Diurnal influences of fasted and non-fasted brisk walking on gastric emptying rate, metabolic responses, and appetite in healthy males

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ABSTRACT

Growing evidence suggests circadian rhythms, nutrition and metabolism are intimately linked. Intermittent fasting (IMF) has become an increasingly popular intervention for metabolic health and combining IMF with exercise may lead to benefits for weight management. However, little is known about the diurnal variation of fasted exercise. This study aimed to investigate the diurnal influences on gastric emptying rate (GER), metabolic responses, and appetite to fasted and non-fasted exercise. Twelve healthy males completed four 45 min walks in a randomised order. Walks were completed in the morning (AM) and evening (PM) and either fasted (FASTED) or after consumption of a standardised meal (FED). GER of a semi-solid lunch was subsequently measured for 2 h using the ^{13}C breath test. Blood glucose concentration, substrate utilisation, and ratings of appetite were measured throughout. Energy intake was also assessed for the following 24 h. GER T_{lag} was slower in PM-FASTED compared to AM-FASTED, AM-FED, and PM-FED (75 ± 18 min vs. 63 ± 14 min, $P = 0.001$, vs. 65 ± 10 min, $P = 0.028$ and vs. 67 ± 16 min, $P = 0.007$). Blood glucose concentration was greater in the FED trials in comparison to the FASTED trials pre-lunch ($P < 0.05$). Fat oxidation was greater throughout exercise in both FASTED trials compared to FED, and remained higher in FASTED trials than fed trials post-exercise until 30 min post-lunch ingestion (all $P < 0.05$). No differences were found for appetite post-lunch ($P > 0.05$) or 24 h post-energy intake ($P = 0.476$). These findings suggest that evening fasted exercise results in delayed GER, without changes in appetite. No compensatory effects were observed for appetite, and 24 h post-energy intake for both fasted exercise trials, therefore, increased fat oxidation holds positive implications for weight management.

1. Introduction

Growing interest in nutrition and the circadian system has produced many insights within recent years, with circadian rhythms, metabolism, and nutrition suggested to be intimately linked (Johnston, Ordovás, Scheer, & Turek, 2016; Wehrens et al., 2017). Intermittent fasting (IMF) has become an increasingly popular dietary strategy for metabolic health and inducing weight loss by increasing insulin sensitivity and fatty-acid mobilization, reducing inflammation, and by creating a state of negative energy balance (Mattson, Longo, & Harvie, 2017). Exercise-induced health benefits alone are favorable for reducing a range of risk factors and preventing the onset of metabolic diseases (Borghouts & Keizer, 2000; Mann, Beedie, & Jimenez, 2014; Rennie, McCarthy, Yazdgerdi, Marmot, & Brunner, 2003; Speakman & Selman, 2003; Steig et al., 2011; Thompson et al., 2001; Whelton, Chin, Xin, & He, 2002). Therefore, combining IMF with exercise may lead to benefits for weight management. Emerging evidence also suggests that morning-loaded energy distribution is a beneficial strategy for weight management

(Garaulet et al. 2013; Jakubowicz, Barnea, Wainstein, & Froy, 2013). Morning calorie consumption was also associated with greater improvements in fasting glucose, insulin and triglycerides, and decreased hunger scores (Jakubowicz et al. 2013; Sutton et al., 2018) and serum lipid levels (Yoshizaki et al., 2014). Therefore, combining eating patterns with exercise that reduce or eliminate eating at particular times of the circadian cycle may result in sustained improvements in human health (Johnston et al., 2016; Longo & Panda, 2016; Mattson et al., 2014).

Many circadian rhythms exist within the human organism that are governed by 'clocks' located centrally and in most peripheral tissues. These central and peripheral clocks are based on clock genes and their protein products (Cermakian & Boivin, 2009). Clock genes in peripheral tissues are primarily regulated by the central 'master clock' located in the suprachiasmatic nuclei (SCN) which is predominantly synchronized by the light/dark cycle (Albrecht, 2012). External factors such as food intake and exercise are also known to influence clock genes (Morris, Yang, & Scheer, 2012). Clock genes have been established in various

organs and tissues, regulating the timing of physiological processes, specifically those involved in the digestion of food, nutrient uptake, and nutrient metabolism (Ruddick-Collins, Johnston, Morgan, & Johnstone, 2018). There has been a considerable amount of interest in the role of clock genes in regulating biochemical pathways and metabolic processes (Marcheva et al., 2013; Sahar & Sassone-Corsi, 2012). However, less attention has been given on examining how the circadian system affects eating patterns combined with exercise, and how this may affect gastric emptying rate (GER) and appetite regulation.

Diurnal variations are evident in gastrointestinal absorption rate and GER, by acting to control food intake differentially at different times of the day. Previous studies have observed slower emptying of the stomach in the evening (Goo, Moore, Greenberg, & Alazraki, 1987; Grammaticos, Doumas, & Koliakos, 2015; Orr, Heading, Johnson, & Kryger, 2004). Thermic effect of food has been shown to follow a time of day variance, with elevated levels in the morning, which may contribute to the diurnal variation observed for GER (Morris et al. 2015a,b). GER influences the release of nutrients into the intestines for absorption, affecting hormonal and metabolite responses essential for nutrient digestion and storage (Romon, Edme, Boulenguez, Lescroart, & Frimat, 1993; Ruddick-Collins et al., 2018). Therefore, GER may play an important role in metabolic health. However, although the abovementioned studies are informative there are some notable limitations, with very low sample size recruited (Goo et al., 1987; Grammaticos et al. 2015), mice studies which may not translate to human physiology (Kentish, O'Donnell, Kennaway, Wittert, & Page, 2014), and none of the aforementioned studies included exercise. Consequently, it is still unknown how circadian variations in GER following subsequent food and energy intake may differentially influence postprandial energy metabolism and on appetite regulation. Particularly, on the diurnal variation of fasted versus fed exercise on gastrointestinal function and appetite. Therefore, there is a largely unmet need to explore how meal timing along with exercise may impact GER, appetite and metabolic health.

The aim of this study was to investigate the effect of brisk walking in the fasted and non-fasted state on metabolic responses, appetite and GER of a subsequent meal at two different times of the day. It was hypothesized that (a) GER would be slower during evening trials in comparison to morning trials (b) evening and morning trials would result in differences in appetite and metabolic responses post-exercise (c) fat oxidation would be higher during fasted exercise, and carbohydrate oxidation would be higher during non-fasted exercise, regardless of time of day and (d) there would be no compensatory effects for appetite post-exercise.

2. Material and methods

2.1. Participants

Twelve recreationally active men (Mean $\pm$ SD; age 25 $\pm$ 3 years; height 178 $\pm$ 6 cm; body mass 83 $\pm$ 12 kg; body fat 21 $\pm$ 6%; body mass index 26 $\pm$ 4 kg/m^2; $\dot{V}O_{2peak}$ 39 $\pm$ 4 ml/kg/min) volunteered to participate in this study. Sample size was determined by a power analysis based on data that would result in a detectable change in GER and fat oxidation with 80% power and at a significance level of 5%. Participants were not taking regular medication or with any known history of respiratory, cardiovascular, or chronic gastrointestinal disease as assessed by a health screen questionnaire. All participants were free from musculoskeletal injury and non-smokers. Participants were also classified as moderate or intermediate chronotypes according to the Munich chronotype questionnaire by Roenneberg, Wirz-Justice, and Merrow (2003). This ensured the exclusion of participants with an early diurnal phase, also known as extreme morning chronotypes and extreme evening chronotypes, since it is known that morning and evening types differ in the daily phase (Roenneberg et al., 2003). Participants recorded a 7-day habitual sleep diary leading up to each trial and the midpoint of sleep (halfway between sleep time and wake time) was

calculated. Participants were not involved in shift work and did not report any disturbances to their normal sleep-wake cycle during the 1 week prior to data collection. All participants were informed of the details of the study both verbally and in writing prior to providing their written informed consent. The study was approved by the Faculty of Science and Engineering Research Ethics and Governance Committee (Reference: SE1617158).

2.2. Preliminary trial

All participants attended a preliminary trial at least 7 days prior to the first experimental trial. During this visit, participants completed a physical activity and dietary habit questionnaire, Munich chronotype questionnaire, Pittsburgh sleep quality questionnaire, and the Epworth sleepiness scale questionnaire (Buysse Reynolds, Monk, Berman, Kupfer 1989). This visit also involved the collection of anthropometric measures of height, weight, body fat percentage, as well as familiarisation of the breath sampling procedures. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer and body mass to the nearest 0.01 kg using electronic scales (GFK 150; Adam Equipment Co. Ltd., Milton Keynes, UK). Body fat percentage was approximated using bioelectrical impedance analysis (Omron BF306; Kyoto, Japan).

Following this, all participants completed a peak oxygen uptake ($\dot{V}O_{2peak}$) test on a motorised treadmill. Initially, the treadmill speed was adjusted until a suitable brisk walking pace was determined. Participants were advised that brisk walking is defined as an exercise intensity yielding a mild shortening of breath yet still enabling to converse. Participants then maintained this speed for 5 min. The speed of the treadmill was then increased to 8–12 km h^{-1} and the gradient increased by 2.5% every 3 min until volitional exhaustion. Expired air was continuously collected using a breath-by-breath gas analyser (Oxycon Pro, CareFusion, Leipzig, Germany) and $\dot{V}O_{2peak}$ was calculated by averaging the rate of oxygen consumption over the final 1 min period. Heart rate was measured continuously using a heart rate monitor (Polar H7, Kempele, Finland) and participants rating of perceived exertion (RPE) (Borg, 1982) was recorded every 3 min.

Before leaving the laboratory, participants were provided with food weighing scales and asked to record their physical activity and food intake in the 24 h before the start of their first experimental trial. Participants were then asked to replicate their activity and diet the day preceding their subsequent trials. In addition, participants were requested to refrain from alcohol consumption, strenuous exercise and caffeine ingestion 24 h before trials.

2.3. Experimental trials

Participants completed four 5 h experimental trials in a randomised crossover fashion; two morning trials fasted (AM-FASTED) and non-fasted (AM-FED), and two evening trials fasted (PM-FASTED) and non-fasted (PM-FED). All morning trials commenced at 08:00 and evening trials commenced at 15:00. Randomisation of trials was achieved using the randomise tool within Microsoft Excel. All trials were separated by at least 7 days.

On the morning trials, participants were required to fast from 00:00 the evening before, and on the evening trials, participants were required to have breakfast and then fast from 07:00 with the exception of plain water consumption. Ninety minutes prior to arrival at the laboratory, participants were asked to drink 500 ml of plain water to ensure an adequate and consistent level of hydration status and not drink anymore water after this point. Upon arrival at the laboratory, participants were asked to empty their bladder before body mass was recorded. Baseline assessments of appetite (hunger, fullness, prospective food consumption (PFC) and satisfaction) were made using 100 mm visual analogue scales (VAS) (Flint, Raben, Blundell, & Astrup, 2000). Expired air samples were also collected for 10 min for the calculation of substrate utilisation. The average $\dot{V}O_2$ and $\dot{V}CO_2$

measurements from the last 5 min of expired air collection was used to calculate fat and carbohydrate oxidation rates using stoichiometric equations (Péronnet & Massicotte, 1991). This sampling method for expired air was adhered to for all resting expired air samples throughout.

Following baseline measurements, participants ingested the test 'breakfast' in FED within a 15 min period, or remained fasted in FASTED. The test 'breakfast' (meal 1) consisted of 30 g of breakfast cereal with 125 mL of semi-skimmed milk, and a croissant, which provided in total 1438 kJ (341 kcal), and contained 10.2 g fat, 48 g carbohydrate and 11.2 g protein. This amount was chosen based on the recommended breakfast serving being of approximately 300–400 kcals (Public Health England, 2018). Participants consumed all of the breakfast within the 15 min window. Post breakfast ratings of appetite and substrate utilisation were measured at the end of the 15 min breakfast period. Participants then rested for 1 h before commencement of the exercise protocol. During this 1 h rest period, further measures of appetite were taken every 15 min and substrate utilisation every 30 min. The exercise protocol involved 45 min of brisk walking on a level motorised treadmill at the speed determined in the preliminary trial (range 5.9–7.0 kmh^{-1}). The relative exercise intensity was $55 \pm 0.8\%$ $\dot{V}O_{2peak}$. Heart rate and RPE were measured every 15 min throughout the exercise, with expired air measured continuously. The last 10 min of each 15 min segment was used to calculate substrate utilisation. After completion of the exercise bout, participants recovered for 30 min (showered if desired) before they ingested a standardised 'lunch' meal (meal 2). The meal was 800 g (2 cans) of vegetable soup (1584 kJ (376 kcal)), containing 6.8 g fat, 66.4 g carbohydrate, 8.8 g protein. Subjective feelings of appetite and substrate utilisation were measured every 15 min post-ingestion for a total period of 2 h. The food served for AM and PM trials were identical. A schematic diagram of the experimental protocol is presented in Fig. 1.

2.4. Blood sampling

Blood glucose concentration was measured via a capillary blood sample from the tip of the finger, with the participant in a seated position. Capillary blood samples were taken at baseline, post breakfast period, pre-exercise, immediately post-exercise, pre-soup ingestion, then every 30 min post-soup ingestion. A 23-gauge single use sterile lancet (Unistik-3, Owen Mumford, Oxford, UK) was used to create a small incision (approx. 3 mm puncture) on the fingertip. From this incision a free-flowing capillary blood sample was collected in microvettes (Hemocue Glucose 201 + Microcuvettes, Ângelholm,

Sweden) containing anticoagulant EDTA, lithium heparin. The blood was analysed immediately using a desktop plasma glucose analyser (Hemocue Glucose 201 + analyser, Ângelholm, Sweden).

2.5. Saliva melatonin sample and analysis

A saliva sample was collected at the beginning of all trials (AM trials 08:00; PM trials 15:00) by the passive drool method, in which the participant allows saliva to pool in his mouth and then drools (rather than spits) through a collection aid into the collection tube (Salimetrics Europe Ltd, Newmarket, Suffolk, UK). Saliva samples were immediately stored at $-80\,°C$ until analysis. On day of analysis, saliva samples were thawed, vortexed and then centrifuged at $1500 \times g$ for 15 min at $4\,°C$. Melatonin concentrations were determined in duplicate using ELISA (Salimetrics, State College, PA, USA).

2.6. Gastric emptying assessment

The vegetable soup contained 100 mg of ^{13}C-sodium acetate for the assessment of GER using the ^{13}C breath test method. A basal end-expiratory breath sample was collected pre-meal ingestion then at every 15 min intervals post-meal ingestion for 2 h. Breath samples were analysed for the ratio of $^{13}CO_2$:$^{12}CO_2$ by non-dispersive infra-red spectroscopy (IRIS Dynamic, Kibion, Germany). The difference in the ratio of $^{13}CO_2$:$^{12}CO_2$ from baseline breath to post-ingestion breath samples are expressed as delta over baseline (DOB). Half-emptying time ($T_{\frac{1}{2}}$) and time of maximum emptying rate (T_{lag}) were calculated utilising the manufacturers integrated software evaluation incorporating equations of a previously described formula (Ghoos et al., 1993).

2.7. Statistical analysis

A three-way (trial (fasted vs. fed) x time of day (AM vs. PM) x time across trial) repeated-measures analysis of variance (ANOVA) was used to assess differences in blood glucose concentration, gastric emptying DOB, substrate oxidation, and VAS ratings. A two-way repeated measures ANOVA was used to assess trial x time of day differences for gastric emptying $T_{\frac{1}{2}}$ and T_{lag} data, melatonin concentration and 24-hour energy intake. A one-way repeated measures ANOVA was used to assess midpoint of sleep and body mass across trials. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse–Geisser corrections were applied for epsilon < 0.75, and the Huynh–Feldt correction adopted for less severe asphericity. Significant *F*-tests were followed by dependent Student's t-Tests or one-

| Baseline | MEAL 1 or FAST | 60 min rest | 45 min brisk walk | 30 min rest | MEAL 2 | Gastric emptying assessment |

0   15   75   120   150   165   180   195   210   225   240   255   270

Time (min)

◇ Blood sample   ✦ Rating of perceived exertion (RPE), and heart rate (HR)
▲ Visual analog scale (VAS)   ✕ Saliva Sample
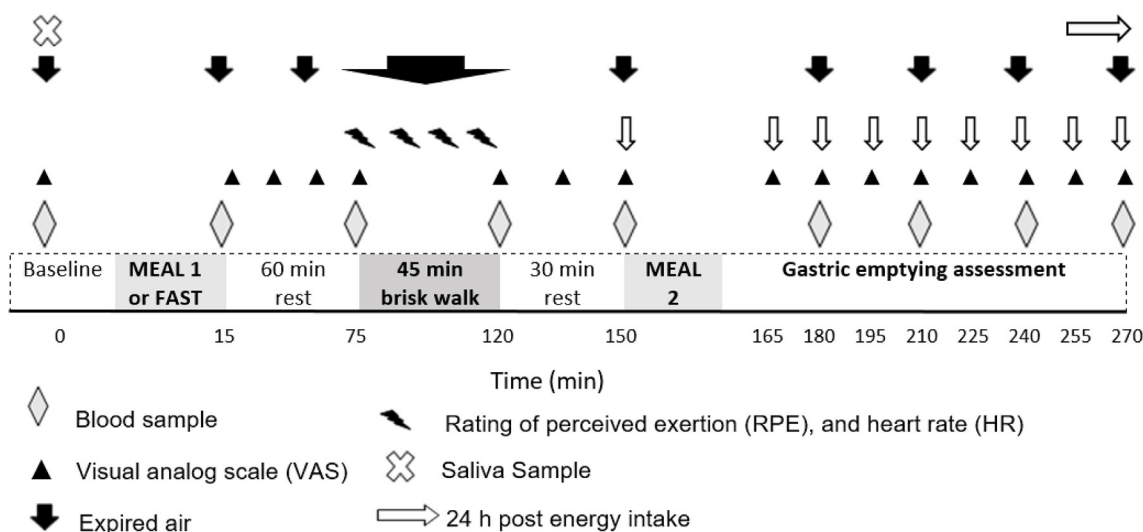⬇ Expired air   ⇨ 24 h post energy intake

Fig. 1. Schematic diagram of the experimental trial protocol.

way repeated ANOVA and Bonferroni adjusted pairwise comparisons as appropriate. All analyses were carried out using IBM SPSS statistics (v25.0 for Windows; SPSS, Chicago, IL). The level of significance was set at $P < 0.05$. Descriptive data are expressed as mean $\pm$ standard deviation (SD).

3. Results

There were no significant differences between trials (AM-FASTED vs. AM-FED vs. PM-FASTED vs. PM-FED) for midpoint of sleep (Mean $\pm$ SD; $02:40 \pm 0.2$ vs. $02:25 \pm 0.4$ vs. $02:32 \pm 0.5$ vs. $02:42 \pm 0.5$ respectively; $P = 0.159$). Sleep-wake times for the four trials were; 22:50–06:30 vs. 22:30–06:20 vs. 22:45–06:20 vs. 22:50–06:35 respectively. There were also no significant differences between trials for pre-trial body mass (82.94 ± 12.53 vs. 82.87 ± 12.55 vs. 82.79 ± 12.47 vs. 82.92 ± 12.55 kg for AM-FASTED, AM-FED vs. PM-FASTED, PM-FED; $P = 0.230$).

3.1. Melatonin

Two factor ANOVA demonstrated a main effect of time of day ($P = 0.002$), no main effect of trial ($P = 0.345$) and no interaction ($P = 0.159$) for salivary melatonin concentration. Salivary melatonin concentration was significantly different between morning and evening trials (AM-FASTED, AM-FED vs. PM-FASTED, PM-FED; 21 ± 6, 23 ± 13 vs. 15 ± 12, 9 ± 5 pg/mL; $P = 0.002$).

3.2. Gastric emptying rate

Two factor ANOVA demonstrated no main effect of time of day ($P = 0.128$), no main effect of trial ($P = 0.111$) and no interaction ($P = 0.430$) for $T_{1/2}$ (Fig. 2a). Two factor ANOVA demonstrated a main effect of time of day ($P = 0.021$), no main effect of trial ($P = 0.256$) and an interaction ($P = 0.023$) for T_{lag} (Fig. 2a). T_{lag} was slower in PM-FASTED compared to AM-FASTED, AM-FED and PM-FED (75 ± 18 vs. 63 ± 14 min, $P = 0.001$, vs. 65 ± 10 min, $P = 0.028$ and vs. 67 ± 16 min, $P = 0.007$). No trial $\times$ time of day X time interaction ($P = 0.088$), time of day effect ($P = 0.073$) or main trial effect ($P = 0.209$) was observed for DOB, although, a main effect for time was found ($P < 0.001$; Fig. 2b). Mean incremental area under curve (iAUC) for DOB were 2633 ± 978 vs. 2541 ± 1082 vs. 3343 ± 840 vs. 2774 ± 613 $^{13}CO_2:^{12}CO_2$ over 5 h for AM-FASTED, AM-FED, PM-FASTED, and PM-FED, respectively. No significant effect of trial ($P = 0.226$), time of day ($P = 0.075$), or interaction effect ($P = 0.177$) was observed.

3.3. Subjective feelings of appetite

A main effect of trial (FASTED vs. FED; $P < 0.001$), time of day ($P = 0.003$) and time ($P < 0.001$) was observed for hunger, although no trial x time of day $\times$ time interaction effect was observed ($P = 0.855$). Subjective feelings of hunger were generally lower during the FED trials compared to the FASTED trials following ingestion of breakfast with a number of time points showing significant differences ($P < 0.05$; Fig. 3a). However, there were no differences in subjective feelings of hunger between trials following ingestion of lunch.

A main effect of trial ($P < 0.001$) and time ($P < 0.001$) was observed for fullness, although no main effect for time of day ($P = 0.057$), or trial X time of day $\times$ time interaction ($P = 0.074$) effect was observed. Subjective feelings of fullness were generally greater during the FED trials compared to the FASTED trials following ingestion of breakfast with a number of time points showing significant differences ($P < 0.05$; Fig. 3b). However, there were no differences in subjective feelings of fullness between trials following ingestion of lunch.

A main effect of trial ($P = 0.008$), time of day ($P < 0.001$) and time ($P < 0.001$) was observed for PFC although no trial X time of
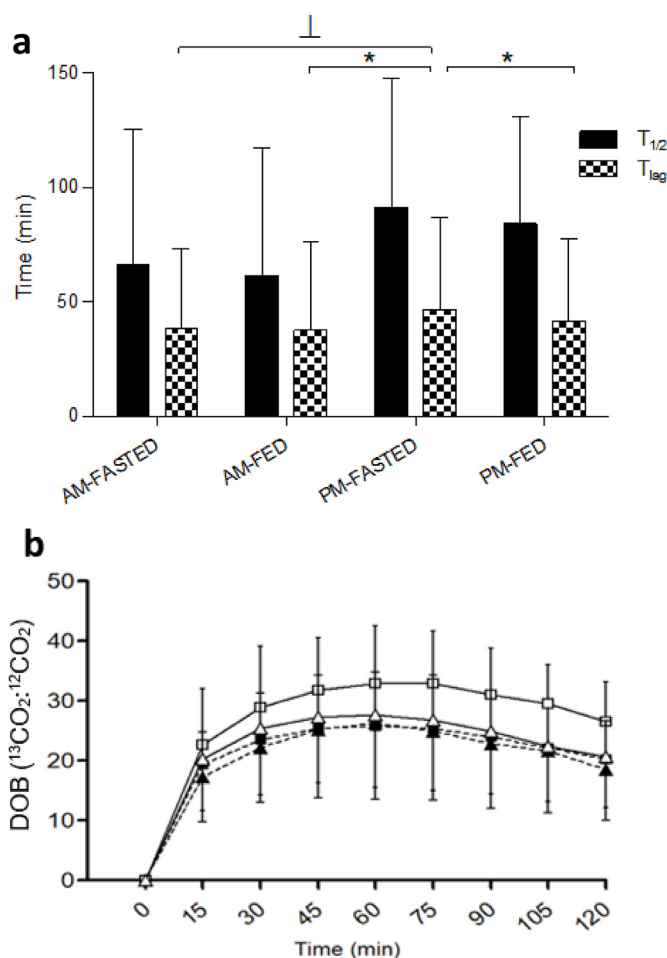

Fig. 2. Gastric emptying assessment, a) Gastric emptying half time ($T_{1/2}$) and time of maximal emptying rate (T_{lag}) b) Gastric emptying delta over baseline (DOB) for all four trials of MEAL 2 (800 g vegetable soup) AM-FASTED (■), AM-FED (▲) PM-FASTED (□) PM-FED (△). *Indicates significance (P < 0.05) versus corresponding condition (i.e. FASTED vs. FED), ⊥ indicates significance versus corresponding time of day (i.e. FASTED AM vs FASTED PM). Values represent mean $\pm$ SD; n = 12.

day $\times$ time interaction effect was observed ($P = 0.577$). Subjective feelings of PFC were generally lower during the FED trials compared to the FASTED trials following ingestion of breakfast with a number of time points showing significant differences (P < 0.05; Fig. 3c). However, there were no differences in subjective feelings of PFC between trials following ingestion of lunch.

A main effect of trial ($P = 0.003$) and time ($P < 0.001$) was observed for food satisfaction, however no main effect for time of day ($P = 0.078$), or trial X time of day $\times$ time interaction effect ($P = 0.679$) was observed. Subjective feelings of food satisfaction were generally greater during the FED trials compared to the FASTED trials following ingestion of breakfast with a number of time points showing significant differences ($P < 0.05$; Fig. 3d). However, there were no differences in subjective feelings of food satisfaction between trials following ingestion of lunch.

3.4. 24 h post energy intake

Two factor ANOVA demonstrated no main effect of time of day ($P = 0.170$), no main effect of trial ($P = 0.564$) and no interaction effect ($P = 0.718$) for 24 h energy intake (Table 1).
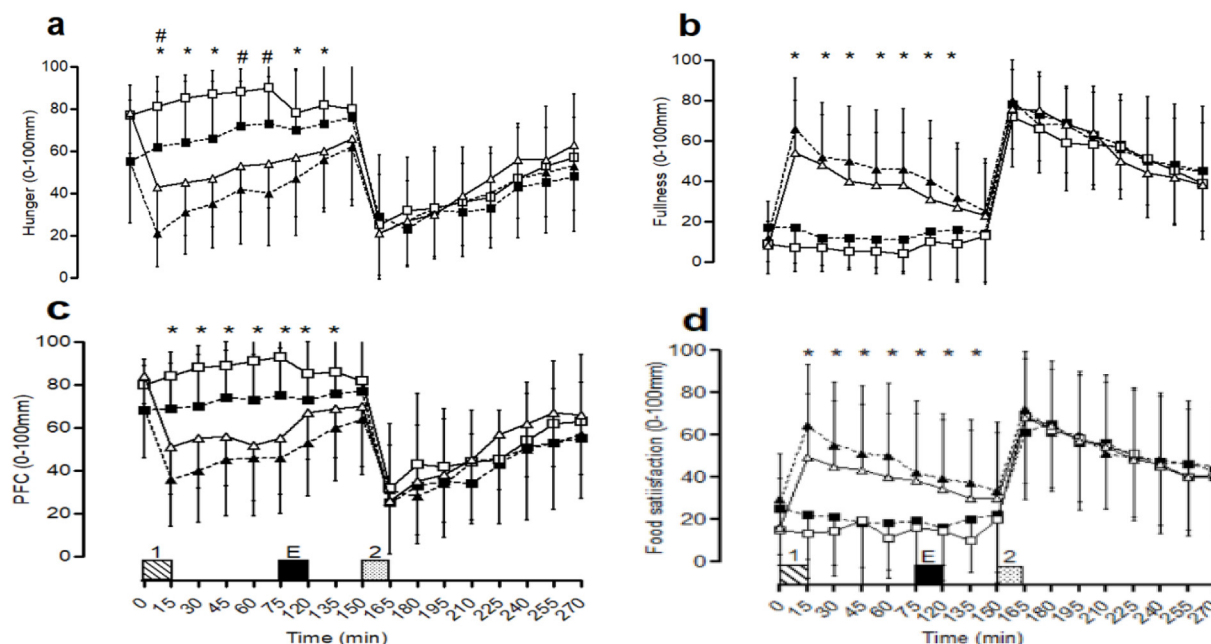
Fig. 3. Appetite ratings during trials, AM-FASTED (■), AM-FED (▲) PM-FASTED (□) PM-FED (Δ). Appetite was assessed by 100 mm visual analogue scale (VAS); a) hunger, b) fullness, c) prospective food consumption (PFC) and d) food satisfaction. Values represent mean ± SD; n = 12. *Indicates significance (P < 0.05) versus corresponding condition (i.e. FASTED vs. FED), # indicates significant difference at one time-point compared to all trials. 1 = Meal 1, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, E = Exercise period, where participants completed a 45 min brisk walk, 2 = Meal 2, where 800 g vegetable soup was ingested.

Table 1
24 h post trial energy intake and macronutrient breakdown for participants (n = 12; mean ± SD).

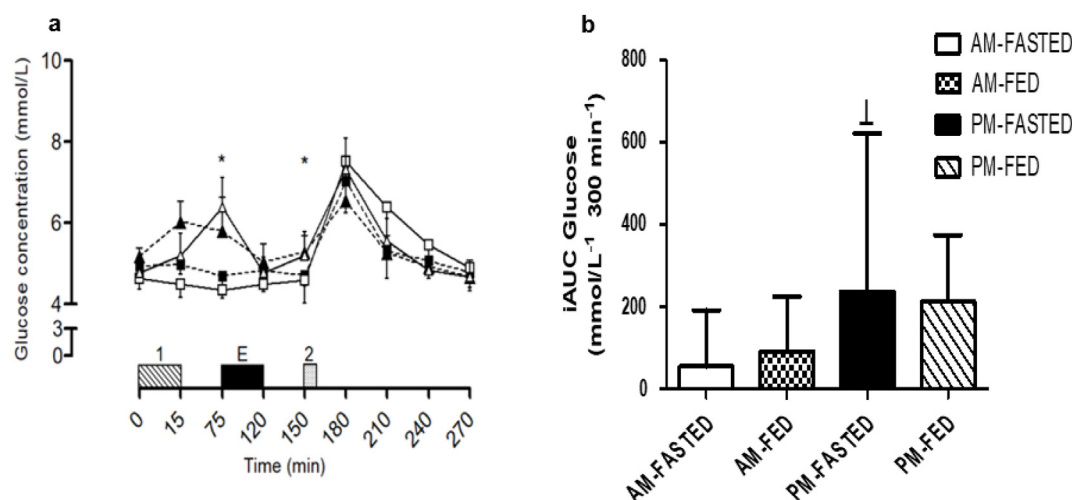|	AM-FASTED	AM-FED	PM-FASTED	PM-FED
Energy intake (kcal)	2789 ± 520	2704 ± 655	2639 ± 668	2490 ± 749
Protein (g)	138 ± 33	145 ± 70	126 ± 54	127 ± 59
Carbohydrate (g)	332 ± 106	296 ± 71	237 ± 119	273 ± 83
Fat (g)	104 ± 43	110 ± 43	134 ± 80	101 ± 49

3.5. Substrate oxidation

A main effect for trial ($P < 0.001$), and time ($P < 0.001$) was observed for CHO oxidation, however, no main effect for time of day ($P = 0.296$) or trial X time of day × time interaction effect was observed ($P = 0.366$; Fig. 4a). CHO oxidation was greater in PM-FED compared to AM-FASTED and PM-FASTED, and AM-FED compared to AM-FASTED throughout exercise at 15 min ($P = 0.004$; $P = 0.007$; $P = 0.021$ respectively), 30 min ($P < 0.001$; $P = 0.003$; $P = 0.006$), and 45 min ($P = 0.001$; $P = 0.001$; $P = 0.005$). CHO oxidation was higher in AM-FED compared to PM-FED and PM-FASTED at 1.5 h after soup ingestion (240 min; $P = 0.005$; $P = 0.022$) (Fig. 4a). Mean iAUC for CHO oxidation was 72.4 ± 34.6 vs. 93.3 ± 30.7 vs. 59.3 ± 33.1 vs. 96.2 ± 31.3 g/min over 5 h for AM-FASTED, AM-FED, PM-FASTED, and PM-FED, respectively. A significant effect of trial ($P = 0.001$) was observed but no significant time of day effect ($P = 0.581$) or interaction effect ($P = 0.368$).

A main effect of trial ($P < 0.001$) and time ($P < 0.001$) was observed for fat oxidation, however, no main effect for time of day ($P = 0.469$) or trial X time of day × time interaction effect was observed ($P = 0.740$; Fig. 4b). Fat oxidation was greater pre-exercise in PM-FASTED compared to PM-FED (0.11 ± 0.04 vs. 0.06 ± 0.02 g/min; $P = 0.003$), and greater throughout exercise for both FASTED compared to FED trials (all $P < 0.05$). At pre-lunch, fat oxidation was also greater in AM-FASTED and PM-FASTED than PM-FED ($P = 0.018$; $P = 0.020$), and AM-FASTED remained higher than PM-FED 30 min
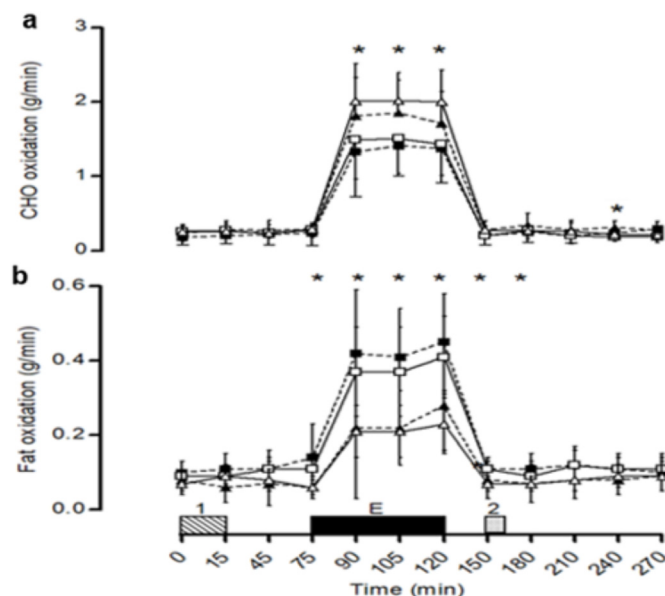
Fig. 4. Substrate utilisation during the trials AM-FASTED (■), AM-FED (▲) PM-FASTED (□) PM-FED (Δ). a) Carbohydrate oxidation and b) fat oxidation. Values represent mean ± SD; n = 12. *Indicates significance (P < 0.05) versus corresponding condition (i.e. FASTED vs. FED), ⊥ indicates significance versus corresponding time of day (i.e. FASTED AM vs FASTED PM). 1 = Meal 1, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, E = Exercise period, where participants completed a 45 min brisk walk, 2 = Meal 2, where 800 g vegetable soup was ingested.

post soup ingestion ($P = 0.041$) (Fig. 4b). Mean iAUC for fat oxidation was 18.8 ± 10.2 vs. 8.7 ± 7.7 vs. 19.5 ± 9.2 vs. 9.3 ± 9.7 g/min over 5 h for AM-FASTED, AM-FED, PM-FASTED, and PM-FED, respectively. A significant effect of trial ($P = 0.002$) was observed with fat oxidation being higher in the FASTED trials compared to FED trials. No

Fig. 5. Blood glucose responses. a) Blood glucose concentrations during trials, AM-FASTED (■), AM-FED (▲) PM-FASTED (□) PM-FED (Δ) and b) Incremental area under curve (iAUC) over the trials. Values represent mean ± SD; n = 12. *Indicates significance (P < 0.05) versus corresponding condition (i.e. FASTED vs. FED). ⊥ indicates significance versus corresponding time of day (i.e. FASTED AM vs FASTED PM). 1 = Meal 1, in which participants ingested a prescribed breakfast during the FED trial and remained fasted during the FASTED trial, E = Exercise period, where participants completed a 45 min brisk walk, 2 = Meal 2, where 800 g vegetable soup was ingested.

time of day ($P = 0.774$), or interaction effect ($P = 0.991$) was observed.

3.6. Blood glucose concentration

A main effect of trial ($P = 0.007$) and time ($P < 0.001$) was observed for glucose concentration, although no main effect for time of day ($P = 0.854$), or trial X time of day X time main interaction effect ($P = 0.058$) was observed. Baseline glucose concentrations were higher in the morning AM-FED trial in comparison to PM-FASTED trial ($P = 0.001$), no further differences during baseline collection. Blood glucose concentration pre-exercise was greater in AM-FED compared to PM-FASTED (5.80 ± 1.30 vs. 4.34 ± 0.31 mmol/L; $P = 0.014$), and greater in PM-FED compared to AM-FASTED and PM-FASTED (6.38 ± 1.15 vs. 4.69 ± 0.58; $P = 0.005$ and 4.34 ± 0.31 mmol/L; $P = 0.001$). No differences between trials were seen post-exercise ($P > 0.05$), however, blood glucose concentration was greater at 150 min pre-lunch in AM-FED compared to AM-FASTED (5.28 ± 0.63 vs. 4.71 ± 0.40 mmol/L; $P = 0.042$) (Fig. 5a). A significant time of day effect ($P = 0.024$) for iAUC for glucose concentrations was observed, but no significant trial ($P = 0.915$), or interaction effect ($P = 0.677$) (Fig. 5b).

4. Discussion

A meal in the evening following fasted exercise elicits a slower maximal gastric emptying rate in comparison to a meal following morning fasted and evening non-fasted exercise. Appetite does not follow a diurnal variation following fasted low intensity exercise, and regardless of the time of day, fasted exercise favours fat oxidation which may help induce a negative energy balance without a subsequent compensatory response in energy intake. This study adds novel insights into the diurnal variation of GER, appetite and metabolism in response to fasted versus fed exercise.

To the authors knowledge, this is the only study that has investigated the diurnal variation of GER response from a subsequent meal following fasted versus fed exercise. Previous studies that have examined the effect of GER between morning and evening have found half time was significantly delayed in the evening (Goo et al., 1987; Grammaticos et al., 2015; Orr et al., 2004). The current study only found a significance in maximal emptying time only, not half time. This may be due to the meal context in the current study in comparison to

others when measuring gastric emptying. Goo et al. (1987) found that in 16 healthy males, only gastric emptying half-times for the evening (20:00) meal were significantly longer for solids but not liquids when compared with morning (08:00) emptying half-times. The present study used a soup meal that contained a large liquid component, which may be an explanation for the lack of difference in half time as a greater delay of emptying with solid food compared with liquids is commonly observed (Hellström, Grybäck, & Jacobsson, 2006). In addition to this, it is well known that variations in gastric emptying can have a major impact on the postprandial glycemic profile, and incretin hormone secretion (Marathe, Rayner, Jones, & Horowitz, 2013; Trahair et al., 2014). Whether a delayed gastric emptying in the evening versus morning would be more beneficial for appetite regulatory hormone in response to weight management is unknown and requires further study. This may be of particular importance for some clinical populations, such as overweight and type 2 diabetes, with research providing a number of strategies to optimise postprandial glycemic control based on modulation of GER (Jones et al., 2001; Marathe et al., 2013; O'Keefe, Abuannadi, Lavie, & Bell, 2011; Phillips, Deane, Jones, Rayner, & Horowitz, 2015). It is suggested that a slower rate of nutrient delivery to the small intestine would be desirable to compensate for the delay in insulin release and the resistance to its actions (Marathe et al., 2013). However, it is difficult to draw accurate comparisons due to no existing studies measuring gastric emptying at different times of day in response to exercise. Therefore, more literature is required to build a clearer understanding, and also to explore whether appetite regulatory hormones are affected between morning and evening exercise.

Similar to gastric emptying, it is well documented that fat, CHO, and glucose metabolism display a time-of-day dependent rhythms, which align with daily rhythms in behaviours, such as sleep/wake, feeding/fasting, and activity cycles (Bailey, Udoh, & Young, 2014; Kalsbeek, La Fleur, & Fliers, 2014; Kessler et al., 2017). Previous evidence has observed higher fat oxidation rates in the evening in comparison to morning (Darvakh, Nikbakht, Shakerian, & Mousavian, 2014), while in contrast, CHO and glucose metabolism are higher in the morning in comparison to the evening (Kessler et. 2017; Qian & Scheer, 2016). However, these conclusions do not translate on to the current study findings, with no time of day effect observed in any of the energy metabolism measures, only between trials (fasted versus. fed exercise). A possible explanation for the lack of time of day variance may be due to the exercise elicited within the current study (55% $\dot{V}O_{2peak}$).

Previous studies that observed a time-of-day variance in fat/CHO oxidation conducted higher exercise intensities (Mohebbi, Azizi, & Tabari, 2011; Suk, Moon, Park, Park, & Shin, 2015). It is thought that during periods of increased physical activity, non-insulin mediated glucose utilisation increases, and the relative contribution of aerobic to anaerobic utilization being dependent upon exercise intensity (Alberts, Johansson, & McArthur, 2006; Calvo et al., 2008; Melzer, 2011; Röhling, Herder, Stemper, & Müssig, 2016; Rose & Richter, 2005). Nevertheless, energy metabolism is predominantly dependent on feeding behaviours, and regardless of time of day, fasted exercise favoured fat oxidation, while eating before exercise elicits a greater CHO oxidation response (Achten & Jeukendrup, 2004; Bachmen, 2016; Iwayama et al., 2017). This corresponds with existing literature, that fasting elicits fat metabolism, while feeding induces a greater CHO metabolism. It would be interesting to examine the time of day effect on energy metabolism with different exercise intensities and/or modes that elicit a greater energy response.

The present study hypothesised that evening and morning trials would result in differences in appetite and metabolic responses post-exercise. However, appetite stabilised across all trials post-exercise, which corresponds with the substrate utilisation and glucose findings. This may be due to a suppression of appetite which has been reported during and briefly following moderate-to-high intensity bouts of running exercise (Broom, Batterham, King, & Stensel, 2008; Vatansever-Ozen, Tiryaki-Sonmez, Bugdayci, & Ozen, 2011). The combined lack of differences in hunger and energy metabolism post exercise, followed by no differences in 24 h post-energy intake, could suggest that regardless of an increased energy expenditure being incurred from exercise there will likely be no compensatory increase in energy intake post-exercise to account for the omission of energy intake prior to exercise. This may, therefore, create a small short-term negative energy balance and if sustained in the long-term, the cumulative effects may have an important role in weight maintenance, which has been found in previous studies from fasted exercise. Previous studies have found that alternate day fasting combined with endurance exercise was effective for weight loss for obese participants following 12-week training programme (Bhutani, Klempel, Kroeger, Trepanowski, & Varady, 2013) and fasting before morning exercise decreased 24 h energy intake (Bachman, Deitrick, & Hillman, 2016). Further research on both the shorter-term effects of an acute bout of exercise and the cumulative effects of frequent fasted exercise at various times of day over a period of time is required to fully understand if compensatory effects occur.

In conclusion, these findings demonstrate that GER is sensitive to time of day variation in response to a meal following fasted exercise. In the postprandial stages, regardless of time of day, appetite, blood glucose concentration, substrate utilisation, and 24 h post energy intake is not sensitive to an acute bout of low-intensity exercise in the fasted state compared to the fed state. Fasted exercise favours fat oxidation, whilst eating before exercise favours CHO oxidation. The indication that no compensatory increase in energy intake will occur post exercise potentially holds positive implications for fasted brisk walking in the long-term control of weight management. Future research is warranted to investigate how appetite regulatory hormones associated with gastric emptying respond to fasted versus fed exercise at different times of the day.

Disclosure

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Conflicts of interest

The authors declare no conflict of interest.

Statement of authors' contributions to manuscript

VJM, AMWY, GHE conceived and designed the experiments; VJM and LRM performed the experiments; VJM analysed the data; VJM wrote the paper with contributions from AMWY and GHE. All authors have read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2019.104411.

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