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Energy intake and appetite in laboratory and free-living conditions may be consistent across menstrual cycle phases

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ABSTRACT

Background: Self-reported dietary intake varies across menstrual cycle phases, but objective assessments of dietary intake together with appetite and resting metabolic rate (RMR) are limited. This study aimed to assess differences in dietary intake, appetite, and RMR during two hormonally-distinct menstrual cycle phases in laboratory and free-living settings.

Methods: Healthy premenopausal females with predictable normal-length menstrual cycles completed two study visits: one in the late-follicular and one in the mid-luteal phase. Menstrual cycle phases were assessed using urinary luteinizing hormone surge and prospective cycle days. Participants consumed a 2-day energy- and macronutrient-balanced run-in diet prior to each visit. RMR was measured with indirect calorimetry, followed by appetite ratings before and after a standardized breakfast, and a food cravings questionnaire. Appetite was also tracked for 2.5 days post-visit in a free-living environment. Ad libitum energy and macronutrient intakes were measured using pre-weighed plus weighing of uneaten food at an in-laboratory lunch meal, as well as during the 2.5-day free-living period.

Results: Eighteen participants were included (age: 21 ± 4 years; body mass index: 21.2 ± 1.5 kg/m²). There were no differences between in-laboratory ad libitum energy or macronutrient intakes, appetite, or food cravings between phases. RMR did not differ between phases, although the mid-luteal phase RMR tended to be higher (104 \pm 218 kcal/day higher; P=0.074). No main or interaction effects for phase or time were observed for free-living dietary intake nor appetite ratings.

Conclusions: Although RMR tended to be increased during the luteal phase, comprehensive appetite and energy intake assessments showed no significant cycle-phase differences in these 18 participants.

1. Introduction

Characterizing energy balance (i.e., dietary energy intake, energy expenditure) and its determinants is crucial for understanding the

mechanisms of body weight regulation (Hall et al., 2012). Ovarian steroid hormones (*i.e.*, estrogens and progesterone) may affect different aspects of energy balance parameters (Hirschberg, 2012; Mauvais-Jarvis et al., 2013). This hormonal influence is particularly evident across the

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Abbreviations: AUC, area under the curve; BMI, body mass index; Kcal, kilocalorie; PFC, prospective food consumption; RMR, resting metabolic rate; VAS, visual analog scale.

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menstrual cycle, during which high estrogen and low progesterone occur in the late-follicular phase and moderate estrogen and high progesterone occur in the mid-luteal phase (Holesh et al., 2024; Reed & Carr, 2000; Stricker et al., 2006). These dynamic hormonal fluctuations may drive changes in energy balance parameters, with a general propensity toward increased hunger, energy intake and resting metabolic rate (RMR) during the mid-luteal phase versus late follicular phase (Barr et al., 1995; Benton et al., 2020; Brennan et al., 2009; Campolier et al., 2016; Lissner et al., 1988; McCarthy et al., 2024; Pelkman et al., 2001; Tucker et al., 2024). Such phase-related variations may contribute to differences in energy balance.

While some previous research has found that hunger, dietary intake, and RMR tend be highest in the mid-luteal phase of the menstrual cycle, many findings are inconsistent (Benton et al., 2020; Rogan & Black, 2023; Tucker et al., 2024). Several key methodological limitations may explain this variability. For example, many studies oversimplify comparisons by grouping data into broad follicular and luteal phases without accounting for the four distinct hormonal environments that occur across the cycle (Allen et al., 2016; Rogan & Black, 2023). Furthermore, many studies have relied on non-validated methods to identify menstrual cycle phases, which can misclassify phases and substantially impact the interpretation of energy balance in different hormonal states (Allen et al., 2016; Chung et al., 2010; Elliott-Sale et al., 2021). Moreover, much of this research has relied on self-reported dietary intake, which is susceptible to bias and measurement error (Bailey, 2021; Burrows et al., 2019; Poslusna et al., 2009). Contextual factors such as differences in weekday versus weekend intake are rarely considered or controlled for, despite their established influence on dietary intake (Li et al., 1999; Rogan & Black, 2023; Yang et al., 2014). Few studies have measured dietary intake together with energy expenditure, limiting the interpretation and generalizability of the findings within the broader framework of energy balance.

This study aimed to address these knowledge gaps by comparing appetite sensations, objectively-measured dietary intake, and RMR between late-follicular versus mid-luteal phases of the menstrual cycle in healthy, normal-weight premenopausal females. Our primary hypothesis was that dietary intake would be lower in the late-follicular phase when compared to the mid-luteal phase. We also hypothesized that hunger, prospective food consumption, and RMR would be higher, while satiety would be lower, in the mid-luteal phase compared to the late-follicular phase.

2. Methods

2.1. Study design

This investigation consisted of three visits, one for screening and baseline documentation, and two study visits – one in the late-follicular phase and one in the mid-luteal phase of the menstrual cycle. Due to logistical constraints, visit order (i.e., late-follicular, mid-luteal) was not randomized. Participants were provided with a two-day energy-balanced diet that they consumed while living in the community before each study visit. Appetite sensations, objective (weighed) dietary intake, and RMR were measured during each of these study visits as described below. Appetite sensations and objective dietary intake were also measured for 2.5 days following each study visit while participants lived in the community.

2.2. Participants

Participants were recruited from the greater Kelowna community (British Columbia, Canada) from May 2022–September 2024. Healthy, premenopausal females aged 18–35 years with normal body weight $(18.5–24.9~{\rm kg/m}^2)$ were eligible. Other major inclusion criteria included: nulliparity; predictable (regular) normal-length and ovulatory menstrual cycles, as indicated by self-reported menstrual cycle length

between 21 and 38 days for the past three cycles and ovulation indicated via serially tested urinary luteinizing hormone (LH) surge; being sedentary or recreationally active, defined as < 300 min of moderate to vigorous intensity exercise per week; not currently pregnant, lactating, nor planning to become pregnant in the next 12 weeks; and ability to fast for 12 h prior to each study visit. Major exclusion criteria included: presence of a major chronic disease (e.g., cardiovascular diseases, diabetes, cancer, thyroid disease, etc.); use of oral contraceptive pills or any hormone-related medication in the last six months; progestin-releasing intrauterine device; or medications in the last half-year that may affect appetite, energy balance, or sleep. Individuals who used tobacco or nicotine, worked night shifts, had a history of extensive weight loss or weight loss surgery, or had food intolerances or allergies that could not be accommodated were also excluded. Other exclusion criteria included current or past history of eating disorders including anorexia nervosa, bulimia, or binge eating disorder (self-report or score >20 on the Eating Attitudes Test - 26 questionnaire (Garner et al., 1982)), current severe depression or history of severe depression within the previous year (self-reported score >30 on the Beck Depression Inventory (Beck et al., 1996) or alcohol or drug abuse (score >2 on the cut-annoved-guilty-eye-opener [CAGE] questionnaire (Brown & Rounds, 1995)). The project was approved by the University of British Columbia Clinical Research Ethics Board (ID: #H22-00874) and all participants provided signed consent prior to enrollment. This study was registered at clinicaltrials.gov (ID: NCT06327087).

2.3. Screening and baseline visit

Individuals interested in participating first completed a questionnaire to determine eligibility. Afterward, participants who were eligible provided written informed consent and attended a screening and baseline visit. During this visit, participants were able to familiarize themselves with the investigator and physical space in which they would complete the study visits, measure body composition, assess menstrual cycle history, and review instructions for assessing ovulation. Height and weight were measured shoeless in light indoor clothing using a stadiometer and digital scale, respectively. Percent body fat was assessed using dual-energy x-ray absorptiometer (Hologic, Horizon DXA system, Auto Whole-Body Fan Beam, version 13.4.2., Bedford, MA, USA).

Each participant provided their menstrual cycle history using a study-specific form to assess menstrual cycle consistency for the past three months and to predict future ovulation and menstrual cycle phases. At the end of the screening/baseline visit, participants were given LH urine test strips with disposable collection cups to prospectively identify ovulation. Using their previous three menstrual cycles, participants were asked to use the LH tests each day during a 7-day period in which ovulation was likely to occur. A positive ovulation test was used to 'anchor' study visits, with the late-follicular phase study visit scheduled 1–5 days before the next predicted or measured LH surge and the mid-luteal phase study scheduled 6–10 days after the LH surge. A minimum of one cycle with a positive urinary LH test was required prior to completing any study visit. Participants were asked to track their LH throughout the entire duration they were enrolled in this study.

2.4. Experimental sessions

2.4.1. Run-in energy balanced diet

Because dietary intake in previous days may impact appetite and dietary intake on subsequent days (Bray et al., 2008), participants were provided with food to support a run-in energy-balanced diet in free-living settings starting two days before each study visit. The energy content of the run-in diets was calculated using the Dietary Reference Intakes (Institute of Medicine, 2005) with the physical activity coefficient selected based on each participant's self-reported activity level (collected at the screening and baseline visit). The macronutrient

content of the run-in diet was 50 % carbohydrates, 30 % fat, and 20 % protein. During the two days before a planned visit, participants were asked to abstain from consuming any foods not provided to them but were permitted to consume non-caloric beverages (e.g., tea, coffee, diet soda). They were instructed to consume all foods provided. If participants did consume non-provided food or calorie-containing beverages, they were instructed to send detailed information about the food (e.g., the nutrition label, photos, description) via email and return the packaging to the study team. Participants were given the same energy- and macronutrient-adjusted meals and snacks to consume before each study visit. Four meals were prepared by a local third-party company in Kelowna (Meal Prep 4 U), each consisting of a protein (e.g., salmon, sirloin strips, chicken), a carbohydrate (e.g., white rice, brown rice, vermicelli), and a vegetable (e.g., broccoli, asparagus, butternut squash). These meals were supplemented with two breakfast meals (e.g., a combination of peanut butter and jelly bagels, granola with yogurt, toast with butter or jam, and egg bites) and various snacks (e.g., granola bars, apples, bananas, trail mix, hummus, yogurt, juice boxes) for each day, based on participant preferences and estimated energy requirements. All foods and beverages were returned to the study team on the day of the in-lab visit to ensure compliance.

2.4.2. Study visits

Before each study visit, participants were asked to refrain from vigorous exercise for 48 h, to avoid alcohol for 24 h, and avoid calorie and caffeine-containing food and beverages for 12 h. Each study visit was scheduled to begin between 7:30–9:30 a.m., with similar start times (within 1 h) for each study visit.

RMR was measured using an indirect calorimeter with a face mask (Parvomedics TrueOne 2400, Murray, Utah). Gas analyzers and the flow meter were calibrated prior to each test according to manufacturer's instructions. Before each test, participants lay supine in a quiet, thermoneutral (20–24 °C) room for 20–25 min. Respiratory gas exchange was measured for 20–25 min. The last 15 min of data were averaged after inspection and exclusion of data that did not meet quality control targets (i.e., minute-by-minute coefficient of variation <10 % for volume of $\rm O_2$ and $\rm CO_2$ and RMR).

Following RMR testing, participants completed a fasting visual analog scale (VAS). Participants were then given a standardized breakfast meal consisting of 25 % of the individual's total daily estimated energy requirements (Institute of Medicine, 2005) with the same macronutrient ratio as the run-in diet. Each breakfast consisted of a combination of toast, butter, egg quiche, fruit, vogurt and/or juice, depending on participant preference and energy and macronutrient requirements. Foods were selected to closely match nutrient profile across participants to minimize differences in the overall macronutrient profile of meals. Participants were instructed to consume this meal in its entirety with no distractions to avoid influences from external stimuli. Participants were permitted to drink water ad libitum during and after the breakfast meal. Participants then rated sensations of hunger, satiety, and prospective food consumption (PFC; i.e., how much food participants thought they could or would eat) (Flint et al., 2000) using the same sliding visual analog scale, which was covertly scored on a scale of 0-100 on a computer or iPad using Research Electronic Data Capture (REDCap). These ratings were completed 30, 60, 90, 120, 150, and 180 min after the breakfast meal. Between recording of VAS and questionnaires, participants could complete light work on their computer, read, work, or watch television; they were asked to do similar activities at both study visits. Area under the curve was calculated to describe differences in appetite sensations between menstrual cycle phases using the trapezoidal method (Allison et al., 1995). Following the 120-min VAS assessment, participants completed the 15-item Food Cravings Questionnaire - State (Cepeda-Benito et al., 2000). Higher scores indicated they had more intense food cravings at that moment. At the end of the 180-min VAS, participants were provided with an in-laboratory lunch meal in which each food was weighed before and after consumption to

objectively measure *ad libitum* dietary intake. This meal provided approximately 1800 kcal, of which approximately 60 % were from carbohydrates, 30 % were from fat, and 10 % were from protein. Items included spaghetti, meatballs (or non-meat alternative), marinara sauce, a green vegetable with butter (e.g., broccoli) or green leafy vegetables, Italian or ranch dressing, a medium apple, a packet of cookies, regular soda, and diet soda. For this meal, participants were asked to consume as much or as little as they would like until they were comfortably full; they could request more of any item. This meal was consumed in a quiet room with no external distractions.

At the completion of each study visit, participants began their freeliving period, in which they were provided pre-weighed food in excess of their estimated energy requirements for the remainder of the study visit day and for two subsequent days. The provided foods consisted of 4-6 pre-prepared meals (examples can be found in Supplementary Table 6) with known energy and macronutrient content from the local company (Meal Prep 4 U), quick breakfast and alternative meal options (e.g., bread and/or frozen egg quiches, instant macaroni and cheese) and several fresh and packaged foods that could serve as snacks or meal supplements (e.g., fresh and canned fruit, granola bars, chips, assorted candies, oatmeal packets, egg bites, brown and white rice, mac and cheese). Although the content of the diet varied slightly based on participant preferences, the total energy provided was approximately 10,500 kcal (~4200 kcal/day). Similar to the in-laboratory lunch meal, participants were instructed to consume as much or as little of the foods and beverages provided as they wanted, and to exclusively consume foods provided by the laboratory. Participants were provided with the same foods for each free-living ad libitum period. To confirm ad libitum dietary intake, participants were instructed to take before and after photographs by telephone of all food and beverages they consumed. Photos were uploaded directly to REDCap using a personal link, which recorded the time in which foods were consumed. At the end of the 2.5day period, participants were asked to bring back all food and wrappers/ containers so all items could be weighed by the research team. Dietary energy and macronutrient intake were assessed using Food Processor Nutrition Analysis Software (version 11.1; ESHA Research, Salem, OR), with entries confirmed by a research assistant. Daily free-living energy intake was expressed as kcal/day and adjusted for RMR (energy intake/ RMR, kcal/day) to better contextualize intake within the broader framework of energy balance, including potential menstrual cycle-related changes in energy expenditure. To explore free-living appetite, participants also provided VAS of hunger, satiety, and PFC in REDCap before and after each meal or snack; these data were averaged across days.

2.5. Statistical analyses

The sample size for this study was determined based on anticipated differences in-laboratory *ad libitum* dietary intake between the latefollicular and mid-luteal phases of the menstrual cycle (primary objective). We anticipated an effect size of .800, consistent with previous research using similar methods that detected differences in energy intake at a single meal between menstrual cycle phases that would be relevant for future nutrition strategies or care (i.e., 141 ± 176 kcal at a single meal (Brennan et al., 2009)). A sample size of n = 14 would provide 80 % power to detect mean differences in energy intake between phases using a two-tailed paired t-test with a significance level (α) of .05.

Statistical analyses were conducted using SPSS (IBM SPSS Statistics, version 29.0.00.0, Chicago, IL, USA). Distribution of the data was assessed using the Shapiro-Wilk test of normality. Linear mixed effect models with unstructured covariance compared outcomes between menstrual cycle phases while maximizing statistical power in the presence of missing data. Models included participants as random effects and menstrual cycle phase (late-follicular or mid-luteal) as a fixed effect; in the case of VAS and free-living appetite and dietary intake data, time (fasting, 30, 60, 90, 120, 150, and 180 min for VAS; days 1, 2, and 3 for

free-living data) and their interaction terms were also included as fixed effects with an unstructured covariance structure. Models with free-living appetite ratings included free-living energy intake as a covariate. To evaluate whether the sequence of visits (i.e., follicular-luteal vs. luteal-follicular) influenced study outcomes, we conducted a sensitivity analysis by including visit order as a covariate in the statistical models for our primary outcomes of interest: in-laboratory energy intake, RMR, mean free-living energy intake, and in-laboratory VAS. In cases where model residuals were not normally distributed, data were log-transformed and re-analyzed, but absolute mean values were presented for ease of translation. Data are presented as mean \pm SD or mean \pm SE unless indicated otherwise.

3. Results

3.1. Participant characteristics

Baseline characteristics of study participants are shown in Table 1. Thirty-one females were enrolled (Fig. 1), although 13 were excluded before the first study visit due to scheduling conflicts (n = 6), exclusionary survey score (n = 3), dietary restrictions (n = 2), and nonresponse (n = 2). Thus, n = 18 were included in this study, and n = 14 had complete data for our primary outcome (in-laboratory *ad libitum* dietary intake) from both study visits. All 18 participants had a positive indication of ovulation test by urine LH surge prior to their first visit (late-follicular or mid-luteal). Visit order was approximately evenly distributed across participants; eight participants had their first visit in the late-follicular phase and ten participants had their first visit in the mid-luteal phase.

3.2. Dietary intake and resting metabolic rate

There were no differences in-laboratory *ad libitum* energy intake (Fig. 2) or macronutrient intake (Supplementary Table 2) between menstrual cycle phases. There were also no main (phase, time) or interaction (phase x time) effects for free-living energy intake (Fig. 3) or macronutrient intake (Supplementary Table 2 and Supplementary Table 4). There were no differences in RMR between phases, although there was a trend towards greater RMR in the luteal phase (104 ± 218 kcal higher in the luteal phase, P = 0.074, Fig. 4 and Supplementary Table 2).

Table 1 Participant characteristics (n = 18).

Characteristic	Mean \pm SD or n (%)
Age (years) ^a	21 [19–24] ^b
Race ^a	
Black	1 (6 %)
White	13 (72 %)
East Asian	2 (11 %)
Middle Eastern	2 (11 %)
BMI (kg/m^2)	21.2 ± 1.5
Percent body fat	25.0 ± 4.5
Percent of students ^a	9 (50 %)
Mean cycle length (days)	30.7 ± 3.0
Length between visits (days)	16 [13–32] ^b
Number of confirmed ovulation tests	
1	5 (28 %)
2	10 (56 %)
3	2 (11 %)
4	1 (6 %)

Data are reported as mean \pm SD or number (%). BMI: body mass index.

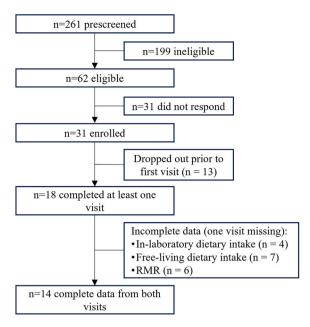


Fig. 1. Flow diagram of the progress of participants. RMR: resting metabolic rate.

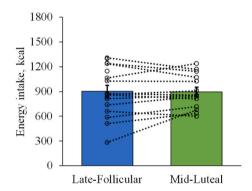


Fig. 2. Mean *ad libitum* energy intake (kcal) at a single meal between the late-follicular and mid-luteal phases of the menstrual cycle. Values are mean \pm SE with individual points representing each participant. N=16 late-follicular, n=14 mid-luteal.

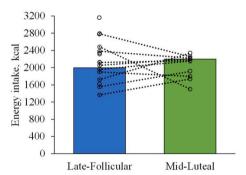


Fig. 3. 2.5-day free-living mean ad libitum energy intake (kcal) between the late-follicular and mid-luteal phases of the menstrual cycle. Values are mean \pm SE with individual points representing each participant. N=10 late-follicular, n=11 mid-luteal.

^a Collected using a study-specific questionnaire.

^b Reported in median [interquartile range].

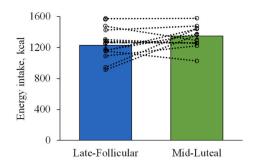


Fig. 4. RMR between the late-follicular and mid-luteal phases of the menstrual cycle. Values are mean \pm SE with individual points representing each participant. N = 13 late-follicular, n = 16 mid-luteal.

3.3. Appetite sensations

VAS ratings of in-laboratory hunger, satiety, and PFC are shown in Fig. 5 and Supplementary Table 1. As expected, there was a main effect

of time on all VAS (all P < 0.05). There were no main effects of menstrual cycle phase nor phase \times time interactions among the VAS, nor were there effects of phase on AUC values of appetite or food cravings score (Supplementary Table 2). There were also no differences in free-living ratings of hunger, satiety, or PFC (Supplementary Table 3). Visit order had no effect on in-laboratory energy intake, RMR, mean free-living energy intake, and in-laboratory VAS (Supplementary Table 5).

4. Discussion

This study adds nuance to the prevailing notion that hunger and energy intake consistently increase during the luteal phase of the menstrual cycle, suggesting that these effects may not be as robust or consistent as previously assumed. Despite common claims in previous research that suggest hormonal fluctuations lead to heightened orexigenic appetite sensations and greater energy intake in the luteal phase (Barr et al., 1995; Brennan et al., 2009; Cheikh Ismail et al., 2009; Gil et al., 2009; Gong et al., 1989; Johnson et al., 1994; Kammoun et al., 2017; Li et al., 1999; Lissner et al., 1988; Martini et al., 1994;

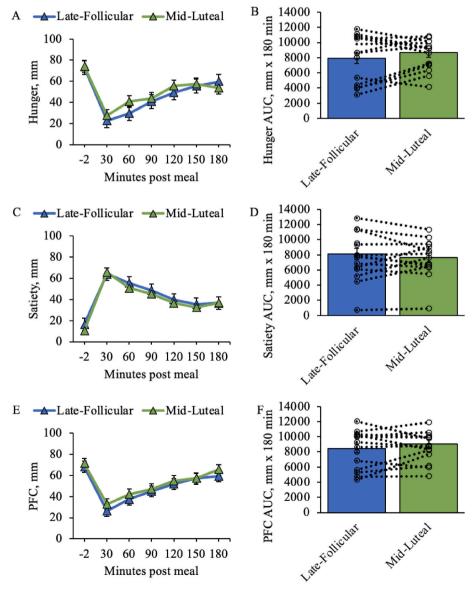


Fig. 5. Time course change of hunger (A), satiety (C), and prospective food consumption (PFC; E) after a standardized meal. Area under the curve (AUC) for hunger (B), satiety (D), and PFC (F) are also included. Values are mean \pm SE with individual points representing each participant. N = 17 late-follicular, n = 17 mid-luteal.

Maury-Sintjago et al., 2022; Pelkman et al., 2001), our findings show no significant cycle phase differences in energy intake nor appetite sensations, whether measured in controlled laboratory conditions or real-world settings. This suggests that the menstrual cycle phase may not exert as pronounced an effect on energy intake as previously suggested, particularly when the luteal phase is indicated by a urinary LH surge and energy intake is measured with high accuracy.

Our findings suggest that differences in dietary intake reported in previous studies may not be solely due to biological differences, but also differences in study design, particularly in the methods used to assess dietary intake and menstrual cycles. A major factor influencing discrepancies across studies is the reliance on self-reported dietary intake, which is prone to underreporting and bias (Burrows et al., 2019) and fluctuations across weekdays and weekends (Yang et al., 2014). Self-reported energy intake has been estimated to be up to 522 kcal/day lower in the follicular phase compared to the luteal phase (Gil et al., 2009). Interestingly, studies using self-reported methods, such as 24-h dietary recalls and food diaries, often report higher energy intake during the luteal phase (Barr et al., 1995; Cheikh Ismail et al., 2009; Elliott et al., 2015; Gil et al., 2009; Johnson et al., 1994; Kammoun et al., 2017; Li et al., 1999; Martini et al., 1994; Maury-Sintjago et al., 2022), while studies utilizing weighed food intake methods report smaller (87-207 kcal/day) or no differences (Campolier et al., 2016; Gong et al., 1989; Lissner et al., 1988). While speculative, it is possible that some of this discrepancy may be due to societal perceptions of dietary intake across the menstrual cycle and media messaging that suggest that it is 'normal' for females to consume more before menstruation because of premenstrual syndrome (PMS) may contribute to self-reported energy intake inaccuracies. It is also possible that physical, emotional, or behavioral symptoms associated with PMS may affect appetite and dietary intake. Some evidence suggests women with PMS experience a larger increase in energy intake in the luteal phase compared to those without PMS (Cross et al., 2001). Others research has found that students with PMS consume more energy overall and have a lower percentage of energy from protein (Mataracı Değirmenci & Erbil, 2024). In contrast, one study reported no differences in energy intake between phases in either PMS or non-PMS groups (Bryant et al., 2006). Whether PMS-related changes in dietary intake stem from shared biological mechanisms (e.g., altered serotonin, gamma-aminobutyric acid (GABA), glutamate, or β-endorphin activity (Cunningham et al., 2009)), behavioral responses (e.g., comfort eating, reduced physical activity), or psychological expectations (e.g., the belief that PMS should affect appetite, similar to perceived impacts on exercise performance (Oester et al., 2024)) remains unclear.

Another important consideration when interpreting our data in the context of the wider literature is menstrual phase classification. Many prior studies have relied primarily on self-reported onset of menses to determine menstrual cycle phase (Brennan et al., 2009; Bryant et al., 2006; Campolier et al., 2016; Cheikh Ismail et al., 2009; Elliott et al., 2015; Gil et al., 2009; Kammoun et al., 2017; Lissner et al., 1988; Maury-Sintjago et al., 2022; Souza et al., 2018), which can be inaccurate due to variability in cycle length, ovulation, and subclinical ovulatory disturbances (Noordhof et al., 2024; Schmalenberger et al., 2021). This is particularly relevant because relying on a fixed 28-day cycle or averaging previous cycles may lead to significant misclassification of menstrual cycle phase by cycle day assessments. Capturing a single day of a participant's cycle may reflect a very different hormonal status than the same cycle day in another participant (Fehring et al., 2006). For example, in a study of 39 healthy premenopausal females, excluding participants with misclassified cycle phases (based on self-report vs. retrospective serum hormone analysis) revealed higher energy and protein intake during the luteal phase (Chung et al., 2010). This study showed that 44 % of cycles could be misclassified when relying on cycle history without more objective techniques, and that including such misclassified cycles may alter conclusions about phase-related differences in energy intake. Studies that objectively confirm menstrual phase through hormonal assessments, as done in this study, are likely to yield

more precise comparisons. Additionally, failure to account for previous day's dietary intake and providing the same breakfast meal to all participants can introduce errors, as prior dietary intake (Bray et al., 2008) and individual differences in energy requirements are key factors influencing subsequent intake (Trumbo et al., 2002).

As assessed by VAS, significant differences in appetite sensations were not observed in either a controlled laboratory or free-living environment. Despite the trends generally aligning with expectations—namely, increased hunger in the luteal phase (Brennan et al., 2009) — our findings are consistent with other research showing no significant difference in appetite VAS between phases (Campolier et al., 2016; McCarthy et al., 2024). However, it is important to consider that VAS may not be sensitive enough to capture the full complexity and depth of appetite regulation (Gibbons et al., 2019), especially considering our study was not powered based on VAS. Furthermore, appetite sensations may not directly relate to self-reported energy intake (Doucet et al., 2003). In free-living conditions, which are inherently challenging to quantify, no phase-specific differences were detected, although the methodology used to assess free-living appetite in this study has not been formally validated.

Previous studies have reported differences in various components of appetite related to food cravings across the menstrual cycle. For example, some evidence suggests that females experience a stronger preference for high-fat and high-sugar foods during the late-luteal phase (McVay et al., 2012) and a greater desire for foods rich in sugar, salt, and fat during the premenstrual period (Souza et al., 2018). Increased explicit wanting for high-fat foods (McNeil et al., 2013), higher consumption of sweet foods (Bowen & Grunberg, 1990), and greater intake of sugar-containing beverages (Fong & Kretsch, 1993) have also been reported in the luteal phase, potentially driven by hormonal fluctuations, such as elevated progesterone level-related increased metabolic rate (Krishnan et al., 2016). Despite these findings, the current study did not observe significant differences in food cravings between phases, although the trends were in the expected direction. This may be due to the limitations regarding the statistical power of our analyses and interpretation of the Food Craving Questionnaire - State, which only partially measures aspects of food cravings, particularly those linked to hedonic or emotional responses, and does not comprehensively account for all dimensions of appetite. Additionally, the insufficient statistical power for this specific portion of the study may have limited the ability to detect significant differences.

We observed a non-significant increase in RMR during the luteal phase that was approximately 100 kcal/day higher than in the follicular phase. Previous studies have reported mixed findings, showing differences ranging from no effect to approximately 50-100 kcal/day greater in the luteal phase (Benton et al., 2020; Elliott et al., 2015; Lariviere et al., 1994). If a significant increase in RMR were to exist, it may be attributed to the thermogenic effects of progesterone, which is greatest in the mid-luteal phase (Baker et al., 2020). For context, free-living energy intake in the current study was approximately 200 kcal/day higher in the luteal phase (although not significant, Supplementary Table 2), exceeding the observed increase in RMR. Persistent discrepancies in energy balance over prolonged periods could potentially lead to weight gain, though it remains unclear whether fluctuations in endogenous sex hormones directly influence long-term weight regulation, or if the increased energy balance observed in the luteal phase is counterbalanced by compensatory mechanisms during the follicular phase. Notably, free-living energy intake expressed as a ratio to RMR was not different between phases in our study.

Strengths of this study include the use of urinary LH peak and cycle day to identify menstrual cycle phases and assess dietary intake in both controlled laboratory and free-living settings, alongside measurements of appetite and RMR—representing a novel approach with rigorous energy balance methodology. However, some limitations should be noted. The study was powered only to detect differences within inlaboratory energy intake, although many previous studies have used

comparable or smaller sample sizes. Although including visit order as a covariate in analyses of several outcomes did not impact our results, we cannot exclude the possibility that order effects may have been present, given randomization or counterbalancing of the cycle phase order for visits was not performed. Future research may also consider randomizing the order of menstrual cycle phase assessments to reduce potential order effects. Furthermore, serum hormone measurements of estrogen and progesterone (which are gold standard for identifying phases in research) (Allen et al., 2016; Elliott-Sale et al., 2021) were not used to verify menstrual phase in this study. Additionally, hormonal markers of appetite, that may vary across menstrual phases, were not measured. During the 2.5-day period, participants were provided with meals and snacks exceeding their caloric needs to minimize boredom, but some evidence suggests that providing large portions of food may lead to spontaneous overeating (Kral & Rolls, 2004). This study did not assess PMS, limiting the ability to explore its potential role in appetite and/or energy intake across the menstrual cycle. Moreover, the study focused solely on healthy, young females with a normal BMI, limiting its generalizability to other populations. Future research may also consider randomizing the order of menstrual cycle phase assessments to reduce potential order effects.

In conclusion, this study provides evidence that the influence of menstrual cycle phase on appetite and energy intake may be more nuanced and variable than commonly assumed. Despite some general tendencies aligning with previous research—such as increased hunger, energy intake, and RMR during the luteal phase—no significant differences were observed in either laboratory or free-living settings. Although these findings should be interpreted in light of the study's limitations, they underscore the importance of accurate cycle phase identification and dietary intake assessment. These results highlight the need for further research to explore the magnitude and consistency of hormonal influences on appetite regulation.

CRediT authorship contribution statement

Miranda Smith: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. Maryam Aghayan: Writing – review & editing, Data curation. Jonathan Little: Writing – review & editing, Methodology, Conceptualization. Jerilynn C. Prior: Writing – review & editing, Methodology, Conceptualization. Tamara R. Cohen: Writing – review & editing, Methodology. Zoë Soon: Writing – review & editing, Methodology. Hephzibah Bomide: Writing – review & editing, Formal analysis. Sarah Purcell: Writing – review & editing, Data curation, Conceptualization.

Ethical statement

This study was performed in accordance with the Declaration of Helsinki. The project was approved by the University of British Columbia Clinical Research Ethics Board (ID: #H22-00874) and all participants provided signed consent prior to enrollment. Approval was initially obtained June 1st, 2022. This study was registered at clinicalt rials.gov (ID: NCT06327087).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2025.108314.

Data availability

Data will be made available on request.

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