Is It Me or My Hormones? Neuroendocrine Activation Profiles to Visual Food Stimuli Across the Menstrual Cycle

- Yardena Arnoni-Bauer, Atira Bick, Noa Raz, Tal Imbar, Shoshana Amos, Orly Agmon, Limor Marko, Netta Levin, and Ram Weiss
- *Journal of Clinical Endocrinology and Metabolism* 102(9):3406-3414
- September 2017
Background

- Obesity is the epidemic of the 21st century
  - Majority of diets fail
  - 40-50% of women are on a diet at any given time

- Food consumption and eating behavior vary across the menstrual cycle
  - Peak consumption in midluteal phase → 250 kcal/day (average)

- What is the physiological mechanism involved in increased food consumption in midluteal phase?

- Objective: to test responses of females with regular cycles during midfollicular and midluteal phase and of users of monophasic oral contraception pills (OCPs) to visual food cues
Methods

- Twenty females with regular cycles
- Twelve females taking monophasic OCPs (>6 months)
  - Feminet or Mercilon
  - Ethynyl estradiol 0.02mg/desogestrel 0.15mg
- Inclusion criteria
  - Age 18-35
  - Stable body weight for past 3 months
  - Normal BMI
  - Not being on active weight loss-oriented dietary regimen
- Eating Attitudes Test [EAT-26]
  - Nonsmoking
  - Right handed
  - Off medications

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<thead>
<tr>
<th>Table 1. Study Participants’ Demographic and Anthropometric Data</th>
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<td><strong>Menstrual (n = 18)</strong></td>
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<td>Fat mass (kg)</td>
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<td>Fat free mass (kg)</td>
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Abbreviation: BMR, basal metabolic rate.
Methods

- **Menstrual group:**
  - 2 visits - *midfollicular and midluteal*
  - *Midfollicular: days 7-12*
  - *Midluteal: days 20-26*
- Uniform testing time: 10AM - 2PM
- **OCP group**
  - 2 visits - *active pill use using same time intervals as menstrual group*
- Each session consisted of 2 MRI scans (fasting vs fed)
  - *Fasting: 10 hrs of fasting prior to scan*
  - *Fed: 20-30 min after glucose ingestion of 75g (300kcal)*
- Blood collected at arrival and 15-20 min after glucose ingestion
- MRIs and blood samples were collected for each group in 4 time points to evaluate the effects of cycle phase and prandial state and their interaction
  - *Follicular fast*
  - *Follicular fed*
  - *Luteal fast*
  - *Luteal fed*
Methods

- Participants were scanned while viewing images of high-calorie sweet foods (e.g., chocolate, cake), high-calorie savory foods (e.g., pizza, lasagna), and non-food options (e.g., computer, hammer)
  - Instructed to think if they wanted what they saw in the visual images and to indicate yes/no by means of the response box to determine reward of food images and maintain attention

- Regions of interest (ROI)
  - Hypothalamus (homeostasis)
  - Amygdala, putamen, insula (reward response)
  - Anterior cingulate cortex, prefrontal cortex, dorsolateral prefrontal cortex (motivation and decision making)
  - Calcarine, lateral occipital cortex (early and high visual regions)
Methods

- Biochemical analyses
  - *Fasting samples:* insulin, progesterone, estrogen, DHEAS, cortisol, triglycerides, C-reactive protein (CRP), HDL-cholesterol, leptin

- Sex hormone levels were used to verify menstrual phase and to establish a correlation between menstrual phase, sex hormones, and reward region activation

- Glucose and insulin were sampled at fasting and 20 minutes following glucose ingestion
# Results

Table 2. Biochemical Parameters of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Follicular Phase (n = 18)</th>
<th>Luteal Phase (n = 18)</th>
<th>OCP Users (n = 11)</th>
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<tr>
<td>Fasting glucose (mg/dL)</td>
<td>82 ± 6</td>
<td>84 ± 9</td>
<td>79 ± 8</td>
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<tr>
<td>Fasting insulin (pmol/L)</td>
<td>39 ± 3</td>
<td>54 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>Fed glucose (mg/dL)</td>
<td>110 ± 2</td>
<td>114 ± 2</td>
<td>115 ± 8</td>
</tr>
<tr>
<td>Fed insulin (pmol/L)</td>
<td>283 ± 256</td>
<td>270 ± 158</td>
<td>457 ± 255</td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td>2.14 ± 1.27</td>
<td>20.15 ± 20.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>150 ± 231</td>
<td>195 ± 242</td>
<td>Undetectable</td>
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<tr>
<td>Testosterone (nmol/L)</td>
<td>1.07 ± 0.24</td>
<td>1.09 ± 0.25</td>
<td>1.05 ± 9</td>
</tr>
<tr>
<td>DHEA-S (µmol/L)</td>
<td>3.45 ± 2.14</td>
<td>3.96 ± 1.84</td>
<td>5.03 ± 1.85&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>TG (mmol/L)</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 1.9</td>
<td>1.2 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.1 ± 0.05</td>
<td>0.1 ± 0.2</td>
<td>0.6 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1 ± 0.3</td>
<td>1 ± 0.2</td>
<td>1.7 ± 0.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>211 ± 85</td>
<td>268 ± 128</td>
<td>577 ± 143&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; TG, triglycerides.

<sup>a</sup>P = 0.004 for luteal vs follicular.

<sup>b</sup>P < 0.001 for luteal vs follicular progesterone.

<sup>c</sup>Cortisol levels tended to be higher in OCP users than in participants with normal cycles (P = 0.036; analysis of variance), as were DHEA (P = 0.024), TG (P < 0.001), CRP (P < 0.001), and HDL-cholesterol (P < 0.001).
Activation patterns in menstrual group

Activation was identified in the hypothalamus only in the luteal fast state.

Reward regions (amygdala, putamen, and insula) and frontal regions showed a substantial effect of hormonal phase, but not of prandial state.

Visual regions showed a relevant dual effect of prandial state and menstrual phase in both regions with no substantial interaction.

Of note, the interaction of cycle phase and prandial state was not statistically significant.
Figure 2. Activation of visual brain areas. Activation maps and food vs control image contrasts are shown. Statistical values were based on the ROI analysis and are presented for each region in the bar charts. In both the calcarine and LOC, uniform activation in all sessions was shown, along with significant effects of menstrual phase (p = 0.018 and p = 0.035, respectively) and prandial state (p < 0.001 and p = 0.004, respectively). In the calcarine, activation in follicular fast was greater compared with follicular fed (**p = 0.01) and activation for luteal fast (**p = 0.004) was greater than luteal fed (p = 0.003). In the LOC, activation in follicular fast was greater compared with follicular fed (**p = 0.05) and activation in luteal fast compared with luteal fed (**p = 0.01).

Figure 3. Activation of reward and frontal brain regions in females on OCPs compared with those with regular cycles. Food vs control contrasts are shown. In the amygdala (a), a significant effect of menstrual phase/ OCP use was shown (p < 0.01), with activation of OCP fast greater than follicular fast (p = 0.001) and OCP fed compared with follicular fed (p = 0.009). In the putamen (b), activation of OCP fast was greater compared with follicular fast (*p = 0.01). In the prefrontal cortex (PFC) (c), activation for OCP fast was greater than follicular fast (**p = 0.004).
Activation patterns in the OCP group

- No substantial differences in ANY brain region were evident between the two visits
- Brain activation patterns in OCP group resembled luteal phase responses of those with regular cycles across all brain regions tested
- Analysis of variance including state and hormonal phase showed no significant effect in hypothalamus
- OCP group activation pattern was similar to the activation pattern in the luteal phase of females with regular cycles and both significantly greater than in the follicular phase
- No significant differences were found when evaluating the fast and fed state
Relation of brain region activation patterns and hormonal/biochemical parameters

- DHEAS significantly correlated with the LOC region (P=0.006), amygdala (P=0.006), and caudate (P=0.009)

- Testosterone associated with amygdala activation (P=0.02) and ventral tegmental area (P=0.01)

- Cortisol concentration significantly correlated with amygdala (P=0.004), insula (P=0.02), caudate (P=0.004), and prefrontal cortex activation (P<0.001)

- Fasting insulin showed significant associations with activation of amygdala (P=0.003), putamen (P=0.01), caudate (P=0.001), hippocampus (P=0.01) and LOC (P=0.02)
Findings

- Cycle phases differ in brain activation in response to food cues
  - *Brain region associated with homeostasis, reward system, executive frontal areas, and afferent visual areas are activated to a different degree during the luteal phase compared with the follicular phase*

- Females on monophasic OCPs (permanently elevated progesterone) - activation patterns in response to food cues are uniform and similar to the activation pattern of luteal phase in women with natural cycles
  - *This hints that sex hormones may be involved in increased caloric intake in luteal phase*

- Progesterone peaks in luteal phase
  - *Progesterone - appetite stimulant, inhibits effects of estrogen, and promotes lipogenesis*

- Tight relation of progesterone levels and reward region activation in females

- Estrogen/progesterone receptors are highly expressed in hypothalamus, amygdala, insula, hippocampus
  - *Activation of reward region may predict high-calorie food choices*
  - *Only high-calorie stimuli induced activation during luteal phase*
Limitations

- 2 groups of females were studied
  - Alternative: single group of normally cycling females that were later put on OCPs
- OCP group only included 11 subjects
- Unable to test plasma levels of synthetic sex hormones of the OCPs
  - Relied on published data on concentrations
- Lean females included in study
  - Need additional studies across the spectrum of adiposity levels
Conclusions

- Hormonal mechanisms may affect the responses of women’s homeostatic, reward/emotional, and attentional brain regions to food cues.
- These findings need further investigation to evaluate eating behavior and caloric intake throughout the menstrual cycle
  - This could have important impact on how diet regimens are developed in the future.
Thank you!
Is It Me or My Hormones? Neuroendocrine Activation Profiles to Visual Food Stimuli Across the Menstrual Cycle

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1Department of Human Metabolism and Nutrition, Braun School of Public Health, Hebrew University, Jerusalem 91120, Israel; 2Functional MRI Unit, Neurology Department, Hadassah Medical Center, Jerusalem 91120, Israel; and 3In Vitro Fertilization Unit, Department of Obstetrics and Gynecology, Hadassah Medical Center, Jerusalem 91120, Israel

Context: Homeostatic energy balance is controlled via the hypothalamus, whereas regions controlling reward and cognitive decision-making are critical for hedonic eating. Eating varies across the menstrual cycle peaking at the midluteal phase.

Objective: To test responses of females with regular cycles during midfollicular and midluteal phase and of users of monophasic oral contraception pills (OCPs) to visual food cues.

Design: Participants performed a functional magnetic resonance imaging while exposed to visual food cues in four time points: fasting and fed conditions in midfollicular and midluteal phases.

Patients: Twenty females with regular cycles and 12 on monophasic OCP, aged 18 to 35 years.

Main Outcome Measures: Activity in homeostatic (hypothalamus), reward (amygdala, putamen and insula), frontal (anterior cingulate cortex, dorsolateral prefrontal cortex), and visual regions (calcarine and lateral occipital cortex).

Setting: Tertiary hospital.

Results: In females with regular cycles, brain regions associated with homeostasis but also the reward system, executive frontal areas, and afferent visual areas were activated to a greater degree during the luteal compared with the follicular phase. Within the visual areas, a dual effect of hormonal and prandial state was seen. In females on monophasic OCPs, characterized by a permanently elevated progesterone concentration, activity reminiscent of the luteal phase was found. Androgen, cortisol, testosterone, and insulin levels were significantly correlated with reward and visual region activation.

Conclusions: Hormonal mechanisms affect the responses of women’s homeostatic, emotional, and attentional brain regions to food cues. The relation of these findings to eating behavior throughout the cycle needs further investigation. (J Clin Endocrinol Metab 102: 3406–3414, 2017)

Food consumption and eating behavior are driven by a complex interaction of hormones and metabolites in multiple brain areas (1) influenced by homeostatic as well as hedonistic signals. Homeostatic cues may signal positive or negative energy balance, whereas hedonistic cues indicate palatability and reward (2). The brain faces multiple hormonal and biochemical signals from organs, but also nonhomeostatic signals from the environment, and coordinates caloric intake and energy expenditure based on the integration of these cues (3). Brain regions

Abbreviations: BMI, body mass index; CRP, C-reactive protein; fMRI, functional magnetic resonance imaging; HDL, high-density lipoprotein; LOC, lateral occipital cortex; OCP, oral contraception pill; ROI, region of interest; TE, echo time; TR, repetition time.

*These authors contributed equally to this study.
involved in homeostasis as well as reward responses have sex hormone receptors for estrogen and progesterone, making these hormones’ active participants in the coordination of eating behavior via affecting gene expression of relevant molecules (4–6).

Studies in primates and humans have shown that eating patterns vary across the menstrual cycle, reaching a peak at the midluteal phase compared with the follicular phase (7, 8). Such fluctuations in food intake in humans reach on average ~250 kcal/d, yet may exceed 500 kcal/d (9), and are considered part of the attempt to meet the needs of the possible pregnancy. However, the physiological mechanism of this phenomenon is unknown. Because obesity is considered the epidemic of the 21st century (10) and because the majority of dietary regimens eventually fail in the long run (11), there is growing interest in unraveling mechanisms that drive and modulate eating behavior (7). To unravel mechanistic insights into the differential eating patterns during the menstrual cycle, we tested hormonal levels and functional magnetic resonance imaging (fMRI) brain responses to visual food cues of lean females with a regular cycle during midfollicular and midluteal phases (12). An additional group of females on monophasic oral contraception pills (OCPs), characterized by a stable elevated progesterone analog level reminiscent of the luteal phase of regularly cycling females, was studied as well. Activity in homeostatic, reward, and decision-making regions as well as in afferent (visual) cortex was assessed. The responses were evaluated in two prandial states (fasting and fed (“fed” representing the period following glucose ingestion).

We postulated that unique brain pattern activity mediated by the hormonal profile may explain the different eating behavior along the menstrual cycle. We postulated that sex hormones, specifically progesterone, modulate brain reward region activation that will be increased in the luteal phase. Furthermore, we anticipated the presence of uniform brain reward activation throughout the cycle in females on monophasic oral contraception, that will be similar the luteal phase of regularly cycling females.

Methods

Subjects

Twenty females with regular cycles and 12 taking monophasic OCPs were recruited. Inclusion criteria for both groups were age (18 to 35 years), stable body weight for the past 3 months, body composition parameters indicating normal weight without increased relative adiposity, not being on an active weight loss–oriented dietary regimen, nonsmoking, right-handed, and off any medications. Participants in the menstrual group had a regular cycle per self-report and were not taking any medications that may affect sex hormone metabolism or mood. Participants in the OCP group had been taking monophasic OCP for longer than 6 months, using either Feminet or Mercilon (both containing ethynyl estradiol 0.02 mg/desogestrel 0.15 mg). Monophasic OCPs suppress endogenous estrogen and progesterone and achieve a progestin to estrogen ratio reminiscent of the natural luteal phase (13). Importantly, the hormonal compounds in these OCPs penetrate the blood brain barrier and bind to the receptors of the endogenous hormones (14, 15). The Hadassah Hebrew University Medical Center Ethics Committee approved the experimental procedure. Written informed consent was obtained from all participants.

Procedure

Each participant in the menstrual group underwent two separate visits in midfollicular and midluteal phase. Midfollicular phase was defined as within days 7 to 12 of the cycle and midluteal phase is defined as days 20 to 26 of the cycle. To eliminate an effect of order of cycle phase, the order of scanning was randomized. To control for effects of circadian rhythm, time of testing was uniform for all participants (10 AM to 2 PM). For control purposes, participants in the OCP group underwent two separate visits both in active pill use using the same time intervals as for the regular cycle group. Each session consisted of two MRI scans in prandial states (“fast” and “fed”). The first scan was after an overnight fast of 10 hours in basal conditions (fast state). The second scan began within 20 to 30 minutes after glucose ingestion of 75 g (300 kcal) (Geffen Medical, Israel) (postprandial state). Blood for biochemical evaluation was collected at arrival and 15 to 20 minutes after glucose ingestion. Blood was collected in chilled tubes, immediately centrifuged, and plasma was stored at ~70°C.

MRIs and blood samples were collected for each group in four time points to evaluate the effects of cycle phase and prandial state and their interaction: (1) follicular fast, (2) follicular fed, (3) luteal fast, and (4) luteal fed (with an identical sampling procedure for the two visits of the participants on OCPs).

Anthropometric and metabolic assessments

On arrival, anthropometric measurements of height and weight were recorded. Subjects filled out questionnaires regarding demographic characteristics, eating attitudes, and behavior [using the Eating Attitudes Test (EAT-26)] (16) and hunger state. The EAT-26 is a commonly implemented screening tool used to measure symptoms and characteristics of eating disorders. A score >20 is an indication of eating disorder pathology (16). Participants completed a visual analog scale (score range −5 to +5, with negative values indicating greater hunger) to confirm state of hunger. Opposite ends of the continuum labeled “not hungry at all” and “extremely hungry.” Participants indicated the point on the line that reflected their current state of hunger.

fMRI data acquisition

Functional and anatomical images were acquired on a 3T Siemens scanner. Blood oxygen level–dependent contrast was acquired with a gradient-echo echo planar imaging sequence using a 12 voltage-channel standard head-coil. To maximize signal in deep brain structures, an advanced shim was implemented before scanning and 6/8ths partial Fourier was used to increase resolution. Images were scanned in the axial position, anteroposterior orientation, repetition time (TR) = 3 seconds,
fMRI paradigm

In each session, participants were scanned while viewing images of high-calorie sweet foods (e.g., chocolate, cake), high-calorie savory foods (e.g., pizza, lasagna), and nonfood objects not associated with eating (e.g., computer, hammer). Visual food cues are established as a valid means to measure neural processing reactions to food behavior (12). Images were collected from open sources on the Internet. Two sets of images were created. Different sets were used for the fed and fast states, counterbalanced between subjects. The same sets were repeated in the next visit. Images in each category were presented in a counterbalanced order in six blocks (108 images each run, 36 images per category), each image appearing for 2.5 seconds with a 0.5-second interstimulus interval. Blocks lasted 18 seconds, followed by a 9-second fixation. Images were presented via a projector onto a screen situated at the far end of the MRI. Images were presented with Presentation software (http://www.neurobs.com/presentation). Within the MRI, subjects were instructed to think if they wanted what they saw in the visual images and to indicate yes/no by means of the response box to determine reward of food images and maintain attention.

fMRI data analysis

Data analysis was performed with BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands, 2000), version 2.8. Preprocessing included slice time correction, head motion correction, and high-pass temporal filtering in the frequency domain (three cycles/total scan time) to remove drifts and to improve the signal-to-noise ratio. The complete dataset was transformed into the Talairach coordinates, 2-normalized, and concatenated (17). The changes in the blood oxygen level–dependent contrast associated with the performance of the visual cues were assessed on a pixel-by-pixel basis using the general linear model (18); the hemodynamic response function was modeled using standard parameters (19).

Analysis was conducted in two approaches. First, activation t-maps were defined by contrasting the food images with the control objects to identify response specific for food. Maps are presented in random effect, $P$ values of .05. To correct for multiple comparisons, effects are reported only for clusters larger than threshold estimator based on Monte Carlo analysis ($P < 0.05$).

Second, regions of interest (ROIs) were anatomically defined to include regions known to be part of the food motivation and reward system as well as visual regions and included:

1. Hypothalamus as a representative of homeostasis
2. Amygdala, putamen, and the insula as representing brain regions associated with the reward response
3. Anterior cingulate cortex, prefrontal cortex, and dorso-lateral prefrontal cortex as representative of motivation and decision-making in frontal regions
4. Calcarine (V1) and the lateral occipital cortex (LOC) representing early and high visual regions (20)

These anatomically defined regions were limited to the noteworthy voxels in the group map for the contrast of food vs objects regardless of cycle and prandial states (ROI details are described in Supplemental Table 1). This was done separately for the menstrual and OCP groups. Regions from the right and left hemispheres were averaged. From these regions, beta values were extracted for each subject in each time point.

Although the maps allowed a broad viewpoint of the activation patterns, statistical claims were based on the direct comparison between the different phases and states done in the ROI analysis.

Biochemical analyses

The metabolic and hormonal profiles of participants were sampled in both fast and fed states in the follicular and luteal cycle phases and in the OCP group. Fasting samples were drawn for insulin, progesterone, estrogen, dehydroepiandrosterone sulfate, cortisol, triglycerides, C-reactive protein (CRP), high-density lipoprotein (HDL)-cholesterol, and leptin. Sex hormone levels were used to verify menstrual phase and establish a correlation between menstrual phase, sex hormones, and reward region activation. Glucose and insulin were sampled at fasting and 20 minutes following glucose ingestion. Glucose was measured in whole blood using a HemoCue glucose analyzer (Hemocue America, Brea, CA). Insulin, leptin, and sex hormones were measured using commercial enzyme-linked immunosorbent assays.

Statistical analysis

Data are presented as means ± standard deviations. Our preliminary data in 10 lean females indicated that in the insula (a representative reward region), the beta activation in fasting in the midluteal and midfollicular phases in response to high carbohydrate food image contrasts were $0.129 \pm 0.048$ vs $0.083 \pm 0.045$, respectively. Assuming this indeed is the difference and the standard deviation is set at 0.05, with an $\alpha$ of 0.05 and power of 80%, we would be able to show a substantial difference using 16 subjects (power of 90% will demand 20 subjects). Because we planned to compare multiple regions as secondary end points, we chose a sample size of 20 participants with regular cycles. We used 12 participants on OCPs as a comparator group. The results were first analyzed for the main effects of cycle phase (follicular vs luteal) and prandial state (fast vs fed) using analysis of covariance. Among those that yielded statistically significant results, post hoc multiple comparisons were performed between paired tests for cycle phase and prandial states and adjusted using the Bonferroni correction. Simple Pearson correlations were used to test relations between parameters. The analysis was performed using SPSS 20.0.

Results

Effects of menstrual phase and prandial state in normal cycling females

In the menstrual group, one participant withdrew during the first scan because of feelings of claustrophobia and another did not complete the scan because of
technical problems at the imaging center. In the OCP group, one participant was excluded because of high ratings on the EAT-26 scale (>20), suggesting the presence of disordered eating. Thus, the results of 18 healthy females with regular cycles and 11 females on monophasic oral contraception were used for the final analysis.

As shown in Table 1, participants in both groups were of normal weight and body mass index (BMI) with no important anthropometric differences between them except for the slightly greater height of participants with regular cycles compared with OCP users \( (P = 0.002) \). There were no reported eating disorders as determined by the EAT-26 questionnaire (average EAT-26 score of 5 for both groups). There were no important group or time effects in comparisons of hunger or valence ratings. Specifically, the hunger scale ratings were \(-2 \pm 2\), \(-3 \pm 2\) for follicular and luteal fast, respectively, and \(3 \pm 5\) and \(2 \pm 5\) for follicular and luteal fed, respectively. Thirteen of 18 participants reported feelings of “wanting” toward images of sweet food in the follicular and luteal fast states, respectively, whereas 10 of 18 and 11 of 18 reported feelings of “wanting” of sweet food in the follicular and luteal fed states \( (p_X^2 = 0.73) \). These results indicate that potential group/time effects were not the result of different degrees of hunger or the subjective desire to eat between the groups or across the time points tested.

Hormonal analysis verified that the self-report of each subject in the menstrual group suited her actual hormonal state. As expected, among females with normal cycles, progesterone levels were significantly greater in the luteal compared with the follicular phase \( (P = 0.001) \), whereas estrogen levels tended to be higher as well, reaching marginal significance \( (P = 0.07) \). Endogenous estrogen and progesterone levels were suppressed and undetectable, as expected in females on OCPs.

As shown in Table 2, the luteal phase was characterized by a higher fasting insulin compared with the follicular phase \( (P = 0.04) \), whereas no difference was observed in the fed state between cycle phases. No substantial differences were observed between other hormonal or biochemical parameters between the follicular and luteal phase. Interestingly, cortisol levels tended to be higher in OCP users \( (P = 0.036) \). Similarly, DHEA \( (P = 0.024) \), triglycerides \( (P < 0.001) \), CRP \( (P < 0.001) \), and HDL-cholesterol \( (P < 0.001) \) concentrations were higher in OCP users.

### Activation patterns in the menstrual group

The relevant axial projections of the maps created for each state and phase are presented for each of the ROIs (Figs. 1 and 2). The full description of activated regions is presented in Supplemental Table 2. Direct comparison between the phases and states was done in the ROI analysis shown in the bar charts.

Activation was identified in the hypothalamus only in the luteal fast state [Fig. 1(a)]. Reward regions (amygdala, putamen, and insula) and frontal regions (anterior cingulate, prefrontal and dorsolateral prefrontal cortices) showed a substantial effect of hormonal phase, but not of prandial state. This was apparent in both map analysis and ROI analysis [Fig. 1(b–d)]. Visual regions (calcarine and LOC) showed a relevant dual effect of prandial state and menstrual phase in both regions, with no substantial interaction (Fig. 2). Of note, the interaction of cycle phase and prandial state was not statistically significant in all of these comparisons.

### Activation patterns in the OCP group

No substantial differences in any brain region were evident between the two visits in the OCP group (Supplemental Fig.). We thus averaged the data from both visits for further analysis.

Brain activation patterns in the OCP group resembled the luteal phase responses of those with regular cycles across all brain regions tested. Analysis of variance including state and hormonal phase (follicular, luteal, or OCP) showed no significant effect in the hypothalamus. In the amygdala [Fig. 3(a)], a significant phase effect \( (P = 0.01) \) was found. Post hoc analysis showed that OCP group activation pattern was similar to the activation pattern in the luteal phase of females with regular cycles and both significantly greater than in the follicular phase. Even in the post hoc analysis, evaluating fast and fed state separately, no significant differences were found between the luteal and OCP groups. In other reward and frontal regions, a similar activation pattern was observed [Fig. 3(b) and 3(c), respectively]. A similar impact of hormonal phase \( (P = 0.02) \) was also observed in both visual areas, with a significant effect of prandial state \( (P < 0.001) \). Of note, the interaction of cycle phases (follicular vs luteal vs OCP users) and prandial state was not statistically significant in all of these comparisons.

### Table 1. Study Participants’ Demographic and Anthropometric Data

<table>
<thead>
<tr>
<th></th>
<th>Menstrual (n = 18)</th>
<th>OCP (n = 11)</th>
<th>P Value</th>
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<tr>
<td>Age (y)</td>
<td>25 ± 3</td>
<td>26 ± 2</td>
<td>0.85</td>
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<tr>
<td>Height (cm)</td>
<td>165 ± 5</td>
<td>161 ± 4</td>
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<td>Weight (kg)</td>
<td>61 ± 8</td>
<td>60 ± 9</td>
<td>0.73</td>
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<td>BMI (Kg/m²)</td>
<td>22 ± 3</td>
<td>23 ± 3</td>
<td>0.37</td>
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<td>BMR (kcal)</td>
<td>1423 ± 81</td>
<td>1418 ± 87</td>
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<td>Body fat (%)</td>
<td>27 ± 6</td>
<td>26 ± 6</td>
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<tr>
<td>Fat mass (kg)</td>
<td>17 ± 6</td>
<td>16 ± 6</td>
<td>0.41</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>44 ± 3</td>
<td>44 ± 4</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Abbreviation: BMR, basal metabolic rate.
Table 2. Biochemical Parameters of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>Follicular Phase (n = 18)</th>
<th>Luteal Phase (n = 18)</th>
<th>OCP Users (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>82 ± 6</td>
<td>84 ± 9</td>
<td>79 ± 8</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>39 ± 3</td>
<td>54 ± 4*</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>Fed glucose (mg/dL)</td>
<td>110 ± 2</td>
<td>114 ± 15</td>
<td>115 ± 8</td>
</tr>
<tr>
<td>Fed insulin (pmol/L)</td>
<td>283 ± 256</td>
<td>270 ± 158</td>
<td>457 ± 255</td>
</tr>
<tr>
<td>Progesterone (nmol/L)</td>
<td>2.14 ± 1.27</td>
<td>20.15 ± 20.10p</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>150 ± 231</td>
<td>195 ± 242</td>
<td>Undetectable</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>1.07 ± 0.24</td>
<td>1.09 ± 0.25</td>
<td>1.05 ± 9</td>
</tr>
<tr>
<td>DHEA-S (μmol/L)</td>
<td>3.45 ± 2.14</td>
<td>3.96 ± 1.84</td>
<td>5.03 ± 1.85c</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 1.9</td>
<td>1.2 ± 0.3c</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>0.1 ± 0.05</td>
<td>0.1 ± 0.2</td>
<td>0.6 ± 0.5c</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1 ± 0.3</td>
<td>1 ± 0.2</td>
<td>1.7 ± 0.4c</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>211 ± 85</td>
<td>268 ± 128</td>
<td>577 ± 143c</td>
</tr>
</tbody>
</table>

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; TG, triglycerides.

*P = 0.004 for luteal vs follicular.

#P < 0.001 for luteal vs follicular progesterone.

*P < 0.001.

Relation of brain region activation patterns and hormonal/biochemical parameters

The two cycle phases differ in some but not all of the profiles of several hormones and biochemical parameters. To decipher the individual contribution of specific hormones to the cycle phase associations described earlier, we evaluated their relation with ROI activation patterns. We used samples from the OCP users as representative of the luteal phase along with samples from the follicular phase of regularly cycling participants.

Dehydroepiandrosterone sulfate was significantly correlated with the LOC region (r = 0.49, P = 0.006), amygdala (r = 0.50, P = 0.006), and caudate (r = 0.47, P = 0.009). Testosterone was associated with amygdala activation (r = 0.42, P = 0.02) and ventral tegmental area (r = 0.47, P = 0.01). Interestingly, cortisol concentration was significantly correlated with amygdala (r = 0.53, P = 0.004), insula (r = 0.42, P = 0.02), caudate (r = 0.53, P = 0.004), and prefrontal cortex activation (r = 0.70, P < 0.001).

Fasting insulin showed significant associations with activation of the amygdala (r = 0.54, P = 0.003), putamen (r = 0.43, P = 0.01), caudate (r = 0.59, P = 0.001), hippocampus (r = 0.47, P = 0.01), and the LOC (r = 0.41, P = 0.02). Partial correlations adjusting for BMI and percent body fat demonstrated a mild attenuation of these associations, which remained statistically significant except for the one with LOC. Postprandial insulin concentrations were not correlated with brain activation patterns. BMI and percent body fat, even within the narrow normal range of these study participants, correlated with activation of the calcarine region (r = 0.36, P = 0.006; r = 0.41, P = 0.002).

Discussion

The discrepant caloric intake behavior in females throughout the menstrual cycle is well known, yet mechanistic explanations for this phenomenon are lacking (7). In this study, we show that cycle phases differ in brain activation in response to food cues. We show that not only brain regions associated with homeostasis, but also the reward system, the executive frontal areas, and the afferent visual areas are activated to a different degree during the luteal compared with the follicular phase in females with regular cycles. However, whereas in the reward and frontal regions, differential activation along the menstrual cycle appeared regardless of prandial state, in the visual regions, a dual effect of prandial state and menstrual phase was observed. Moreover, we show that in females on monophasic OCPs, characterized by a permanently elevated progesterone concentration, activation patterns in response to food cues are uniform and similar to the activation patterns of the luteal phase in females with natural cycles. These observations, along with the correlations between hormonal levels and specific brain activation profiles, may hint to the involvement of sex hormones in modulating increased caloric intake during the luteal phase.

Obesity is becoming a major public health problem and has been declared a “worldwide epidemic” (10, 21). In parallel, there is an increased demand for effective and sustainable dietary regimens to address this major public health problem. Dieting is specifically common among young women and it is estimated that 40% to 50% of women are on a diet at any given time (22). Importantly, the long-term success rates of dietary interventions combined with behavioral modification, even in intensive
clinical trials, is very modest (11), and less than 25% of weight loss is sustained at 5 years (23). Because women have more difficulty losing weight (24), it is crucial to identify the molecular modifiers of eating behavior: the obvious candidates are sex hormones. Specifically, progesterone, peaking in the luteal phase, which has appetite-stimulating effects, inhibits the effect of estrogen and promotes lipogenesis (25) and may explain these observations. And indeed, our data clearly demonstrate a tight relation of progesterone levels and reward region activation in females, as seen by the difference of phase in normal cycles and the uniform activation across the cycle in those on OCPs as well as in simple correlations. Note further that estrogen and progesterone receptors are highly expressed not only in the hypothalamus, but also in brain areas involved in emotion and cognition, such as the amygdala, ventral tegmental area, insula, and hippocampus (4, 26, 27). As activation of specific brain regions shown in this analysis, such as the amygdala and insula, has been shown to predict high-calorie food choices (28), these findings may potentially have implications regarding caloric intake and eating behavior. Similar to our study results, Frank et al. (29) also highlighted the impact of sex hormones on reward region activation during females’ menstrual cycle. Using a different visual stimulus pattern of high- and low-calorie food images, they showed that only the high-calorie stimuli induced activation during the luteal phase.

The primary and secondary visual cortical regions presented a phase-dependent differential activation pattern that was even more interesting. In addition to the substantial effect of the menstrual phase, the prandial state also affected brain activation patterns. Satiety is known to influence the visual region (29). The menstrual

Figure 1. Activation of the hypothalamus, amygdala, insula, and ACC in response to visual food cues. Activation maps and food vs control image contrasts are shown. Activation patterns in the follicular and luteal phases were analyzed in the fast and fed states. Statistical values were based on the ROI analysis and are presented for each region in the bar charts. In the hypothalamus (a), uniform activation was detected in the luteal fast state with fasting luteal activation being significantly greater than fasting follicular (*P = 0.02). In the amygdala (b), a significant effect of menstrual phase with luteal showing greater activation than follicular was noted (P = 0.001). Activation in luteal fast (**) was significantly greater than follicular fast (P = 0.009) and activation for luteal fed (***) was significantly greater than follicular fed (P = 0.007) in the amygdala for the contrast of food vs objects. In the insula (c), a similarly significant effect of menstrual phase was detected (P = 0.05). Activation at luteal fast was significantly greater than follicular fast (ΔΔP = 0.03). In the ACC (d), a significant effect of the menstrual phase was detected (P = 0.012). Activation in luteal fast was significantly greater than follicular fast (ΔΔΔP = 0.02) and activation in luteal fed was significantly greater than follicular fed (ΔΔΔP = 0.04). ACC, anterior cingulate cortex.
phase effect is in agreement with the known effect of sex hormones on visual regions (30, 31). van der Laan et al. (32) have shown in a meta-analysis of functional neuroimaging studies that the LOC as well as the insula and the lateral orbitofrontal cortex were the most concurrent brain regions activated in response to visual food stimuli. They speculated that the consistent activation of the LOC in response to visual food cues that are emotionally salient leads to heightened attention and thereby more extensive visual processing (33). The amygdala and the anterior cingulate, which are known to project back to the visual cortex, have been proposed as the mediators of this process (34). In our study, similar effects were found also in lower visual areas (calcarine), thus extending these “top-down” effects even further. Alonso-Alonso et al. (35) also reported different patterns of visual cortical activity along the menstrual cycle in response to visual food stimuli. Looking at early and late follicular phases (low vs high estrogen), they suggested that estradiol may reduce food intake by decreasing sensitivity to food cues in the ventral visual pathway under conditions of energy deprivation. From a different viewpoint, insulin showed an association with visual region activation in our lean subjects and has previously been shown to be associated with activation of corticolimbic regions in response to visual food cues in obese individuals but not in lean ones (36, 37), suggesting again a potential effect mediated by homeostatic factors on attentiveness along cortical hierarchy.

Limitations of this study include that two groups of females were studied instead of a single group of normally cycling women later put on OCPs. The OCP group included only 11 subjects but, because the results were based on two statistically indifferent scans for each subject, we believe those results are solid. We were not able to sample plasma levels of the synthetic sex hormones of the OCPs and had to rely on the published data of their concentrations. That endogenous sex hormones were totally suppressed in all participants (below the level of detection of the assay) indicates that compliance of the OCP was adequate. Despite studying a population of young women with BMI within the normal lean range, we
we do not expect this to reflect on our results. Finally, it is important to emphasize that, in the current study, regional brain differences were reported, and the reverse inference from brain activation to cognitive states is only speculative.

To conclude, our findings imply that hormonal mechanisms may affect the responses of women’s homeostatic, emotional, and attentional brain regions to food cues. The relation of these findings with eating behavior and caloric intake throughout the cycle need further investigation. Such findings may have implications on the design of future diet regimens based on the menstrual cycle rather than on a generic approach.

**References**


13. Siekmann L, Siekmann A, Bidlingmaier F, Bril K, Albringer M. Gestodene and desogestrel do not have a different influence on


