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Short communication

Physiological predictors of leptin vary during menses and ovulation in healthy women

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ABSTRACT

Although research has shown interactions between the reproductive system and energy homeostasis, it is not clear how environmental or behavioral factors may factor into these associations. Here we aimed to determine how changes in reproductive state (i.e., phase of the menstrual cycle) and other behavioral and physiological factors may influence leptin levels in healthy women, as well as how sexual activity may play a role in leptin modulation. We collected serum and saliva from 32 healthy women and measured leptin, estradiol, and progesterone. Participants also completed surveys of demographics, health and sexual behaviors, and physical activity. Leptin was predicted by meals per day and missed meals at both menses and ovulation. However, estradiol and physical activity were stronger predictors of leptin at menses, while sexual activity was a stronger predictor of leptin at ovulation. These findings suggest that predictors of serum leptin, and possibly energy storage and expenditure, vary across the menstrual cycle.

1. Introduction

Leptin, a hormone produced primarily by adipose tissue, acts as a signal to help regulate metabolism [1]. It serves a particularly important role as a signal of energy stores, which moderates food intake and physical activity [2]. Leptin also plays an important role in reproduction by stimulating hypothalamic release of gonadotropin-releasing hormone, the hormone responsible for stimulating downstream release of sex steroids from the gonads. For example, female mice that are deficient in leptin production genes are not only morbidly obese, they are also sterile; however, leptin treatment can restore their fertility [3]. Women with unexplained infertility have been found to have significantly lower serum leptin than case-control healthy fertile women [4], and high leptin levels can predict negative outcomes during assisted reproductive cycles [5]. There are leptin receptors present in tissues throughout the body, including in the brain and reproductive organs [6,7], further suggesting leptin may play a role in mediating reproduction.

Numerous studies have shown that in healthy pre-menopausal females, leptin levels are highest during the late follicular and luteal phases of the menstrual cycle and lowest during the early follicular phase [8,9], suggesting interactions with sex steroid hormones. However, the associations between leptin and sex hormones are not consistent across the literature. This may be because many studies have focused on the role of sex steroids alone in explaining the changes in leptin across the menstrual cycle, not accounting for the environmental or social context in which those hormones are released. Importantly, some research suggests a possible role for sexual activity on women's endocrine function. Low leptin may be linked to low sex desire in women [10], potentially through its interactions with melanocyte-stimulating hormone [11]. Although much research has shown interactions between the reproductive system and energy homeostasis, it is not clear how environmental or behavioral factors may factor into these associations. By using data collected from multiple time points during a menstrual cycle, we aimed to determine how changes in reproductive state (i.e., phase of the menstrual cycle) and other behavioral and physiological factors may influence leptin levels in healthy women, as well as how sexual activity may play a role in leptin modulation.

2. Materials and methods

The data presented here were collected as part of a larger study of the effects of sexual behavior on healthy women's immune and endocrine function across the menstrual cycle. See [12,13] for full details of

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the parent study; relevant aspects of the study are reviewed briefly below.

2.1. Participants

Study procedures were approved by the Indiana University Institutional Review Board, and participants provided informed consent. Thirty-five healthy women were recruited from the community; of these, three dropped out, leaving a final sample of $N = 32$. All participants reported regular menstrual cycles every 26–34 days. Exclusion criteria were any self-reported use of hormonal medications or immunoactive medications (e.g., antibiotics); pregnancy or lactation within the past 12 months; or history of sexual transmitted infections, significant gynecologic health conditions (e.g., endometriosis) or any medical condition with ongoing immune effects (e.g., cancer). Women reporting occasional ($< 1\times$/wk) use of over-the-counter antihistamines or analgesics were included.

Sexually abstinent participants ($N = 17$) included women who reported no partnered genital sexual activity within the past 3 months, although women with a lifetime history of sexual activity could be included. Sexually active participants ($N = 15$) included women who reported penile-vaginal intercourse at least once a week with only one male partner, who used either condoms or non-hormonal intrauterine devices. We did not include nor exclude participants from the sexually active group on the basis of non-intercourse activity (e.g., anal sex, oral sex). Based on a small literature suggesting some differences in diet and physical activity patterns between heterosexual vs. homosexual women [14–16] – including differences in *changes* in diet and physical activity over the menstrual cycle [17] – for this initial, exploratory analysis we considered only women who identified as heterosexual. Sexually active and abstinent women were similar in terms of age, body fat percentage, and body mass index (see Section 2.5 for more information).

2.2. Timing of laboratory sessions to menstrual cycle phase

To minimize the potential effect of circadian rhythms on women's leptin and other hormone measures, all data were collected in afternoon sessions (between 12 and 7pm). Participants completed two laboratory visits: within 2 days of onset of menstrual bleeding (the "menses" time point) and within 2 days of a spike in luteinizing hormone (LH), indicating likely ovulation (the "ovulation" time point). Date of ovulation was confirmed via daily tests for urine LH (Onestep Urine Ovulation Test; BlueCross Biomedical) [18,19]. One of the goals of the parent study was to follow changes in markers of inflammation [20]; given data suggesting that venipuncture may itself influence inflammation [21], serum samples were limited to the two most important defining features of the menstrual cycle, namely, menses and ovulation.

2.3. Serum and saliva collection and assay

During laboratory visits, participants provided unstimulated saliva samples, which were assayed for progesterone and estradiol. Participants also provided whole blood samples via standard venipuncture, assayed for leptin; these samples were allowed to coagulate at room temperature for 30–40 min, spun down, and serum was drawn off. Saliva and serum samples were immediately frozen at −80 °C 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 [22]. 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; estradiol, 0.1 pg/mL; and leptin, 0.42 ng/mL.

2.4. Assessment of demographics, health behaviors, and other factors

Participants completed a demographics survey and validated questionnaires on diet, physical activity, and other health behaviors (see http://hdl.handle.net/2022/21874 for more details).

2.5. Statistical analyses

We performed all statistical analyses in R v. 3.2.2 (R Core Team, 2015). Two-tailed *t*-tests were used to compare salivary estradiol and serum leptin levels across groups. Pearson's correlations were run on salivary estradiol and serum leptin levels across groups. We then used generalized linear mixed models (GLMMs), selecting the model that best fit the data using model comparison with Akaike's Information Criterion (AIC) (see http://hdl.handle.net/2022/21874 for information on model choice). For all analyses, we verified the assumptions of linear modeling via plots of fitted values vs. residuals.

We did not include body mass index (BMI) in our analysis, as serum leptin levels were highly correlated with individual BMI (menses: $r^2 = 0.647$, $p = 4.273e{-}07$; ovulation: $r^2 = 0.406$, $p = .0006$); including this variable would have resulted in significant collinearity and thus inaccurate model estimates [22–24]. Sexually active and abstinent women were, however, 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 had an average BMI of 26.143 (SD = 0.896), and 4 (12.5%) fell in the "obese" range and had an average BMI of 31.840 (SD = 2.127), indicating this sample was less overweight/obese than national averages [25]. The average BMI of women in the "normal" range was 22.006 (SD = 1.937). Women were also 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 = .151).

We used Cohen's f^2 as an index of effect sizes, and set our threshold for interpreting effect sizes as follows: very small, < 0.10; moderate, 0.10–0.20; large, 0.20–0.40; and very large, > 0.40 (equivalent to Cohen's d < 0.20; 0.20–0.50; 0.50–0.80, and > 0.80, respectively). We also reported *p*-values in Tables 1 and 2, however, because of our small sample size, these values should be considered less reliable estimates than effect sizes.

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 effect at $p < .05$.

	Value	Std. Error	DF	t-value	p-value	Cohen's f^2
(Intercept)	140.514	32.365	15	4.341	.001	
Age	0.101	0.412	15	0.245	.810	−0.030
Age of Menarche	−0.668	2.009	15	−0.333	.744	−0.028
Diet	−9.992	16.433	15	−0.608	.552	−0.020
Estradiol	9.484	4.261	15	2.226	.042*	0.117
Intense Exercise	−2.623	1.505	15	−1.743	.102	0.062
Meals per Day	−10.846	4.786	15	−2.266	.039*	0.122
Missed Meals	−10.145	4.379	15	−2.317	.035*	0.128
Physical Activity	−5.939	2.623	15	−2.265	.039*	0.122
Progesterone	−0.014	0.043	15	−0.321	.753	−0.028
Sexual Activity	6.190	4.618	15	1.341	.200	0.025
Weight Stability	−9.838	8.507	15	−1.156	.266	0.010