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1	<b>Title:</b> Impact of exercise timing on perceived appetite and food reward in early and late				
2	chronotypes: an exploratory study in a male Saudi sample				
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There is very limited evidence on the influence of diurnal exercise timing on appetite control, and none on food reward or how an individual's chronotype could moderate such effects. We examined the impact of acute exercise timing on perceived appetite and food reward in young Saudi adults with early or late chronotypes. Forty-five young adults (23±4 years; BMI=25.1±4.0 kg/m²) completed the Morningness–Eveningness Questionnaire (MEQ) and were divided into early (score =  $59\pm5$ ) or late (score = 41±6) chronotypes. Participants attended the laboratory after ≥4h fast on two occasions for an AM (8:00-10:00) and PM (17:00-19:00) 30-min moderate-intensity cycling bout in a randomized counterbalanced order. Appetite ratings and food reward (Arab Leeds Food Preference Questionnaire) were measured before and after exercise. An acute exercise-induced decrease in hunger was found, which appeared to be dependent upon diurnal timing and chronotype, with hunger being more suppressed after AM exercise in the early chronotypes and after PM exercise in the late chronotypes. There was greater wanting for low-fat sweet foods after AM exercise relative to PM exercise, whereas there was greater wanting for high-fat sweet food and sweet relative to savoury food after PM exercise compared to AM exercise. These preliminary findings suggest that diurnal timing of exercise impacts food preferences, and that chronotype may influence the appetite response to an exercise bout at different times of day.

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Keywords: Exercise timing; chrono-exercise; appetite control; food reward; Saudi adults

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- Abbreviations:
- 40 HFSA, high-fat savoury; HFSW, high-fat sweet, LFPQ, Leeds Food Preference Questionnaire; LFSA,
- 41 low-fat savoury; LFSW, low-fat sweet

#### 1. Introduction

The influence of behavioural temporal patterns such as sleep, food intake and exercise on obesity and health status has gained interest in the scientific community, the latter two increasingly so. These patterns differ on a geographical level (e.g. the timing of the largest meal differs between Northern and Southern Europe (Almoosawi, Vingeliene, Karagounis, & Pot, 2016)) as well as on an individual level. An individual's chronotype reflects preferences in the timing of sleep, physical activity and eating, typically defined as morning, neither/intermediate, and evening chronotypes (Horne & Ostberg, 1976). Earlier food intake timing has been shown to associated with better weight loss outcomes in studies from Southern Europe and the Middle East (Garaulet et al., 2013; Jakubowicz, Barnea, Wainstein, & Froy, 2013). Underlying mechanisms may be related to appetite control and eating behaviour. Indeed, we recently reported diurnal variations in food reward and appetite response to early vs late meal timing in a sample of British young adults (Beaulieu, Oustric, Alkahtani, et al., 2020). Early meal timing was associated with reduced appetite, increased perceived fillingness and reduced liking and wanting for high-fat food. We did observe overall chronotype differences such that a later chronotype was associated with a greater BMI, weaker suppression of appetite and greater wanting but not liking for high-fat food. Other studies have suggested an impact of chronotype on eating behaviours (Schubert & Randler, 2008) and that chronotype moderates the relationship between meal timing and BMI (Xiao, Garaulet, & Scheer, 2019). Therefore, in studying the impact of behavioural temporal patterns in the context of appetite and obesity, it is important to consider the influence of an individual's chronotype.

There is considerably more evidence surrounding the impact of meal timing in health and disease (e.g. (Flanagan, Bechtold, Pot, & Johnston, 2021)) than there is for exercise timing. In fact, at this point in time, there appears to be more reviews than empirical evidence on the topic. Earlier evidence of exercise timing has been in the context of athletic performance, with its peak occurring in the late afternoon in general (Aoyama & Shibata, 2020). Considering that multiple factors (e.g. skills, cognitive abilities, diet, muscle fatigue) affect sport performance, chronotype is a major determinant of diurnal variation in athletic performance, with early chronotypes peaking earlier in the day and later chronotypes peaking later in the day (Facer-Childs & Brandstaetter, 2015). Early chronotypes seem to have lower diurnal variability in performance than late chronotypes, i.e. time of day seems to affect performance to a greater degree in late chronotypes. This is reflected by ratings of perceived exertion, which are typically lower in response to self-paced morning exercise in morning chronotypes compared to evening chronotypes (Rossi, Formenti, Vitale, Calogiuri, & Weydahl, 2015).

In the context of obesity, some suggest that earlier exercise timing might be beneficial for weight control (Chomistek, Shiroma, & Lee, 2016; Creasy et al., 2021; Schumacher, Thomas, Raynor, Rhodes, & Bond, 2020), with intervention studies showing greater weight loss after morning exercise training relative to afternoon exercise training (Alizadeh, Younespour, Rajabian Tabesh, & Haghravan, 2017; Willis, Creasy, Honas, Melanson, & Donnelly, 2020), but not always (Creasy et al., 2022; Teo et al., 2021). As reviewed by Blankenship and colleagues (2021), the current state of evidence lacks randomized controlled trials that are adequately powered, and of sufficiently high exercise dose and duration to detect differences in body composition between morning and evening exercise. Furthermore, how exercise timing might impact mechanisms of appetite control and energy balance regulation also remains to be fully understood. A few acute studies have investigated this area (Alizadeh, Mostafaee, Mazaheri, & Younespour, 2015; Larsen et al., 2019; Maraki et al., 2005; O'Donoghue, Fournier, & Guelfi, 2010). Overall, these studies suggest minimal impact of exercise timing on perceived appetite and energy intake. However, it is important to highlight that they did not consider how chronotype may influence these responses, nor assess the impact of exercise timing on food preferences and reward. Therefore, the aim of this exploratory study was to investigate the effect of diurnal timing of moderate-intensity exercise on perceived appetite and

food reward in healthy Saudi adults according to their morningness-eveningness score.

## 2. Methods

## 2.1 Participants

Inclusion criteria included male participants aged 18 to 39 years at the time of signing informed consent (in order to count Metabolic Equivalents (METs) based on this age category), regular sleep patterns (7-9 hours per night), regular meal patterns including breakfast, lunch and evening dinner consumption, and BMI of  $18.5-39.9~{\rm kg/m^2}$ . Exclusion criteria included history of eating disorders including binge eating, taking any medication or supplements known to affect appetite or weight within the past month and/or during the study, known food allergies or food intolerance, smokers and those who have recently ceased smoking (<6 months), BMI <  $18.5~{\rm kg/m^2}$  or >  $39.9~{\rm kg/m^2}$ , volunteers having lost significant amount of weight in the previous 6 months ( $\pm~4{\rm kg}$ ), volunteers who exercise <1 day or >5 days per week or have significantly changed their physical activity patterns in the past 6 months or who intend to change them during the study, and participants who do shift work.

109 Participants who were eligible and expressed their interest to participate in the study completed 110 Horne & Ostenberg's (1976) Morningness-Eveningness Questionnaire (MEQ), which has been 111 translated and validated among Saudi adults (BaHammam, Almistehi, Albatli, & AlShaya, 2011). 112 Participants and research assistants were not informed of the MEQ score/chronotype of the 113 participants. During data analysis, participants were divided into two groups based on the median of 114 MEQ score. This research was approved by the Internal Review Board (IRB) at King Saud University 115 (E-19-3965), and all participants provided written informed consent prior to taking part. 116 117 2.2 Study Design 118 Study design was a crossover within independent groups. All participants were required to visit the 119 laboratory two times to participate in an exercise session after overnight fasting in the morning 120 session and after 4 hours of fasting in the evening session. The AM exercise session was performed 121 between 8:00-10:00 and the PM exercise session was performed between 17:00-19:00. Participants 122 started either AM or PM exercise in a randomised counterbalanced order. The two exercise sessions 123 were done on the same day of the week, with a wash out of 7 days (i.e. if a participant did the AM 124 exercise on Tuesday morning, they did the PM exercise on the following Tuesday). There was a 125 preliminary visit to understand the process of the study and sign the consent form. 126 127 2.3 Experimental sessions 128 Participants were asked to attend the laboratory after a regular day in terms of physical activity, 129 food intake and sleep time, and re-schedule the appointment if there was a sudden change in their 130 habitual lifestyle in the 24 hours preceding the two exercise sessions. On the day of evening exercise 131 session, participants were expected to wake up at their usual time in the morning, and were 132 required to carry out their usual day avoiding any additional effort, extra coffee consumption or 133 change in their habitual diet habit. 134 135 Upon arrival, participants were seated for 5 minutes, completed an appetite sensations 136 questionnaire, followed by the Arabic version of the Leeds Food Preference Questionnaire (LFPQ; 137 more below). They then completed a 30-minute exercise session according to the below description. 138 Five minutes post-exercise, participants repeated the appetite sensations and LFPQ tasks. 139 Participants then consumed a Greek drinking yoghurt (mixed berry flavour), which consisted of 250 140 mL and contained 231 kcal including 27 g protein and 33 g carbohydrate. Participants were required 141 to consume the entire drink. Fifteen minutes post-exercise, participants completed a final set of 142 appetite sensations, and this was the end of session.

143 144 2.3.1 Exercise bout 145 During each experimental session, participants did 30 minutes of exercise on a Monark bike at 146 moderate intensity of 4.8 METs, and the period of 30 minutes has been determined based on our 147 pilot study to confirm that all participants can complete the exercise session properly. Moderate-148 intensity exercise based on METs is the intensity that is required to expend 3 to 6 METs (Jetté, 149 Sidney, & Blümchen, 1990), and has been categorized based on age such that moderate-intensity 150 exercise for people between 20 to 39 years requires METs between 4.8 and 7.1 (Garber et al., 2011). 151 According to the 2011 Compendium of Physical Activities, stationary cycling at 51 – 89 watts is 152 considered a light-to-moderate effort at 4.8 METs (Ainsworth et al., 2011). Oxygen consumption and 153 METs were estimated individually according to participants' weight based on the American College 154 of Sport Medicine cycle ergometry equation (Whaley, Brubaker, Otto, & Armstrong, 2006). 155 Participants repeated exactly the same bout of exercise on both trials. 156 157 2.3.2 Appetite sensations 158 Ratings of hunger, fullness and desire to eat were assessed with 100-mm visual analogue scales 159 before, after and 15-min after the exercise bout using an Arabic paper-based questionnaire. 160 161 2.3.3 Food reward 162 Food reward was measured before and after exercise using the Arabic version of the Leeds Food 163 Preference Questionnaire (Alkahtani, Dalton, Abuzaid, Obeid, & Finlayson, 2016). Scores of implicit 164 wanting and explicit liking for high-fat savoury (HFSA; chicken burger, falafel, creamy pasta, French 165 fries), low-fat savoury (LFSA; carrot, cucumber, lettuce, steamed rice), high-fat sweet (HFSW; ice 166 cream, cheesecake, chocolate bar, basbosah) and low-fat sweet (LFSW; dates, watermelon, rock 167 melon, jelly sweets) food categories were computed. Fat bias scores were computed by subtracting 168 the mean low-fat scores from the mean high-fat scores and taste bias scores were computed by 169 subtracting the mean savoury scores from the mean sweet scores. Therefore, positive scores 170 indicate greater reward for high-fat relative to low-fat foods and sweet relative to savoury foods, 171 respectively. Implicit wanting was assessed by asking the participants to select as fast as possible 172 which of two foods from the four food categories "they most want to eat now". Scores for implicit

wanting were computed from mean response times adjusted for frequency. Explicit liking was

determined by asking participants to rate the extent to which they liked each food ("How pleasant

would it be to taste this food now?") using a 100-point visual analogue scale (Oustric et al., 2020).

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### 2.4 Statistical analyses

Data were visually inspected for normality and outliers before analysis. Baseline differences between groups (early vs late chronotype) were determined with independent samples t-tests, and differences between time-of-day conditions (AM vs PM exercise), groups (early vs late chronotype) and time (pre, post and 15-min post exercise) were determined with 3-way repeated measures ANOVA using SPSS v26. Data are presented as means  $\pm$  SD or mean difference ( $M_{\Delta}$ ) and [95% confidence interval], alongside partial eta squared ( $\eta^2 p$ ) as effect size. Due to the preliminary and exploratory nature of this study and its numerous comparisons, we have avoided referring to any outcome as 'statistically significant' on the basis of a particular p-value and have interpreted our findings more descriptively.

#### 3. Results

### 3.1 Participant characteristics

Participant characteristics are shown in Table 1. By design, early chronotypes had greater MEQ scores than late chronotypes (p < 0.001), but there were no other differences between chronotype groups nor was chronotype associated with BMI (r=0.076, p=0.619). According to the MEQ scoring criteria (Horne & Ostberg, 1976), the early chronotype group had 12 morning types (MEQ score  $\geq$ 59) and 11 neither types (MEQ score 42-58), and the late chronotype group had 11 neither types and 11 evening types (MEQ score  $\leq$ 31).

**Table 1.** Participant characteristics

	All	Early Chronotypes	Late Chronotypes	
	(n=45)	(n=23)	(n=22)	
Age (years)	23 ± 4	23 ± 3	$24\pm4$	
Weight (kg)	$\textbf{75.3} \pm \textbf{12.5}$	$78.6 \pm 12.2$	$\textbf{72.0} \pm \textbf{12.2}$	
BMI (kg/m²)	$25.1 \pm 4.0$	$25.6 \pm 4.1$	$24.6 \pm 4.0$	
MEQ score	$\textbf{50} \pm \textbf{10}$	$59\pm5$	$41\pm6\text{*}$	

Data are mean ± SD

MEQ; Morningness–Eveningness Questionnaire.

\*Early vs. late p < 0.001.

# 3.2 Exercise bout parameters

Overall, the cycle ergometer resistance was 1.46  $\pm$  0.19 kg and the exercise energy expenditure was 190  $\pm$  27 kcal. After controlling for body weight, there were no differences between groups in terms

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206
        Heart rate was greater in PM than AM (129 \pm 16 bpm vs 126 \pm 16 bpm, respectively; p = 0.044, \eta^2p =
207
        0.091), and there were no differences between groups nor condition by group interaction (both p \ge 1
208
        0.511, \eta^2 p \le 0.010). With regards to ratings of perceived exertion, the average RPE was 12 ± 2, and
209
        there were no effects of condition nor condition by group interaction (both p \geq 0.327, \eta^2p \leq0.02).
210
        The early chronotypes had greater ratings of perceived exertion than the late chronotypes (M_{\Delta} = 0.8
211
        [95% CI -0.1, 1.7]; p=0.085, \eta^2p = 0.067).
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213
        3.3 Appetite sensations
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        Pre-exercise ratings of hunger, fullness and desire to eat did not differ between AM vs PM (p ≥
215
        0.512).
216
217
        Hunger was overall greater in the early chronotypes relative to the late chronotypes (M_{\Delta} = 9 mm
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        [95% CI 0.4, 17]; p=0.041, \eta^2p = 0.094). While hunger appeared to decrease post-exercise and return
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        to baseline shortly after (p = 0.067, \eta^2 p = 0.063), there was no effect of condition (p = 0.711, \eta^2 p =
220
        0.003). Interestingly, as shown in Figure 1, in the early chronotypes hunger appeared to decrease to
221
        a greater extent in response to AM (M_{\Delta} = 11 mm [95% CI -4, 27]) than PM exercise (M_{\Delta} = 2 mm [95%
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        CI-14, 18]), whereas in the late chronotypes hunger appeared to decrease to a greater extent in
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        response to PM (M_{\Delta} = 12 mm [95% CI -4, 28]) than AM exercise (M_{\Delta} = 3 mm [95% CI -13, 18]).
224
225
        Fullness was overall lower in the early chronotypes relative to the late chronotypes (M_{\Delta} = 9 mm
226
        [95% CI 1, 17]; p=0.032, \eta^2 p = 0.102). There was a main effect of time (p = 0.015, \eta^2 p = 0.099), with
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        post-exercise fullness being greater than pre-exercise (M_{\Delta} = 11 mm [95% CI 1, 21]; p=0.027) and 15-
228
        min post-exercise (M_{\Delta} = 6 mm [95% CI -0.7, 14]; p=0.088) ratings. There was no effect of condition
229
        nor interactions (p \geq 0.314, \eta^2p \leq 0.027).
230
231
        Desire to eat was overall greater in the early chronotypes relative to the late chronotypes (M_{\Delta} = 9
232
        mm [95% CI -0.2, 19]; p=0.054, \eta^2p = 0.084). While there appeared to be a main effect of time, with
233
        desire to eat decreasing post-exercise and returning to baseline afterwards (p = 0.085, \eta^2p = 0.058),
234
        there was no effect of condition nor interactions (p \geq 0.114, \eta^2p \leq 0.050).
235
236
        [Figure 1 here]
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of exercise energy expenditure (early 198  $\pm$  28 kcal vs late 182  $\pm$  24 kcal; p=0.547,  $\eta^2$ p = 0.009).

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3.4 Food reward

239 Two extreme outliers were excluded from some of the wanting analysis due to being above 3rd 240 quartile + 3\*interquartile range or below 1st quartile - 3\*interquartile range, identified via boxplot 241 (see Table 2). There were no apparent pre-exercise differences in wanting and liking between AM 242 and PM, except for liking HFSW which was greater in PM relative to AM ( $M_{\Delta}$  = 9 mm [95% CI -0.2, 243 19]; p=0.055). 244 245 There were no condition, time nor group effects for wanting HFSA and LFSW. Wanting LFSA was 246 overall greater in AM compared to PM ( $M_{\Delta}$  = 12.8 [95% CI 3.6, 22.0]; p=0.008,  $\eta^2$ p = 0.162). 247 However, a condition by time interaction (p=0.048,  $\eta^2$ p = 0.092) revealed that wanting LFSA was 248 only greater in AM vs PM post-exercise ( $M_{\Delta}$  = 17.8 [95% CI 8.9, 26.7]; p<0.001). Wanting HFSW 249 appeared to be greater overall in PM vs AM ( $M_{\Delta} = 7.6$  [95% CI -1.5, 16.7]; p=0.098,  $\eta^2 p = 0.062$ ) and 250 this seemed to be driven by a larger difference post-exercise ( $M_{\Delta}$  = 12.8 [95% CI 1.9, 23.7]; p=0.023) 251 than pre-exercise ( $M_{\Delta}$  = 2.5 [95% CI -10.1, 15.0]; p=0.693). Wanting fat bias ( $M_{\Delta}$  = 10.1 [95% CI -1.3, 252 21.5]; p=0.080,  $\eta^2$ p = 0.071) and taste bias (M<sub>\(\Delta\)</sub> = 10.3 [95% CI -1.4, 22.0]; p=0.082,  $\eta^2$ p = 0.070) were 253 overall greater in PM compared to AM, indicating greater preference for high-fat relative to low-fat 254 and to sweet relative to savoury food, respectively. For taste bias, this appeared to be driven by a 255 larger difference post-exercise ( $M_{\Delta}$  = 17.8 [95% CI 2.9, 32.6]; p=0.020) than pre-exercise ( $M_{\Delta}$  = 2.9 256 [95% CI -11.3, 17.0]; p=0.685). 257 258 Liking HFSA was greater overall in the early chronotypes relative to the late chronotypes ( $M_{\Delta}$  = 13 259 mm [95% CI -0.6, 26]; p=0.061,  $\eta^2$ p = 0.079), and in PM relative to AM (M<sub>\Delta</sub> = 7 mm [95% CI -0.6, 15]; 260 p=0.069,  $\eta^2$ p = 0.075). Liking LFSA was greater in the early chronotypes than the late chronotypes 261  $(M_{\Delta} = 18 \text{ mm} [95\% \text{ CI 5, 30}]; p=0.006, \eta^2 p = 0.164)$ . Liking HFSW was greater overall in the early 262 chronotypes relative to the late chronotypes ( $M_{\Delta}$  = 20 mm [95% CI 7, 33]; p=0.004,  $\eta^2$ p = 0.177), in PM relative to AM (M $_{\Delta}$  = 11 mm [95% CI 3, 19]; p=0.005,  $\eta^2 p$  = 0.166), and pre-exercise relative to 263 264 post-exercise (M<sub> $\Delta$ </sub> = 6 mm [95% CI -0.3, 12]; p=0.060,  $\eta^2$ p = 0.080), but there were no interactions. 265 Liking LFSW was greater overall in the early chronotypes relative to the late chronotypes ( $M_{\Delta}$  = 18 266 mm [95% CI 8, 28]; p=0.001,  $\eta^2$ p = 0.237), in PM relative to AM ( $M_{\Delta}$  = 5 mm [95% CI 0.4, 10]; 267 p=0.034,  $\eta^2$ p = 0.101), and pre-exercise relative to post-exercise (M<sub>\Delta</sub> = 4 mm [95% CI -0.3, 9]; 268 p=0.051,  $\eta^2$ p = 0.085), but there were no interactions. Liking fat bias was greater overall in PM 269 relative to AM ( $M_{\Delta}$  = 5 mm [95% CI -0.2, 11]; p=0.056,  $\eta^2$ p = 0.082), indicating greater liking for high-270 fat relative to low-fat food. Liking taste bias was greater overall pre-exercise compared to post-271 exercise ( $M_{\Delta}$  = 5 mm [95% CI -0.2, 11]; p=0.059,  $\eta^2$ p = 0.081), indicating greater liking for sweet 272 relative to savoury food.

Table 2. Wanting and liking scores for foods varying in sweet taste and fat content in response to morning (AM) and evening (PM) exercise in early vs late
 chronotype groups.

	Early Chronotypes (n=23)			Late Chronotypes (n=22)				
	Pre AM	Post AM	Pre PM	Post PM	Pre AM	Post AM	Pre PM	Post PM
Wanting HFSA	19.4 ± 36.6	26.0 ± 40.3	19.5 ± 42.7	20.3 ± 39.4	3.4 ± 43.8 <sup>b</sup>	14.5 ± 47.0 <sup>b</sup>	12.0 ± 39.8 <sup>b</sup>	15.2 ± 37.4 <sup>b</sup>
Wanting LFSA	-11.9 ± 35.7ª	-10.1 ± 31.0 <sup>a</sup>	-18.9 ± 25.3ª	-21.9 ± 23.3ª	-9.7 ± 45.2 <sup>b</sup>	0.7 ± 37.9 <sup>b</sup>	-18.4 ± 29.9 <sup>b</sup>	-23.1 ± 33.8 <sup>b</sup>
Wanting HFSW	-1.0 ± 33.4	-3.4 ± 33.8	12.2 ± 27.2	12.1 ± 25.6	9.9 ± 38.4	-2.6 ± 33.1	1.6 ± 52.2	7.4 ± 41.0
Wanting LFSW	-5.9 ± 26.8ª	-12.3 ± 25.3ª	-12.3 ± 22.4ª	-10.2 ± 23.5ª	-3.0 ± 19.1	-10.4 ± 42.2	5.5 ± 38.0	-0.7 ± 26.4
Wanting fat bias	18.4 ± 50.6	22.6 ± 40.3	31.6 ± 33.8	32.4 ± 31.8	13.8 ± 49.1 <sup>b</sup>	11.2 ± 51.6 <sup>b</sup>	14.8 ± 48.2 <sup>b</sup>	27.6 ± 41.7 <sup>b</sup>
Wanting taste bias	-6.1 ± 33.7ª	-15.0 ± 46.9ª	-0.6 ± 43.3 <sup>a</sup>	0.8 ± 40.5 <sup>a</sup>	6.8 ± 41.4	-13.0 ± 52.0	7.1 ± 47.4	6.8 ± 40.5
Liking HFSA	56 ± 28	49 ± 28	60 ± 29	60 ± 27	41 ± 33	40 ± 31	44 ± 32	48 ± 28
Liking LFSA	49 ± 25	46 ± 28	52 ± 22	56 ± 23	32 ± 24	35 ± 22	32 ± 20	34 ± 27
Liking HFSW	57 ± 31	45 ± 27	69 ± 23	65 ± 23	38 ± 31	34 ± 30	44 ± 33	40 ± 28
Liking LFSW	55 ± 22	48 ± 23	57 ± 17	60 ± 15	39 ± 24	32 ± 22	43 ± 20	36 ± 22
Liking fat bias	4 ± 27	-1 ± 25	10 ± 23	4 ± 23	4 ± 19	3 ± 21	7 ± 16	10 ± 15
Liking taste bias	4 ± 24	-1 ± 27	7 ± 19	4 ± 21	2 ± 14	-4 ± 20	5 ± 36	-3 ± 24

AM, morning exercise; PM, evening exercise; HFSA, high-fat savoury; LFSA, low-fat savoury; HFSW, high-fat sweet; LFSW, low-fat sweet. Positive bias scores indicate greater wanting or liking for high-fat relative to low-fat (fat bias) or for sweet relative to savoury (taste bias). an=22. bn=21.

#### 4. Discussion

In the context of appetite control, there is a paucity of research examining the impact of diurnal exercise timing. In the current study, we examined the appetite response to early vs late exercise in individuals varying in chronotype. Overall, our findings revealed a ~10-mm greater reduction in hunger in response to acute AM exercise compared to PM exercise in the early chronotype group, whereas a similar magnitude of reduction in hunger was found in response to PM exercise compared to AM exercise in the late chronotype group. There was also greater wanting for LFSA foods after AM exercise compared to PM exercise, whereas there was greater wanting for HFSW foods and sweet preference (i.e. taste bias) after PM exercise compared to AM exercise. The liking response to exercise was not impacted by exercise timing per se but we did observe overall effects of time-of-day, chronotype and exercise. Finally, we found no relationship between chronotype and BMI.

The suppression of appetite in response to moderate-to-vigorous exercise is a common occurrence and has been termed 'exercise-induced anorexia' (King, Burley, & Blundell, 1994). Previous diurnal timing studies had not reported any effect of exercise on appetite suppression (Alizadeh et al., 2015; Larsen et al., 2019; Maraki et al., 2005; O'Donoghue et al., 2010). Specifically, Maraki et al. (2005) showed that a one-hour aerobic and muscle conditioning exercise class in the morning (8:15) or in the evening (19:15) in low-active healthy weight females did not result in differences in appetite (both exercise sessions increased appetite) nor in daily self-reported energy intake. O'Donoghue et al. (2010) also did not find any impact of exercise timing on daily ad libitum food intake (buffets) in physically active men in response to a 45-min treadmill run at 75% VO<sub>2max</sub> at 7:00 or 17:00. Similarly, Alizadeh et al. (2015) did not find any differences in daily self-reported energy intake in response to a 30-min treadmill exercise at ventilatory threshold between 8:00-10:00 vs 14:00-16:00 in inactive women with obesity; however, satiety ratings were greater in response to morning exercise. Finally, Larsen et al. (2019) also found that high-intensity interval exercise performed in the morning (6:00), afternoon (14:00) or early evening (19:00) did not impact appetite nor energy intake, despite some differences in the ghrelin response to the exercise bouts.

These prior studies had not considered if an individual's chronotype impacts the appetite response to an exercise bout earlier vs later in the day. The current study suggests that it might, with earlier exercise appearing to suppress hunger to a greater extent in early chronotypes than later exercise and vice versa for late chronotypes. These potential exercise timing-chronotype differences warrant further investigation. Interestingly, such exercise timing-chronotype differences have been observed previously with exercise performance and exertion outcomes with self-paced exercise (Rae,

Stephenson, & Roden, 2015; Rossi et al., 2015). The implications for such an appetite-suppressing response among early and late chronotypes for weight control remains to be fully understood.

In terms of wanting and liking, previous studies investigating the effect of acute exercise on food reward had not considered time-of-day differences of exercise and have shown, with the same LFPQ methodology, equivocal results with large individual variability (Beaulieu, Oustric, & Finlayson, 2020). The current study suggests that performing exercise results in greater wanting for LFSA foods after early exercise whereas in greater wanting for HFSW and sweet preference after later exercise, independent of chronotype. The liking response to exercise timing was not influenced by chronotype (i.e. no interactions) but overall liking was greater later than earlier in the day, in early compared late chronotypes and decreased after exercise. Previous studies have shown that regardless of time of day, an increase in reward for HFSW food in response to acute exercise is associated with compensatory eating after exercise (Finlayson, Bryant, Blundell, & King, 2009) and to lower-thanexpected weight loss after 12 weeks of training (Finlayson et al., 2011). Therefore, it seems important to mitigate any increase in HFSW food reward in response to an acute exercise bout. As with our previous study on meal timing (Beaulieu, Oustric, Alkahtani, et al., 2020), it appears that overall, preference for high-fat food is greater later in the day than earlier in the day. It remains unknown whether performing exercise earlier in the day could promote better food choices during the remainder of the day and how an individual's chronotype might impact this. We hope this will be addressed in future studies.

Unlike our previous study in a British sample (Beaulieu, Oustric, Alkahtani, et al., 2020), and others (Sun, Gustat, Bertisch, Redline, & Bazzano, 2020; Xiao et al., 2019) where it was found that evening chronotype was associated with a higher BMI, in the current study there was a lack of association between chronotype and BMI. However, this lack of association between chronotype and BMI is supported by a recent systematic review with meta-analysis examining chronotype differences in energy intake, anthropometric parameters and other health status outcomes (Lotti, Pagliai, Colombini, Sofi, & Dinu, 2022). In the meta-analysis on BMI, which included 33 studies with 158,680 morning types and 54,552 evening types, there were no differences between the two chronotypes; however, heterogeneity was extremely high with an I² of 96%. Five studies suggested no difference in body fat percentage either, with an I² of 0%. In terms of energy intake, 16 studies also suggested no differences between chronotypes, but again with high heterogeneity with an I² of 89%. Nevertheless, it was still found that evening chronotypes had a worse cardiometabolic profile, with higher fasting blood glucose, HbA1c, LDL cholesterol and triglycerides and greater risk of diabetes,

cancer and depression (Lotti et al., 2022). Clearly, more work is needed to understand the health implications of being an evening chronotype, and the links between chronotype, eating behaviours and obesity.

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As one of the few studies to investigate the impact of diurnal exercise timing on perceived appetite, and the first to assess food reward and consider chronotype in this context, several considerations and limitations need to be addressed. While we accounted for chronotype, we did not account for habitual time of exercise of the participants, which may be important. While not related to appetite, both chronotype and habitual training time-of-day differences were observed in the performance and exertion response to early vs late exercise (Rae et al., 2015). This means that the chronotype effect may be mitigated when habitual exercise timing does not coincide with preferred exercise timing (e.g. an evening type habitually exercising in the morning finds morning exercise easier than an evening type habitually exercising in the evening). If this also affects appetite responses is unknown. In Saudi Arabia where the average temperature in the summer ranges between 42 – 46°C, individuals may prefer to exercise in the early morning or in the evening. Understanding the impact of exercise timing on appetite and body composition among habitual and non-habitual exercisers requires further acute and chronic studies. Future acute/observational studies should therefore consider habitual exercise timing as well as chronotype as part of their demographic information. Another consideration for chronotype is our approach to grouping our participants. Due to the difficulty of recruiting 'true' morning and evening chronotypes, a similar median-split approach to grouping participants was employed as our previous study (Beaulieu, Oustric, Alkahtani, et al., 2020). Accordingly, half of our groups contained 'neither' types which may have impacted our results. Future studies should aim to recruit 'true' morning and evening types to confirm our results. Additionally, our assessment window only included the timing around the exercise bout itself and lacked a test meal afterwards; therefore, future studies should assess effects throughout the day and also over time, i.e. in a training study, and on food intake/postprandial appetite to assess exercise-meal timing effects. And finally, we have used the standard Arabic version of the Leeds Food Preference Questionnaire. Our preliminary unpublished electronic survey showed that healthy adult men prefer to have cheesecake (HFSW), burger (HFSA) and steamed rice (LFSA) in the evening. It may be hard to have similar food selection for the assessment of morning and evening food preference. This requires further validation and investigation.

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To conclude, this is the first study examining both perceived appetite and food reward in response to acute exercise timing while considering the influence of chronotype. It is important to highlight

the preliminary and exploratory nature of this study, which we hope will set the stage for future studies in the area. The study revealed that the appetite response to early vs late exercise may be influenced by an individual's chronotype. Our results suggest that hunger suppression after exercise may be heightened at a time in line with an individual's chronotype (i.e. with early exercise in early chronotypes and vice versa for late chronotypes). The food reward response to exercise may also differ according to time of day, with wanting for high-fat sweet foods and sweet food preference greater after later exercise than earlier exercise, regardless of chronotype. Given the increasing interest of the topic, we hope these findings will elicit further work in the area and address the questions and considerations this study has raised. Understanding how diurnal exercise timing and chronotype affect appetite control and energy balance has implications for improving the efficacy of exercise in the context of weight management.

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#### **Author contributions**

**GF**: Conceptualization; **GF**, **SA**, **MH**: Study methodology; **SA**, **AH**: Data curation; **GF**, **KB**: Data analysis; **KB**: Writing – original draft; **All**: Writing – review & editing.

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#### **Conflicts of interest**

The authors report no conflicts of interest.

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540	Figure Captions
541	
542	Figure 1. Hunger (A), fullness (B) and desire to eat (C) in response to morning (AM) and evening (PM)
543	exercise in early vs late chronotype groups.
544	