Title: Effects of Morning Vs. Evening Exercise on Appetite, Energy Intake, Performance and Metabolism, in Lean Males and Females.

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Abstract

Exercise is an important component of a weight management strategy. However, little is known about whether circadian variations in physiological and behavioural processes can influence the appetite and energy balance responses to exercise performed at different times of the day. This study compared the effects of morning and evening exercise on appetite, post-exercise energy intake, and voluntary performance. In randomised, counterbalanced order, 16 healthy males and females (n=8 each) completed two trials, performing morning exercise at 10:30 (AMEx) or evening exercise at 18:30 (PMEx). Exercise consisted of 30 min steady-state cycling (60% $\dot{V}O_{2peak}$), and a 15-min performance test. A standardised meal (543 ± 86 kcal) was consumed 2-h before exercise and *ad-libitum* energy intake was assessed 15 min after exercise, with subjective appetite measured throughout. Absolute *ad-libitum* energy intake was 152 ± 126 kcal greater during PMEx (P<0.001), but there was no differences in subjective appetite between trials between trials immediately pre-exercise, or immediately before the post-exercise meal (P≥0.060). Resting energy expenditure (P<0.01) and carbohydrate oxidation (P<0.05) were greater during AMEx, but there were no differences in substrate oxidation or energy expenditure during exercise (P≥0.155). Exercise performance was not different between trials (P=0.628). In conclusion, acute morning and evening exercise prompt similar appetite responses, but post-exercise *ad-libitum* energy intake is greater following evening exercise. These findings demonstrate discordant responses between subjective appetite and *ad-libitum* energy intake but suggest that exercise might offset circadian variations in appetite. Longer-term studies are required to determine whether exercise timing may impact adherence and weight management outcomes to exercise interventions.

Trial registration NCT04742530, 8th February 2021.

Keywords: Circadian rhythm, Energy intake, Exercise, Substrate oxidation, Appetite

Introduction

Long-term weight management is notoriously difficult, with many people experiencing progressive weight gain throughout adult life (Østbye et al. 2011). It is imperative that early action is taken by lean individuals to prevent weight gain, as compensatory mechanisms strongly counteract an energy deficit by increasing appetite and/or reducing energy expenditure (Hill et al., 2012). This makes it more difficult to achieve weight loss and reverse obesity once established. As evidenced by continuously increasing obesity rates (Cobiac & Scarborough, 2021), there is a need to explore possible ways in which current weight management strategies might be optimised to increase their efficacy.

Physical exercise holds well-established benefits to metabolic health (Mancilla et al., 2021; Moholdt et al., 2021; Motahari-Tabari et al., 2015), but its effects on weight management are equivocal. Exercise-only interventions in people living with overweight and obesity typically result in trivial weight loss after 1–2 years (Franz et al. 2007). However, regular exercise is associated with reduced risk of becoming overweight or obese in the future (Dorling et al. 2018), with studies in lean individuals indicating that energy balance parameters respond to aerobic exercise in a manner that may be conducive to the maintenance of an energy deficit (Cox, 2017).

Most studies assessing the effect of exercise on energy balance position exercise in the morning, as the overnight fast permits easier control over factors that may influence metabolism or performance, such as pre-exercise activity and food intake. However, there is increasing evidence that the diurnal timing of exercise can influence responses (Alizadeh et al., 2015; Alizadeh et al., 2017; Willis et al., 2020), likely due to interactions between exercise, nutrient intake, and circadian physiology. The circadian system is governed by the central circadian ‘clock’, situated in the suprachiasmatic nuclei, which responds primarily to the daily light and dark cycle (Potter, 2016). Peripheral circadian clock genes located in tissues including fat, muscle, and liver are primarily regulated by the central clock, although peripheral zeitgebers such as exercise and food intake are known to independently influence these clock genes (Chaix et al 2016; Smith and Betts, 2022). The circadian system plays an integral role in regulating key physiological processes involved in energy balance, such as hormone secretion, eating behaviour and metabolism (Smith and Betts, 2022; Parr et al., 2020). For example, appetite peaks in the evening, coinciding with the time of the day typically associated with the largest intake of energy (Smith and Betts, 2022).

Due to the potential for exercise to influence circadian physiology, the diurnal timing of exercise could mediate effects on metabolism, appetite, and subsequent food intake (Arciero et al., 2022; Smith and Betts, 2022), although direct evidence assessing this is limited. Existing evidence suggests that there are no differences in appetite following aerobic exercise conducted in the morning or evening in healthy-weight women (Maraki et al., 2005), and men (McIver et al. 2019), although greater satiety has been reported following morning, compared to afternoon exercise, in women classified as overweight (Alizadeh et al., 2015). Despite inconsistent appetite findings, none of these studies observed differences in acute energy intake after exercise taking place at different times of day (Alizadeh et al., 2015; Maraki et al., 2005; McIver et al., 2019; O'Donoghue et al., 2010). Infrequent appetite measurements, limited sensitivity and accuracy due to self-reported assessments of food intake, and/or minimal dietary control prior to exercise sessions may, however, limit these findings. As such, the acute effects of diurnal exercise timing on appetite and energy intake remain unclear.

Adherence to exercise training is a well-known barrier to achieving the health benefits associated with exercise (Aronne et al., 2021). As such, it is important to understand whether exercising at different times of day influences subjective feelings of appetite and post-exercise energy intake. These findings will provide insight into the potential for the timing of exercise to influence weight maintenance in the long term.

Therefore, the aim of this study was to assess the acute effects of morning and evening cycling exercise on appetite, post-exercise *ad-libitum* energy intake, substrate metabolism, voluntary performance, and subjective responses to exercise, in young, healthy males and females.

Methods

Participants

Sixteen healthy participants [eight males and eight females (Table 1)] were recruited to the study. Participants were weight stable (self-reported >6 months), not currently dieting, not taking any medication, and were recreationally active (completing more than 1 h but less than 10 h structured exercise per week). Participants were not restrained, disinhibited, or hungry eaters, identified by responses to a three-factor eating questionnaire (TFEQ) (Stunkard & Messick, 1985). Female participants were either regular monophasic combined oral contraceptive users (n=3; use for ≥6 months before commencing the study), or eumenorrheic (n=5; self-reported) and not using a hormonal contraceptive. All participants completed a health screening questionnaire and provided written informed consent before commencing the study.

The sample size for this study was estimated from G*Power 3.0.10 software. Sample size was based on our primary outcome variable, *ad-libitum* energy intake. Using previous data from our laboratory (Slater et al., 2022a), with statistical power of 0.90 and α of 0.05 estimated that 15 participants would be required to reject the null hypothesis. A total of 16 participants were tested to counterbalance the study.

The study was approved by the Nottingham Trent University Ethical Advisory Committee; ethics application number: 670. ClinicalTrials registration no: NCT04742530. This is one of two studies published as part of this clinical trial. A separate study has been published elsewhere (Slater *et al.*, 2022b) comparing the effects of fed-state (including data from 15 participants presented here) and fasted-state evening exercise.

Table 1. Participant baseline characteristics

Characteristic	Overall (n = 16)	Males (n = 8)	Females (n = 8)
Age (y)	25 ± 3	25 ± 2	24 ± 4
Body Mass (kg)	71 ± 12	81 ± 8	61 ± 5
Height (m)	1.74 ± 0.11	1.83 ± 0.06	1.65 ± 0.05
BMI ($kg \cdot m^{-2}$)	23 ± 2	24 ± 2	23 ± 2
Body fat (%)	20 ± 7	14 ± 3	26 ± 3
$\dot{V}O_{2peak}$ ($mL \cdot kg \cdot min^{-1}$)	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 ($kcal \cdot day^{-1}$)[b]	1557 ± 265	1754 ± 237	1395 ± 77

Values are means ± SD

[a] Three-factor eating questionnaire (Stunkard & Messick, 1985)

[b] Estimated via predictive equation (Mifflin *et al.*, 1990)

Study design

Each participant completed two preliminary trials, followed by two experimental trials which involved exercising at 10:30 (AMEx) or 18:30 (PMEx). Trials were completed in a randomised, counterbalanced order, and were separated by ≥4 days. To control for fluctuations in appetite across the menstrual cycle (Buffenstein *et al.*, 1995), eumenorrheic women completed both experimental trials in the follicular phase (3–14 days after the onset of menstruation – self-reported using a menstrual cycle questionnaire, with day 0 representing the first day of menstruation) and oral contraceptive users completed all trials between days 4–17 of the pill-taking phase.

Preliminary trials

During the first preliminary trial, participants' mass and height were measured, with body fat estimated from skinfold thickness (biceps, triceps, sub-scapula supra-iliac; Durnin & Womersley, 1974). A discontinuous incremental exercise test on an electronically braked cycle ergometer (Lode Corival, Groningen, Netherlands), involving 4-min incremental stages separated by approximately 5-min of rest, was performed until volitional exhaustion to determine $\dot{V}O_{2peak}$. Heart rate (Polar V800, Kempele, Finland), rating of perceived exertion (RPE; Borg, 1982), and 1-min expired gas samples were collected during the final minute of each increment. After a self-selected rest period, participants were familiarised with the 15-min performance test to be used in experimental trials. Participants completed a second preliminary trial at least 48-h after the first, in which they were familiarised with the cycling protocol and the *ad-libitum* meal. Participants selected the ergometer handlebar and saddle position in preliminary trials, and this remained constant for the experimental trials.

Standardisation

Following appropriate training in how to accurately complete a food and activity diary, participants recorded all food intake and physical activity during the 24-h prior to the first experimental trial and repeated this before the second experimental trial. Strenuous physical activity and alcohol intake were prohibited during this period and adherence with these instructions was confirmed verbally before each experimental trial. Participants arrived at the laboratory for experimental trials via motorised transport.

Standardised meals were prepared by researchers and provided to participants to consume at home. Each meal was designed to provide a percentage of estimated energy requirements (EER), determined by multiplying resting metabolic rate (Mifflin *et al.*, 1990) by a physical activity level of 1.7, to account for the exercise component of the trial. Participants were given clear, written instructions on when to consume each meal, as well as strict instructions to consume nothing other than what was provided. Adherence was ensured through regular contact with participants via telephone messaging.

The evening before each experimental trial, participants consumed a pre-prepared dinner (30% of EER) at 20:30, consisting of tuna/chicken sandwiches, crisps, and chocolate (814 ± 129 kcal, 72.5 ± 11.1 g carbohydrate, 36.8 ± 6.9 g protein, 41.0 ± 6.2 g fat, 4.1 ± 0.6 g fibre). During AMEx, participants consumed a breakfast (20% of EER) consisting of porridge, cereal bars, and yoghurt (543 ± 86 kcal, 93.2 ± 15.7 g carbohydrate, 14.5 ± 1.0 g protein, 11.2 ± 1.9 g fat, 5.5 ± 0.9 g fibre) at 08:30, two hours prior to commencing exercise at 10:30. During PMEx, a breakfast (08:30; as above), lunch (11:30; identical to dinner), and a snack (16:30; identical to breakfast), were consumed prior to commencing exercise at 18:30 (Figure 1).

Laboratory protocol

Participants completed baseline subjective appetite questionnaires two hours prior to arriving at the laboratory (0 h). Participants arrived at the laboratory at 10:00 (AMEx) or 18:00 (PMEx) and were fitted with a heart rate monitor. An expired gas sample was collected after 20 min of supine rest, following which participants completed subjective appetite, mood, and exercise readiness questionnaires immediately prior to commencing exercise (2 h) at 10:30 (AMEx) or 18:30 (PMEx). Participants cycled at 60% $\dot{V}O_{2peak}$ for the first 30-min, with heart rate and RPE measured every 5-min and 2-min expired gas samples collected every 10-min. Participants then rested for 3-min before commencing a 15-min all-out performance test requiring them to complete as much work as possible within the allotted 15-min. Fifteen minutes after the cessation of exercise (3 h) subjective appetite was assessed and a meal consisting of pasta, tomato sauce and extra virgin olive oil was provided to assess *ad-libitum* energy intake (1.25 ± 0.01 $kcal \cdot g^{-1}$, 69% carbohydrate, 11% protein, 18% fat, and 2% fibre). The meal was consumed in an isolated room to avoid distractions, with food and water provided in excess of expected consumption. Participants were permitted 20-min to eat until they felt *"comfortably full and satisfied"* but were instructed to remain in the room for the entire 20-min period. All participants reported that they had ceased eating within the allotted time during all trials. Energy and water intake were determined by weighing before and after consumption. Subjective appetite was assessed 10-min after termination of the meal, after which participants were permitted to leave the laboratory. Participants were required to abstain from further food and drink intake for 2-h after leaving the laboratory, with a final subjective appetite questionnaire completed at 14:00 (AMEx) or 22:00 (PMEx) (5.5 h) (Figure 1). Adherence to this was checked via hourly telephone contact with participants.

[Figure 1 appears here]

Subjective responses

Participants rated their feelings of hunger, fullness, desire to eat (DTE), prospective food consumption (PFC), and nausea on digital visual analogue scales (VAS) that were sent to their personal mobile telephone at 0, 2, 3, 3.5 and 5.5 h. Additional questions to assess motivation to exercise, readiness to exercise, tiredness, energy, and likelihood of skipping the exercise outside of the study were added to the pre-exercise questionnaire (2 h). All VAS were designed and administered using SurveyMonkey.com and comprised of a 0 to 100 sliding scale with written anchors of "not at all/no desire at all/none at all" and "extremely/a lot" placed at 0 and 100, respectively. Participants also completed a paper-based Positive and Negative Affect Schedule (PANAS; Watson *et al.*, 1988) before commencing exercise.

A paper-based, shortened version of the Physical Activity Enjoyment Scale (PACES-8) was completed immediately after exercise to measure enjoyment of the exercise sessions (Kendzierski & DeCarlo, 1991; Raedeke, 2007). The PACES-8 uses a series of eight, sevenpoint bipolar scales which participants use to rate their agreement with one of the two statements at either end of the scale (*e.g.,* “I enjoyed it” – “I hated it”).

Exercise performance

The ergometer was set in linear mode, with the linear factor (L) calculated using the formula: $L = W/(rpm)^2$ to elicit a workload (*W*) of 85% $\dot{V}O_{2peak}$ at the participants’ preferred cadence (rpm) as identified during the $\dot{V}O_{2peak}$ test. Power output could be increased and decreased by participants voluntarily increasing or decreasing their cadence (Jeukendrup et al., 1995; Metcalfe et al., 2021). Participants were instructed to complete as much work as possible within 15-min and were blinded to all outcome measures, except time remaining displayed on a digital clock. No encouragement was provided, and standardised instructions were given to participants before each trial. Work completed and heart rate were recorded every minute, and RPE was recorded every 2-min. Performance outcomes were total work completed, mean power, mean HR, mean RPE.

Expired gas samples

A 5-min expired gas sample was collected into a Douglas bag immediately prior to exercise following 20-min of supine rest (Compher *et al.,* 2006). During the steady-state cycling, 2-min expired gas samples were collected between 8–10, 18–20, and 28–30 min. Samples were assessed for oxygen and carbon dioxide concentrations (1400 Series, Servomex, East Sussex, UK), volume (Harvard Dry Gas Meter, Harvard Ltd, Kent, UK), and temperature. Substrate oxidation rates were calculated using stoichiometric equations (Jeukendrup & Wallis, 2005).

Statistical analyses

Data were analysed using SPSS v26.0 (IBM, Chicago, USA). All data were checked for normality of distribution using a Shapiro-Wilk test. For subjective appetite-related variables, area under the curve (AUC) values were calculated using the trapezoidal method from arrival at the laboratory (2 h) until the end of the trial (5.5 h). Data containing one factor (baseline measurements, energy/water intake, AUC values, total energy expenditure, exercise performance, and pre-/post-exercise subjective responses) were analysed using paired samples *t*-tests for normally distributed data or Wilcoxon Signed-Rank tests for non-normally distributed data. Data containing two factors (subjective appetite) were analysed using repeated-measures ANOVA. Where ANOVA main effects were significant, *post-hoc* paired samples *t*-tests, or Wilcoxon Signed-Rank tests, with Holm-Bonferroni correction were conducted. In addition, sex was entered as a between-participants factor in repeated-measures ANOVA to test for sex-by-trial-by-time interactions and sex-by-trial interactions. Data sets were determined to be statistically different when $P < 0.05$. Data are presented as mean ± SD, unless otherwise stated. Where appropriate, to supplement key findings, effect sizes (Cohen's *dz*) were calculated for within-measures comparisons; small effect (0.2-0.49), medium effect (0.5-0.79) and large effect (>0.8) (Cohen, 1988).

Results

Sex analysis and laboratory conditions

There were no sex-by-trial interaction effects for any measurement (all P>0.05). Therefore, male and female data are presented together.

Laboratory conditions differed between trials, with temperature lower (19.6 ± 2.0 vs. 21.9 ± 1.7 °C) and pressure higher (756 ± 6 vs. 751 ± 8 mmHg) during AMEx vs. PMEx (P<0.05), although there was no difference in humidity (39 ± 9 and 36 ± 9 %; P=0.057).

Ad-libitum energy and water intake

Energy intake following exercise was greater during PMEx (835 ± 379 kcal) compared to AMEx (683 ± 325 kcal; dz = 1.20; P<0.001) (Figure 2). There was no effect of trial order on energy intake (P=0.367). There was no difference in water intake between trials (P=0.210).

[Figure 2 appears here]

Subjective appetite responses

There were trial (P<0.05), time (P<0.01) and interaction (P<0.05) effects for fullness, DTE and nausea (P<0.01). Hunger and PFC showed time (P<0.001) and interaction (P<0.001) effects, but no effect of trial (P>0.065).

Hunger, DTE, PFC, and nausea at 0 h were greater, and fullness was lower during AMEx (P<0.05). There were no further differences in appetite (P≥0.060), except for PFC being greater at 3.5 h during AMEx (P<0.05).

There were no differences between trials in AUC for hunger (P=0.646), fullness (P=0.793), DTE (P=0.460), PFC (P=0.737) or nausea (P=0.057; Figure 3).

[Figure 3 appears here]

Exercise responses

During the 30-minute steady state exercise, there were no differences for mean $\dot{V}O_2$ ($P=0.629$), mean heart rate ($P=1.000$) or mean RPE ($P=0.835$). During the 15-minute performance test, total work completed ($dz = 0.12$; $P=0.628$), mean power ($P=0.393$), mean RPE ($P=0.806$) and mean heart rate ($P=0.970$) were not different between trials (Table 2). There was no trial order effect for total work completed ($P=0.811$).

Immediately pre-exercise, participants reported higher motivation ($dz = 0.56$; $P<0.05$), lower readiness ($dz = 1.15$; $P<0.001$) to exercise, during AMEx compared to PMEx. There was no difference between trials for tiredness ($dz = 0.51$; $P=0.071$) and energy ($dz = 0.45$; $P=0.089$) or likelihood of skipping the session outside of the study ($dz = 0.44$; $P=0.103$). The pre-exercise PANAS questionnaire revealed no differences in positive ($dz = 0.33$; $P=0.207$) or negative ($dz = 0.12$; $P=0.647$) affect between trials. The post-exercise PACES-8 questionnaire revealed participants enjoyed both trials to a similar extent ($dz = 0.16$; $P=0.528$) (Table 2).

Table 2. Pre-, During and Post-Exercise Responses

	AMEx	PMEx	Pairwise comparison
Pre-exercise questionnaire (PANAS[a])			
Positive affect	27 ± 8	26 ± 8	*P*=0.207
Negative affect	12 ± 3	12 ± 3	*P*=0.647
30-minute steady-state period			
Mean $\dot{V}O_2$ (%)	52.6 ± 5.6	51.8 ± 5.9	*P*=0.629
Mean heart rate (bpm)	140 ± 13	140 ± 14	*P*=1.000
Mean RPE[b]	11 ± 2	11 ± 2	*P*=0.835
15-minute performance test			
Total work completed (kJ)	164 ± 62	163 ± 61	*P*=0.628
Mean power (W)	185 ± 69	188 ± 67	*P*=0.393
Mean heart rate (bpm)	170 ± 11	170 ± 10	*P*=0.970
Mean RPE[b]	14 ± 1	14 ± 1	*P*=0.806
Post-exercice questionnaire (PACES-8[c])			
Score (%)	60 ± 13	59 ± 12	*P*=0.528

Values are means ± SD

[a] PANAS questionnaire (Watson *et al.*, 1988)

[b] Rating of perceived exertion (RPE) (Borg, 1982)

[c] PACES-8 questionnaire (Kendzierski & DeCarlo, 1991; Raedeke, 2007)

Substrate oxidation and energy expenditure

Pre-exercise resting carbohydrate oxidation rate was greater during AMEx (0.18 ± 0.09 vs. 0.14 ± 0.06 $g \cdot min^{-1}$; dz = 0.71; P<0.05), but there was no difference between trials for resting fat oxidation rate (0.08 ± 0.03 vs. 0.09 ± 0.03 $g \cdot min^{-1}$; dz = 0.46; P=0.141). Consequently, resting energy expenditure was also greater during AMEx (1.48 ± 0.26 kcal·min^{-1} vs. 1.40 ± 0.26 kcal·min^{-1}; *dz* = 0.09; *P*>0.05). There were no differences between trials for carbohydrate oxidation (*dz* = 0.12; *P*=0.633), fat oxidation (*dz* = 0.38; *P*=0.155), or energy expenditure (*dz* = 0.30; *P*=0.245) during steady-state exercise (Figure 4).

[Figure 4 appears here]

Discussion

This study found that post-exercise energy intake was greater after acute cycling exercise performed in the evening compared to the morning, despite no post-exercise differences in subjective appetite. Substrate oxidation during steady-state exercise and performance during a 15-min all-out performance test were also not different between morning and evening exercise. These findings suggest that post-exercise energy intake is greater after evening exercise compared to morning exercise, however, evening exercise may offset the elevations in evening appetite which are typical of the diurnal western appetite profile. Longer-term studies are required to determine whether manipulating the timing of exercise elicits differential effects on indices of weight management.

Most research exploring the effects of exercise on appetite and energy intake are performed in the morning, as this reduces the potential for prior food intake and activity to influence study results. However, it is likely that many individuals are unable to exercise in the morning or prefer to exercise in the evening. Circadian variations in metabolism, appetite and energy intake are well established (Smith and Betts, 2022), but there is limited understanding of how exercise may influence these variables, or how these variables might be differentially affected by exercise performed at different times of day. Only a small number of studies have assessed the effects of exercise timing on energy intake, with research in lean populations particularly scarce. O'Donoghue *et al.*, (2010) assessed 24 h energy intake using standardised laboratory eating procedures in a group of lean males, with 45 min of treadmill running performed at either 07:00 or 17:00. This study found no difference in energy intake over the trial period, indicating that exercise timing does not affect *ad-libitum* energy intake. Whilst these findings contrast the present study, it is important to note that each eating opportunity (breakfast, lunch, dinner, and snacks) comprised of a different selection of foods, and energy intake was grouped by time of day. Thus, a direct comparison of post-exercise energy intake could not be ascertained from this study. Running also cause greater gastrointestinal discomfort than cycling (Peters *et al.*, 2000), which may influence pre-exercise eating behaviours. The present study builds on O'Donoghue *et al.*, (2010), finding that evening cycling exercise, performed after standardised feeding, increases energy intake at the post-exercise meal by ~150 kcal, compared to cycling exercise performed in the morning.

Maraki et al. (2005) found no differences in post-exercise energy intake in healthy females following a 1 h aerobic exercise class performed in the evening or the morning. Additionally, no differences in 24 h energy intake were found in response to morning or afternoon aerobic exercise in women classified as overweight (Alizadeh et al., 2015), and no differences in 48 h post-exercise energy intake were found when men classified as overweight performed 30 min high-intensity exercise in the morning, afternoon, or evening (Larsen et al., 2019). The energy intake data from these studies were estimated from self-reported food diaries, which have inherent limitations (Dhurandhar et al. 2015). For example, self-reported food diaries can be compromised by the reporting of socially desirable food intakes and can also be burdensome for participant to complete (Ortega et al., 2015). Thus, enhanced sensitivity of the laboratory-based measures of energy intake in the present study may have allowed for the detection of increased post-exercise energy intake following evening compared to morning exercise (Blundell et al. 2010).

Our findings align with quintessential western energy intake patterns, whereby energy intake is typically greater in the evening compared to the morning or afternoon, commonly observed across Northern Europe (Huseinovic et al., 2016), and the United States (Kant, 2018). Interestingly, the time of day in which food is consumed may influence the degree of satiation it elicits. De Castro (2004) reported that energy intake in the evening is less satiating than in the morning, which can result in overall increased energy intake in the evening. The effect of exercise on this pattern of appetite and food intake is not well established and given that exercise is an important peripheral zeitgeber for the circadian system (Basti et al., 2021), the timing of exercise is likely to influence metabolic pathways that govern food intake behaviour (Parr et al., 2020). A recent study in participants with overweight or obesity utilised the intake-balance method to assess energy intake (Racette et al., 2012), calculated using changes in body energy stores (measured via dual-energy X-ray absorptiometry) and total energy expenditure (measured by doubly-labelled water). This 15-week pilot study found that 3 evening exercise sessions per week reduced daily energy intake by 21 kcal, whereas the same exercise performed in the morning increased daily energy intake by 99 kcal (Creasy et al., 2022). These findings conflict with the current study but may suggest that lean individuals and individuals living with overweight or obesity exhibit different eating behaviours in response to morning and evening exercise. It is also possible that changes in energy intake occurs at eating occasions other than the meal immediately following exercise, although the previously discussed findings from O'Donoghue et al., (2010) refute this. Longer-term exercise training studies investigating exercise timing in lean individuals are required to elucidate this further.

Appetite demonstrates circadian variability, with hunger typically lowest in the morning and peaking in the evening (Smith and Betts, 2022), corresponding with western eating behaviours (Huseinovic et al., 2016). The current study found no differences in subjective appetite following acute exercise performed in the morning or evening. These findings agree with studies performed in lean males (McIver et al. 2019), lean females (Maraki et al. 2005) and individuals with overweight or obesity (Alizadeh et al. 2015; Larsen et al., 2019). This suggests that exercise may offset appetite to a similar level, regardless of the time of day in which exercise takes place, and that positioning exercise in the evening may offset circadian-related increase in appetite (Smith and Betts, 2022). However, subjective appetite does not always predict subsequent energy intake (Clayton et al. 2014; James et al. 2015), and indeed the current study found greater energy intake after evening exercise, despite no differences in post-exercise subjective appetite. It may be that consistent training at a specific time of day is required to engender a change in eating behaviour. Whilst acute exercise may alter appetite, this may be an insufficient stimulus to affect food intake. It should also not be ruled out that a change in diurnal appetite profile would not affect eating behaviour outside of a controlled laboratory environment in which abundant food is available to eat.

Acute exercise is known to transiently suppress appetite in an effect termed ‘exercise-induced anorexia’ (Deighton and Stensel, 2014). This acute effect of exercise on appetite may have acted to override circadian appetite profiles, possibly masking any differences in appetite between trials in the present study. However, this effect is typically found following exercise of a higher intensity (>60% $\dot{V}O_{2peak}$) than used in the current study (Broom et al., 2017) and is short-lived, persisting only for 30–60 minutes after exercise (Dorling et al., 2018). Few studies have assessed appetite responses to evening exercise. McIver et al. (2019) found no time-of-day differences in subjective appetite immediately after walking-based exercise, and no differences up to 2 h after consuming a post-exercise standardised meal. Other studies have similarly found no acute differences in appetite following a range of exercise modes performed at different times of the day, including high-intensity interval training (Larsen et al., 2019), aerobic exercise to music (Makari et al., 2005) and aerobic locomotion (Alizadeh et al., 2015). Taken together with the current study, there is potential for evening exercise to be operationalised as a tool to offset the naturally occurring rise in evening appetite, although further research is required. Analysis of hormones involved in appetite regulation (such as acylated ghrelin, GLP-1 and PYY) may provide additional insight into appetite responses to exercise timing. We intended measure these in the present study, but, due to the study taking place during a UK lockdown period to reduce transmission of COVID-19, we removed blood sampling as a preventative measure.

No differences in exercise performance were found between morning and evening exercise. Anaerobic exercise performance is typically enhanced in the evening compared to the morning (Chtourou and Souissin, 2012), a response likely mediated by diurnal rhythms in several physiological and metabolic pathways. For example, elevated oxygen uptake kinetics and increased energy efficiency have been evidenced during a 30-second all-out cycling performance test in the evening compared the morning (Souissi et al, 2007). In addition, muscle strength and oxidative capacity have been shown to rise over the course of the day in healthy participants (Atkinson and Reilly, 1996 and Van Moorsel 2016), and evening resistance exercise produces favourable anabolic hormonal profiles in weight-trained men when compared to the morning (Bird and Tarpenning, 2004). However, when the exercise duration is extended, such as in our study, the diurnal rhythm in exercise performance appears diminished. For example, previous work utilising a similar 15-min cycling performance test to the present study also found no difference in average power output and total work completed, irrespective of whether the test was performed in the morning, afternoon, or evening (Dalton et al, 1997). Findings from the current study, therefore, suggest that short duration exercise performance may not be impacted by time-of-day effects.

Resting carbohydrate oxidation and energy expenditure were greater before morning exercise compared to evening exercise, agreeing with previous findings which have demonstrated circadian rhythms in substrate metabolism and energy expenditure (Rynders et al., 2020). This increased ability to oxidise carbohydrate may help to explain why glycaemic control is improved in the morning compared to the evening (Jackubowicz et al., 2013). It is interesting, however, that these diurnal differences did not persist during exercise, with no differences in substrate oxidation or energy expenditure being observed. Exercise is a key external zeitgeber to the circadian system (Parr et al., 2020), and this study indicates that the increase in metabolic rate during exercise can supersede diurnal patterns in metabolism, although we did not assess the duration for which these effects persisted post-exercise. Previous evidence suggests that exercise performed in the evening potentiates improvements in glycaemic control in people with type-2 diabetes (Mancilla at al., 2021; Moholdt et al., 2021; Savikj et al., 2019), which is possibly due to the ability of exercise to influence diurnal metabolism. Specifically, exercise increases insulin sensitivity (Bird and Hawley, 2017), meaning there may be benefits to positioning exercise in the evening, when insulin sensitivity is at its worst (Parr et al., 2020). Further research is required to understand how exercise performed at different times of the day impacts metabolism to help determine the therapeutic potential for exercise and nutrient timing to achieve optimal benefit to health.

Pre-exercise mood (assessed using the PANAS questionnaire) and enjoyment of the exercise sessions (assessed using the PACES-8 questionnaire) were not different between trials in the present study. However, participants reported increased motivation, but reduced readiness, prior to morning versus evening exercise. It is possible that alternative priorities emerge throughout the day which compete with the motivation to exercise (Schumacher et al., 2020), potentially explaining why motivation is typically greatest in the morning (Benedetti et al., 2015). Although seemingly contrasting, the findings of reduced readiness prior to morning exercise may be a product of its early placement within the day, leaving less time to prepare physically and mentally for the upcoming session. In accordance with this idea, Maraki et al. (2005) found that morning exercise was perceived to require more effort than evening exercise. These findings suggest that exercise timing may influence subjective outcomes, which have the potential to influence adherence in the long-term.

Exercise is generally considered an important intervention for weight loss (Franz et al., 2007) and weight management (Blankenship et al., 2021). Despite this, chronic exercise interventions for weight management are often less effective than would be anticipated based on predictive equations (Martin et al., 2019). This is likely due to compensatory alterations in energy balance behaviours such as increased energy intake and/or reductions in energy expenditure (Blankenship et al., 2021). Recent studies have revealed that the diurnal timing of exercise might influence outcomes, with afternoon/evening exercise appearing to enhance metabolic benefits (Arciero et al., 2022; Mancilla et al., 2021; Moholdt et al., 2021; Savijk et al., 2019), whereas preliminary evidence supports the efficacy of morning exercise for weight management (Alizadeh et al., 2017; Chomistek et al., 2016; Willis et al., 2020), although findings are equivocal (De Blasio et al., 2010; Mancilla et al., 2021). Therefore, whilst metabolic and weight management outcomes to exercise interventions appear to differ according to the time-of-day in which exercise is performed, more long-term randomised controlled studies are required to substantiate a superior exercise time for optimising metabolic and weight management outcomes. Based on our current understanding, it has been suggested that exercise timing which aligns with an individual's schedule and/or preference is likely to be of greater importance than circadian considerations, ultimately determining adherence and long-term success (Mansingh and Handschin, 2022).

In summary, this study found that whilst appetite sensations responded similarly to acute exercise in the morning and evening, post-exercise *ad-libitum* energy intake was greater following evening exercise. In addition, exercise timing did not affect performance during a 15-min all-out performance test. These findings demonstrate a disconnect between subjective appetite and *ad-libitum* energy intake but provide some evidence that exercise can offset circadian-related appetite profiles. Long-term studies are required to determine whether exercise timing can be operationalised as a tool to support appetite regulation and weight maintenance.

Author Contribution

DJC, LJJ, WJAM, TS, JH, RMJ and IV conceived and designed the study. WJAM, TS and MGP performed data collection and analysis. WJAM, DJC, JH and TS wrote the manuscript, with review and editing provided by LJJ, RMJ and IV. All authors approved the final version of the manuscript.

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Declaration of Competing Interests

None.

Data Availability

Data will be made available on request.

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Figure Captions

Figure 1: Schematic representation of study design. Time of day (ToD) in which trials took place during AMEx and PMEx is indicated on the timeline. Indicates a standardised meal. Indicates a subjective appetite questionnaire, with additional questions relating to subjective feeling toward exercise provided pre- and post-exercise. ▼ Indicates an expired gas sample collection. Indicates exercise, with the shaded bar representing 30 min steady-state and hatched bar representing the 15-min performance test. Indciates the post-exericse *ad-libitum* meal.

Figure 2. Energy intake (kcal) at the post-exercise *ad-libitum* meal. The bars display mean values, with vertical error bars representing standard deviation. The lines display individual participants' dinner energy intake for each experimental trial. * Significantly different to PMEx ($P < 0.05$).

Figure 3. Subjective appetite responses for (a) hunger, (b) fullness, (c) prospective food consumption (PFC), (d) desire to eat (DTE), (e) nausea, and (f) total area under the curve (AUC) between 2–5.5 h for each appetite variable. Data are mean, with vertical error bars representing standard deviation. * Significant difference between AMEx and PMEx ($P < 0.05$).

Figure 4. Energy expenditure at rest (left panel) and during steady-state exercise (right panel). Contribution of carbohydrate (CHO) and fat oxidation to total energy expenditure is displayed as white and grey bars, respectively. Due to equipment error with one participant, resting data are presented for n=15. Data are mean, with vertical error bars representing standard deviation. * Significant difference between AMEx and PMEx ($P < 0.05$).