Supplementary Electronic Appendix, accompanying article: Physiological Predictors of Leptin Vary During Menses and Ovulation in Healthy Women, by K.E. Sylvia, T.K. Lorenz, J.R. Heiman & G.E. Demas; under consideration.

Demographics and Health Measures

At the first laboratory session, participants completed a demographics survey, including information about age at first menstrual period (menarche), birthdate (from which exact age on the day of the study session was calculated), racial/ethnic background, and level of education.

At both laboratory sessions, participants completed a battery of surveys about their health behaviors. Dieting was assessed via yes/no screener ("are you currently dieting or restricting your food or calorie intake?"), as was their subjective weight stability over the last week ("has your weight stayed within about 5 pounds within the last week?"). We also assessed relative spacing of meals, which may influence leptin production [1,2]. Number of meals per day and number of missed/skipped meals per week were assessed with a Likert response scale ranging from 1 – 4+ meals/day, and 1 – 3+ meals/week, respectively.

A broad assessment of dietary quality in the last week was also assessed. These measures included number of days in the last week in which the participant had 5 servings of fruit and vegetables, and the number of servings of omega-3 rich foods, anti-oxidant rich foods, and meat per week. For specific food categories (e.g., omega-3 rich foods), participants were provided with a list of examples of foods within the category and what constitutes a serving. Finally, we assessed number of alcohol units consumed in the last week; a unit of alcohol was defined according the standards set by the US Dietary Guidelines [3].

Sexual activity was tracked via time-stamped self-report diaries; from these, we found that women reported the frequency of intercourse at menses as at ovulation ($F(1,3) = 0.08$, $p = 0.96$).

Energy expenditure was assessed via computer-administered version of the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH; [4]). The SQUASH is a validated, structured assessment of physical activity over the last week, including separate indices for light or incidental activity (such as walking or light household work) and intense exercise (such as running). The SQUASH has been cross-validated with accelerometers and computerized activity monitors, and shown excellent test-retest reliability and reproducibility [4]. Scores on the SQUASH reflect the week's sum total number of minutes spent on each task, scaled by relative intensity of that activity (i.e., the metabolic equivalent of task). Hours spent sleeping and sleep quality was assessed via the Pittsburgh Sleep Quality Index (PSQI; [5]), a well-validated measure used in both healthy and clinical populations [6].

Finally, immediately prior to complete saliva and blood samples, participants completed a questionnaire about their day, including time at waking and time of last meal. Each questionnaire was marked for time at sample collection; from these data, hours since waking and hours since last meal were calculated.

Model Parameters

- Sexual Activity Group (Abstinent or Active)
- Menstrual Phase (menses or ovulation)
- Fasting (hours since last meal at time of blood/saliva sampling)
- Awake (hours since waking)
- Cortisol (salivary, ug/mL)
- Leptin (serum, pg/mL)

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- Body Mass Index (BMI)
- Body Fat Percentage
- Estradiol (salivary, pg/mL)
- Progesterone (salivary, pg/mL)
- Age (years)
- Race
- Age of Menarche (years)
- Dieting (yes or no)
- Weight stability
- Missed meals in last week
- Meals per day in last week
- Meals at home in last week
- Servings of Omega-3 foods in last week
- Servings of fruits and vegetables in last week
- Metabolic Equivalent of Task in last week (Physical Activity Score)
- Metabolic Equivalent of Task of intense exercise (not including incidentals like walking to work)

Serum and Saliva Collection and Assay

During laboratory visits, participants provided unstimulated saliva samples. Participants also provided whole blood samples via standard venipuncture; these samples were allowed to coagulate at room temperature for 30-40 minutes, spun down, and serum was drawn off. Saliva and serum samples were immediately frozen at -80C and stored frozen until assayed.

Saliva samples were assayed for progesterone and estradiol, and serum samples for leptin, with commercially available enzyme-linked immunosorbent (ELISA) kits and procedures recommended by the kit manufacturers (saliva kits: Salimetrics; serum kit: American Laboratory Products Company (Alpco)). Leptin assays were conducted in serum as there is not yet consensus as to the meaningful interpretation of salivary leptin; however, the validity of assays for salivary progesterone, and estradiol are relatively more robust [7]. Intra-assay and inter-assay coefficients of variance were 0.54% – 6.35%, and 2.24 – 11.12% respectively. Sensitivity limits for the assays were as follows: progesterone, 5.0 pg/mL; and estradiol, 0.1 pg/mL; leptin, 0.42 ng/mL.

Assessment of Demographics, Health Behaviors, and Other Factors

At both laboratory visits, participants were measured for anthropometrics including body composition using the Tanita Fitscale 585F. All participants completed urine tests for human chorion gonadotropin; no participant was found to have evidence of pregnancy during the study. All participants provided information on stress and health across the menstrual cycle by means of weekly surveys; no participant reported significant traumatic events, illness, or injury during the study period. Also, participants reported on symptoms experienced in the past week, including those commonly associated with premenstrual syndrome (PMS) such as bloating, acne, and irritability. Scores on this measure were significantly higher at menses than at ovulation ($F(1, 53.1) = 10.65$, $p = 0.002$). However, even at menses, participants reported an average score of

1.36, corresponding to being bothered by these symptoms between "not at all" to "several days" in the past week, suggesting lack of evidence for significant PMS in this sample.

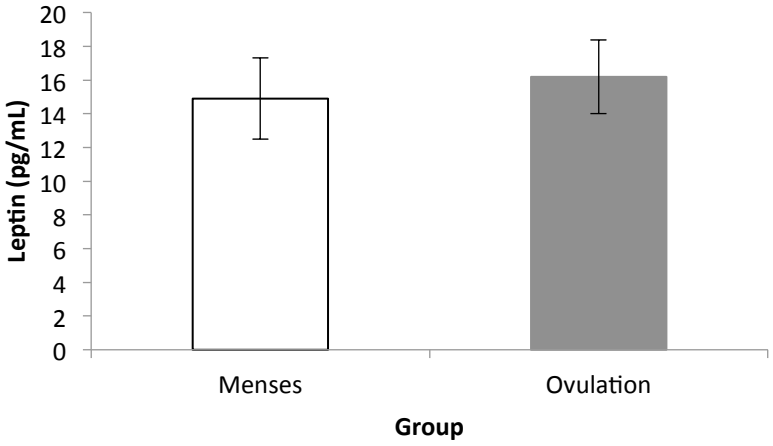
Statistical Analyses

To choose the model of best-fit, we used an iterative process in which we explored a number of models, by systematically adding or deleting explanatory variables and their interaction effects to determine a balance between parsimony of explanation and a good-fitting model [8]. We began by adding all off the potential variables into the model and then eliminating insignificant parameters. Once particular parameters were deleted they were added back into the model at a later point to verify the model was the best-fit (i.e., modified version of a stepwise regression).

Age, Body Mass Index (BMI), and Body Fat Percentage Comparisons

Sexually active and abstinent women were similar in terms of age (sexually active M =24.96, SD = 7.22; sexually abstinent M = 22.16, SD = 2.92, t(30) = 1.47, p = 0.151). They were also similar in terms of body fat percentage and body mass index. The mean percent body fat in sexually active females was 27.64%, (SD = 5.66), and in abstinent females, the mean percent body fat was 26.02% (SD = 8.67). In sexually active females, average BMI was 23.530 (SD = 3.192), and in sexually abstinent females, the average BMI was 23.960 (SD = 4.761). 6 women (18.75%) fell in the "overweight" range and 4 (12.5%) fell in the "obese" range of BMI, indicating this sample was less overweight/obese than national averages [9].

Figures



Supplementary Figure 1. Concentrations of leptin during menses and ovulation. There were no effects of treatment, time, or their interaction on leptin. Bar heights represent means ± SEM.

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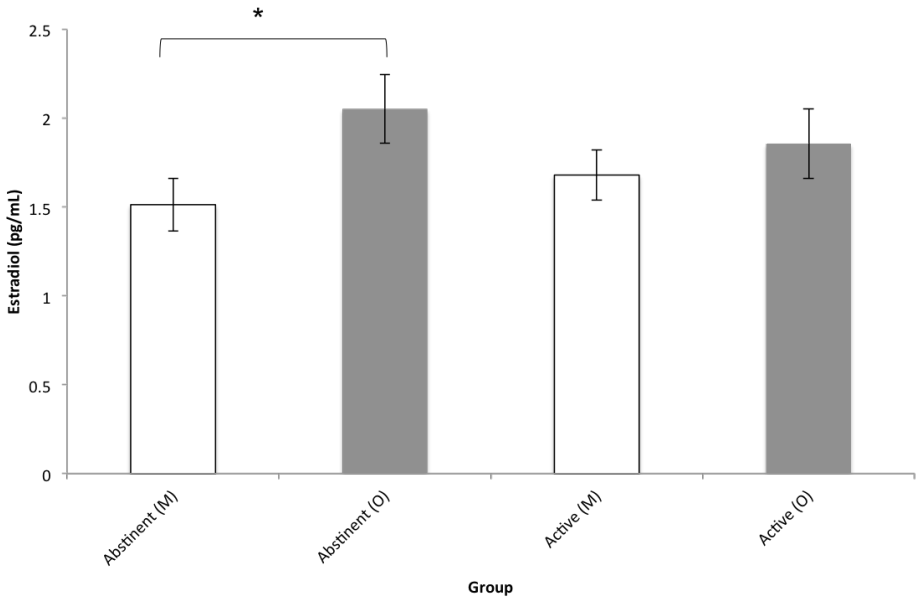

Supplementary Figure 2. Concentrations of estradiol during menses and ovulation, in abstinent and active women. Salivary estradiol was significantly higher during ovulation in abstinent women. Bar heights represent means ± S.E.M. An asterisk (*) indicates statistically significant differences between group means at $p < 0.05$.

References in Supplementary Index

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