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 (Carlson and others, 2007; Stote and others, 2007). 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 (US Department of Health & Human Services and US Department of Agriculture, 2015).

Energy expenditure was assessed via computer-administered version of the Short Questionnaire to Assess Health-enhancing physical activity (SQUASH; (Wendel-Vos and others, 2003). 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 (Wendel-Vos and others, 2003). 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; (Buysse and others, 1989), a well-validated measure used in both healthy and clinical populations (Carpenter and Andrykowski, 1998).

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)

Supplementary Electronic Appendix, accompanying article: <u>Physiological Predictors of Leptin Vary Across the Menstrual Cycle in Healthy Women</u>, by K.E. Sylvia, T.K. Lorenz, & G. E. Demas; under consideration at *The American Journal of Human Biology.*

- 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)

Figures
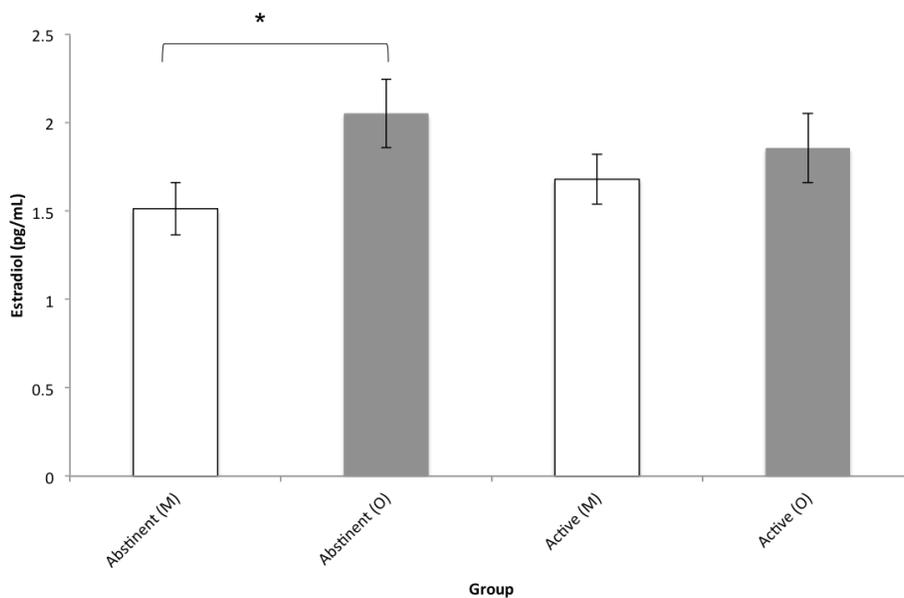
Supplementary Figure 1. Concentrations of estradiol during menses and ovulation, in abstinent and active women. Salivary estradiol was significantly higher during ovulation in abstinent women. Bar heights represent means ± S.E.M. An asterisk (*) indicates statistically significant differences between group means at $p < 0.05$.

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Tables

Table 1. Predictors of serum leptin levels at menses estimated using the best-fit model. Model AIC value = 178.749 (model > 2 AIC values less than other models). An asterisk (*) indicates statistically significant differences between group means at $p < 0.05$.

	Value	Std. Error	DF	t-value	p-value	Cohen's f^2
(Intercept)	140.514	32.365	15	4.341	0.001	
Age	0.101	0.412	15	0.245	0.810	-0.030
Age of Menarche	-0.668	2.009	15	-0.333	0.744	-0.028
Diet	-9.992	16.433	15	-0.608	0.552	-0.020
Estradiol	9.484	4.261	15	2.226	0.042*	0.117
Intense Exercise	-2.623	1.505	15	-1.743	0.102	0.062
Meals per Day	-10.846	4.786	15	-2.266	0.039*	0.122
Missed Meals	-10.145	4.379	15	-2.317	0.035*	0.128
Physical Activity	-5.939	2.623	15	-2.265	0.039*	0.122
Progesterone	-0.014	0.043	15	-0.321	0.753	-0.028
Sexual Activity	6.190	4.618	15	1.341	0.200	0.025
Weight Stability	-9.838	8.507	15	-1.156	0.266	0.010

Table 2. Predictors of serum leptin levels at ovulation estimated using the best-fit model. Model AIC value = 141.752 (model > 2 AIC values less than other models). An asterisk (*) indicates statistically significant differences between group means at $p < 0.05$.

	Value	Std. Error	DF	t-value	p-value	Cohen's f^2
(Intercept)	92.189	18.764	12	4.913	0	
Age	-5.265	1.943	12	-2.71	0.019*	0.22
Age of Menarche	-14.951	8.258	12	-1.81	0.095	0.084
Diet	-5.186	5.48	12	-0.946	0.363	-0.004
Estradiol	4.866	3.255	12	1.495	0.161	0.046
Intense Exercise	-0.86	1.347	12	-0.638	0.535	-0.023
Last Meal	-0.501	0.469	12	-1.068	0.306	0.005
Meals per Day	-13.732	3.383	12	-4.06	0.002*	0.48
Missed Meals	-9.208	3.634	12	-2.534	0.026*	0.19
Physical Activity	-0.401	1.857	12	-0.216	0.833	-0.037
Progesterone	-0.03	0.028	12	-1.068	0.307	0.005
Sexual Activity	8.64	2.475	12	3.492	0.004*	0.364
Weight Stability	1.235	5.916	12	0.209	0.838	-0.037

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