Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women

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FOLLOWING MEAL INGESTION, the presence of nutrients in the small intestine results in slowing of gastric emptying (46), which increases gastric distension and modulates the release of a number of gastrointestinal hormones, including cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) (13). These effects are important in the acute regulation of energy intake (27, 35). Studies evaluating appetite and energy intake, accordingly, frequently utilize a “preload” paradigm (5, 13, 51), whereby subjects are presented with a standardized test meal of variable macronutrient composition, volume, and/or energy density and subsequent energy intake is assessed. Healthy, lean, young, male subjects are traditionally used in such studies, since they have been reported to have a greater capacity to adjust energy intake in response to a nutrient preload compared with healthy women, elderly men, and obese individuals (44, 47), although even within this group there is substantial interindividual variability (14). However, in our recent study in which healthy men consumed a glucose preload on 3 separate days, acute energy intake was shown to be highly reproducible and associated with reproducible patterns of gastric emptying (39). It is unknown whether this applies to women.

One of the major reasons that women are used less frequently in research studies assessing gastrointestinal function, appetite, and energy intake is the perceived confounding effect of the menstrual cycle on these parameters. There is evidence from studies in both animals (8, 30) and humans (26, 34, 36) that fluctuations in hormone levels over the menstrual cycle affect energy intake, such that energy intake is less during the follicular phase, when estrogen is relatively high, and increased during the luteal phase, when progesterone is at its peak. There is also evidence from studies in rats that estrogen increases the sensitivity to the inhibitory effect of CCK on energy intake (17), consistent with the concept that energy intake is reduced when plasma estrogen is higher. It is not known whether the effects of the menstrual cycle on energy intake in humans are related to changes in the release of CCK.

There is persuasive evidence that both the rate of gastric emptying (46) and intragastric meal distribution (27) affect energy intake. Accordingly, differences in energy intake across the menstrual cycle may potentially be related to changes in gastric emptying. However, previous studies that have assessed the effect of the menstrual cycle on gastric emptying have yielded conflicting information (4, 10, 19, 23, 38). The rate of gastric emptying is recognized as a major determinant of postprandial blood glucose homeostasis, so that when gastric emptying is relatively slower, the initial (28) and potentially the overall (45) glycemic response to a carbohydrate-containing meal is reduced. In addition, enteral (as opposed to intravenous) glucose stimulates the release of the incretin hormone, GLP-1, the release of which is dependent on the rate of gastric emptying into the small intestine (40, 42), and the so-called “incretin effect” accounts for ~50% of the rise in plasma insulin following oral glucose. Hence if there are changes in
gastric emptying during the menstrual cycle these have the potential to affect glycemia, with implications for the diagnosis of diabetes by oral glucose tolerance testing (16, 25).

Our study was designed to evaluate the hypotheses that in healthy women 1) gastric emptying of a glucose preload would be slower and blood glucose, plasma GLP-1, insulin and CCK responses, hunger, and energy intake would be less during the follicular compared with the luteal phase; 2) the reduction in hunger and energy intake during the follicular phase would be related to slower gastric emptying; and 3) gastric emptying, blood glucose and plasma hormone concentrations, hunger, and energy intake would be reproducible when assessed twice within a particular phase of the menstrual cycle, i.e., during the follicular phase.

SUBJECTS AND METHODS

Subjects

Nine healthy women, aged 31 ± 1 (range 26–38) years, of normal body weight for their height [body mass index 21 ± 0.5 (range 19–24) kg/m²], participated in the study. The number of subjects included was based on power calculations derived from previous work addressing within-subject variability of energy intake (39). Assuming a within-subject standard deviation of 553 kJ, a mean difference in energy intake between phases of 700 kJ is detectable with a sample size of nine subjects at 80% power and a Bonferroni-adjusted significance level of 5%, consistent with the reported differences in energy intake between the follicular and luteal phases (32, 36). Subjects were unrestrained eaters [scoring <12 on the eating restraint section (Factor 1) of the Three Factor Eating Questionnaire (50)], had no significant gastrointestinal symptoms, disease (including diabetes), or surgery, and were not taking any medication known to affect gastrointestinal function or appetite. Subjects who regularly consumed >20 g of alcohol or smoked >10 cigarettes per day were excluded. In addition, subjects who used contraceptive medication (e.g., oral contraceptive pill, Depo-Provera, Norplant, hormone-releasing IUDs), who had clinically significant premenstrual syndrome and/or premenstrual dysphoric disorder [as assessed by a modified premenstrual symptom screening tool (49)], or who were pregnant (as assessed by a pregnancy test on a urine sample) were excluded. All subjects were required to confirm a regular menstrual cycle for at least 3 consecutive months. Subjects were instructed to consume their usual diets, to maintain their usual exercise levels, and to avoid heavy exercise throughout the study. The study protocol was approved by the Royal Adelaide Hospital Human Research Ethics Committee, and all experiments were carried out in accordance with the Declaration of Helsinki. All subjects provided informed, written consent prior to their enrollment. To distract from the aim of assessing energy intake, subjects were informed that the study would evaluate the effects of a glucose drink on gastric emptying and blood glucose concentrations. The investigator who performed the studies and analyzed the data (I. M. Brennan) was blinded to the phase of the menstrual cycle on each study day; this information was obtained, and study visits were coordinated, by investigators (K. L. Feltrin, T. J. Little) who were not involved in primary data analysis.

Protocol

Each subject participated on three occasions, twice during the follicular (between days 6 and 12; “FOL-P1” and “FOL-P2”), and once during the luteal (between days 18 and 24; “LUT-P”), phase of the menstrual cycle (where day 1 is the first day of menstrual bleeding). On each of the three visits, serum levels of the ovarian hormones estradiol (E2) and progesterone (P4) were measured. To exclude potential order effects, the study was conducted in randomized fashion, with visits scheduled across consecutive menstrual cycles.

To standardize study conditions, subjects were provided with a “ready-to-eat” dinner [Beef Lasagne (2,472 kJ), McCain Foods, Wendouree, Victoria, Australia] to be consumed at 1900 on the evening prior to each study day, after which time they were required to fast. On each study day, subjects attended the laboratory in the Discipline of Medicine, Royal Adelaide Hospital, at either ~0800 or 1100, i.e., for logistical reasons, two subjects were studied on each day; each subject attended at the same time of day on each visit. Upon arrival an intravenous cannula was inserted into an antecubital vein in one arm for blood sampling and kept patent with 0.9% saline. Subjects were seated comfortably in an upright position for the duration of the study. At t = −15 min, an image of the fasted stomach was acquired by three-dimensional (3D) ultrasonography, a baseline blood sample was collected, and a visual analog scale questionnaire (VAS) assessing perceptions of appetite (hunger and fullness) and gastrointestinal symptoms (nausea and bloating) was completed. At t = −2 min, the subject consumed a preload consisting of 50 g glucose dissolved in 300 ml water (200 kcal, 0.17 g/ml) within 2 min. The glucose preload employed in this study was chosen since we have demonstrated that gastric emptying of this drink can be assessed accurately by 3D ultrasonography (18). At t = 0 min, immediately following ingestion of the preload, another 3D image of the stomach was acquired, a blood sample was collected, and a VAS was completed. Subsequently, 3D ultrasound scans, blood samples, and VAS were obtained at 15-min intervals until t = 90 min. At t = 90 min, the subject was presented with a standardized, cold, buffet-style meal, with food in excess of what they would be anticipated to consume, and invited to eat until comfortably full, for up to 30 min (i.e., t = 90–120 min) (15). A final blood sample was collected and a VAS was completed following the meal (t = 120 min), after which the intravenous cannula was removed and the subject was allowed to leave the laboratory.

Measurements

Gastric emptying and intragastric meal distribution. Gastric emptying was assessed by 3D ultrasonography (Logiq 9 ultrasound system; GE Healthcare Technologies, Sydney, NSW, Australia). This technique allows evaluation of total, proximal, and distal gastric volumes (i.e., gastric emptying and intragastric meal distribution) (18). Although scintigraphy may be regarded as the “gold standard” for the measurement of gastric emptying in clinical and research studies (7), the associated radiation burden limits its use, and we have recently validated the use of 3D ultrasonography against scintigraphy as an accurate measure of gastric emptying of liquids (18). For 3D positioning and orientation measurement, a transmitter was placed next to the subject, and a 3D sensor was attached to a 3.5C broad spectrum 2.5–4 MHz convex transducer. All metal objects were removed from both the subject and surrounding area to avoid interference during acquisition. 3D scans of the total stomach were taken to evaluate total gastric volume and intragastric volume distribution by use of EchoPAC-3D software (GE Vingmed Sound, Horten, Norway). The raw data (original scan planes) were used for 3D reconstructions of the stomach. The proximal and distal gastric segments were separated by vertically slicing the 3D stomach reconstruction from the incisura angularis at the lesser gastric curvature sagitally toward the greater curvature (39). Total, proximal, and distal gastric volumes at each time point were derived and expressed as percentages of the volumes at t = 0 min (volume immediately following preload ingestion), with total gastric volume at t = 0 min defined as 100%. Gastric emptying profiles were then constructed, and the time at which 50% of the glucose had emptied from the stomach [50% gastric emptying time (T50)] was derived (18).

Blood glucose; plasma GLP-1, insulin, and CCK; and serum estradiol and progesterone concentrations. Venous blood glucose concentrations (mmol/l) were determined immediately via a portable glucometer (Medisense Precision, Abbott Laboratories, Bedford, MA). This technique has a coefficient of variation of 2.1–5.6%, and its...
accuracy has been confirmed in our laboratory by the hexokinase technique (24).

For the measurement of plasma GLP-1 (pmol/l), plasma insulin (mU/l), and plasma CCK (pmol/l) concentrations, blood samples (10 ml) were collected into ice-chilled EDTA-treated tubes containing 400 KIU aprotinin per milliliter blood (Trasylol, Bayer Australia, Pyrmble, Australia). Plasma was separated by centrifugation (3,200 rpm, 15 min, 4°C) within 30 min of collection and stored at −70°C until assayed. Total GLP-1 was measured by radioimmunoassay (GLPIT-36HK, Linco Research, St. Charles, MO). The intra-assay and interassay coefficients of variation (CVs) were 6.7 and 7.8%, respectively, with a detection limit of 3 pmol/l. Plasma insulin was measured by enzyme-linked immunosorbent assay (Diagnosics Systems Laboratories, Webster, TX). The intra-assay and interassay CVs were 2.6 and 6.2%, respectively, with a detection limit of 0.26 mU/l. Plasma CCK was determined by radioimmunoassay following ethanol extraction. The intra-assay and interassay CVs were 6.2 and 14.8%, respectively, with a detection limit of 2.5 pmol/l (35).

For the measurement of serum estradiol (pmol/l) and serum progesterone (nmol/l) concentrations, blood samples (8 ml) were collected into serum clot activator tubes. Estradiol and progesterone concentrations were both determined by the Institute of Medical and Veterinary Sciences (Royal Adelaide Hospital, Adelaide, South Australia, Australia) by chemiluminescent microparticle immunoassay (ARCHITECT System, Abbott Laboratories, Abbott Park, IL). For estradiol, the intra-assay and interassay CVs were 1.8 and 2.3%, respectively, with a detection limit of 37 pmol/l. For progesterone, the intra-assay and interassay CVs were 1.5 and 2.1%, respectively, with a detection limit of 1 nmol/l.

Appetite perceptions and energy intake. Hunger and fullness were evaluated by using validated VAS (41). Nausea and bloating were also assessed. Other perceptions including happiness and drowsiness were assessed to distract the subject from the main purpose of the questionnaire but were not formally evaluated. Each VAS consisted of a 100-mm horizontal line, where 0 mm represented “sensation not felt at all” and 100 mm “sensation felt the greatest.” The subject was asked to place a vertical mark along the line to indicate the strength of each sensation.

The amount (g) of food consumed at the buffet meal was determined by weighing the meal before and after consumption. Energy intake (kJ) and macronutrient composition (% energy from fat, carbohydrate, and protein) were measured by use of commercially available software (Foodworks 3.01, Xyris Software, Highgate Hill, QLD, Australia) (15).

Statistical Analysis

Areas under the curve (AUC) for gastric emptying, proximal and distal gastric volumes, blood glucose, and plasma hormone concentrations were calculated by use of the trapezoidal rule. Repeated-measures analysis of variance (ANOVA) was used to evaluate variables measured over time (% retention for total, proximal, and distal gastric volumes, blood glucose and plasma hormones, and VAS scores), with time and visit as factors. One-way ANOVA was used to analyze energy intake. Post hoc paired comparisons, adjusted for multiple comparisons by Bonferroni’s correction, were performed when ANOVAs revealed significant effects. Blood glucose and plasma hormone concentrations at t = 90 and 120 min were compared using Student’s paired t-test. Relationships between gastric emptying with blood glucose and hormone concentrations and between energy intake with gastric emptying, T50, hormone concentrations and scores for hunger were calculated by the method described by Bland and Altman (2). Only r values >0.5 were considered physiologically relevant. Intrasubject reproducibility (i.e., the agreement within each individual’s data) between FOL-P1 and FOL-P2 for T50, energy intake, and AUCs for gastric emptying, proximal and distal gastric volumes, blood glucose, and plasma hormone concentrations were evaluated by determining intraclass correlation coefficients, r. An r ≥ 0.8 was considered to indicate “excellent” agreement, 0.8 > r ≥ 0.7 to indicate “good” agreement, and 0.7 > r ≥ 0.5 to indicate “moderate” agreement (39). Intrasubject variability in energy intake within and between phases was assessed by calculating the CVs (CV = standard deviation/mean * 100%). Statistical significance was accepted at P < 0.05. Data are presented as mean values ± SE.

RESULTS

The study protocol was well tolerated, and all subjects completed all visits. Subjects reported regular menstrual cycles ranging 25–30 days in length. Although there were no differences in serum estradiol concentrations (mean concentrations (pmol/l); FOL-P1: 499 ± 85, FOL-P2: 466 ± 72, LUT-P: 544 ± 73; mean differences (pmol/l); FOL-P1 vs. LUT-P: 45 ± 133; FOL-P2 vs. LUT-P: 78 ± 124; FOL-P1 vs. FOL-P2: 33 ± 59), there were significant differences in progesterone concentrations between study days (mean concentrations (nmol/l); FOL-P1: 2 ± 0, FOL-P2: 2 ± 1, LUT-P: 48 ± 4; P < 0.05; mean differences (nmol/l); FOL-P1 vs. LUT-P: 46 ± 4; FOL-P2 vs. LUT-P: 46 ± 4; FOL-P1 vs. FOL-P2: −1 ± 1).

There were no differences in baseline values between the 0800 and 1100 visits, nor did the timing of the commencement of the studies affect any of the outcome measures (data not shown). No subject reported premenstrual gastrointestinal symptoms.

Gastric Emptying

Total gastric emptying. The profile of gastric emptying approximated an overall linear pattern on all study days (Fig. 1A), so that the volume of glucose retained in the stomach decreased...
progressively over time [time effect: \(F(6,48) = 136.10, P < 0.001\)]. There was a significant effect of the phase of the menstrual cycle on gastric emptying \([F(2,16) = 3.89, P < 0.05]\) (Table 1). Gastric emptying was slower during FOL-P1 and FOL-P2 compared with LUT-P \((P < 0.05)\), with no difference between FOL-P1 and FOL-P2 [mean AUC differences (\% \cdot \text{min})]; FOL-P1 vs. LUT-P: 762 ± 313; FOL-P2 vs. LUT-P: 718 ± 250; FOL-P1 vs. FOL-P2: 45 ± 261]. There was also a significant effect of the phase of the menstrual cycle on the 50% gastric emptying time, \(T_{50}\) \([F(2, 16) = 5.20, P < 0.05]\), so that the \(T_{50}\) was greater during FOL-P1 (76 ± 4 min) and FOL-P2 (75 ± 5 min) compared with LUT-P (61 ± 6 min) \((P < 0.05)\), with no difference between FOL-P1 and FOL-P2 [mean differences (min)]; FOL-P1 vs. LUT-P: 17 ± 5; FOL-P2 vs. LUT-P: 18 ± 4; FOL-P1 vs. FOL-P2: −1 ± 2].

**Intragastric meal distribution.** There was no significant effect of the phase of the menstrual cycle on the volume of glucose retained in the proximal or distal stomach (Fig. 1B), although the mean values for volumes in the proximal stomach were greater during FOL-P1 and FOL-P2 compared with LUT-P. The volume of glucose retained in the proximal [time effect: \(F(6,46) = 82.52, P < 0.05\)] and distal stomach [time effect: \(F(6,46) = 5.60, P < 0.05\)] decreased progressively over time, reflecting total gastric emptying.

**Blood Glucose and Plasma Hormone Concentrations**

Examples of differences in the magnitude of responses in blood glucose and plasma GLP-1 and insulin concentrations from three subjects, i.e., individuals with smaller, moderate, or larger responses, following consumption of the glucose solution, are provided in Fig. 2. There were uniformly no differences in plasma CCK concentrations in response to the glucose solution (data not shown).

**Blood glucose.** There was no difference in baseline blood glucose concentrations between visits. There was a treatment-by-time interaction \([F(12, 96) = 1.95, P < 0.05]\) (Fig. 3A) so that after the glucose load, blood glucose concentrations were less during FOL-P1 and FOL-P2 compared with LUT-P between \(t = 30–90\) min \((P < 0.01)\), with no difference between FOL-P1 and FOL-P2. There was substantial difference in the magnitude of peak blood glucose concentrations during FOL-P1 and FOL-P2 (~7.6 mmol/l for both) compared with LUT-P (~9.4 mmol/l). The AUC between \(t = 0–90\) min (Table 1) was less during FOL-P1 and FOL-P2 compared with LUT-P \((P < 0.05)\), with no difference between FOL-P1 and FOL-P2 [mean AUC differences (mmol\(\cdot\)l\(^{-1}\)\cdot min\(^{-1}\)); FOL-P1 vs. LUT-P: 92 ± 50; FOL-P2 vs. LUT-P: 109 ± 56; FOL-P1 vs. FOL-P2: 17 ± 38]. Following the buffet meal \((t = 120\) min), blood glucose concentrations returned to baseline values during FOL-P1 and FOL-P2, but not LUT-P.

**Plasma GLP-1.** There was no difference in baseline plasma GLP-1 concentrations between visits. There was a treatment-by-time interaction \([F(12, 96) = 1.95, P < 0.05]\) (Fig. 3B) so that after the glucose load plasma GLP-1 concentrations were less during FOL-P1 and FOL-P2 compared with LUT-P between \(t = 15–75\) min \((P < 0.01)\), with no difference between FOL-P1 and FOL-P2. The AUC between \(t = 0–90\) min (Table 1) was less during FOL-P1 and FOL-P2 compared with LUT-P [mean AUC differences (pmol\(\cdot\)l\(^{-1}\)\cdot min\(^{-1}\)); FOL-P1 vs. LUT-P: 340 ± 140; FOL-P2 vs. LUT-P: 297 ± 109; FOL-P1 vs. LUT-P: −43 ± 84]. Plasma GLP-1 concentrations increased following the buffet meal (time effect: \(P < 0.01\)), with no difference between visits.

**Plasma insulin.** There was no difference in baseline plasma insulin concentrations between visits. There was a treatment-by-time interaction \([F(12, 96) = 1.83, P < 0.05]\) (Fig. 3C) so that after the glucose load plasma insulin concentrations were less during FOL-P1 and FOL-P2 compared with LUT-P between \(t = 15–90\) min \((P < 0.05)\), with no difference between FOL-P1 and FOL-P2. The AUC between \(t = 0–90\) min (Table 1) was less during FOL-P1 and FOL-P2 compared with LUT-P \([mean AUC differences (\mu U\(\cdot\)l\(^{-1}\)\cdot min\(^{-1}\)); FOL-P1 vs. LUT-P: 1,073 ± 608; FOL-P2 vs. LUT-P: 1,184 ± 456; FOL-P1 vs. FOL-P2: 111 ± 334]. Plasma insulin concentrations increased following the buffet meal (time effect: \(P < 0.01\)), with no difference between visits. Plasma CCK. There was no difference in baseline CCK concentrations between visits and no effect of the phase of the menstrual cycle on overall plasma CCK concentrations or the AUC between \(t = 0–90\) min (Table 1) [mean AUC differences (pmol\(\cdot\)l\(^{-1}\)\cdot min\(^{-1}\)); FOL-P1 vs. LUT-P: 11 ± 11; FOL-P2 vs. LUT-P: 7 ± 13; FOL-P1 vs. FOL-P2: 5 ± 11] (Fig. 3D). Plasma CCK rose slightly within 15 min of glucose ingestion (time effect: \(P < 0.01\)) and subsequently reached a plateau. Plasma CCK increased further following the buffet meal (time effect: \(P < 0.01\)), with no difference between visits.

**Appetite and Energy Intake**

**Appetite.** There was a trend for baseline hunger scores to be less during FOL-P1 and FOL-P2 compared with LUT-P \((P = 0.07)\). There was a treatment-by-time interaction \([F(12, 96) = 3.10, P < 0.01]\) (Fig. 4). Hunger was less during FOL-P1 and FOL-P2 over the entire study period compared with LUT-P \((P < 0.01)\), with no difference between FOL-P1 and FOL-P2 (Table 1) [mean AUC differences (mm-min)]; FOL-P1 vs. LUT-P: 1,133 ± 420; FOL-P2 vs. LUT-P: 981 ± 360; FOL-P1 vs. FOL-P2: 485 ± 358]. There was no difference in baseline scores and no effect of the menstrual cycle on scores for fullness, nausea, or bloating, which all increased less than 10% from baseline (data not shown).

**Energy intake.** There was a significant effect of the menstrual cycle on both the amount eaten (g) \([F(2, 16) = 5.35, P <

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Table 1. Mean AUC values for gastric emptying, blood glucose, plasma GLP-1, plasma insulin and plasma CCK concentrations and hunger during the follicular and luteal phases of the menstrual cycle

<table>
<thead>
<tr>
<th></th>
<th>FOL-P1</th>
<th>FOL-P2</th>
<th>LUT-P</th>
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<tbody>
<tr>
<td>Gastric emptying, % \cdot \text{min}</td>
<td>6,705 ± 318*</td>
<td>6,659 ± 216*</td>
<td>5,711 ± 188</td>
</tr>
<tr>
<td>Blood glucose, mmol(\cdot)l(^{-1})\cdot min(^{-1})</td>
<td>651 ± 30*</td>
<td>634 ± 42*</td>
<td>743 ± 49</td>
</tr>
<tr>
<td>Plasma GLP-1, pmol(\cdot)l(^{-1})\cdot min(^{-1})</td>
<td>1,081 ± 99†</td>
<td>1,124 ± 116†</td>
<td>1,421±100</td>
</tr>
<tr>
<td>Plasma insulin, \mu U(\cdot)l(^{-1})\cdot min(^{-1})</td>
<td>4,162 ± 403*</td>
<td>4,052 ± 394*</td>
<td>5,235±519</td>
</tr>
<tr>
<td>Plasma CCK, pmol(\cdot)l(^{-1})\cdot min(^{-1})</td>
<td>455 ± 37</td>
<td>449 ± 37</td>
<td>444 ± 32</td>
</tr>
<tr>
<td>Hunger, mm(\cdot)min</td>
<td>3,513 ± 332†</td>
<td>3,831 ± 593†</td>
<td>4,812±2431</td>
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</tbody>
</table>

Values are means ± SE; \(n = 9\). AUC, area under the curve; GLP-1, glucagon-like peptide-1; FOL-P1 and FOL-P2, first and second follicular phase visits, respectively; LUT-P, luteal phase. * vs. LUT-P: \(P < 0.05\), † vs. LUT-P: \(P < 0.01\).
0.05] and energy consumed (kJ) \([F(2, 16) = 8.17, P < 0.05]\) at the buffet meal (Table 2). Both were less during FOL-P1 and FOL-P2 compared with LUT-P (\(P < 0.05\) for both), with no difference between FOL-P1 and FOL-P2 [mean differences in amount eaten (g); FOL-P1 vs. LUT-P: 70 ± 63; FOL-P2 vs. LUT-P: 72 ± 89; FOL-P1 vs. FOL-P2: 2 ± 84; mean differences in energy intake (kJ); FOL-P1 vs. LUT-P: 588 ± 245; FOL-P2 vs. LUT-P: 766 ± 230; FOL-P1 vs. FOL-P2: 178 ± 130]. Differences ranged from −174 to 854 kJ for FOL-P1-FOL-P2, from 674 to 1,966 kJ for LUT-FOL-P1 and from 18 to 1,937 kJ for LUT-P-FOL-P2 (Fig. 5). There was no difference between visits in the percentage of macronutrients consumed (Table 2).

Relationships Between Hormones and Blood Glucose With Gastric Emptying

There were inverse relationships between plasma GLP-1 at \(t = 45, 60, 75,\) and 90 min (\(r > -0.60, P < 0.05\) for all) and blood glucose and plasma insulin at \(t = 60, 75,\) and 90 min (\(r > -0.60, P < 0.05\) for all), but not plasma CCK concentrations, with the volume of glucose remaining in the stomach at these times. There was an inverse relationship between \(T_{50}\) with serum progesterone but not estradiol (\(r > -0.60, P < 0.05\)).

Relationships Between Energy Intake With Gastric Emptying and Hunger

There were inverse relationships between energy intake with the amount of glucose remaining in the stomach at \(t = 90\) min (i.e., immediately prior to the buffet meal), \(T_{50}\), and scores for hunger at \(t = 90\) min (\(r > -0.60, P < 0.05\) for all).

Relationships Between Hunger and Energy Intake With Hormones and Blood Glucose

There were significant relationships between energy intake with blood glucose concentrations at \(t = 45, 60, 75,\) and 90 min
(r > 0.50, P < 0.05 for all) (Fig. 6A), and plasma GLP-1 concentrations at t = 15, 30, 45, and 75 min (r > 0.50, P < 0.05 for all) (Fig. 6B), but not with plasma insulin and CCK concentrations. There was no relationship between blood glucose, plasma GLP-1, insulin, or CCK concentrations with scores for hunger at any time point. There was a relationship between scores for hunger at t = 90 min and energy intake with serum progesterone (r > 0.60, P < 0.05 for both), but not serum estradiol, concentrations.

**Intrasubject Reproducibility Between FOL-P1 and FOL-P2**

There was excellent agreement between FOL-P1 and FOL-P2 for T50 (r = 0.81), AUCs of plasma CCK profiles (r = 0.91), and energy intake (r = 0.94), good agreement for AUCs of hunger (r = 0.71) and plasma GLP-1 (r = 0.70) profiles, and moderate agreement for AUCs of plasma insulin (r = 0.65) and blood glucose (r = 0.50) profiles. In contrast, there was no agreement for AUCs of proximal (r = 0.11) and distal (r = 0.12) gastric volume profiles. Intrasubject variability of energy intake was less during FOL-P1 and FOL-P2 (CV = 6.6%) compared with FOL-P1 vs. LUT-P (CV = 15.0%) and FOL-P2 vs. LUT-P (CV = 16.0%).

**DISCUSSION**

Our observations establish that in healthy women after ingestion of a 50-g glucose preload, 1) gastric emptying is slower and glycemic, plasma GLP-1, and insulin responses, hunger and energy intake are less during the follicular compared with the luteal phase; 2) energy intake and the glucose, plasma GLP-1, and insulin responses are related to gastric emptying; and 3) these parameters are reproducible when assessed twice within one phase of the menstrual cycle, i.e., the follicular phase.

To our knowledge, this is the first study that quantified the effects of the menstrual cycle on acute energy intake prospectively within the laboratory setting. Our observation that energy intake

<table>
<thead>
<tr>
<th>Table 2. Mean values for food intake during the follicular and luteal phases of the menstrual cycle</th>
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<tr>
<td>FOL-P1</td>
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<tr>
<td>Energy intake, kJ</td>
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<tr>
<td>Weight of food, g</td>
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<tr>
<td>Protein, %</td>
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<tr>
<td>Fat, %</td>
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<tr>
<td>Carbohydrate, %</td>
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</table>

Data are mean values ± SE, n = 9; * vs. LUT-P; P < 0.05.
The relationship between the rate of gastric emptying and appetite remains to be defined, the observed regulation of energy intake (12, 27, 51). Although mechanisms are related to varying patterns of gastric emptying, although this is in appetite and energy intake observed across the menstrual cycle, in particular interest is that our study demonstrates that the changes in appetite and luteal phases (32, 36), whereas our study quantified acute energy intake observed between the follicular and luteal phases were not associated with changes in plasma CCK concentrations. It would, therefore, be of interest to evaluate variations across the menstrual cycle is consistent with existing literature, which includes studies in both animals (8, 30) and humans (26, 34, 36). Specifically, in our study, both the amount eaten (g) and energy consumed (kJ) at the buffet meal were less during the follicular compared with the luteal phase, with a substantial mean difference in energy intake of 700 kJ. Previous studies have observed a difference of 660–1,000 kJ in daily (24-h) energy intake, using dietary recall methods, between the follicular and luteal phases (32, 36), whereas our study quantified acute energy intake following a nutrient preload. There is evidence that daily energy expenditure fluctuates across the menstrual cycle, i.e., energy expenditure is lower in the latter half of the follicular phase and higher during the luteal phase (48, 52). Therefore, an adjustment of appetite and energy intake ensures that energy balance is maintained. In contrast to others (32, 36), we did not observe any effect of the menstrual cycle on the amount of energy derived from individual macronutrients. It should, however, be recognized that this may reflect the composition of our buffet-style meal, i.e., a meal that contains a selection of foods that vary substantially in their macronutrient composition is likely to be more suitable to evaluate effects on macronutrient distribution or food choice.

We have demonstrated that gastric emptying of glucose is slower during the follicular (T50 ~75 min), compared with the luteal (T50 ~60 min), phase. This observation contrasts with previous studies that have reported either no difference in the rate of emptying of the solid and liquid phases of a mixed meal between the follicular and luteal phases (10, 23) or slower gastric emptying of a solid meal during the luteal phase (19). In some studies gastric emptying was evaluated with low-nutrient meals, e.g., beef soup and mashed potato (~99 kcal) (19), which are known to empty rapidly from the stomach, since they do not stimulate mechanisms that retard gastric emptying effectively. Of particular interest is that our study demonstrates that the changes in appetite and energy intake observed across the menstrual cycle are related to varying patterns of gastric emptying, although this is not surprising given that intragastric factors are important in the regulation of energy intake (12, 27, 51). Although mechanisms underlying the observed effects of the menstrual cycle on gastric emptying and appetite remain to be defined, the observed relationship between the rate of gastric emptying with the serum progesterone concentration suggests that changes in sex steroids, particularly progesterone, are likely to be important.

It is well established that there is a close relationship between the initial rise in blood glucose and gastric emptying after an oral glucose load and carbohydrate-containing meals (22, 28). In some (20, 45), but not all (21), studies the total glycemic response to a meal was also diminished when gastric emptying was slowed. In this study, the observed direct relationship between the initial and overall glycemic response and the rate of gastric emptying was, accordingly, predictable, given that even modest changes in gastric emptying of glucose are associated with substantial changes in the glycemic response (22, 43). Our study has demonstrated for the first time that oral glucose-induced GLP-1 release is greater during the luteal compared with the follicular phase of the menstrual cycle and is related to more rapid gastric emptying. Given the observed changes in gastric emptying, this is not surprising since previous studies have shown that the stimulation of GLP-1 (and the other incretin hormone, glucose-dependent insulinotrophic polypeptide) are dependent on the rate of entry of glucose into the small intestine (6, 40, 42). Hence the observed reduction in insulinemia during the follicular phase is likely to reflect the decreases in both glucose and GLP-1 secretion. CCK has an established role in appetite regulation (11, 37) and mediates, at least in part, the effects of nutrients on gastrointestinal motility and energy intake (29, 37). Glucose, compared with both fat or protein (33), stimulates plasma CCK only modestly, and it is, therefore, not surprising that the differences in gastric emptying, hunger, and energy intake observed between the follicular and luteal phases were not associated with changes in plasma CCK concentrations. It would, therefore, be of interest to evaluate...
potential variations in CCK release across the menstrual cycle by using test meals known to stimulate CCK potently. Alternatively, given the evidence, albeit from animal studies, that the sensitivity to CCK is increased when estrogen is high (17), it is possible that although concentrations of CCK did not change during the follicular phase, subjects may be more sensitive to the effects of CCK.

Although we did not measure plasma ghrelin in this study, it has been reported previously that plasma ghrelin concentrations remain unchanged over the menstrual cycle (9).

Buffet-style meals that contain a range of food items, varying in macronutrient composition and provided in excess of what subjects would be expected to consume, are used frequently to assess energy intake in the laboratory setting (1, 3). It has been suggested that the availability of a meal in excess could result in spontaneous overeating (31), thereby confounding the capacity to detect small changes in energy intake in response to a treatment. On the other hand, individuals may experience a sense of boredom when presented with the same meal on multiple occasions, resulting in a reduction in energy intake at later visits because of disinterest. Few studies have assessed the potential variability that may occur when energy intake is assessed repeatedly using a buffet-style meal (1, 39), and, to our knowledge, no study has assessed this in women. We have now demonstrated that energy intake is highly reproducible when young adult women are assessed twice during the follicular phase of the menstrual cycle. This observation supports the use of a buffet-style meal as a reliable measure of energy intake, since providing subjects with the same foods repeatedly did not appear to influence energy intake.

Some limitations of our study warrant discussion. Firstly, since we have only evaluated healthy, lean women, our observations may not be applicable to other female subject groups, i.e., under- or overweight and obese. Although the number of subjects included was based on power calculations derived from our previous work (39), and the observed differences in primary endpoints appear clear cut, it is possible that potential differences in intragastric meal distribution remained undetected.

**Perspectives and Significance**

We have demonstrated that in healthy women the reduction in energy intake during the follicular phase is related to slower gastric emptying and reduced hunger. Furthermore, during the follicular phase the glycemic and plasma GLP-1 and insulin responses to an oral glucose load are attenuated and related to the slower rate of gastric emptying. Hence our observations strongly suggest that the menstrual cycle should be controlled for in research studies investigating gastrointestinal function, appetite and energy intake, glycemia, and gastrointestinal hormone concentrations, i.e., female subjects included in multiple visit research studies should ideally be assessed during the same phase of the menstrual cycle. The substantial effects of the menstrual cycle on glycemia secondary to gastric emptying may have important implications for oral glucose tolerance testing for the diagnosis of diabetes mellitus in premenopausal women; our data suggest that consideration should be given to the timing of oral glucose tolerance tests, since it is likely that more subjects will be diagnosed with impaired glucose tolerance and diabetes during the luteal phase. Further studies are required to assess the time course of changes in variables across the menstrual cycle, and longer term studies are desirable to better characterize the significance of our findings.

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