<u>No changes in energy intake, resting and physical activity energy expenditure, or food</u> <u>reinforcement across the menstrual cycle</u>

By: Jessica McNeil, Jamesason D. Cameron, and Éric Doucet

McNeil J, Cameron JD, Doucet E. No changes in energy intake, resting and physical activity energy expenditure, or food reinforcement across the menstrual cycle. In: Gosselin M (Ed), Menstrual cycle: signs and symptoms, psychological/behavioral changes abnormalities, Chapter 5. Nova Science Publishers, New York, USA, 2013; 121-138.

\*\*\*© 2013 Nova Science Publishers, Inc. Reprinted with permission. No further reproduction is authorized without written permission from Nova Science Publishers. This version of the document is not the version of record. Figures and/or pictures may be missing from this format of the document. \*\*\*

# Abstract:

Background: Energy intake (EI) and physical activity energy expenditure (PAEE) have been previously evaluated across the menstrual cycle with food and physical activity journals. To our knowledge, the direct assessments of EI, macronutrient intake, resting energy expenditureEE (REE) and PAEE have not been studied across the menstrual cycle within the same study design. Furthermore, no study has related these factors to possible variations in the severity of the premenstrual syndrome (PMS) and food reinforcement across the cycle. Methods: Seventeen women (Body mass index: 22.3±1.6 kg/m<sup>2</sup>; Body fat-DXA: 28.5±6.8%) participated in three identical sessions during distinct phases of the menstrual cycle: Early follicular, Late follicular/ovulation and Mid-luteal (confirmed by basal temperature and plasma gonadotropins, estradiol and progesterone levels). EI was measured inside the laboratory and under free-living conditions with food menus and food journals, respectively. REE and PAEE were measured with indirect calorimetry and accelerometers, respectively. Also measured were body fat mass (DXA), the severity of PMS, leptin and the relative- reinforcing value (RRV) of preferred foods. Results: No differences in body fat mass, REE, PAEE and leptin were noted across the menstrual cycle. Furthermore, no changes in measured and reported energy, carbohydrate, lipid and protein intakes, as well as the RRV of preferred foods were noted across the cycle. Differences in the severity of PMS (25±10, 19±11, 25±10 points; p<0.05) across phases were noted. However, the severity of PMS and food reinforcement did not coincide with energy and macronutrient intakes. Conclusions: Taken together, these results suggest that the menstrual cycle may not be of practical concern when assessing food intake and physical activity patterns under the methodological conditions presented in this study.

**Keywords:** menstrual cycle | energy intake | resting energy expenditure | physical activity energy expenditure | food reinforcement | premenstrual syndrome

# **Book chapter:**

# Introduction

It has been previously observed that energy intake (EI) decreases during the late follicular and ovulation phases of the menstrual cycle, which are characterized by higher levels of estradiol, while EI tends to increase during the luteal phase, at which time levels of both estradiol and progesterone are elevated [1]. A large variation can however be observed when comparing the caloric intake values previously reported, with increases in EI ranging from  $\approx$ 364-2092 kilojoules (kj) during the luteal phase in comparison to the follicular phase. As for macronutrient intake across the menstrual cycle, most studies [2-7] noted similar results: increases in absolute intake of all macronutrients during the luteal phase.

As for resting energy expenditure (REE), a mean increase in basal metabolic rate of 15% following ovulation has been previously noted [8]. However, not all participants had demonstrated an increase in basal metabolic rate following ovulation [8].

As for physical activity energy expenditure (PAEE), no variation has been noted across the menstrual cycle with physical activity journals [2]. Lastly, no significant changes in body weight or body fat percentage across the cycle have been previously noted in lean women [2; 3; 9].

Certain secondary factors may in part explain the possible variations in EI, REE and PAEE across the menstrual cycle. Among those are the occurrence and severity of the premenstrual syndrome (PMS). Approximately 50% of women suffer from a minimal level of distress related to PMS, from which 31%, 14% and 8% reported low, moderate and severe levels of distress, respectively [10]. Women who also reported more severe PMS symptoms consumed on average more calories, and had more frequent episodes of overeating and cravings for sweet-fatty foods during the late luteal phase [11; 12]. Additionally, the relative-reinforcing value (RRV) of food, a specific aspect of appetitive motivation which can be generally portrayed as the amount of work/effort one individual is willing to do in order to obtain a certain type of food [13], has not been previously evaluated across the menstrual cycle. The RRV of food is an objective measure that may in part explain the possible variations in energy and snack intake across the cycle, especially at times during which women may be more prone to episodes of overeating [14]. Finally, variations in leptin levels, a hormone that is secreted by adipocytes and circulates in the plasma at concentrations relative to fat mass [15], have been noted across the menstrual cycle; with values  $\approx$ 35-60% higher during the early to mid-luteal phase in comparison to the early follicular phase [9; 16-19].

The objective of this study was to measure EI, macronutrient intake, REE and PAEE across the menstrual cycle within the same study design. In an effort to increase the accuracy of menstrual cycle phase determination, plasma levels of LH, FSH, estradiol and progesterone, as well as basal temperature were assessed at the start of every experimental session. We hypothesized that energy, carbohydrate, lipid and preferred snack intakes would be highest during the mid-luteal phase; coinciding with more severe PMS symptoms and greater reinforcement for snack foods. We also hypothesized that leptin levels would be higher during the mid-luteal phase. Lastly, we hypothesized that no changes in body weight, body fat percentage, REE and PAEE would occur across the cycle.

### **Materials and Methods**

## Participants

A total of 18 women completed this study. However, one participant was excluded from the analysis due to her measured LH, estradiol and progesterone levels being well below the normal range [20], suggesting that an anovulatory cycle may have occurred. And so, the results of 17 participants are presented herein. Participants had to be between the ages of 18-40 years, non-smokers, weight stable ( $\pm 2$  kg), have a 24-34 day menstrual cycle, not taking prescribed medications, and not taking hormonal contraceptives (e.g. pill, patch, injection, intra-uterine device) within the past six months. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all the procedures involving human participants were approved by the University of Ottawa ethics committee. Written informed consent was also obtained from all participants.

## **Design and Procedure**

A preliminary session was held to determine whether participants corresponded to the inclusion criteria. Following this, three identical experimental sessions were conducted during the early follicular (days 1-5 inclusively), late follicular/ovulation (days 11-14 inclusively) and mid-luteal (days 21-26 inclusively) phases.

These days were based on a 28-day cycle and each participant was asked to count the number of days (length) of her menstrual cycle for at least one month prior to testing; permitting us to tailor the times of testing for each participant according to the length of her cycle. Plasma LH, FSH, estradiol and progesterone levels, as well as basal temperature, aided in confirming each menstrual cycle phase. Lastly, the participants arrived at the laboratory at 8h00 following a 12-hour overnight fast prior to the start of each experimental session.

Participants had also been instructed to not consume any alcohol or engage in any type of structured physical activity (e.g. playing sports or training) for at least 24 hours prior to the start of each session. Anthropometric measurements were performed during the preliminary and each experimental session, while all other measurements described below were performed during the three experimental sessions.

### Anthropometric Measurements

Standing height was measured, without shoes, to the nearest centimeter using a Tanita HR-100 height rod (Tanita Corporation of America, Inc, Arlington Heights, IL). Body weight and body composition were measured using a standard beam scale (HR-100; BWB-800AS, Tanita Corporation, Arlington Heights, IL., USA) and DXA scanner (Lunar Prodigy, General Electric, Madison, WI, USA), respectively. During each experimental session, anthropometric measurements were taken at 9h00. The coefficient of variation and correlation for body fat percentage measured by DXA scanner in 12 healthy participants were 1.8% and r = 0.99, respectively.

### Blood Sample

A single blood sample was drawn from the antecubital vein of the nondominant arm between 9h00 and 9h30 to determine the plasma levels of estradiol, progesterone, FSH, LH and leptin. Each blood sample was placed into a tube containing ethylenediaminetetraacetic acid (EDTA) and was centrifuged at 3500 rpm at 40C immediately after the blood was drawn and stored at -800C until assayed. LH and FSH levels were assayed with a two step "sandwich" chemiluminescent assay (CIA) using the Beckman Coulter Dxl Unicel 800 (Beckman Coulter Canada Incorporated, Mississauga, Ontario, Canada).

Progesterone levels were assayed by means of an electro-chemiluminescent immunoassay (ECLIA) system, Elecsys 2010 disk system (Roche Diagnostics, Indianapolis, Indiana, USA). As for estradiol analyses, a carbonyl metallo immunoassay (CMIA) procedure was employed with an Architect estradiol reagent kit (Abbott Laboratories, Abbott Park, Illinois, USA). 100 test reagent packs were used to analyze gonadotropins, estradiol and progesterone levels. Leptin levels were assayed with a dual range enzyme-linked immunosorbent assay (ELISA) human leptin kit (Millipore Corporation, Billerica, Massachusetts, USA). Leptin concentrations were determined as the average of duplicate determinations and the duplicate coefficient of variation was 7.4%.

#### **Temperature Measurements**

Basal temperature was measured orally with a digital thermometer (rapid digital thermometer, BD, Franklin Lakes, NJ, USA) between 9h00 and 9h30.

#### Energy Intake

EI and the macronutrient composition of foods during each laboratory session were evaluated with a validated food menu [21]. This food menu contains 62 items, which includes breakfast items, snacks, hot meals, caloric beverages and water. The participants were handed a copy of the food menu on six different occasions throughout the day (9h30, 10h30, 12h30, 14h30, 15h30 and 16h30), at which time they were able to choose the type of foods and beverages from the menu that they may want to consume. The chosen foods and beverages were then prepared and served to the participants in a sufficient amount (two portions of each item). The prepared foods were weighed in grams before serving, using an electronic scale (Scout Pro SP2001, Ohaus Corporation, Pine Brook, N.J.), and after the participants were done eating. The macronutrient composition of foods consumed were analyzed with nutritional labels and the Food Processor SQL software (version 9.6.2; ESHA Research, Salem, OR). At the end of each experimental session, a weighed food journal [22] was given to the participants to record food and beverage intakes outside of the laboratory for three consecutive days following each session. Participants were asked to report, as accurately as possible, the foods and beverages consumed (i.e. brand name and type of food/recipe), the location and time at which they were consumed, and the quantities for each, estimated using standard household items (e.g. cups, tablespoons). Finally, participants were asked to provide, if possible, recipes and/or dietary information for all items consumed.

Pleasantness Ratings of Foods and Beverages Consumed

Participants were asked to draw a vertical line on a 150 millimeter visual analogue scale (VAS) [23], reflecting their appreciation for all foods consumed during each experimental session. The question asked on each VAS was: "How pleasant is the taste of this food?" The pleasantness rating of each item was performed in order to determine whether participants enjoyed/liked the foods and beverages consumed, and evaluate whether this may alter with menstrual cycle phase.

# Energy Expenditure

REE was measured using indirect calorimetry (Vmax encore 29N, Viasys respiratory care Incorporated, Palm Springs, California, USA) at 8h30, for 30 minutes. The participants rested in a supine position for 30 minutes prior to the start of REE measurement. The coefficient of variation and correlation for the REE measured by the Vmax encore 29N system in 12 healthy participants were 5.1% and r = 0.94, respectively. As for PAEE, this was estimated with a small, water resistant, omnidirectional accelerometer (Actical Accelerometer, Bio-Lynx Scientific Equipment, Montreal, Quebec, Canada) for seven consecutive days following each session. Participants were asked to wear the accelerometer at all times (including in water), from the time they wake until the time they went to sleep. The accelerometer was worn on the right hip (anterior to the iliac crest), and secured with an elastic belt with the arrow pointing up, because that placement, when evaluated along with lower leg or foot, upper leg, head and trunk, lower arm or hand, and upper arm placements, was the best predictor of energy expenditure (r = 0.92 - 0.97) [24]. The accelerometers used in this study were previously validated with doubly labelled water measurements [25].

## Shortened Premenstrual Assessment Form

The occurrence and severity of PMS was evaluated with the shortened premenstrual assessment form [26] at 9h30. This questionnaire is used to classify the subjective changes in certain mood and physical symptoms (i.e. affect, water retention and pain), based on a six-point visual analogue scale (1 = no change and 6 = extreme change), seen or felt by the participants at the time of measurement using a ten category (symptom) chart.

# Relative-Reinforcing Value of Food

The RRV of a preferred snack food versus a preferred vegetable or fruit was measured with the Behavioral Choice Task [13] using progressive ratios for responding, as previously described [27]. A small sample of each participant's favorite snack and favorite fruit/vegetable was presented to them prior to the test at 11h30. and tThey were then asked to consume both samples. Following this, the participants earned points by working for the two personalized food items of choice. The participants then received a specific amount of each food item at 13h00 based on their point distribution during the test; a ratio of 1 slice/piece ( $\approx$  4-5 grams) of the preferred food was given for each point earned towards that food reinforcer. Preferred snack and vegetable/fruit intakes were also measured.

### Statistical Analyses

Statistical analyses were performed using SPSS software (version 17.0; SPSS Inc, Chicago, IL). A two-way repeated measures ANOVA was used to determine the main effects of menstrual cycle phase (early follicular, late follicular/ovulation and mid-luteal) on the components of dietary intake (total amount of energy (kJ), protein (kJ), carbohydrate (kJ) and lipid (kJ)) for the in-laboratory sessions and the three-day weighed food journals, hormone levels (FSH, LH, estradiol, progesterone and leptin), REE, PAEE, reported PMS, as well as the RRV of snack and fruit/vegetable and the associated consumption of each preferred food. ANOVA Bonferroni tests were used to evaluate where significant differences existed. Bivariate correlations were used to determine the strength of the relationship between measured and reported energy and macronutrient intakes with reported PMS and the RRV of food points, button presses and the consumption of preferred snacks and fruits/vegetables. Values are presented as means  $\pm$  standard deviations. Differences with *p*-values < 0.05 were considered statistically significant.

## Results

## Characteristics of the Participants

The characteristics of the participants are shown in Table 1. The average age, height and cycle length of the participants were  $22.4\pm3.2$  years,  $164.2\pm0.06$  centimeters and  $28.1\pm3.8$  days, respectively. There were no significant differences in body weight, body mass index, body fat percentage and fat mass across the menstrual cycle. However, a significant difference was noted in fat-free mass across the menstrual cycle, where fat-free mass was higher during the early follicular phase in comparison to the late follicular/ovulation phase (p<0.05).

Table 1. Characteristics of the participants measured during each menstrual cycle phase for one
complete menstrual cycle

	Early Follicular		Late Follicular/Ovulation		Mid-Luteal		Phase	
	Mean	SD	Mean	SD	Mean	SD	(p-value)	
Body weight (kg)	60.5	7.5	60.3	7.4	60.4	7.4	NS	
BMI (kg/m <sup>2</sup> )	22.4	1.8	22.3	1.6	22.3	1.6	NS	
Fat mass (%)	28.0	6.7	28.3	6.9	28.2	6.9	NS	
Fat mass (kg)	17.1	5.9	17.3	6.1	17.3	6.1	NS	
Fat-free mass (kg)	42.9	3.2	42.5	3.3	42.7	3.2	0.019	

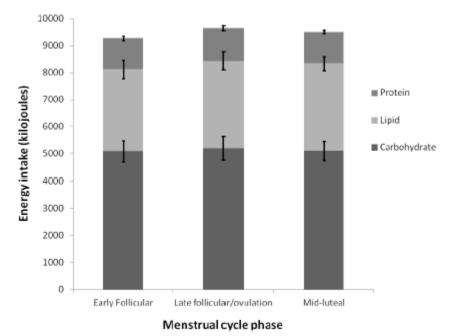
Note: BMI, body mass index; SD, standard deviation; kg, kilogram; m, meter.

### Hormone Levels and Basal Temperature

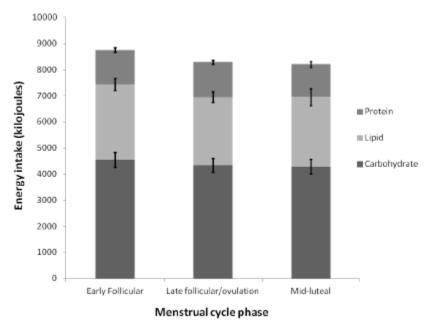
No significant difference was noted in basal temperature across the menstrual cycle ( $36.1\pm0.4^{\circ}C$ ,  $36.2\pm0.4^{\circ}C$ ,  $36.2\pm0.4^{\circ}C$ ; p=NS). However, significant differences were noted for FSH ( $5\pm2$ ,  $6\pm2$ ,  $4\pm2$  IU/L; p<0.0001), LH ( $4\pm3$ ,  $9\pm7$ ,  $6\pm6$  IU/L; p<0.05), estradiol ( $93\pm74$ ,  $284\pm294$ ,  $445\pm154$  pmol/L; p<0.005) and progesterone ( $2.7\pm1.0$ ,  $2.4\pm1.0$ ,  $37.6\pm25.2$  nmol/L; p<0.0001) levels.

As expected, FSH and LH levels were higher during the late follicular/ovulation phase, in comparison to the mid-luteal (p<0.0001) and early follicular (p<0.05) phases. Estradiol levels were higher during the midluteal phase than the early follicular phase (p<0.0001).

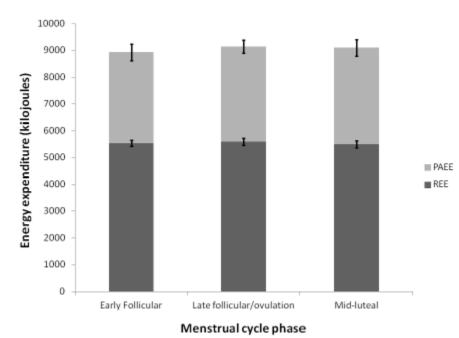
Progesterone levels were also highest during the mid-luteal phase, when compared to the early follicular (p<0.0001) and late follicular/ovulation (p<0.0001) phases. No significant differences in leptin concentrations ( $12.2\pm10.1$ ,  $11.3\pm11.1$ ,  $11.5\pm11.0$  ng/ml; p=NS) across the menstrual cycle were noted.



**Figure 1.** Measured (In-lab from 9h30 to 17h30) energy and macronutrient intakes across the menstrual cycle. Values are presented as means for 17 women with standard errors of the mean represented by vertical bars.



**Figure 2.** Reported (three-day weighed food journal) energy and macronutrient intakes across the menstrual cycle. Values are presented as means for 17 women with standard errors of the mean represented by vertical bars.



**Figure 3.** Resting energy expenditure (REE) (measured for 30 minutes) and daily physical activity energy expenditure (PAEE) (measured for seven days) across the menstrual cycle. Values are presented as means for 17 women with standard errors of the mean represented by vertical bars.

EI, Macronutrient Intake, REE, PAEE and Pleasantness Ratings

As shown in Figure 1, no significant differences were noted for measured (in-laboratory) energy, carbohydrate, lipid and protein intakes across the menstrual cycle. No significant differences in total reported (three-day weighed food journal) energy, carbohydrate, lipid and protein intakes were also noted across the cycle (Figure 2). Additionally, Figure 3 demonstrates that there were no significant differences in REE and PAEE across the menstrual cycle. Lastly, no significant difference in the pleasantness ratings of foods and beverages consumed were noted between menstrual cycle phase ( $122\pm17$ ;  $124\pm14$ ,  $120\pm16$  mm; p=NS).

The Occurrence and Severity of PMS and the RRV of Food

A significant difference in the severity of PMS symptoms was noted across the menstrual cycle  $(25\pm10, 19\pm11, 25\pm10 \text{ points}; p<0.05)$ . More specifically, a significant difference in the severity of PMS was noted between the late follicular/ovulation and mid-luteal phases (p<0.05).

However, no correlations were noted between reported and measured energy and macronutrient intakes with the severity of PMS. As for food reinforcement, no significant differences were found in snack points, fruit/vegetable points, snack button presses, fruit/vegetable button presses and percentage of snack points earned across the menstrual cycle (Table 2)

No correlations were found between reported and measured energy and macronutrient intakes with all variables of RRV of food and preferred food intakes. A trend was noted for preferred

snack intake across the menstrual cycle (Table 2). However, no significant difference was noted in preferred fruit/vegetable intake across the cycle. Positive correlations were noted between preferred fruit intake and fruit points during the early follicular (r=0.510, p<0.05), late follicular/ovulation (r=0.647, p<0.01) and mid-luteal (r=0.730, p<0.01) phases, as well as between preferred fruit intake and fruit button presses during the late follicular/ovulation (r=0.620, p<0.01) and midluteal (r=0.688, p<0.01) phases. Positive correlations were also noted between preferred snack intake and snack points (r=0.510, p<0.05), as well as snack button presses (r=0.534, p<0.05), but only during the early follicular phase. Lastly, no significant correlations were noted between all variables of RRV of food and the severity of PMS for all tested phases of the menstrual cycle (results not shown).

	Early Follicular		Late Follicular/Ovulation		Mid-Luteal		Phase
	Mean	SD	Mean	SD	Mean	SD	(p-value)
Snack points	8	5	9	5	10	5	NS
Fruit/vegetable points	12	5	11	5	10	5	NS
Snack button presses	89	68	111	74	126	61	NS
Fruit/vegetable button presses	77	31	67	34	60	28	NS
% snack presses	39.4	26.7	46.2	27.2	52.4	22.6	NS
Snack intake (kJ)	431	515	414	347	699	598	0.06
Fruit/vegetable intake (kJ)	247	213	218	121	243	163	NS

**Table 2.** Relative-reinforcing value of food computer task results and preferred snack and fruit/vegetable intakes measured during each menstrual cycle phase for one complete menstrual cycle

Note: SD, standard deviation; kJ, kilojoule.

#### Discussion

Our results show no differences in measured and reported energy, carbohydrate, lipid and protein intakes across the menstrual cycle, while only a trend was observed in preferred snack intake across the cycle. Additionally, the severity of PMS and food reinforcement were not related to energy and macronutrient intakes. As for preferred snack intake, this was only positively correlated with snack points and snack button presses during the early follicular phase. Thus, our results do not support our initial hypothesis. Leptin did not increase during the mid-luteal phase, which also rejects one of our hypotheses. Lastly, no changes in body weight, body fat percentage, REE and PAEE were noted across the menstrual cycle, thereby accepting our final hypothesis.

No significant variations were noted in basal temperature across the menstrual cycle. However, greater levels of FSH and LH during the late follicular/ovulation phase, as well as higher levels of progesterone during the mid-luteal phase are suggestive that ovulation did occur.

As for anthropometric measurements, no variations in body weight, body mass index, body fat percentage and fat mass were seen across the menstrual cycle, which is in accordance with existing literature [2; 3; 9]. However, a significant difference in fat-free mass was noted between the early follicular and late follicular/ovulation phases. This difference may be due to increases in water retention during the early follicular phase [28], since drops in progesterone levels prior to the start of menses have shown to increase water and salt retention [29] at this time [29].

No significant differences were noted in energy and macronutrient intakes across the menstrual cycle when directly assessed inside the laboratory, as well as reported with three-day weighed food journals. Many studies which have previously evaluated variations in energy and macronutrient intakes across the menstrual cycle have noted significantly higher EI [2-7; 30-32], lipid [2; 3; 5; 7] and carbohydrate [3; 6] intakes during the luteal phase. However, the use of different methodologies and testing times and/or frequencies to assess EI may explain the divergence in the results obtained by these studies. A few studies [3; 6; 7] which noted higher variations in EI have employed dietary recall methods or food journals, and have measured this variable on two occasions (follicular and luteal phases). On the other hand, studies which have directly measured EI inside the laboratory for more than one meal found smaller variations in the latter across the cycle [32; 33]. For instance, Lissner et al. [32] noted an increase of 364 kJ in the luteal phase when compared to the follicular phase. Fong and Kretsch [33] also directly measured ad libitum energy and macronutrient intakes inside the laboratory during four phases of the menstrual cycle (menses, follicular, ovulation and luteal) in nine lean women. They noted a trend in carbohydrate intake but no significant difference in EI across the menstrual cycle, which is in accordance with our results. The pleasantness ratings of foods consumed were relatively high (81, 83 and 80% rating on VAS) and showed no significant variations across the menstrual cycle, suggesting that the foods consumed inside the laboratory were overall well appreciated during each session. As for measurements of REE, no significant differences were noted across the menstrual cycle. The highest REE values in this study were noted during the late follicular/ovulation phase, but this was not significant. As for daily PAEE, this study extends results from a previous study that measured PAEE with questionnaires [2], by providing objective measures of PAEE with accelerometers.

Even though many studies [9; 16-19] have noted significant variations in leptin levels across the menstrual cycle, other studies [34-36] have noted no variation in this hormone across the cycle, which is in agreement with our results. The discrepancy between these studies may be in part related to the frequency at which measurements of leptin were taken. The studies which measured leptin levels on four or more occasions across the cycle reported significant variations of this hormone [9; 16-19]. Some of these studies [9; 16-18] even measured leptin from blood samples taken every two-three days for one entire menstrual cycle. On the other hand, the present study and others [34-36] which noted no variation in leptin only assayed blood samples for leptin on three occasions across the cycle, thus suggesting that frequent measurements of leptin may be needed to pick-up significant variations in this hormone across the menstrual cycle.

Reported PMS symptoms were less severe during the late follicular/ovulation phase, which is in accordance with other studies [11; 12; 37]. Despite reporting more severe PMS symptoms during the mid-luteal phase, EI was not higher during this phase. Along those lines, Bryant *et al.* [38] found no significant differences in EI during the follicular and luteal phases in women who reported suffering from PMS, when compared to women who reported not suffering from PMS. Additionally, although not significant, the women who reported suffering from PMS consumed more calories during the follicular phase, which is similar to the findings of the current study even though PMS symptoms were not greater at this time.

The present study is one of the first to measure the RRV of preferred foods across the menstrual cycle and relate these results to energy and macronutrient intakes. No significant differences were noted in snack and fruit/vegetable points, snack and fruit/vegetable button presses, percentage of snack points earned and fruit/vegetable intake, while only a trend was noted in preferred snack intake across the cycle. These results thus suggest that preferred foods varying in energy density may not necessarily be more reinforcing or sought after during certain phases of the menstrual cycle.

There are limitations to this study. The present findings are limited to a small sample, where the results of only 17 women were analysed. Direct measurements of energy and macronutrient intakes were only taken for 1 day (8h00-17h30) inside the laboratory for each phase. Evening snacking was also not measured on these days, meaning that we are unable to draw conclusions based on the direct assessment of 24-hour EI. Further measurements of appetite, including hunger and satiety, with VAS were not performed, limiting our interpretation of potential variations in appetite across the cycle.

In conclusion, no differences were noted in EI, macronutrient intakes, REE and PAEE across the menstrual cycle. The measurement of these factors within the same individuals provides a better idea of the non-significant variations in EI, REE and PAEE which occur across the menstrual cycle. Taken together, this suggests that the menstrual cycle may not be of practical concern when assessing food intake and physical activity patterns under the methodological conditions presented in this study.

## Acknowledgments

The authors would like to thank the participants for their devoted participation. É. Doucet is a recipient of a CIHR/Merck-Frosst New Investigator Award, CFI/OIT New Opportunities Award and of an Early Research Award. The authors declare no conflict of interest.

# References

- 1. Dye L and Blundell JE (1997) Menstrual cycle and appetite control: implications for weight regulation. *Hum. Reprod.* 12, 1142-1151.
- 2. Johnson WG, Corrigan SA, Lemmon CR, Bergeron KB and Crusco AH (1994) Energy regulation over the menstrual cycle. *Physiol. Behav.* 56, 523-527.
- 3. Li ET, Tsang LB and Lui SS (1999) Menstrual cycle and voluntary food intake in young Chinese women. *Appetite* 33, 109-118.
- 4. Lyons PM, Truswell AS, Mira M, Vizzard J and Abraham SF (1989) Reduction of food intake in the ovulatory phase of the menstrual cycle. *Am. J. Clin. Nutr.* 49, 1164-1168.
- 5. Tarasuk V and Beaton GH (1991) Menstrual-cycle patterns in energy and macronutrient intake. *Am. J. Clin. Nutr.* 53, 442-447.
- 6. Dalvit-McPhillips SP (1983) The effect of the human menstrual cycle on nutrient intake. *Physiol. Behav.* 31, 209-212.

- 7. Martini MC, Lampe JW, Slavin JL and Kurzer MS (1994) Effect of the menstrual cycle on energy and nutrient intake. *Am. J. Clin. Nutr.* 60, 895-899.
- 8. Solomon SJ, Kurzer MS and Calloway DH (1982) Menstrual cycle and basal metabolic rate in women. *Am. J. Clin. Nutr.* 36, 611-616.
- 9. Riad-Gabriel MG, Jinagouda SD, Sharma A, Boyadjian R and Saad MF (1998) Changes in plasma leptin during the menstrual cycle. *Eur. J. Endocrinol.* 139, 528-531.
- 10. Angst J, Sellaro R, Merikangas KR and Endicott J (2001) The epidemiology of perimenstrual psychological symptoms. *Acta Psychiatr Scand* 104, 110-116.
- 11. Both-Orthman B, Rubinow DR, Hoban MC, Malley J and Grover GN (1988) Menstrual cycle phase-related changes in appetite in patients with premenstrual syndrome and in control subjects. *Am. J. Psychiatry* 145, 628-631.
- 12. Dye L, Warner P and Bancroft J (1995) Food craving during the menstrual cycle and its relationship to stress, happiness of relationship and depression; a preliminary enquiry. J. *Affect Disord.* 34, 157-164.
- 13. Lappalainen R and Epstein LH (1990) A behavioral economics analysis of food choice in humans. *Appetite* 14, 81-93.
- 14. Frank TC, Kim GL, Krzemien A and Van Vugt DA (2010) Effect of menstrual cycle phase on corticolimbic brain activation by visual food cues. *Brain Res.* 1363, 81-92.
- 15. Murphy KG and Bloom SR (2004) Gut hormones in the control of appetite. *Exp. Physiol.* 89, 507-516.
- 16. Al-Harithy RN, Al-Doghaither H and Abualnaja K (2006) Correlation of leptin and sex hormones with endocrine changes in healthy Saudi women of different body weights. *Ann. Saudi Med.* 26, 110-115.
- 17. Hardie L, Trayhurn P, Abramovich D and Fowler P (1997) Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. *Clin. Endocrinol. (Oxf)* 47, 101-106.
- Mannucci E, Ognibene A, Becorpi A, Cremasco F, Pellegrini S, Ottanelli S, Rizzello SM, Massi G, Messeri G and Rotella CM (1998) Relationship between leptin and oestrogens in healthy women. *Eur. J. Endocrinol.* 139, 198-201.
- 19. Thong FS, McLean C and Graham TE (2000) Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. *J. Appl. Physiol.* 88, 2037-2044.
- 20. Bermant G and Davidson JM (1974) *The biological bases of sexual behavior*. New York: Harper Row.
- 21. McNeil J, Riou ME, Razmjou S, Cadieux S and Doucet E (2012) Reproducibility of a food menu to measure energy and macronutrient intakes in a laboratory and under real-life conditions. *Br. J. Nutr.*, 1-9.

- Bingham SA, Cassidy A, Cole TJ, Welch A, Runswick SA, Black AE, Thurnham D, Bates C, Khaw KT, Key TJ and et al. (1995) Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br. J. Nutr.* 73, 531-550.
- 23. Flint A, Raben A, Blundell JE and Astrup A (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int. J. Obes. Relat. Metab. Disord.* 24, 38-48.
- 24. Bouten CV, Sauren AA, Verduin M and Janssen JD (1997) Effects of placement and orientation of body-fixed accelerometers on the assessment of energy expenditure during walking. *Med. Biol. Eng. Comput.* 35, 50-56.
- 25. Goris AH, Meijer EP, Kester A and Westerterp KR (2001) Use of a triaxial accelerometer to validate reported food intakes. *Am. J. Clin. Nutr.* 73, 549-553.
- 26. Allen SS, McBride CM and Pirie PL (1991) The shortened premenstrual assessment form. J. *Reprod. Med.* 36, 769-772.
- 27. Saelens BE and Epstein LH (1996) Reinforcing value of food in obese and non-obese women. *Appetite* 27, 41-50.
- 28. Johnson WG, Carr-Nangle RE and Bergeron KC (1995) Macronutrient intake, eating habits, and exercise as moderators of menstrual distress in healthy women. *Psychosom Med.* 57, 324-330.
- 29. Frye CA and Demolar GL (1994) Menstrual cycle and sex differences influence salt preference. *Physiol. Behav.* 55, 193-197.
- 30. Brennan IM, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilcore D, Wishart JM, Jones KL, Horowitz M and Feinle-Bisset C (2009) Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. *Am J. Physiol. Gastrointest Liver Physiol.* 297, G602-610.
- 31. Gong EJ, Garrel D and Calloway DH (1989) Menstrual cycle and voluntary food intake. *Am. J. Clin. Nutr.* 49, 252-258.
- Lissner L, Stevens J, Levitsky DA, Rasmussen KM and Strupp BJ (1988) Variation in energy intake during the menstrual cycle: implications for food-intake research. *Am. J. Clin. Nutr.* 48, 956-962.
- 33. Fong AK and Kretsch MJ (1993) Changes in dietary intake, urinary nitrogen, and urinary volume across the menstrual cycle. *Am. J. Clin. Nutr.* 57, 43-46.
- 34. Capobianco G, de Muro P, Cherchi GM, Formato M, Lepedda AJ, Cigliano A, Zinellu E, Dessole F, Gordini L and Dessole S (2010) Plasma levels of C-reactive protein, leptin and glycosaminoglycans during spontaneous menstrual cycle: differences between ovulatory and anovulatory cycles. *Arch. Gynecol. Obstet.* 282, 207-213.
- Mills PJ, Ziegler MG and Morrison TA (1998) Leptin is related to epinephrine levels but not reproductive hormone levels in cycling African-American and Caucasian women. *Life Sci.* 63, 617-623.

- 36. Teirmaa T, Luukkaa V, Rouru J, Koulu M and Huupponen R (1998) Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. *Eur. J. Endocrinol.* 139, 190-194.
- 37. Cross GB, Marley J, Miles H and Willson K (2001) Changes in nutrient intake during the menstrual cycle of overweight women with premenstrual syndrome. *Br. J. Nutr.* 85, 475-482.
- 38. Bryant M, Truesdale KP and Dye L (2006) Modest changes in dietary intake across the menstrual cycle: implications for food intake research. *Br. J. Nutr.* 96, 888-894.