Published in final edited form as:

Womens Reprod Health (Phila). 2018 ; 5(3): 170–182. doi:10.1080/23293691.2018.1490079.

Salivary Afternoon Cortisol and Relationship Status in Healthy Women with Regular Menstrual Cycles

M. Claire Wilson[a], **Tierney K. Lorenz**[b,c,d], **Julia R. Heiman**[a,c,d]

[a]Department of Psychological and Brain Sciences, Indiana University, Bloomington

[b]Department of Psychology, University of North Carolina at Charlotte

[c]Center for the Integrative Study of Animal Behavior, Indiana University, Bloomington

[d]Kinsey Institute, Indiana University, Bloomington

Abstract

Although ovarian hormones and social relationships are known to interact with HPA axis regulation, evidence for systematic covariation with basal salivary cortisol levels remains mixed. As part of a larger study, in this analysis we pursued two questions. First, do baseline cortisol concentrations consistently vary across the menstrual cycle? Second, do cortisol levels differ by relationship status? We collected afternoon saliva samples at four points across the menstrual cycle from 14 single and 18 monogamously partnered women, ages 18 to 48, who were not taking hormonal medications. Samples taken in the lab yielded significantly higher cortisol concentrations than samples provided at home; the two were thus considered separately. No significant differences were observed across lab-session (menses vs. ovulation) or at-home (follicular vs. luteal) levels. This finding converges with studies of awakening salivary, urinary, and plasma cortisol, which suggest that, in healthy women, menstrual schedules do not affect systematic shifts in basal cortisol. Contrary to expectations, single and partnered women did not differ in overall cortisol levels. Future research would benefit from examining potential links between cortisol, relationship status, and sexual activity.

Keywords

cortisol; menstrual cycle; relationship status; stress; ELISA

As the end product of the hypothalamic-pituitary-adrenal (HPA) axis, cortisol is known to increase for both psychological and energetic stressors and to coordinate a variety of adaptive response mechanisms. Peripherally, cortisol orchestrates anti-inflammatory processes and helps to suppress cytotoxicity and restore homeostasis after an immune challenge (Elenkov, 2004). In response to stress, cortisol mobilizes energy stores, aids in tissue repair, and enhances the norepinephrine reactivity of smooth muscles that regulate blood pressure (Widmaier, Raff, & Strang, 2014). The activation of these stress systems also

Address correspondence to Tierney K. Lorenz, Ph.D., Department of Psychology, University of North Carolina at Charlotte, 9105 University Road, Charlotte, NC 28223, USA: tlorenz@uncc.edu.

Conflicts of Interest: The authors have no conflicts to declare.

suppresses non-urgent functions, such as feeding and reproduction (Widmaier et al., 2014). Accordingly, interactions between cortisol and the female reproductive system are well-documented. HPA axis activity has been linked to the inhibition of reproduction at the levels of gonadotropin-releasing hormone, luteinizing hormone, estradiol and progesterone synthesis, and ovarian function (Kalantaridou et al., 2004; Nepomnaschy et al., 2007). Reproductive hormones also feed back into the regulation of cortisol, as estrogen stimulates and progesterone counteracts multiple HPA axis targets (Wirth et al., 2007). Yet, although sex steroid hormones, such as estrogen and progesterone, exhibit systematic monthly variations in accordance with the menstrual cycle, evidence for menstrual variations in cortisol remains mixed.

Some studies have shown that women in the luteal phase exhibit enhanced cortisol reactivity to both physical (Andreano, Arjomandi, & Cahill, 2008; Roca et al., 2003) and psychosocial (Kirschbaum et al., 1999) stressors. These findings accord with reports of more dysphoric mood, dysregulated sleep, and sympathetic responsivity in the luteal phase (Baker & Driver, 2007; Ossewaarde et al., 2010; Symonds et al., 2004). The luteal phase is also marked by high levels of progesterone, a hormone thought to be functionally antagonistic to cortisol in freely cycling women (Hellhammer, Wust, & Kudielka, 2009; Wirth et al., 2007). Nonetheless, additional studies have shown either greater salivary cortisol responses to stress in the follicular phase (Maki et al., 2015) or no difference in either phase (Duchesne & Pruessner, 2013; Ossewaarde et al., 2010).

Mixed results are not uncommon for cortisol reactivity between participants exposed to external stressors. One commonly offered explanation is that differences in the basal functioning of the HPA axis affect the speed and strength of hormone responses. Yet, studies of awakening baseline cortisol in both plasma (Bloch et al., 1998) and saliva (Kudielka & Kirschbaum, 2003; Wolfram, Bellingrath, & Kudielka, 2011) have failed to show evidence of variation by menstrual cycle phase. A longitudinal field study of 25 Mayan women also documented that morning urinary cortisol did not significantly differ from day to day, unless women with longer than average cycles were considered (Nepomnaschy et al., 2011).

Two potential sources of variation stand out here: sample timing and type. As for the first, it is widely held that baseline cortisol concentrations taken in the afternoon are more stable (both within and between individuals) than those sampled in the morning (Hansen, Garde, & Persson, 2008; Jessop & Turkkner-Cobb, 2008). Cortisol's well-established diurnal rhythm flattens out by the afternoon or early evening. As such, concentrations obtained at earlier sampling times may be confounded by interindividual variability in the awakening spike (the upswing) and the latency to decline (the downswing; Adam & Gunnar, 2001). Accounting for cortisol's diurnal rhythm, whether by collecting multiple samples throughout the day or restricting between-subjects comparisons to the more stable afternoon nadir, may make the difference between washout and detected effects. For instance, in a sample of 35 freely cycling women who provided two saliva samples 14 days apart, Liening and colleagues (2010) found no support for basal cortisol differences by menstrual cycle phase. Yet, sampling times varied by participant and do not appear to have been accounted for in the regressions, such that high morning levels and low evening levels may have been pooled together within menstrual cycle time points. By contrast, Bao et al. (2004) collected multiple

salivettes across multiple days of a single menstrual cycle. Subtle differences in diurnal cortisol rhythms, including shallower troughs and reduced overall amplitudes, emerged during the luteal phase. This result may support the notion of blunted HPA function late in the menstrual cycle, especially for women under chronic stress (Adam & Gunnar, 2001; Baker & Driver, 2007).

A second inconsistency in the literature stems from the type of sample collected. Though urine, blood, and hair samples are common choices, their assays often fail to distinguish between bound and free cortisol. This free or "bioavailable" cortisol is able to diffuse across cell barriers and directly influence the function of target tissues (Widmaier et al., 2014). As salivary samples contain the cortisol that successfully diffused through the salivary glands out of systemic circulation, they serve as a good indicator of biologically active cortisol as opposed to overall HPA secretion (Hellhammer et al., 2009). This distinction may help to explain disparate results by sample type. In a small sample of young women, Symonds et al. (2004) reported that total daily volumes of *urinary* cortisol were significantly lower during the luteal phase, but neither morning nor evening concentrations of *salivary* cortisol differed across the menstrual cycle. As less free cortisol is retained in a 24-hour urine sample than in saliva, their discrepant results draw into question whether the observed reduction in urinary cortisol was truly due to reduced HPA activity (Hellhammer et al., 2009).

In addition, many researchers have expressed concern that drawing blood can evoke an anticipatory stress response, such that serum or plasma cortisol may be ill-suited for capturing true baselines across individuals (Russell et al., 2012). By contrast, salivary samples are minimally invasive, can be easily collected in a variety of contexts, and do not require researcher intervention (Hansen et al., 2008). Although important covariates, such as recent food intake, smoking, and time since waking, must be considered (Hansen et al., 2008), afternoon saliva samples may be more appropriate than morning and blood measures for comparisons of basal cortisol.

Taken together, methodological variance in sampling time, type, and other circumstances has precluded scientific consensus as to whether cortisol regularly fluctuates in accordance with menstrual phase. Yet assumptions that women's mental states are driven by robust hormone changes across the menstrual cycle remain popular. Data on the presence and strength of basal cortisol differences are of importance to the study of women's psychology and physiology. Menstrual cycle variation has proven to be a concern in research that makes use of heart rate variability (e.g., Sato & Miyake, 2004), neuroimaging (e.g., Ossewaarde et al., 2010), and event-related potentials (e.g., O'Reilly et al., 2004). Whether it should be accounted for in cortisol research remains uncertain. The first aim of the current study was thus to characterize basal cortisol variation across the menstrual cycle with afternoon saliva samples.

A second, exploratory aim was to investigate cortisol variability alongside a subject of burgeoning interest in the field of social endocrinology: intimate partnerships. A large sample of men from the Cebu Longitudinal Health and Nutrition Survey showed co-regulation of cortisol and testosterone that varied by relationship status (Gettler, Mcdade, & Kuzawa, 2011). The authors argued for a life history perspective, whereby individual

differences in basal cortisol reflect differential prioritization of energetic demands: living independently, improving one's fitness and social status, and competing for potential mates (higher cortisol) vs. enjoying the health and social benefits of a secured partner, maintaining relationship stability, and caring for children (lower cortisol). Consistent with this perspective, recent evidence from 122 young adults showed that single men and women exhibit higher baseline salivary cortisol than do those in relationships, in part as a function of the partnered individuals' perceived relationship stability (Maestripieri et al., 2013). In a study of 70 mothers of toddlers, single mothers had higher evening salivary cortisol than partnered mothers (Adam & Gunnar, 2001). Multiple (though not all) experiments have furthermore demonstrated the attenuating role of intimate partner support on cortisol response and recovery after an acute laboratory stressor (Ditzen et al., 2007; Meuwly et al., 2012). It is thus possible that intimacy in stable relationships serves as a buffer to daily life stress (Ditzen, Hoppmann, & Klumb, 2008).

Thus, we sought to explore the relationships between basal cortisol, the menstrual cycle, and relationship status among healthy, free-cycling women. We first examined whether salivary afternoon cortisol concentrations exhibit consistent variation across cycle phases. Given the discordant findings of the literature reviewed above, we considered two competing hypotheses: cortisol would either (a) show no change across menstrual cycle phases or (b) differ during the luteal phase, as suggested by studies of stress reactivity and diurnal variation. We also considered whether cortisol levels (and any cycle-related variations therein) would differ by relationship status. We hypothesized that partnered participants would exhibit lower basal cortisol than single women. We additionally explored the possibility of an interaction between menstrual cycle phase and relationship status, although no specific prediction was made.

Method

Participants

Thirty-five women were initially recruited from the community via flyers and screened for eligibility both over the phone and in person. Initial power analyses supported that sample sizes of 12 participants per group would have adequate power (85%) to detect small effect sizes for repeated measures effects (i.e., significant differences in degree of change across the menstrual cycle). Participants had regular menstrual cycles between 26 and 34 days in length and no more than 2–3 days difference in length between cycles; none had missed a menstrual period in the last 6 months. Exclusion criteria included any self-reported use of hormonal or immunomodulatory medications, regular use of any other medications, pregnancy or lactation within the past 12 months, and any chronic health conditions. For the purposes of the larger study, single women were all sexually abstinent within the last 4 months, although some reported masturbation and others had a prior history of partnered genital sexual activity. All partnered women reported their relationships to be heterosexual and monogamous (i.e., one male partner, no extrapair sex).

Three women withdrew from the study after their first sessions. Thus, a total of 32 women were included in the present analysis. Sample characteristics are summarized in Table 1. Participants had a mean age of 23.32 years (SD = 5.71, range = 18 to 48) and an average

BMI of 23.69 (SD = 3.95, range = 17 to 34). Fourteen women (44%) were single and had been sexually abstinent for the last 4 months. The remaining 18 women (56%) were partnered and reported an average relationship length of 4.14 years (SD = 7.19, range = 1 month to 29 years). The majority of participants (66%) reported their race/ethnicity as White. Average salivary progesterone in the luteal phase was within the range for ovulatory cycles (M = 210.5 pg/mL; Finn et al., 1988), and all participants showed a significant increase in progesterone from ovulation to the luteal phase (for details, see Lorenz, Heiman, & Demas, 2015). Thus, there was evidence that all participants' cycles were ovulatory.

Procedure

We collected observational data at each phase of the menstrual cycle. Each participant attended two laboratory sessions: one within 2 days after the onset of menstruation, and another within 2 days of ovulation. Menstruation was determined by self-report. Ovulation was dually confirmed by both self-report (i.e., using the first day of menstrual bleeding and typical cycle length to estimate a date) and urine tests for luteinizing hormone (i.e., to detect a surge that typically occurs 24–36 hours prior to ovulation; One Step Urine Ovulation Test, BlueCross Biomedical Co., Ltd.). Tests were completed at home between 1PM and 5PM on the days preceding each participant's expected date of ovulation. If she obtained a positive test before this date, she was scheduled for her second laboratory session within 48 hours. If no test was positive by the expected date, the session was postponed until a positive result was obtained.

During the laboratory visits, we instructed each participant in how to provide a passive drool saliva sample of at least 3 milliliters. These saliva samples and a variety of other measurements were also taken as part of a larger study of variations in immune system activity across the menstrual cycle (Lorenz, Demas, & Heiman, 2015; Lorenz, Heiman, & Demas, 2015), including a sample of whole blood during lab sessions, which was collected via standard venipuncture procedures. During her follicular (7–10 days after menses) and luteal (7–10 days after ovulation) phases, each participant provided saliva samples (but not blood) at home according to the same instructions given during the lab sessions. At-home samples were stored in the participant's freezer and transported to the lab in Styrofoam boxes lined with ice packs. All study procedures were approved by the Institutional Review Board at Indiana University. Written and signed informed consent was obtained from all women prior to beginning the study, and all were compensated $30 for their participation.

As afternoon cortisol is more stable than morning levels (Hansen et al., 2008), participants were instructed to complete all saliva samples between 12PM and 7PM. Only 11% of samples fell outside this range. Twelve saliva samples (two follicular, one ovulation, and nine luteal phase) were missing (9% of the total data). Women were instructed to refrain from eating, chewing gum, smoking, and drinking anything but water for at least an hour prior to providing saliva. Noncompliance for each of these criteria was below 5%. For statistical control, participants recorded the time at which they had awakened each day that a saliva sample was given. The number of hours since waking was calculated from these reports (mean = 7.16, SD = 3.34) and included as a covariate.

Saliva samples were stored at –80°C until analysis, and no sample was subjected to more than two freeze-thaw cycles. We used commercially available ELISA kits to assay the samples for cortisol according to manufacturer protocol (Salimetrics, Inc.). Intra-assay coefficients of variance ranged from 0.54% to 4.24%. Inter-assay coefficients of variance were 2.99% and 8.77% for the high and low controls, respectively.

Statistical Analyses

We used a mixed-effects linear model to characterize cortisol variation by the predictors of interest. We entered menstrual cycle phase (treated as a repeated measure), relationship status, and their interaction as fixed effects. We also entered time since waking as a covariate and specified a heterogeneous compound symmetry covariance structure. Analyses were performed in IBM SPSS Statistics 23.0. We adopted an α threshold of 0.05 for determining statistical significance.

Results

Noncompliance to study procedures was low. Nevertheless, measures with the potential to influence cortisol assays (e.g., eating or drinking during the hour preceding the saliva sample) were tested for inclusion as covariates. No such variable demonstrated substantial benefit to the model by either *p*-value or Akaike's Information Criterion (AIC). Although time since waking also yielded statistically insignificant effects, we decided to retain this variable as a covariate. Including hours since waking yielded the lowest AIC of all tested models, and it is widely regarded as a standard covariate for cortisol analyses (Hansen et al., 2008).

As depicted in Figure 1, raw basal cortisol values at each phase of the menstrual cycle exhibited an unexpected pattern of variance. A two-tailed t-test showed that the samples provided during the laboratory sessions significantly differed from those provided at home, $t_{114} = 2.38$, $M_{diff} = .046$, SE = .019, Cohen's $d = .445$, $p = .02$; however, given that sample setting was collinear with menstrual cycle phase, the two could not be compared as independent or interacting predictors. As prior literature could not easily account for this unexpected variance as a naturally occurring pattern, we agreed that the most parsimonious explanation for the difference in menstruation and ovulation vs. follicular and luteal phase samples was a difference in sampling methodology – most notably, that laboratory sessions involved a blood draw, whereas at-home samples did not. Thus, for all subsequent analyses, lab-session and at-home samples were considered separately.

There were no significant main effects of menstrual cycle phase on baseline cortisol, controlled for time since waking. Among the samples provided in the laboratory, we observed no significant difference in cortisol at menstruation vs. ovulation ($M_{diff} = –.00113$, SE = .025), such that cycle phase was not a significant predictor in the model, $F_{1,30.9} = .002$, Cohen's $f^2 = .002$, $p = .96$. Similarly, at-home samples (follicular vs. luteal phase) did not differ in basal cortisol concentrations ($M_{diff} = .015$, SE = .017), nor was the effect of menstrual cycle phase significant, $F_{1,9.81} = .78$, Cohen's $f^2 = .021$, $p = .40$. There was also no significant mean difference nor main effect of relationship status (single vs. partnered women) for either lab-session ($M_{diff} = .044$, SE = .031), $F_{1,32.73} = 2.14$, Cohen's $f^2 = .044$,

$p = .15$, or at-home ($M_{diff} = –.018$, SE = .029), $F_{1,9.45} = .39$, Cohen's $f^2 = .018$, $p = .55$, samples. Last, we found no significant interaction between menstrual cycle phase and relationship status, whether the samples were provided in the laboratory, $F_{1,31.9} = .26$, $p = .62$, or at home, $F_{1,12.13} = .08$, $p = .78$.

Discussion

Afternoon saliva samples from 32 healthy women showed no meaningful variation in cortisol levels as a function of menstrual cycle phase or relationship status. Effect sizes of these null results were very small (range = .002 to .044), although the sample sizes were adequately powered to detect small effect sizes (recommendations for Cohen's f^2 deem an effect of .02 small and .15 medium; Lakens, 2013). Thus, even if there were consistent variations in afternoon cortisol by either of our predictors of interest, these effects would be subtle and likely to be overshadowed by other factors, such as individual differences in stress reactivity. These results challenge the common perception that changes in psychological variables are necessarily driven by robust hormonal fluctuations across the menstrual cycle.

Our results did indicate significant variation by the setting in which cortisol was sampled. Studies with children have shown differential cortisol baselines in the laboratory vs. at home, which may reflect a stress response to the novel experimental setting (Jessop & Turner-Cobb, 2008). A small study of adult male volunteers also documented elevated salivary cortisol on the order of a two- to five-fold increase when obtained in the hospital vs. at home (Scheer et al., 2002). Whereas the follicular and luteal phase values observed in these data were in line with those typically reported for afternoon saliva samples provided at home (e.g., Schreiber et al., 2006), menstrual and ovulatory cortisol concentrations were higher and more comparable to baseline levels reported by laboratory studies of the Trier Social Stress Test (e.g., Kirschbaum et al., 1999). In addition, saliva samples have been noted for the advantage of pain-free sampling, as serum measurements may evoke distress in anticipation of the blood draw (Hansen et al., 2008; Russell et al., 2012). The present analysis is derived from a larger study that entailed additional laboratory measures. Participants who attended the laboratory sessions provided saliva samples immediately before undergoing venipuncture, of which they were fully informed ahead of time. The cortisol values obtained from these different methods thus exhibited a distinct disparity in line with the differences reported in previous literature.

We did not find evidence that salivary afternoon cortisol consistently varies across the menstrual cycle, although, of course, methodological considerations warrant caution. This finding converges with studies of awakening salivary, plasma, and urinary levels (Bloch et al., 1998; Kudielka & Kirschbaum, 2003; Nepomnaschy et al., 2011; Wolfram et al., 2011), which suggests that, in healthy women, menstrual cycles do not systematically impact basal cortisol. However, researchers should continue to consider cycle-related shifts in clinical populations believed to be at risk for atypically high or low cortisol (e.g., major depression, possibly premenstrual dysphoric disorder; Roca et al., 2003; Staufenbiel et al., 2013) and/or women under chronic personal, professional, or energetic stress (Adam & Gunnar, 2001; Baker & Driver, 2007; Nepomnaschy et al., 2007; Ditzen et al., 2008).

That single and partnered women did not significantly differ in overall cortisol levels contradicts prior research, which has shown that women and men show a correlation between relationship status and cortisol, both in basal levels and in reactivity (Gettler et al., 2011; Maestripieri et al., 2013). These associations are thought to reflect differential psychological and energetic demands upon those who are single vs. in a relationship. A large body of research has considered the theorized buffering effect of romantic involvement, particularly physical intimacy, to daily stressors (Adam & Gunner, 2001; Ditzen et al., 2008). Stable partnerships may confer greater social, financial, and emotional security for both individuals, and spouses' basal cortisol levels are often correlated (Schreiber et al., 2006). Individual differences in environmental stressors, mood disorders, attachment style, and relationship functioning complicate the buffering hypothesis and highlight the variability inherent in such correlations (Maestripieri et al., 2013; Meuwly et al., 2012). It is possible that one or more of these unmeasured variables confounded any main effect of relationship status in the present analysis. Indeed, the partnered women in this study reported a variety of relationship arrangements (see Table 1), although the duration of the partnerships was not correlated with our measures of interest.

Another potential explanation lies in the recruitment strategy for the larger study from which the present analysis was derived, whereby all of the single women were sexually abstinent (i.e., no partnered sexual activity within the past 4 months). We believe it is noteworthy that the majority of prior studies examined sexually active individuals or did not consider sexual activity a variable of interest. Social endocrinology researchers have hypothesized that, beyond the psychological benefits of having a romantic partner, relationship status may be related to lowered cortisol and testosterone via a decrease in sexual partner diversity and the associated demands of attracting and competing for mates. For instance, similar to the current study, Maestripieri and colleagues (2014) unexpectedly found that single young men had *lower* testosterone if they were also less sexually experienced or "promiscuous" than men who were partnered. As Gettler and colleagues (2011) found longitudinal co-regulation of testosterone and cortisol in the context of relationships, it is possible that cortisol tracks similar differences in sexual promiscuity insofar as it reflects energy devoted to mating effort and independent self-maintenance. This perspective would predict that single individuals tend to have higher baseline cortisol than partnered individuals only if they are pursuing new sexual partners. In short, the conflation of sexual activity and relationship status in the present data may have obscured the true effect of relationship status on cortisol levels in healthy women.

Several limitations to the present study must be noted. As detailed previously, the probable effect of salivary sampling setting and our inability to include this difference as a covariate in the model restricted the analysis to examining differences within lab-session values (menses vs. ovulation) and at-home samples (follicular vs. luteal) separately. We must acknowledge that there are alternative explanations for this variance, such as confounded group differences in susceptibility or early-life exposure to stress (Schreiber et al., 2006; Staufenbiel, 2013). Given the physiological and behavioral correlates of contiguous menstrual cycle phases, it would also have been ideal to examine cortisol variation across all four time points rather than across two models that contain two time points each. Nonetheless, comparisons between the follicular and luteal phases alone are common in

psychophysiological research on women's menstrual cycles (e.g., Maki et al., 2015). In addition, due to the vastly dissimilar endocrine and immune processes at play during menstruation and ovulation (Lorenz et al., 2015; Nepomnaschy et al., 2007), their very similar basal concentrations of cortisol in the present study make a strong case for the independence of metabolic and gonadal hormone variation among healthy, premenopausal women (Wirth et al., 2007).

Other methodological additions might have strengthened our study's results. Beyond blood, urine, and saliva, there has been increased attention to cortisol measurement from hair samples as an indication of long-term stress exposure (Russell et al., 2012). This method may have been well-suited for capturing between-subjects differences by stable characteristics, such as relationship status. However, its use would have hindered our aim of characterizing cortisol variations within the span of a single menstrual cycle. Hair cortisol, much like serum samples, would also have failed to distinguish bound and free cortisol. To keep focus on short-term changes while ruling out the possibility of floor effects in downstream cortisol, it might have been ideal to supplement our ELISA assays with a finer-grained analysis strategy (e.g., liquid chromatography – mass spectrometry) or else to include parallel measures of other bioavailable glucocorticoids (e.g., cortisone; Hellhammer et al., 2009; Russell et al., 2012). Alternatively, on the data collection side, it might have been helpful to include one or more salivary measurements during the morning hours. This approach would have allowed for a direct comparison of the two widely used sampling times (i.e., morning peak vs. afternoon nadir) as well as helped to control for interindividual variation in diurnal cortisol rhythms (e.g., Bao et al., 2004). Considering hair cortisol, employing supplemental analysis methodologies, or collecting multiple salivary samples may thus have strengthened or nuanced the conclusions that could be drawn from the present study.

Our participants were predominantly White, young, and heterosexual, which limits the generalizability of these results. Our study design required participants to have regular menstrual cycles (i.e., self-reported length of 26–34 days, no missed periods in the last 6 months), although women with irregular or particularly long cycles might have yielded greater cortisol variability, as suggested by Nepomnaschy and colleagues (2011). Oral contraceptives have received attention for their association with elevated baseline cortisol and decreased cortisol reactivity (Hansen et al., 2008; Kirschbaum et al., 1999; Wirth et al., 2007), but hormonal birth control users were not included in the present study. Future researchers interested in relationship status and menstrual cycle changes in cortisol might want to examine interactive group differences in the effects of and/or propensity to use hormonal contraceptives, especially different types (e.g., pills, implants, intrauterine devices).

Conclusion

In the present analysis, we used afternoon salivary cortisol to assess patterns of basal cortisol across the menstrual cycle and relationship status. Ovulation was confirmed using validated biomarkers, and women were stringently selected for regular cycles, no significant health complications, and no use of medications that could alter normal variation in hormones or

cycling. The results of our study add to the literature that indicates that menstrual cycle phase may not need to be taken into account in studies of basal cortisol measurements. Exceptions may still apply for clinical populations and women with irregular cycle lengths, which researchers should continue to explore. Although we found no difference between single and partnered women in the present study, future research would benefit from examining potential links between cortisol, relationship status, and sexual activity.

Acknowledgments

We thank Dr. Gregory Demas for his contributions to and guidance throughout this study, as well as Yuqing Jiao for statistical consultation. This work was partially funded by the Office of the Vice Provost of Research at Indiana University through the Collaborative Research and Creative Activity Funding Award, and partially by the American Psychological Foundation's Henry P. David Award for Research in Human Reproductive Behavior and Population Studies. Dr. Lorenz was supported by grant T32HD049336-09 from the National Institute of Child Health and Human Development.

References

Adam EK, & Gunnar MR (2001). Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. Psychoneuroendocrinology, 26, 189–208. [PubMed: 11087964]

Andreano JM, Arjomandi H, & Cahill L (2008). Menstrual cycle modulation of the relationship between cortisol and long-term memory. Psychoneuroendocrinology, 33, 874–882. [PubMed: 18468808]

Baker FC, & Driver HS (2007). Circadian rhythms, sleep, and the menstrual cycle. Sleep Medicine, 8, 613–622. [PubMed: 17383933]

Bao AM, Ji YF, Van Someren EJ, Hofman MA, Liu RY, & Zhou JN (2004). Diurnal rhythms of free estradiol and cortisol during the normal menstrual cycle in women with major depression. Hormones and Behavior, 45, 93–102. [PubMed: 15019795]

Bloch M, Schmidt PJ, Su TP, Tobin MB, & Rubinow DR (1998). Pituitary-adrenal hormones and testosterone across the menstrual cycle in women with premenstrual syndrome and controls. Biological Psychiatry, 43, 897–903. [PubMed: 9627744]

Ditzen B, Hoppmann C, & Klumb P (2008). Positive couple interactions and daily cortisol: On the stress-protecting role of intimacy. Psychosomatic Medicine, 70, 883–889. [PubMed: 18842747]

Ditzen B, Neumann ID, Bodenmann G, von Dawans B, Turner RA, Ehlert U, & Heinrichs M (2007). Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women. Psychoneuroendocrinology, 32, 565–574. [PubMed: 17499441]

Duchesne A, & Pruessner JC (2013). Association between subjective and cortisol stress response depends on the menstrual cycle phase. Psychoneuroendocrinology, 38, 3155–3159. [PubMed: 24055042]

Elenkov IJ (2004). Glucocorticoids and the Th1/Th2 balance. Annals of the New York Academy of Sciences, 1024, 138–146. [PubMed: 15265778]

Finn MM, Gosling JP, Tallon DF, Madden AT, Meehan FP, & Fottrell PF (1988). Normal salivary progesterone levels throughout the ovarian cycle as determined by a direct enzyme immunoassay. Fertility and Sterility, 50, 882–887. [PubMed: 3060380]

Gettler LT, Mcdade TW, & Kuzawa CW (2011). Cortisol and testosterone in Filipino young adult men: Evidence for co-regulation of both hormones by fatherhood and relationship status. American Journal of Human Biology, 23, 609–620. [PubMed: 21638512]

Hansen ÅM, Garde AH, & Persson R (2008). Sources of biological and methodological variation in salivary cortisol and their impact on measurement among healthy adults: A review. Scandinavian Journal of Clinical and Laboratory Investigation, 68, 448–458. [PubMed: 18609093]

Hellhammer DH, Wust S & Kudielka BM (2009). Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology, 34, 163–171. [PubMed: 19095358]

Jessop DS, & Turner-Cobb JM (2008). Measurement and meaning of salivary cortisol: A focus on health and disease in children. Stress, 11, 1–14. [PubMed: 17853059]

Lakens D (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and anovas. Frontiers in Psychology, 4, 863 1–12. [PubMed: 24324449]

Liening SH, Stanton SJ, Saini EK, & Schultheiss OC (2010). Salivary testosterone, cortisol, and progesterone: Two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels. Physiology & Behavior, 99, 8–16. [PubMed: 19833145]

Kalantaridou SN, Makrigiannakis A, Zoumakis E, & Chrousos GP (2004). Stress and the female reproductive system. Journal of Reproductive Immunology, 62, 61–68. [PubMed: 15288182]

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, & Hellhammer DH (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosomatic Medicine, 61, 154–162. [PubMed: 10204967]

Kudielka BM, & Kirschbaum C (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology, 28, 35–47. [PubMed: 12445835]

Lorenz TK, Demas GE, & Heiman JR (2015). Interaction of menstrual cycle phase and sexual activity predicts mucosal and systemic humoral immunity in healthy women. Physiology & Behavior, 152, 92–98. [PubMed: 26394125]

Lorenz TK, Heiman JR, & Demas GE (2015). Sexual activity modulates shifts in TH1/TH2 cytokine profile across the menstrual cycle: An observational study. Fertility & Sterility, 104, 1513–1521. [PubMed: 26385401]

Maestripieri D, Klimczuk AC, Seneczko M, Traficonte DM, & Wilson MC (2013). Relationship status and relationship instability, but not dominance, predict individual differences in baseline cortisol levels. PLOS ONE, 8, e84003 1–7. [PubMed: 24358324]

Maestripieri D, Klimczuk A, Traficonte D, & Wilson MC (2014). Ethnicity-related variation in sexual promiscuity, relationship status, and testosterone levels in men. Evolutionary Behavioral Sciences, 8, 96–108.

Maki PM, Mordecai KL, Rubin LH, Sundermann E, Savarese A, Eatough E, & Drogos L (2015). Menstrual cycle effects on cortisol responsivity and emotional retrieval following a psychosocial stressor. Hormones and Behavior, 74, 201–208. [PubMed: 26187711]

Meuwly N, Bodenmann G, Germann J, Bradbury TN, Ditzen B, & Heinrichs M (2012). Dyadic coping, insecure attachment, and cortisol stress recovery following experimentally induced stress. Journal of Family Psychology, 26, 937–947. [PubMed: 23127351]

Nepomnaschy PA, Altman RM, Watterson R, McConnell DS, & England BG (2011). Is cortisol excretion independent of menstrual cycle day? A longitudinal evaluation of first morning urinary specimens. PLOS ONE, 6, e18242. [PubMed: 21483825]

Nepomnaschy PA, Sheiner E, Mastorakos G, & Arck PC (2007). Stress, immune function, and women's reproduction. Annals of the New York Academy of Sciences, 1113, 350–364. [PubMed: 17978283]

O'Reilly MA, Cunningham CJ, Lawlor BA, Walsh CD, & Rowan MJ (2004). The effect of the menstrual cycle on electrophysiological and behavioral measures of memory and mood. Psychophysiology, 41, 592–603. [PubMed: 15189482]

Ossewaarde L, Hermans EJ, van Wingen GA, Kooijman SC, Johansson IM, Bäckström T, & Fernández G (2010). Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. Psychoneuroendocrinology, 35, 47–55. [PubMed: 19758762]

Roca CA, Schmidt PJ, Altemus M, Deuster P, Danaceau MA, Putnam K, & Rubinow DR (2003). Differential menstrual cycle regulation of hypothalamic-pituitary-adrenal axis in women with premenstrual syndrome and controls. Journal of Clinical Endocrinology & Metabolism, 88, 3057–3063. [PubMed: 12843143]

Russell E, Koren G, Rieder M, & Van Uum S (2012). Hair cortisol as a biological marker of chronic stress: Current status, future directions, and unanswered questions. Psychoneuroendocrinology, 37, 589–601. [PubMed: 21974976]

Sato N, & Miyake S (2004). Cardiovascular reactivity to mental stress: Relationship with menstrual cycle and gender. Journal of Physiological Anthropology and Applied Human Science, 23, 215–223. [PubMed: 15599065]

Scheer FA, Van Paassen B, Van Montfrans GA, Fliers E, Van Someren EJ, Van Heerikhuize JJ, & Buijs RM (2002). Human basal cortisol levels are increased in hospital compared to home setting. Neuroscience Letters, 333, 79–82. [PubMed: 12419485]

Schreiber JE, Shirtcliff E, Van Hulle C, Lemery-Chalfant K, Klein MH, Kalin NH, Essex MJ, & Goldsmith HH (2006). Environmental influences on family similarity in afternoon cortisol levels: Twin and parent-offspring designs. Psychoneuroendocrinology, 31, 1131–1137. [PubMed: 16997489]

Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, & van Rossum EF (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. Psychoneuroendocrinology, 38, 1220–1235. [PubMed: 23253896]

Symonds CS, Gallagher P, Thompson JM, & Young AH (2004). Effects of the menstrual cycle on mood, neurocognitive and neuroendocrine function in healthy premenopausal women. Psychological Medicine, 34, 93–102. [PubMed: 14971630]

Widmaier EP, Raff H, & Strang KT (2014). Vander's human physiology (13th ed.). New York: McGraw-Hill.th

Wilson MC, Zilioli S, Ponzi D, Henry A, Kubicki K, Nickels N, & Maestripieri D (2015). Cortisol reactivity to psychosocial stress mediates the relationship between extraversion and unrestricted sociosexuality. Personality and Individual Differences, 86, 427–431.

Wirth MM, Meier EA, Fredrickson BL, & Schultheiss OC (2007). Relationship between salivary cortisol and progesterone levels in humans. Biological Psychology, 74, 104–107. [PubMed: 16904811]

Wolfram M, Bellingrath S, & Kudielka BM (2011). The cortisol awakening response (CAR) across the female menstrual cycle. Psychoneuroendocrinology, 36, 905–912. [PubMed: 21237574]

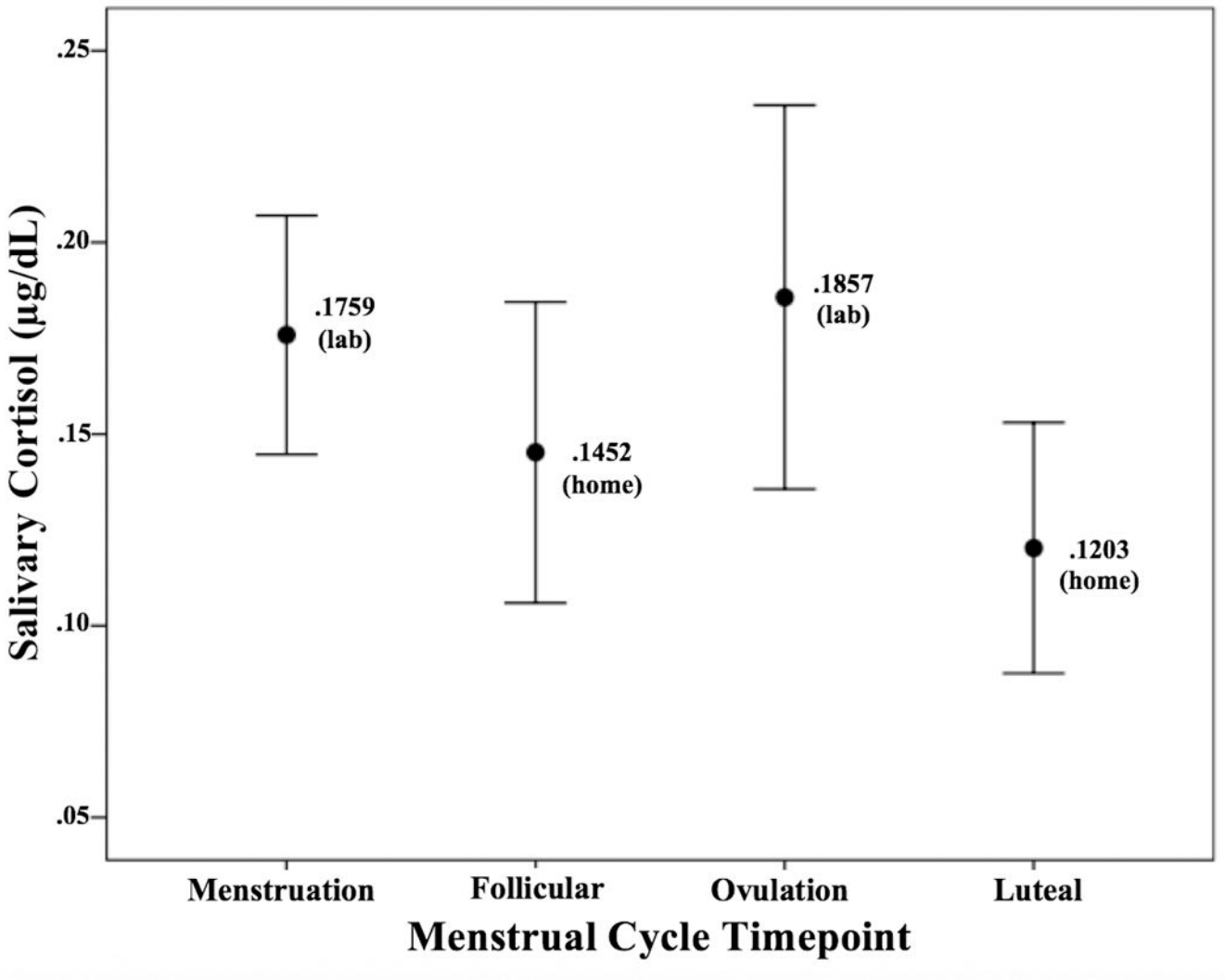

Figure 1.
Raw mean cortisol concentrations in µg/dL at each of four points across the menstrual cycle (displayed with 95% confidence intervals).

Table 1

Demographic characteristics of the analysis sample.

	Single Women		Partnered Women	
	Mean	*SD*	*Mean*	*SD*
Age in years	22.42	3.08	24.06	7.14
Body mass index	24.31	4.63	23.21	3.27
Cortisol in μg/dL	.1682	.1241	.1528	.0911
Time since waking	7.10	3.97	7.22	2.74
Relationship length in years	–	–	4.14	7.19
	Count	*Percent*	*Count*	*Percent*
Race/ethnicity				
White/Caucasian	8	25%	13	41%
East/South/Southeast Asian	3	9%	2	6%
Mixed/Other	3	9%	3	9%
Relationship status[1]				
Single, no sexual partner	14	44%	–	–
Non-committed relationship	–	–	1	3%
Dating someone local	–	–	8	25%
Dating someone long-distance	–	–	2	6%
Cohabiting or married	–	–	6	19%

[1]One woman did not report relationship status. Supplementary analyses were conducted excluding this participant and the participant in a non-committed relationship. As the results were not significantly affected either way, we chose to include them with the partnered group, given their reports of sexual activity with a single partner at all four time points.