) may aid in regulating energy balance. For example,
38 compared to consuming breakfast before exercise, fasted exercise produces either no change
39 (Bachman et al., 2016; Gonzalez et al., 2013; Griffiths et al., 2020) or a small increase
40 (Edinburgh et al., 2019) in lunch energy intake, but facilitates a lower overall energy intake
41 (breakfast plus lunch), and lowers 24 h energy intake (Bachman et al., 2016; Edinburgh et al.,
42 2019). Additionally, fasted morning exercise increases fat oxidation (Edinburgh et al., 2019;
43 Gonzalez et al., 2013), which may drive adaptations leading to improved markers of
44 metabolic health (Robinson et al., 2015).

45 Almost all research on fasted exercise has been undertaken in the morning because the
46 overnight fast offers a practical and convenient opportunity to achieve a fasted state without
47 the need to skip meals. The response to fasted exercise at other times of day is not well

48 researched. There is evidence that evening exercise is associated with a reduced perception of
49 effort (Maraki et al., 2005), and may improve glycaemic control more than morning exercise
50 (Moholdt et al., 2021). These diurnal differences may be explained by the circadian system,
51 which regulates several endogenous processes, including macronutrient metabolism, appetite,
52 and components of energy balance, in 24 h oscillations (Smith and Betts, 2022). Therefore,
53 findings from overnight-fasted exercise might not translate to exercise performed later in the
54 day.

55 Only one study has examined the energy intake responses to fasted-state exercise performed
56 at a time of day other than the morning. McIver et al. (2019a) showed similar 24 h energy
57 intakes following fed- and fasted-state exercise commencing in the morning or early-evening,
58 indicating fasted exercise may reduce daily energy intake, irrespective of the time of day. The
59 amount of exercise performed and motivation to exercise are, however, important to
60 maximise both the energy deficit achieved and the positive health outcomes from exercise
61 training (Foulds et al., 2014). Skipping breakfast has been shown to reduce voluntary exercise
62 performance (Clayton and James, 2016), but the effect of fasting on evening exercise
63 performance is unknown.

64 The present study examined the effects of fasting for 7 h before evening cycling exercise on
65 post-exercise *ad-libitum* energy intake, appetite, voluntary exercise performance, and
66 substrate oxidation in healthy, recreationally active males and females.

67 **METHODS**

68 **Participants**

69 Sixteen healthy, recreationally active ($<10 \text{ h}\cdot\text{wk}^{-1}$) males and females ($n=8$ each) completed
70 the study (Table 1), which was conducted in accordance with the Declaration of Helsinki and

71 approved by the Nottingham Trent University Ethical Advisory Committee; ethics
72 application number: 670. Clinical Trials registration: NCT04742530. Herein, we describe the
73 first of two studies completed as part of this clinical trial. A separate study will be published
74 elsewhere comparing the effects of fed-state evening exercise (including the data from 15
75 participants presented here) to fed-state morning exercise. Participants were not restrained,
76 disinhibited, or hungry eaters, determined by the three-factor eating questionnaire (Stunkard
77 and Messick, 1985). Female participants were regular monophasic combined oral
78 contraceptive users (≥ 6 months use before commencing the study; $n=3$) or eumenorrheic
79 (self-reported; $n=5$), and not using a hormonal contraceptive. Participants completed health
80 screening questionnaires and provided written informed consent before commencing the
81 study. The sample size for this study was estimated for the primary outcome variables of
82 voluntary exercise performance and energy intake using G*Power 3.1 software. Using an α of
83 0.05, β of 0.8 and data from a previous study (Clayton et al., 2015), it was estimated that 15
84 participants would be required to detect a 5% difference in voluntary exercise performance,
85 and 12 participants to detect a 15% difference in energy intake. Secondary outcome variables
86 include substrate oxidation, appetite, and subjective responses to exercise.

87 **Study design**

88 Participants completed two preliminary trials, followed by two experimental trials (completed
89 between February–July 2021 in Nottingham Trent University laboratories) in randomised (by
90 drawing trial orders for participants out of a bag), counterbalanced, cross-over order, and
91 were separated by ≥ 4 days. To control for fluctuations in appetite associated with sex
92 hormone concentrations (Buffenstein et al., 1995), eumenorrheic women completed
93 experimental trials in the follicular phase (3–14 days after the onset of menstruation – self-
94 reported) and oral contraceptive users completed all trials between days 4–17 of the pill-
95 taking phase. This was individually standardised within a 4-day period for each female

96 participant. Experimental trials involved consuming a 24 h standardised diet before an
97 exercise session at 18:30. Exercise consisted of 30-min steady-state cycling and a 15-min all-
98 out performance test, which required participants to complete as much work as possible
99 within the allotted time. In FAST, participants ceased food intake at 11:30 and commenced
100 exercise after a 7 h fast. In FED, participants consumed a pre-exercise meal at 16:30 and
101 commenced exercise after a 2 h fast.

102 **Preliminary trials**

103 During the first preliminary trial, participants' body mass and height were measured, before
104 body fat percentage was estimated by measuring skinfold thickness (Durnin and Womersley,
105 1974). Cycling VO_{2peak} was determined during a discontinuous incremental exercise test on an
106 electronically braked cycle ergometer (Lode Corival, Netherlands). The test involved 4-min
107 incremental stages separated by ~5 min rest until volitional exhaustion. Heart rate, rating of
108 perceived exertion (RPE; Borg, 1982), and 1-min expired gas samples were collected during
109 the final minute of each increment. After adequate rest, participants completed the 15-min
110 performance test. During the second preliminary trial, participants were familiarised with the
111 exercise protocol and the *ad-libitum* meal.

112 **Pretrial standardisation**

113 Participants recorded food intake and habitual physical activity during the 24 h prior to the
114 first experimental trial and replicated this before the second experimental trial. Strenuous
115 physical activity and alcohol intake were prohibited during this period, with adherence
116 confirmed verbally before each trial. Participants arrived at the laboratory via motorised
117 transport.

118 **Protocol**

119 Participants consumed a standardised dinner at 20:30 the evening before trial days, a
120 breakfast at 08:30, and a lunch at 11:30. In FED, participants consumed a standardised pre-
121 exercise meal at 16:30, which was replaced with a prescribed volume of water in FAST.
122 Participants arrived at the laboratory at 18:00 and measures of subjective appetite, mood, and
123 exercise readiness were completed. After 20 min supine rest, a 5-min expired gas sample was
124 collected. Exercise commenced at 18:30, with 30-min steady-state cycling (60% VO_{2peak}).
125 During exercise, heart rate and RPE were measured every 5 min, with 2-min expired gas
126 samples collected every 10 min. After 3-min rest, participants commenced a 15-min all-out
127 performance test. An *ad-libitum* pasta meal was served 15 min after the cessation of exercise,
128 and participants were permitted 20 min to eat. Participants then left the laboratory and were
129 instructed to consume nothing other than the prescribed water and to refrain from engaging in
130 exercise until after completing the final subjective appetite questionnaire at 08:30 the
131 following day. Adherence to this was confirmed via text messaging.

132 **Exercise performance test**

133 The ergometer was set in linear mode, with the linear factor (L) calculated using the formula:
134 $L = W/(\text{rpm})^2$ to elicit a workload (W) of 85% VO_{2peak} at the participants' preferred cadence
135 identified during the VO_{2peak} test. Power output could be increased and decreased with an
136 increase or decrease in cadence. Participants completed as much work as possible within 15
137 min and were blinded to all outcome measures, except time remaining. No encouragement
138 was provided, and standardised instructions were provided before each trial. Work completed
139 (kJ) and heart rate were recorded every minute, and RPE was recorded every 2 min from the
140 first minute.

141 **Standardised meals**

142 Participants were provided with weighed meals and water to be consumed at home, with
143 clear, written guidelines on timing of intake and instruction to consume nothing else.
144 Participants were regularly contacted via text messaging to encourage adherence with these
145 instructions. Meals were designed to provide a percentage of estimated energy requirements
146 (EER; resting metabolic rate [Mifflin et al., 1990] multiplied by a physical activity level of
147 1.7).

148 Standardised dinner and lunch meals were identical (30% EER), consisting of tuna/chicken
149 sandwiches prepared by the researchers (white bread (Hovis, UK), tuna chunks in brine
150 (Princess, UK)/chicken breast chunks (Bernard Matthews, UK), and full-fat mayonnaise
151 (Hellmann's, UK)), ready salted crisps (Walkers, UK), and chocolate (Cadbury, UK).
152 Standardised breakfast and pre-exercise meals were also identical (20% EER), consisting of
153 instant porridge oats (Oatso Simple Golden Syrup, Quaker, UK), cereal bars (Strawberry
154 Nutri-Grain, Kellogg's, UK), and yoghurt (Ski Strawberry, Nestlé, UK) (Table 2). Water
155 intake was provided at $30 \text{ mL}\cdot\text{kg}^{-1}$ body mass during trials, distributed into 5 equal volumes
156 consumed: 1) between waking and lunch (<11:30); 2) during lunch (11:30–12:00); 3) early
157 afternoon (12:00–17:30); 4) 1 h before exercise (17:30); and 5) between the *ad-libitum* meal
158 and sleep (>20:00).

159 ***Ad-libitum* meal**

160 Energy and water intake were determined by weighing food and water before and after
161 consumption. The *ad-libitum* meal was homogenous, providing $1.25 \pm 0.01 \text{ kcal}\cdot\text{g}^{-1}$ (69%
162 carbohydrate, 11% protein, 18% fat, and 2% fibre), and consisted of pasta, tomato sauce and
163 olive oil. The meal was provided in excess of expected consumption, and participants ate in
164 isolation to eliminate distractions until they felt "*comfortably full and satisfied*". Water was

165 available *ad-libitum*. Participants remained in the booth for the 20-min period, and all
166 participants reported they had ceased eating within this time in all trials.

167 **Expired gas samples**

168 A 5-min expired gas sample was collected into a Douglas bag immediately pre-exercise
169 following 20 min of supine rest. During steady-state cycling, 2-min expired gas samples were
170 collected between 8–10, 18–20, and 28–30 min. Samples were assessed for oxygen and
171 carbon dioxide concentrations (MiniHF 5200, Servomex, UK), volume (Harvard Dry Gas
172 Meter, Harvard Ltd., UK), and temperature. Substrate oxidation rates were calculated using
173 stoichiometric equations (Jeukendrup and Wallis, 2005).

174 **Subjective responses**

175 Participants rated their subjective feelings of hunger, fullness, desire to eat (DTE),
176 prospective food consumption (PFC), and nausea on digital visual analogue scales (VAS) that
177 were sent to their personal mobile telephone at each timepoint (0, 2, 3, 3.5, 5, 7, 8, 10, 11,
178 11.5, 13.5, and 24 h). Additional subjective feelings of motivation to exercise, readiness to
179 exercise, tiredness, and energy were added to the pre-exercise questionnaire (10 h). All VAS
180 were designed and administered using SurveyMonkey.com and comprised of a 0 to 100
181 sliding scale with written anchors of “not at all”/“no desire at all”/“none at all” and
182 “extremely”/“a lot” placed at 0 and 100, respectively. Participants also completed a paper-
183 based Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) pre-exercise.

184 A paper-based, shortened version of the Physical Activity Enjoyment Scale (PACES-8) was
185 completed immediately post-exercise to measure enjoyment of exercise sessions (Raedeke,
186 2007). The PACES-8 uses a series of eight, seven-point bipolar scales which participants use

187 to rate their agreement with one of the two statements at either end of the scale (*e.g.*, “I
188 enjoyed it” – “I hated it”).

189 **Statistical analyses**

190 Data were analysed using SPSS v26.0 (IBM, USA). All data were checked for normality of
191 distribution using a Shapiro-Wilk test. For subjective appetite-related variables, area under
192 the curve (AUC) values were calculated using the trapezoidal method and averaged over time
193 in response to breakfast (0–3 h), lunch (3–7 h), pre-exercise meal (7–11 h), and *ad-libitum*
194 meal (11–24 h). Data containing one factor were analysed using paired samples *t*-tests or
195 Wilcoxon Signed-Rank tests as appropriate. Data containing two factors were analysed using
196 repeated-measures ANOVA, with significant main effects followed by *post-hoc* paired
197 samples *t*-tests, or Wilcoxon Signed-Rank tests, with Holm-Bonferroni correction. Because
198 fluctuations in circulating sex hormone concentrations can influence appetite and energy
199 intake in females, sex was entered as a between-participants factor in repeated-measures
200 ANOVA to test for sex-by-trial-by-time interactions and/or sex-by-trial interactions. Due to
201 equipment issues, heart rate data is missing for one participant. Data sets were considered
202 statistically different when $P < 0.05$. Data are presented as mean \pm 1SD, unless stated. Where
203 appropriate, effect sizes (Cohen’s *d_z*) were calculated (Cohen, 1988).

204 **RESULTS**

205 **Energy intake**

206 *Ad-libitum* energy intake post-exercise was 99 ± 162 kcal greater during FAST ($d_z = 0.61$; P
207 < 0.05), but cumulative energy intake across the day was 443 ± 128 kcal lower during FAST
208 than FED ($d_z = 3.42$; $P < 0.001$; Table 2).

209 There was a sex-by-trial interaction effect for *ad-libitum* energy intake ($P < 0.001$), with
210 greater energy intake during FAST than FED in males ($+203 \pm 122$ kcal; $d_z = 1.67$; $P < 0.01$),
211 but not females (-5 ± 129 kcal; $d_z = 0.04$; $P = 0.919$; Figure 1).

212 **Subjective appetite responses**

213 There were trial ($P < 0.01$) and time ($P < 0.01$) main effects and a trial-by-time interaction (P
214 < 0.001) effect for hunger, fullness, DTE, and PFC. Participants reported increased hunger,
215 DTE, and PFC, and reduced fullness, in the period following the pre-exercise meal until
216 immediately before the post-exercise *ad-libitum* meal (16:30–19:30) during FAST ($P < 0.05$).
217 Nausea showed a main effect of time ($P < 0.01$), and a trial-by-time interaction effect ($P <$
218 0.05), but no main effect of trial ($P = 0.149$). Nausea tended to be greater immediately pre-
219 exercise in FAST ($P = 0.06$; Figure 2).

220 AUC for hunger, DTE, PFC, and nausea were all greater, and fullness was lower, between the
221 pre-exercise meal and the *ad-libitum* meal in FAST ($P < 0.01$). No further AUC differences
222 were shown between trials in response to breakfast ($P \geq 0.398$), lunch ($P \geq 0.458$) or *ad-*
223 *libitum* meal ($P \geq 0.464$; Figure 3).

224 **Energy expenditure and substrate oxidation**

225 At rest, carbohydrate oxidation was lower (0.04 ± 0.03 vs 0.13 ± 0.06 g·min⁻¹; $d_z = 1.25$; $P <$
226 0.001) and fat oxidation was higher (0.11 ± 0.02 vs 0.09 ± 0.03 g·min⁻¹; $d_z = 0.67$; $P < 0.01$)
227 in FAST. Energy expenditure at rest was lower in FAST (1.3 ± 0.2 kcal·min⁻¹ vs 1.2 ± 0.2
228 kcal·min⁻¹; $d_z = 0.67$; $P < 0.001$). There was a sex-by-trial interaction effect for resting
229 energy expenditure ($P < 0.05$), which was lower in FAST than FED in males (1.5 ± 0.2
230 kcal·min⁻¹ vs 1.3 ± 0.2 kcal·min⁻¹; $d_z = 1.12$; $P < 0.05$) but was not different between trials in
231 females (1.2 ± 0.1 kcal·min⁻¹ vs 1.1 ± 0.1 kcal·min⁻¹; $d_z = 0.14$; $P = 0.602$).

232 During steady-state exercise, total fat oxidation was greater ($+3.25 \pm 1.99$ g; $d_z = 1.64$; $P <$
233 0.001), and total carbohydrate oxidation was lower (-9.16 ± 5.80 g; $d_z = 1.58$; $P < 0.001$) in
234 FAST (Figure 4). Total energy expenditure in the steady-state exercise was lower in FAST ($-$
235 6 ± 8 kcal; $d_z = 0.59$; $P < 0.05$).

236 **Exercise performance and responses**

237 Work completed during the 15-min performance test was 5 ± 8 kJ lower during FAST ($d_z =$
238 0.62 ; $P < 0.05$; Figure 5).

239 Mean VO_2 achieved during steady-state exercise was lower in FAST ($57.9 \pm 5.6\%$ VO_{2peak} vs.
240 $59.0 \pm 6.1\%$ VO_{2peak} ; $P < 0.01$). Mean heart rate ($P = 0.079$) and RPE ($P = 0.806$) were not
241 different between trials during the 30-min steady-state bout. Mean heart rate during the
242 performance test was lower in FAST ($P < 0.05$), but RPE was not different between trials (P
243 $= 0.739$).

244 Laboratory temperature ($P = 0.212$), humidity ($P = 0.702$), and pressure ($P = 0.442$) were not
245 different between trials.

246 **Exercise subjective responses**

247 Participants reported lower pre-exercise motivation, energy, and readiness to exercise in
248 FAST ($P < 0.001$), although tiredness was not different between trials ($P = 0.270$). The
249 PANAS questionnaire revealed lower positive affect pre-exercise in FAST ($P < 0.05$), but
250 negative affect was not different between trials ($P = 0.238$). Mean score on the PACES-8
251 questionnaire was lower in FAST ($P < 0.01$), suggesting that the exercise session was
252 enjoyed less in FAST (Table 3).

253 **DISCUSSION**

254 We showed that fasting for 7 h before evening exercise increased *ad-libitum* energy intake by
255 ~100 kcal compared to exercise performed 2 h after eating, but this did not compensate for
256 the omission of a pre-exercise meal. Accordingly, net energy intake was lower when evening
257 exercise was performed following a 7 h fast. However, fasting before evening exercise
258 reduced performance by ~3.8%, and was associated with reduced motivation and exercise
259 enjoyment. Further study is required to determine whether fasting before evening exercise
260 can be used chronically to assist in weight and health management, or whether it's associated
261 negative perceptions impede long-term success.

262 Most studies explore fasted-state exercise in the morning due to the convenience of extending
263 the overnight fast. However, morning exercise is not always convenient or possible, so this
264 study assessed the metabolic and behavioural responses to fasted-state exercise in the
265 evening. Previously, McIver et al. (2019a) showed that fasting for 9 h before exercising at
266 17:00 increased appetite pre-exercise, but post-exercise appetite was not different to fed-state
267 exercise. This aligns with some (Gonzalez et al., 2013; McIver et al., 2019b), but not all
268 (Bachman et al., 2016; Griffiths et al., 2020) morning fasted-state exercise studies. Findings
269 from the present study are in-line with the latter, demonstrating elevated appetite extending
270 into the post-exercise period. Interestingly, post-exercise energy intake was ~100 kcal
271 (~13%) greater, which contrasts the results of studies where exercise is performed in the
272 morning (Bachman et al., 2019; Gonzalez et al., 2013; Griffiths et al., 2020). As such, the
273 present study provides novel data suggesting a potential disparity in post-exercise energy
274 intake responses between morning and evening fasted-state exercise, with evening fasted-
275 state exercise appearing to provoke compensatory eating which is not typically found with
276 morning fasted-state exercise, although further studies directly comparing morning and
277 evening fasted-state exercise are still needed.

278 Interestingly, this increase in energy intake was driven predominantly by males, with
279 seemingly no such compensation occurring in females. Appetite and energy intake responses
280 to acute exercise are generally similar between males and females (Dorling et al., 2018),
281 although only a small number of studies have directly compared males and females.
282 Moreover, nutrient-exercise interactions have not been considered (Frampton et al., 2022), so
283 the sex-specific responses to fasted-state exercise are unknown. Our findings suggest that
284 fasted-state evening exercise may provoke a smaller compensatory energy intake response in
285 females, potentially making it a more effective weight management strategy for females than
286 males. Sex hormones may influence appetite and energy intake (Buffenstein et al., 1995). We
287 attempted to control this by conducting trials in the same phase of the menstrual or pill-taking
288 cycle. However, we were unable to standardised this to the exact day within the phase, and
289 we did not measure hormones directly, both of which can be considered limitations of the
290 present study. Sex hormone concentrations may still fluctuate within the same cycle phase
291 (Buffenstein et al., 1995), meaning larger sample size studies of both males and females with
292 measurement of ovarian hormone concentrations are required to further explore these
293 preliminary findings.

294 Despite post-exercise energy intake being greater following fasted-state evening exercise, this
295 increase only compensated for ~18% of the pre-exercise meal in FED. Therefore, energy
296 intake over the course of the entire day was ~443 kcal lower in the fasted trial. Energy intake
297 was only measured at a single post-exercise meal, so it is possible that further energy intake
298 compensation may occur later in the evening or during the subsequent day. Consistent with
299 other studies (Bachman et al., 2016; McIver et al., 2019a; McIver et al., 2019b; Griffiths et
300 al., 2020), differences in appetite were abolished after the post-exercise meal, implying that
301 future eating behaviour may not differ between trials. Indeed, studies tracking energy intake
302 for up to 24 h post-exercise demonstrate that the reduction in energy intake caused by fasting

303 (meal skipping) is not compensated for in this time period (McIver et al., 2019a; Edinburgh et
304 al., 2019; Bachman et al., 2016). Additionally, recent work suggests energy intake increases
305 in anticipation of energy restriction (James et al., 2020) and/or exercise (Barutcu et al., 2021),
306 but this could not be assessed in the present study, as food intake was controlled to ensure
307 similar metabolic conditions at the start of trials.

308 We showed that prior fasting for 7 h increased fat oxidation by 3.25 g during 30 min evening
309 exercise. Exercising after a 10–14 h overnight fast increases fat oxidation (Edinburgh et al.,
310 2019; Gonzalez et al., 2013), which if performed regularly, may drive adaptations leading to
311 improved markers of metabolic health (Robinson et al., 2015). Despite circadian variations in
312 several metabolic processes (Smith and Betts, 2022), the present study, and previous work
313 (McIver et al., 2019a), show that a shorter, 7–9 h fasting period during the afternoon also
314 increases fat oxidation during evening exercise. However, it must be noted that longer fasting
315 durations that include the overnight fast, and shorter fasting durations such as that used in the
316 present study, likely elicit differences in metabolism beyond changes in substrate oxidation.
317 For example, plasma glycerol concentrations (a marker of lipolysis) increase in direct
318 proportion to the duration of the fast (Montain et al., 1991), meaning the metabolic effects of
319 a shorter period of afternoon fasting may not necessarily mimic those of an overnight fast.
320 Future studies should seek to explore whether elevated fat oxidation during fasted-state
321 evening exercise improves markers of metabolic health.

322 The main benefits from exercise are likely to be driven by the volume and intensity of
323 exercise performed (Foulds et al., 2014). This is especially important when time for exercise
324 is often curtailed by other commitments (Cerin et al., 2010). We found that fasting before
325 evening exercise reduced subjective ratings of motivation, readiness, and energy immediately
326 prior to exercise, indicating a suboptimal psychological state for maximising the volume or
327 intensity of voluntary exercise. Accordingly, the amount of work completed during the 15-

328 min performance test was reduced by 3.8% with fasting. Eating, particularly carbohydrate,
329 appears to enhance aerobic performance >60 min due partially to increased endogenous
330 carbohydrate stores (Aird et al., 2018), but effects on aerobic exercise <60 min are less
331 conclusive (Mears et al., 2018; Galloway et al., 2014). Recent evidence suggests that the
332 perception of consuming nutrients prior to exercise using an energy-free “placebo” meal
333 (Mears et al., 2018; Naharudin et al., 2020) or the suppression of hunger (Naharudin et al.,
334 2021), might improve performance. Therefore, the awareness of consuming nutrients and/or
335 subjective responses during the fed-state exercise trial may have increased self-selected
336 intensity during the performance test.

337 The absolute difference between trials for work completed was very small (~6 kcal), possibly
338 due to the short duration (15 min) and high intensity (85% $\text{VO}_{2\text{peak}}$) of the selected test. This
339 reduction in performance is unlikely to manifest in meaningful change to energy balance.
340 However, if motivation to exercise and self-selected duration and/or intensity of exercise are
341 curtailed, as this reduction in performance might imply, this could dramatically impact the
342 success of exercise training programmes. Additionally, given that exercise enjoyment may be
343 an important predictor of long-term adherence to exercise interventions (Raedeke, 2007), the
344 finding of reduced exercise enjoyment in the present study provides further insight into
345 possible challenges with incorporating fasted-state evening exercise into a weight
346 management programme.

347 The present study provides novel insight into the effects of fasting before evening exercise,
348 but it is not without limitations. Firstly, the absence of an overnight-fasted trial precludes the
349 direct comparison of morning and evening fasted-state exercise. Secondly, participants were
350 required to consume standardised meals and undergo instructed fasting periods in the absence
351 of experimenter supervision. Although regular contact was made via text messaging to
352 increase compliance, full adherence with these instructions cannot be assumed. Thirdly, the

353 study was conducted in lean and healthy participants, meaning the results cannot be directly
354 extrapolated to other population groups, particularly individuals with overweight or obesity,
355 who may respond differently to fasting-based interventions (Gonzalez et al., 2018). Finally,
356 this study investigated a single exposure, and compensatory energy intake was only assessed
357 at a single timepoint. As such, it is not known whether our acute findings would persist after
358 multiple exposures within a free-living setting, with greater opportunity for compensatory
359 energy balance behaviours to occur.

360 **CONCLUSION**

361 This study showed that fasting for 7 h prior to evening exercise may be an effective method
362 of reducing net energy intake, whilst also increasing fat oxidation. The chronic success of this
363 intervention may, however, be compromised by elevations in appetite and reductions in
364 voluntary performance, as well as reductions in the motivation to exercise and the enjoyment
365 of exercise sessions. Future studies are required to explore whether regular fasted-state
366 evening exercise can be used by lean and healthy individuals as a method of managing body
367 weight and/or composition in the long-term. Additionally, exploring the effects of this
368 intervention on indices of energy balance and metabolic health within overweight/obese
369 populations represents an important avenue for future research.

370 **Acknowledgments**

371 The authors would like to thank Terrance Campion and Beverley Armstrong for their
372 assistance during data collection.

373 **Authorship**

374 All authors contributed to the study conception and design; Tommy Slater, William J A
375 Mode, Mollie G Pinkney and David J Clayton completed data collection; Tommy Slater,
376 William J A Mode and David J Clayton analysed the data; the first draft of the manuscript

377 was written by Tommy Slater; and all authors critically reviewed previous versions of the
378 manuscript. All authors have read and approved the final manuscript.

379 **Conflict of interest**

380 LJJ is part of the National Institute for Health Research's Leicester Biomedical Research
381 Centre, which is a partnership between University Hospitals of Leicester NHS Trust,
382 Loughborough University, and the University of Leicester. This report is independent
383 research by the National Institute for Health Research. The views expressed in this
384 publication are those of the authors and not necessarily those of the NHS, the National
385 Institute for Health Research, or the Department of Health. LJJ has current/previous funding
386 from Entrinsic Beverage Company LLP, Herbalife Europe Ltd, Bridge Farm Nurseries,
387 Decathlon SA, PepsiCo Inc., Volac International, has performed consultancy for PepsiCo Inc.
388 and Lucozade, Ribena Suntory, and has received conference fees from PepsiCo Inc. and
389 Danone Nutricia. In all cases, monies have been paid to LJJ's institution and not directly to
390 LJJ.

391 CS has no conflicts of interest to declare as they relate directly to the topic of this study.
392 More generally, potential and perceived conflicts of interest over the last few years include:
393 Research funding from the UK Ministry of Defence, Natural Alternatives International,
394 English Institute of Sport, NHS Nottingham City, Birmingham City University, Coventry
395 University and GlaxoSmithKline HPL (all as PI) and Fundação de Amparo à Pesquisa do
396 Estado de São Paulo (Brazil), Ciência sem Fronteiras (Brazil), British Milers Club, Irish
397 Research Council and NHS Nottingham City (as Co-I). Honoraria have been received from
398 the Gatorade Sport Science Institute, UK Dairy Council, Guru Performance Ltd, International
399 Society of Sports Nutrition, English Institute of Sport, GlaxoSmithKline HPL and Nutrition
400 X. Other 'in kind' research support has been received from Natural Alternatives International

401 in the form of supplements for research, support to attend a conference and payment of open
402 access page charges.

403 **Funding Sources**

404 Tommy Slater is supported by a PhD studentship awarded by Nottingham Trent University.

405 **Protocol**

406 Trial registration: 8th February 2021 (<https://clinicaltrials.gov/ct2/show/NCT04742530>)

References

- Aird, T. P., Davies, R. W., & Carson, B. P. (2018). Effects of fasted vs fed-state exercise on performance and post-exercise metabolism: A systematic review and meta-analysis. *Scandinavian Journal of Medicine & Science in Sports*, 28(5), 1476–1493. <https://doi.org/10.1111/sms.13054>
- Bachman, J. L., Deitrick, R. W., & Hillman, A. R. (2016). Exercising in the fasted state reduced 24-hour energy intake in active male adults. *Journal of Nutrition and Metabolism* 2016. <https://doi.org/10.1155/2016/1984198>
- Barutcu, A., Briasco, E., Moon, J., Stensel, D. J., King, J. A., Witcomb, G. L., & James, L. J. (2021). Planned morning aerobic exercise in a fasted state increases energy intake in the preceding 24 h. *European Journal of Nutrition*, 60(6), 3387–3396. <https://doi.org/10.1007/s00394-021-02501-7>
- Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise*, 14(5), 377–381. <https://doi.org/10.1249/00005768-198205000-00012>
- Buffenstein, R., Poppitt, S. D., McDevitt, R. M., & Prentice, A. M. (1995). Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiology & Behavior*, 58(6), 1067–1077. [https://doi.org/10.1016/0031-9384\(95\)02003-9](https://doi.org/10.1016/0031-9384(95)02003-9)
- Cerin, E., Leslie, E., Sugiyama, T., & Owen, N. (2010). Perceived barriers to leisure-time physical activity in adults: an ecological perspective. *Journal of Physical Activity and Health*, 7(4), 451–459. <https://doi.org/10.1123/jpah.7.4.451>

- Clayton, D. J., & James, L. J. (2016). The effect of breakfast on appetite regulation, energy balance and exercise performance. *Proceedings of the Nutrition Society*, 75(3), 319–327. <https://doi.org/10.1017/S0029665115004243>
- Clayton, D. J., Barutcu, A., Machin, C., Stensel, D. J., & James, L. J. (2015). Effect of breakfast omission on energy intake and evening exercise performance. *Medicine & Science in Sports & Exercise*, 47(12), 2645–2652. <http://doi.org/10.1249/MSS.0000000000000702>
- Cohen, J. (1988). *Statistical power analysis for the behavioural sciences* (2nd ed.). Routledge Academic.
- Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., & Smith, B. K. (2009). American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine & Science in Sports & Exercise*, 41(2), 459–471. <https://doi.org/10.1249/MSS.0b013e3181949333>
- Dorling, J., Broom, D. R., Burns, S. F., Clayton, D. J., Deighton, K., James, L. J., King, J. A., Miyashita, M., Thackray, A. E., Batterham, R. L., & Stensel, D. J. (2018). Acute and chronic effects of exercise on appetite, energy intake, and appetite-related hormones: the modulating effect of adiposity, sex, and habitual physical activity. *Nutrients*, 10(9), 1140. <https://doi.org/10.3390/nu10091140>
- Durnin, J. V., & Womersley, J. V. G. A. (1974). Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *British Journal of Nutrition*, 32(1), 77–97. <https://doi.org/10.1079/BJN19740060>

- Edinburgh, R. M., Hengist, A., Smith, H. A., Travers, R. L., Betts, J. A., Thompson, D., Walhin, J-P., Wallis, G. A., Hamilton, L. D., Stevenson, E. J., Tipton, K. D., & Gonzalez, J. T. (2019). Skipping breakfast before exercise creates a more negative 24-hour energy balance: A randomized controlled trial in healthy physically active young men. *The Journal of Nutrition*, 149(8), 1326–1334. <https://doi.org/10.1093/jn/nxz018>
- Foulds, H. J., Bredin, S. S., Charlesworth, S. A., Ivey, A. C., & Warburton, D. E. (2014). Exercise volume and intensity: a dose–response relationship with health benefits. *European Journal of Applied Physiology*, 114(8), 1563–1571. <https://doi.org/10.1007/s00421-014-2887-9>
- Frampton, J., Edinburgh, R. M., Ogden, H. B., Gonzalez, J. T., & Chambers, E. S. (2022). The acute effect of fasted exercise on energy intake, energy expenditure, subjective hunger and gastrointestinal hormone release compared to fed exercise in healthy individuals: a systematic review and network meta-analysis. *International Journal of Obesity*, 46(2), 255–268. <https://doi.org/10.1038/s41366-021-00993-1>
- Galloway, S. D., Lott, M. J., & Toulouse, L. C. (2014). Preexercise carbohydrate feeding and high-intensity exercise capacity: Effects of timing of intake and carbohydrate concentration. *International Journal of Sport Nutrition and Exercise Metabolism*, 24(3), 258–266. <https://doi.org/10.1123/ijsnem.2013-0119>
- Gonzalez, J. T., Veasey, R. C., Rumbold, P. L., & Stevenson, E. J. (2013). Breakfast and exercise contingently affect postprandial metabolism and energy balance in physically active males. *British Journal of Nutrition*, 110(4), 721–732. <https://doi.org/10.1017/S0007114512005582>
- Gonzalez, J. T., Richardson, J. D., Chowdhury, E. A., Koumanov, F., Holman, G. D., Cooper, S., Thompson, D., Tsintzas, K., & Betts, J. A. (2018). Molecular adaptations of

adipose tissue to 6 weeks of morning fasting vs. daily breakfast consumption in lean and obese adults. *The Journal of Physiology*, 596(4), 609–622. <https://doi.org/10.1113/JP275576>

Griffiths, A., Deighton, K., Shannon, O. M., Boos, C., Rowe, J., Matu, J., King, R., & O'Hara, J. P. (2020). Appetite and energy intake responses to breakfast consumption and carbohydrate supplementation in hypoxia. *Appetite*, 147, 104564. <https://doi.org/10.1016/j.appet.2019.104564>

Hill, J. O., Wyatt, H. R., & Peters, J. C. (2012). Energy balance and obesity. *Circulation*, 126(1), 126–132. <https://doi.org/10.1161/CIRCULATIONAHA.111.087213>

James, R., James, L. J., & Clayton, D. J. (2020). Anticipation of 24 h severe energy restriction increases energy intake and reduces physical activity energy expenditure in the prior 24 h, in healthy males. *Appetite*, 152, 104719. <https://doi.org/10.1016/j.appet.2020.104719>

Jeukendrup, A. E., & Wallis, G. A. (2005). Measurement of substrate oxidation during exercise by means of gas exchange measurements. *International Journal of Sports Medicine*, 26, S28–S37. <https://doi.org/10.1055/s-2004-830512>

King, N. A., Hopkins, M., Caudwell, P., Stubbs, R. J., & Blundell, J. E. (2008). Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss. *International Journal of Obesity*, 32(1), 177–184. <https://doi.org/10.1038/sj.ijo.0803712>

Maraki, M., Tsofliou, F., Pitsiladis, Y. P., Malkova, D., Mutrie, N., & Higgins, S. (2005). Acute effects of a single exercise class on appetite, energy intake and mood. Is there a time of day effect? *Appetite*, 45(3), 272–278. <https://doi.org/10.1016/j.appet.2005.07.005>

- Martin, C. K., Johnson, W. D., Myers, C. A., Apolzan, J. W., Earnest, C. P., Thomas, D. M., Rood, J. C., Johannsen, N. M., Tudor-Locke, C., Harris, M., & Hsia, D. S. (2019). Effect of different doses of supervised exercise on food intake, metabolism, and non-exercise physical activity: The E-MECHANIC randomized controlled trial. *The American Journal of Clinical Nutrition*, 110(3), 583–592. <https://doi.org/10.1093/ajcn/nqz054>
- McIver, V. J., Mattin, L. R., Evans, G. H., & Yau, A. M. (2019a). Diurnal influences of fasted and non-fasted brisk walking on gastric emptying rate, metabolic responses, and appetite in healthy males. *Appetite*, 143, 104411. <https://doi.org/10.1016/j.appet.2019.104411>
- McIver, V. J., Mattin, L. R., Evans, G. H., & Yau, A. M. (2019b). The effect of brisk walking in the fasted versus fed state on metabolic responses, gastrointestinal function, and appetite in healthy men. *International Journal of Obesity*, 43(9), 1691–1700. <https://doi.org/10.1038/s41366-018-0215-x>
- Mears, S. A., Dickinson, K., Bergin-Taylor, K., Dee, R., Kay, J., & James, L. J. (2018). Perception of breakfast ingestion enhances high-intensity cycling performance. *International Journal of Sports Physiology and Performance*, 13(4), 504–509. <https://doi.org/10.1123/ijsp.2017-0318>
- Mifflin, M. D., St Jeor, S. T., Hill, L. A., Scott, B. J., Daugherty, S. A., & Koh, Y. O. (1990). A new predictive equation for resting energy expenditure in healthy individuals. *The American Journal of Clinical Nutrition*, 51(2), 241–247. <https://doi.org/10.1093/ajcn/51.2.241>
- Moholdt, T., Parr, E. B., Devlin, B. L., Debik, J., Giskeødegård, G., & Hawley, J. A. (2021). The effect of morning vs evening exercise training on glycaemic control and serum

metabolites in overweight/obese men: a randomised trial. *Diabetologia*, 64(9), 2061–2076. <https://doi.org/10.1007/s00125-021-05477-5>

Monnier, L., Schlienger, J. L., Colette, C., & Bonnet, F. (2021). The obesity treatment dilemma: Why dieting is both the answer and the problem? A mechanistic overview. *Diabetes & Metabolism*, 47(3), 101192. <https://doi.org/10.1016/j.diabet.2020.09.002>

Montain, S. J., Hopper, M. K., Coggan, A. R., & Coyle, E. F. (1991). Exercise metabolism at different time intervals after a meal. *Journal of Applied Physiology*, 70(2), 882–888. <https://doi.org/10.1152/jappl.1991.70.2.882>

Naharudin, M. N., Adams, J., Richardson, H., Thomson, T., Oxinou, C., Marshall, C., Clayton, D. J., Mears, S. A., Yusof, A., Hulston, C. J., & James, L. J. (2020). Viscous placebo and carbohydrate breakfasts similarly decrease appetite and increase resistance exercise performance compared with a control breakfast in trained males. *British Journal of Nutrition*, 124(2), 232–240. <https://doi.org/10.1017/S0007114520001002>

Naharudin, M. N., Yusof, A., Clayton, D. J., & James, L. J. (2021). Starving Your Performance? Reduced Preexercise Hunger Increases Resistance Exercise Performance. *International Journal of Sports Physiology & Performance*, 17(3), 458–464. <https://doi.org/10.1123/ijsp.2021-0166>

Raedeke, T. D. (2007). The relationship between enjoyment and affective responses to exercise. *Journal of Applied Sport Psychology*, 19(1), 105–115. <https://doi.org/10.1080/10413200601113638>

Robinson, S. L., Hattersley, J., Frost, G. S., Chambers, E. S., & Wallis, G. A. (2015). Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *Journal of Applied Physiology*, 118(11), 1415–1422. <https://doi.org/10.1152/jap.00058.2015>

- Smith, H. A., & Betts, J. A. (2022). Nutrient timing and metabolic regulation. *The Journal of Physiology*, 600(6), 1299–1312. <https://doi.org/10.1113/JP280756>
- Stunkard, A. J., & Messick, S. (1985). The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of Psychosomatic Research*, 29(1), 71–83. [https://doi.org/10.1016/0022-3999\(85\)90010-8](https://doi.org/10.1016/0022-3999(85)90010-8)
- Thompson, D., Peacock, O. J., & Betts, J. A. (2014). Substitution and compensation erode the energy deficit from exercise interventions. *Medicine & Science in Sports & Exercise*, 46(2), 423–423. <https://doi.org/10.1249/mss.0000000000000164>
- Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology*, 54(6), 1063–1070. <https://doi.org/10.1037/0022-3514.54.6.1063>

Tables

Table 1. Participant baseline characteristics

Characteristic	Overall (<i>n</i> = 16)	Males (<i>n</i> = 8)	Females (<i>n</i> = 8)
Age (y)	25 ± 3	25 ± 2	24 ± 4
Weight (kg)	70.9 ± 12.1	80.6 ± 8.3	61.2 ± 4.9
Height (m)	1.74 ± 0.11	1.83 ± 0.06	1.65 ± 0.05
BMI (kg·m ⁻²)	23.3 ± 1.9	24.1 ± 2.0	22.6 ± 1.6
Body fat (%)	20 ± 7	14 ± 3	26 ± 3
VO _{2peak} (mL·kg ⁻¹ ·min ⁻¹)	39 ± 6	43 ± 6	36 ± 5
Dietary restraint ^a	8 ± 3	6 ± 3	9 ± 3
Dietary disinhibition ^a	5 ± 3	5 ± 3	5 ± 3
Hunger ^a	5 ± 3	5 ± 3	4 ± 2
Estimated resting metabolic rate (kcal·day ⁻¹) ^b	1557 ± 265	1754 ± 237	1395 ± 77

Values are means ± SD

^a Three-factor eating questionnaire (Stunkard and Messick, 1985)

^b Estimated via predictive equation (Mifflin et al., 1990)

Table 2. Macronutrient composition of each meal

	Carbohydrate (g)	Protein (g)	Fat (g)	Fibre (g)	Energy (kcal)
Breakfast					
FAST	93.2 ± 15.7	14.5 ± 1.0	11.2 ± 1.9	5.5 ± 0.9	543 ± 86
FED					
Lunch					
FAST	72.5 ± 11.1	36.8 ± 6.9	41.0 ± 6.2	4.1 ± 0.6	814 ± 129
FED					
Pre-Exercise Meal					
FAST	0	0	0	0	0
FED	93.2 ± 15.7	14.5 ± 1.0	11.2 ± 1.9	5.5 ± 0.9	543 ± 86
<i>Ad-Libitum</i> Post-Exercise Meal					
FAST	152.1 ± 60.3	23.9 ± 9.5	17.9 ± 7.2	8.4 ± 3.3	882 ± 350*
FED	135.0 ± 48.4	21.2 ± 7.6	15.9 ± 5.7	7.4 ± 2.7	783 ± 281
Total					
FAST	317.7 ± 82.4	75.3 ± 16.3	70.1 ± 14.4	18.0 ± 4.6	2239 ± 533*
FED	393.8 ± 80.9	87.1 ± 14.6	79.3 ± 14.3	22.5 ± 4.5	2682 ± 519

Data are mean ± SD

* Values are significantly different from FED ($P < 0.05$).

Table 3. Pre- and Post-Exercise Subjective Responses

	FAST	FED
PANAS Positive Affect ^a	22 ± 6*	26 ± 6
PANAS Negative Affect ^a	13 ± 3	12 ± 3
PACES-8 Score ^b (%)	49 ± 12*	57 ± 13

Values are means ± SD

* Values are significantly different from FED ($P < 0.05$).

^a PANAS questionnaire (Watson et al., 1988)

^b PACES-8 questionnaire (Raedeke, 2007)

Figures

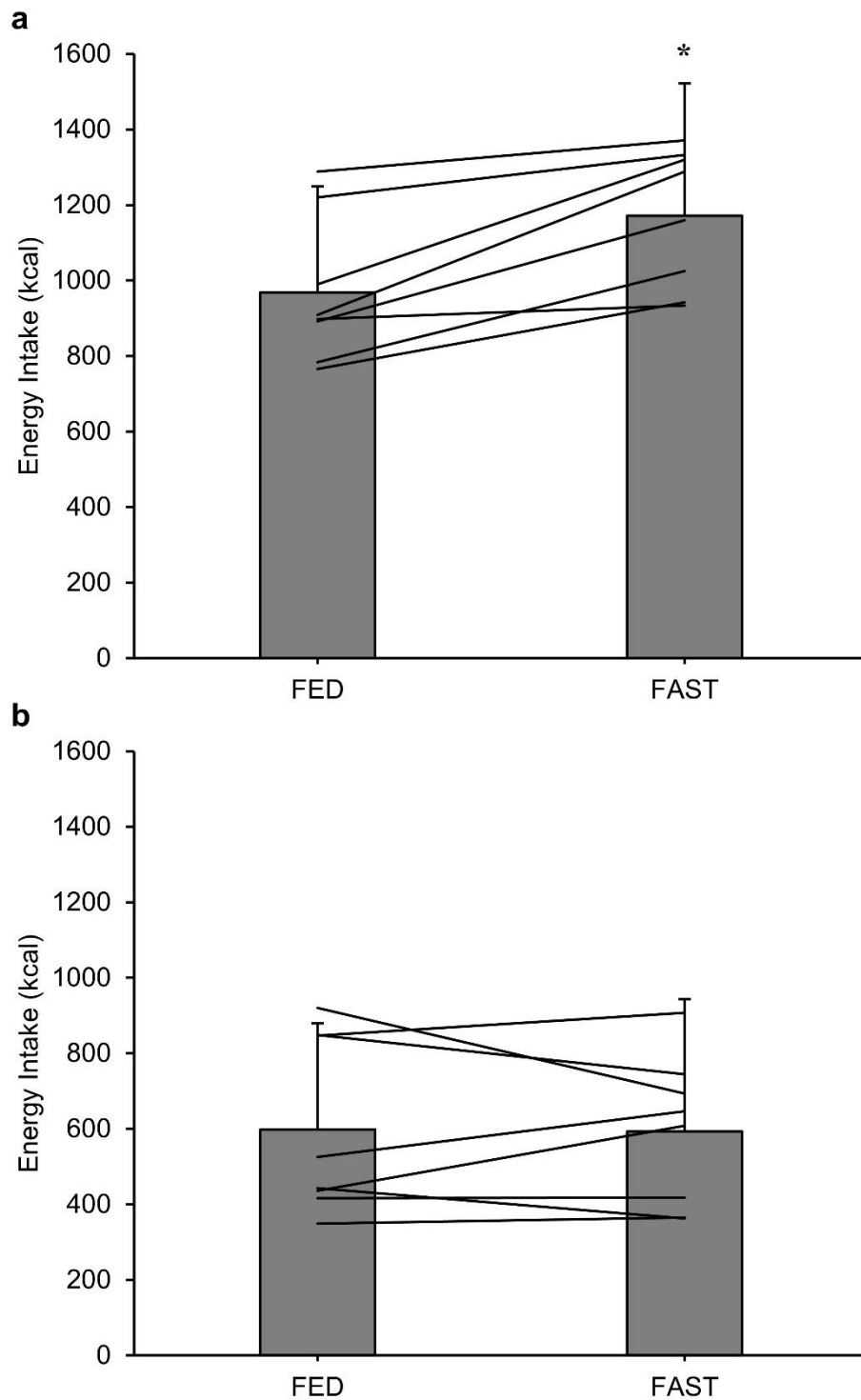


Figure 1. Energy intake (kcal) at the *ad-libitum* meal for (a) males ($n=8$), and (b) females ($n=8$). The bars display mean values, with vertical error bars representing SD. The lines display individual participants' *ad-libitum* energy intake for each experimental trial. * Significant difference between FED and FAST ($P < 0.05$).

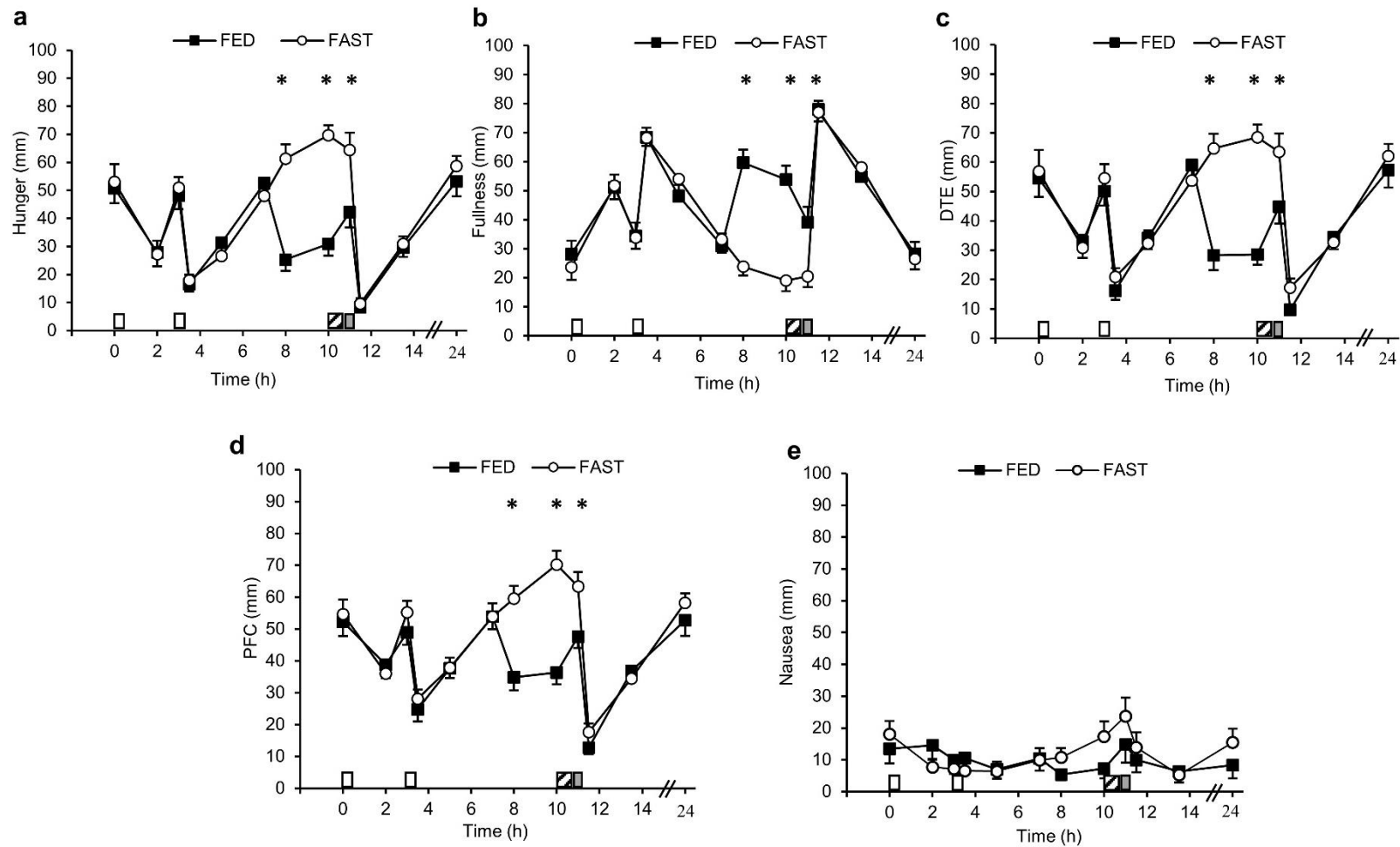


Figure 2. (a) Hunger, (b) fullness, (c) desire to eat (DTE), (d) prospective food consumption (PFC), and (e) nausea in FED and FAST. Data are mean \pm SEM. White rectangles represent standardised meals; grey rectangle represents *ad-libitum* meal; diagonal striped rectangle represents exercise. * Significant difference between FED and FAST ($P < 0.05$).

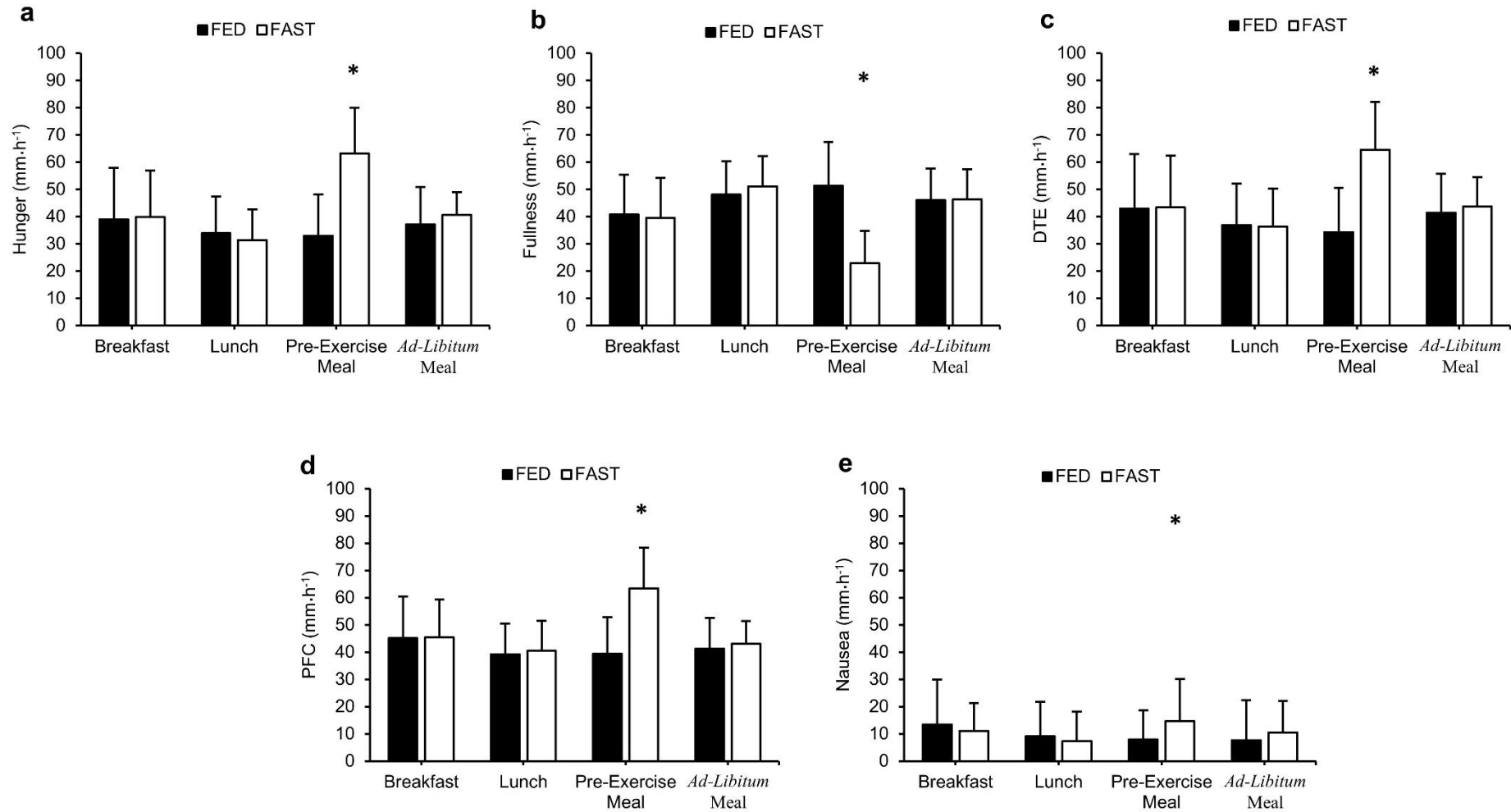


Figure 3. (a) Hunger, (b) fullness, (c) desire to eat (DTE), (d) prospective food consumption (PFC), and (e) nausea time-averaged area under the curve (AUC) in FED and FAST. Data are mean \pm SEM. * Significant difference between FED and FAST ($P < 0.05$)

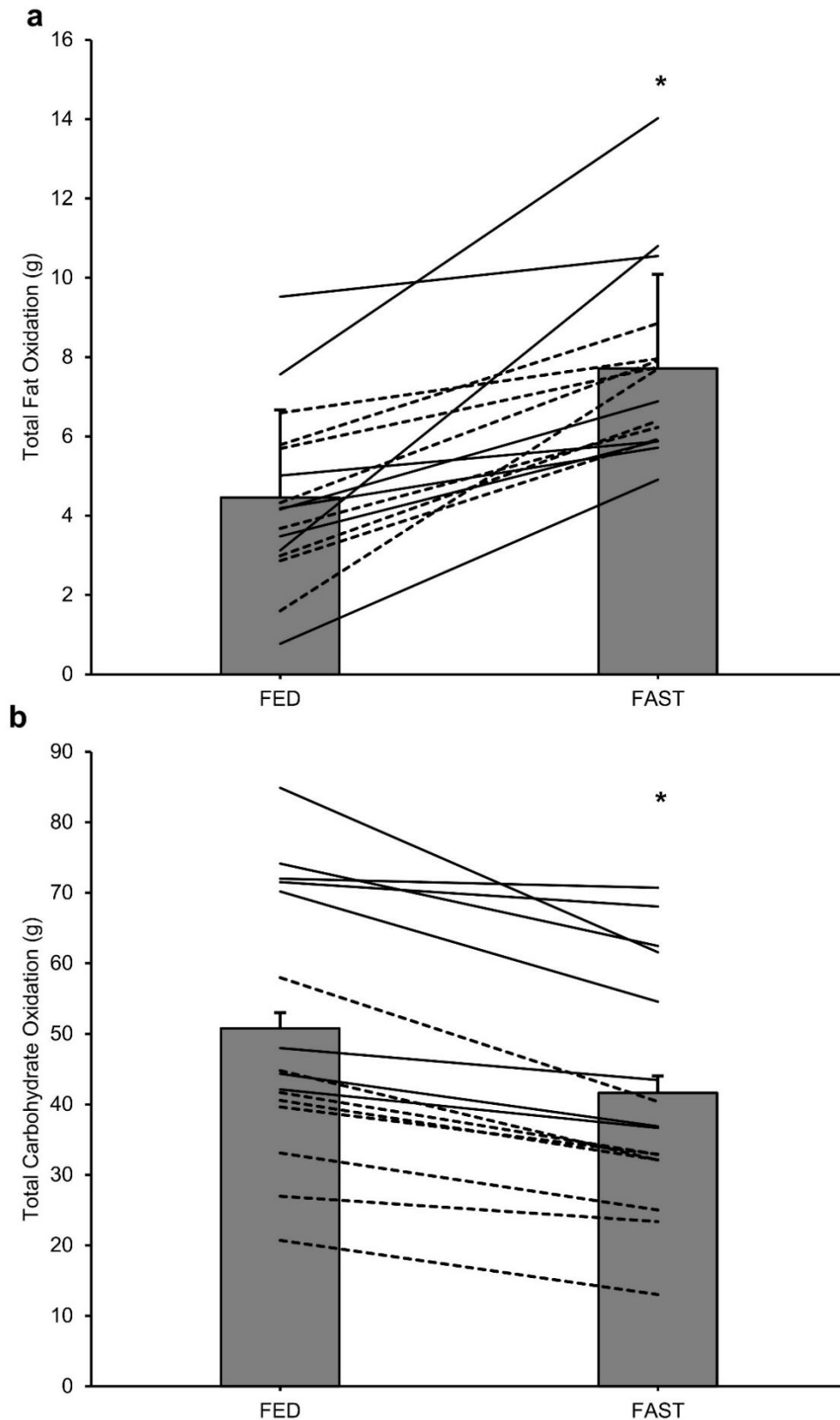


Figure 4. (a) Total fat oxidation (g), and (b) total carbohydrate oxidation (g) during the 30-min steady-state bout of cycling in FED and FAST. Data are mean \pm SD. The lines display individual participants' substrate oxidation during each experimental trial (dotted line: females; block line: males). * Significant difference between FED and FAST ($P < 0.05$).

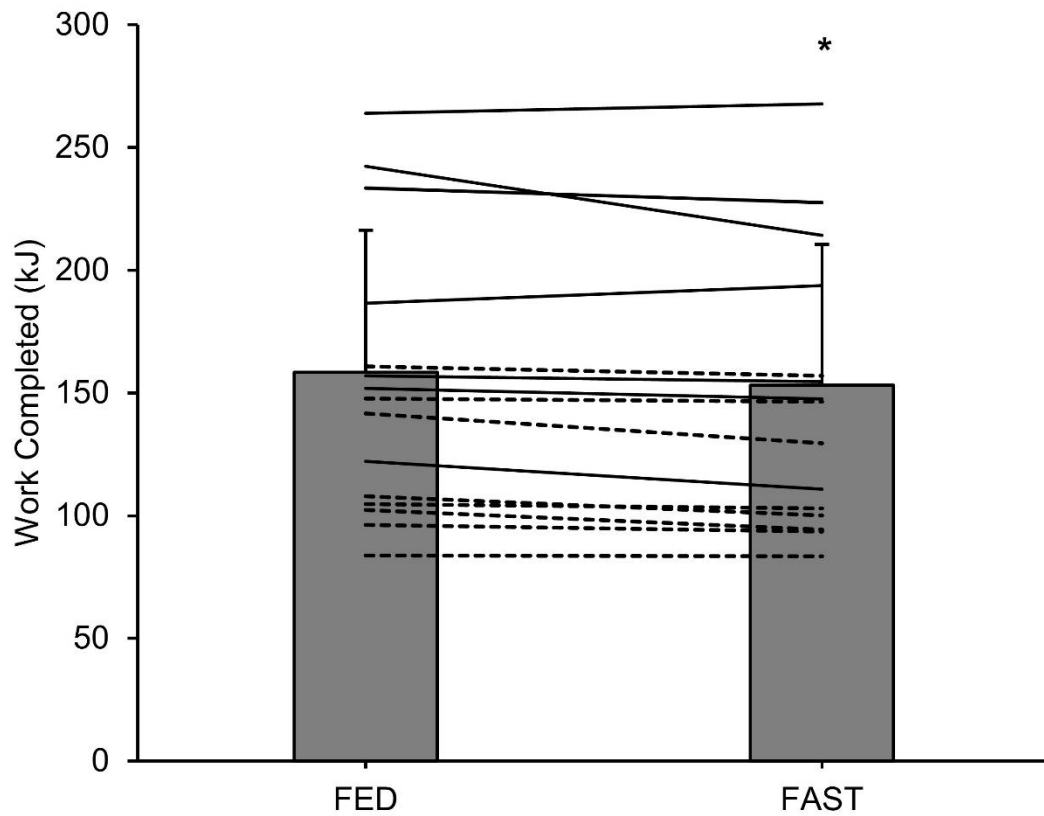


Figure 5. Total work completed (kJ) during the 15-min exercise performance test in FED and FAST. Data are mean \pm SD. The lines display individual participants' completed work during each experimental trial (dotted line: females; block line: males). * Significant difference between FED and FAST ($P < 0.05$).