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Short communication

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

Kristyn E. Sylvia[a,b,*], Tierney K. Lorenz[b,c], Julia R. Heiman[b,c,d], Gregory E. Demas[a,b,c]

[a] Department of Biology, Indiana University, Bloomington, IN 47405, USA
[b] Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington, IN 47405, USA
[c] Kinsey Institute for Research on Sex, Gender, and Reproduction, Indiana University, Bloomington, IN 47405, USA
[d] Psychological and Brain Sciences, Indiana University, Bloomington, IN 47405, USA

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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

* Corresponding author at: Department of Biology, Indiana University, 1001 E. 3rd Street, Bloomington, IN 47405, USA.
E-mail address: ksylvia@indiana.edu (K.E. Sylvia).

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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

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 effect at $p < .05$.

	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	.019*	0.22
Age of Menarche	−14.951	8.258	12	−1.81	.095	0.084
Diet	−5.186	5.48	12	−0.946	.363	−0.004
Estradiol	4.866	3.255	12	1.495	.161	0.046
Intense Exercise	−0.86	1.347	12	−0.638	.535	−0.023
Last Meal	−0.501	0.469	12	−1.068	.306	0.005
Meals per Day	−13.732	3.383	12	−4.06	.002*	0.48
Missed Meals	−9.208	3.634	12	−2.534	.026*	0.19
Physical Activity	−0.401	1.857	12	−0.216	.833	−0.037
Progesterone	−0.03	0.028	12	−1.068	.307	0.005
Sexual Activity	8.64	2.475	12	3.492	.004*	0.364
Weight Stability	1.235	5.916	12	0.209	.838	−0.037

3. Results and discussion

The effect of time on leptin levels was very small and statistically non-significant ($t_{64.894} = 0.512=$, $p = .611$), indicating that there was no change in leptin from menses to ovulation. In the following models, we included individual subject as a random effect to account for individual differences. See Tables 1 and 2 for parameter estimates and model fit parameters. In both the menses and ovulation time points, the final best-fit model indicated the following variables would predict higher leptin: being sexually active (vs. sexually abstinent), higher salivary estradiol, lower progesterone, lower age at menarche, fewer average number of missed meals, fewer average meals per day, lower self-reported dieting, lower average physical activity score, and lower average intense exercise. At menses, the best-fit model also included weight instability (self-reported, > 5 pounds change in the last week) and higher age. At ovulation, the best-fit model included fewer hours since last meal, but (in contrast to the menses data) also included weight *stability* (self-reported, < 5 pounds change in the last week) and *lower* age.

As expected, measures of energy intake and expenditure generally had moderate to very large effect sizes in predicting leptin concentrations at both time points. Specifically, the effect of missed meals was moderate during both menses and ovulation (menses: Cohen's $f^2 = 0.128$; ovulation: Cohen's $f^2 = 0.190$), and the effect of total meals per day in predicting leptin was moderate at menses and very large at ovulation (menses: Cohen's $f^2 = 0.122$; ovulation: Cohen's $f^2 = 0.480$). Additionally, the effect of physical activity in predicting leptin was moderate during menses but very small at ovulation (menses: Cohen's $f^2 = 0.122$; ovulation: Cohen's $f^2 = -0.037$).

More surprisingly, other physiological and behavioral variables seemingly unrelated to energy intake and expenditure differed substantially in their effect at menses and ovulation. In particular, the effect of serum estradiol in predicting leptin during menses was more than twice that of the effect during ovulation (menses: Cohen's $f^2 = 0.117$; ovulation: Cohen's $f^2 = 0.046$). At menses, higher estradiol predicted higher leptin ($r_s = 0.360$, n = 32, $p = .036$), but the same pattern was not seen during ovulation ($r_s = 0.\ 268$, n = 32, $p = .139$) (Fig. 1). Also, the magnitude of the effects of sexual activity (menses: Cohen's $f^2 = 0.025$; ovulation: Cohen's $f^2 = 0.372$) and age (menses: Cohen's $f^2 = -0.030$; ovulation: Cohen's $f^2 = 0.305$) in predicting leptin were more than ten times greater at ovulation than at menses.

Because the effects of both sexual activity and estradiol differed across time points, we separately tested if sexual activity group predicted estradiol across phases. Sexually abstinent women had significantly higher levels of salivary estradiol at ovulation relative to menses ($t_{29.852} = -2.328$, $p = .027$); however, there were no differences by cycle phase among sexually active women ($t_{26.262} = -0.729$, $p = .472$). See http://hdl.handle.net/2022/21874 for full results.

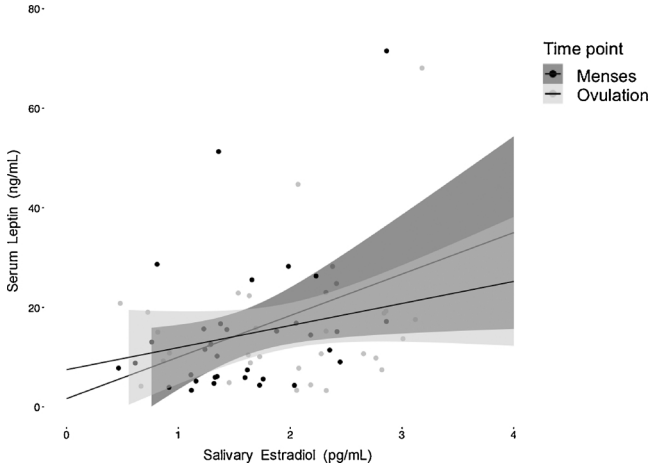

Fig. 1. The relationship between salivary estradiol and serum leptin in healthy women at menses (black dots) and at ovulation (gray dots). At menses, salivary estradiol and serum leptin had a stronger positive relationship ($r_s = 0.360$, n = 32, $p = 0.036$) than at ovulation ($r_s = 0.\ 268$, n = 32, $p = 0.139$), though these relationships were affected by other physiological factors included in the GLMMs.

Leptin plays a vital role in modulating metabolism. As reproduction is more likely to occur when resources are readily available, markers of energy storage such as leptin can play an important role in reproductive health. Disruptions of leptin have been shown to contribute to reproductive dysfunction (e.g., *in vitro* fertilization failures, pituitary suppression, polycystic ovary syndrome) [8,26]. In the present study, we found that measures of energy use and storage (e.g., meals, exercise) predicted leptin, but so too did sex steroid hormones and sexual activity. We observed differential predictors of leptin across menstrual cycle phases, suggesting that reproductive state may also interact with energy homeostasis.

Previous studies have determined that there are higher leptin levels during the luteal phase than the follicular phase, and that leptin levels rise steadily throughout the menstrual cycle [8,27]. We found that leptin levels were higher (albeit not significantly so) during ovulation than during menses [8], but we further found that estradiol was a stronger predictor during menses than at ovulation. One explanation is that during ovulation, estradiol plays an important role in the LH surge, which ultimately triggers the maturation of the ovum [28]. Because estradiol is significantly elevated around the time of ovulation, other systems in the body (e.g., energy regulation) may downregulate *response* to estradiol *signaling* during this time. However, at menses, levels of steroid hormones are generally low overall [29], suggesting that a small rise in estradiol during this phase may be a stronger signal for leptin production.

Interestingly, sexual activity status (active vs. abstinent) predicted leptin in these healthy women, but only at ovulation. There has been significant interest in how sexual activity status may influence reproductively-relevant hormones, such as testosterone [30], estrogen [31], and oxytocin [32]. There has been less attention paid to the possibility that sexual activity may similarly influence hormones in systems more indirectly related to reproduction. However, energy regulation is an important factor in signaling readiness for reproduction [33]; for example, in timing menarche [34] and promoting ovulation [35]. Sexual activity is a critical first step in reproduction – without sexual activity, conception will not occur. As such, the body may consider sexual activity status alongside energetic signals (such as leptin) to engage processes that prepare for possible reproduction. For example, recent research suggests sexual activity may predict changes in immune response across the menstrual cycle, biasing towards those aspects of immunity that promote conception (e.g., T-helper type 2 cell production) [13]. Sexual activity has been shown to predict differential

reproductive hormone profiles across the menstrual cycle [31], which may in turn alter metabolic hormones such as leptin. Alternatively, these interactions may be managed via central nervous system processing, as specific aspects of both sexual stimulation and feeding behaviors are coordinated via the ventromedial hypothalamus [36,37]. Finally, it is possible that sexual activity status is related to some psychosocial or behavioral factor (such as diet) that may influence leptin production. For example, one study found that women who were sexually active ate significantly less protein and drank more alcohol during their menstrual phase than women who were sexually abstinent [17].

In our sample, we found stronger associations between sexual activity and leptin at ovulation. Previous studies have suggested that leptin plays an important role during ovulation, and that leptin signaling contributes to the implantation process, as it increases cytokines and adhesion molecules in the endometrium [38]. Speculatively, it is possible that sexual activity may exert greater influence on leptin production during fertile windows within the menstrual cycle (i.e., just before and during ovulation). Taken together with these studies, our findings suggest a role for leptin in understanding interactions between immunity and reproduction in healthy women.

All of the women in the present study were healthy and had no evidence of reproductive dysfunction; further research is needed to establish if these effects would play out similarly in a clinical population. However, animal models have provided evidence suggesting that there is a relatively narrow range of leptin concentrations that are capable of maintaining appropriate function of the reproductive axis and providing proper signals to other physiological systems of the body.

This was an exploratory analysis, and as such, there were a number of limitations that must be considered when interpreting our findings. Because we had limited sampling within the menstrual cycle, future research would benefit from collection of data at more time points over the course of the cycle; however, we were able to collect hormonal data and physiological measures at ovulation, which is an extremely important and understudied time point. Also, further studies sampling across multiple cycles would provide insight into variation across cycles. Because we had a limited range of BMI in the females in this study, for clinical application, it would be helpful to extend to a sample with more overweight and obese women. Our sample size was limited and it is possible that we were underpowered to detect some effects. However, we were able to detect small significant effects (e.g., the effect of estradiol on leptin at menses: Cohen's $f^2 = 0.117$). Therefore, although a larger sample would be needed to detect smaller effects, effects smaller than those reported here may not be clinically meaningful. Finally, in this initial exploration of the effect of sexual activity on leptin, we restricted analyses to one aspect of sexual behavior (namely, penile intercourse) in one kind of sexual relationship (monogamous, heterosexual relationships). To better understand the mechanisms by which sexual activity may influence leptin, we must extend this work into a broader consideration of sexuality: how might these effects play out in women who have sex with women, or women who have multiple partners? Similarly, how might contraception (particularly hormonal contraception) influence how sexuality interacts with leptin? Better understanding of the conditions under which sexual behavior leads to changes in leptin − and more broadly, energy homeostasis – may ultimately uncover new routes of behavioral management of metabolic disease.

Since leptin provides a signaling link between energy homeostasis, reproduction, and immune function, the more we understand about what regulates leptin in the body, the better we can predict how the hormone may affect other vital aspects of physiology, such inflammation, angiogenesis, and glucose availability [8,9]. We found factors predicting leptin vary across the menstrual cycle, suggesting that in order to accurately assess leptin's effects, both menstrual phase and behavioral factors (such as sexual activity) should be taken into account. Further studies can begin to manipulate these physiological factors in order to determine the precise cross-talk taking place between reproductive and metabolic systems.

Conflicts of interest

The authors have no conflict of interest to declare.

Author contributions

KES and TKL analyzed the data and drafted the manuscript. TKL designed the study, and directed implementation and data collection. GED and JRH edited the manuscript for intellectual content and provided critical comments on the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.repbio.2018.01.011.

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