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The role of ovarian steroid hormones in mood

Deborah H. Schwartz [a], Sarah E. Romans [b,e], Soumia Meiyappan [b], Mary Jane De Souza [c], Gillian Einstein [a,b,d,*]

[a] Department of Psychology, University of Toronto, Canada
[b] Women's College Research Institute, Women's College Hospital, Toronto, Canada
[c] Faculty of Physical Education and Health, University of Toronto, Canada
[d] Dalla Lana School of Public Health; University of Toronto, Canada
[e] Department of Psychological Medicine, University of Otago Wellington, New Zealand

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ABSTRACT

Fluctuations in ovarian hormones across the menstrual cycle have long been considered a determinant of mood in women. The majority of studies, however, use menstrual cycle phase as proxy for hormone levels. We measured ovarian hormone levels directly in order to examine the relationship between daily hormone levels and mood in non-help-seeking women. Participants ($n = 19$) provided daily information about their positive and negative moods, and collected their first morning-voided urine for 42 days, which was analyzed for estrogen and progesterone metabolites (E1G and PdG). The independent contributions of daily E1G, PdG, stress, physical health, and weekly social support, were calculated for 12 daily mood items, and composite measures of positive and negative mood items, using linear mixed models. E1G or PdG contributed to few mood items: E1G measured 2 days prior contributed negatively to the model for Motivation, while E1G measured 3 days prior contributed negatively to Getting Along with Others, and E1G measured 4 days prior contributed negatively to Anxiety. PdG, measured the same day and 1 day prior, contributed positively to the models of Irritability, and PdG measured 5 days prior contributed positively to Difficulty Coping. By contrast, the variables stress and physical health contributed significantly to all the mood items, as well as both composite positive and negative mood measures. These findings demonstrate that, compared to stress and physical health, ovarian hormones make only a small contribution to daily mood. Thus, fluctuations in ovarian hormones do not contribute significantly to daily mood in healthy women.

Introduction

Ovarian steroids, with the menstrual cycle as their proxy, are often seen as a determinant of mood in women. The late luteal or premenstrual phase with its drop in estrogens and progestagens is perceived as a time of increased irritability and negativity (Richardson, 1995). However, studies examining a possible relationship between the menstrual cycle or hormone levels and mood in non-help-seeking women have yielded inconsistent findings. While some studies have shown increased negative mood during the premenstrual phase (Freeman et al., 1996; Van Goozen et al., 1997) or decreased positive mood in the premenstrual phase (Cohen et al., 1987) others have shown a cyclical pattern of negative mood that is not confined specifically to the premenstrual period (Hardie, 1997; Sveindottir and Backstrom, 2000). Other studies find no menstrual phase-related changes in mood at all (Almagor and Ben-Porath, 1991; Charette et al., 1990).

Differences in methods of assessing both mood as well as the menstrual cycle phase may account for divergent results. Discrepancies in premenstrual-linked mood changes have been shown to depend on the scale used (Natale and Albertazzi, 2006). Studies that use the menstrual cycle phase as a proxy for directly measured ovarian hormone levels may fail to capture variability within predefined phases (Bellem et al., 2011; Hampson and Young, 2008). Such studies are also liable to unknowingly include anovulatory cycles, resulting in menstrual cycle phases that are not representative of actual hormone levels.

Few studies, in fact, examine directly the relationship between daily hormones and daily mood. Often hormones or mood are only measured on a subset of days during the menstrual cycle (Abplanalp et al., 1979; Fox et al., 2008; Laessle et al., 1990; Rapkin et al., 2011; Stoddard et al., 2007). Composite measures of hormones, such as averages, may be used instead of directly assessing the relationship between daily hormone levels and daily mood (Stoddard et al., 2007). The few studies directly comparing daily measurements of hormones and mood in non-help-seeking women for the duration of at least once complete menstrual cycle have in fact not found a correlation between either estradiol or progesterone and mood (Redei and Freeman, 1995).

* Corresponding author at: Department of Psychology, 100 St. George St., Toronto, ON, Canada M5S 3G3.
E-mail address: gillian.einstein@utoronto.ca (G. Einstein).

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Failure to recognize a time lag between hormone change and resulting mood changes may lead to divergent results. Genomic steroid actions are, by definition, slow and prolonged (McEwen and Alves, 1999), suggesting that hormones measured the same day as mood may not reflect their impact on mood until a few days later. Meaden et al. (2005) found a worsening mood during the first 1 to 2 days of menses, rather than in the premenstrual period and explained these results by hypothesizing a lag between hormonal changes and resulting mood symptomology.

Given the divergent results of methodologies and outcomes generally pointing away from a direct influence of ovarian steroids on mood, we wondered whether psychosocial factors might play a stronger role in determining mood. In order to address this question we blinded participants to the object of the study and assessed the relationship between daily levels of estrogen and progesterone metabolites with 12 individual daily mood items – some positive as well as negative – as well as the composites of positive and negative mood items, in a randomly recruited community sample of non-help-seeking women. To better understand the role of psychosocial factors in women's mood we included three other measures in our models: Social Support, Stress and Physical Health. These have been shown to have substantial influence on mental health (Helliwell and Putnam, 2004; McEwen, 2001; Romans et al., 2009) and were used as comparison variables against which to assess the strength of the association between ovarian hormones and daily mood. We included additional models to explore whether hormone levels from 1 to 5 days prior might also influence daily mood. Finally, we explored whether any hormone–mood relationships could be consistently mapped onto menstrual cycle phases, as determined by direct measurement of estrogen and progesterone metabolites.

Materials and methods

Mood in Daily Life study

The present study is a sub-study of a larger project, Mood in Daily Life (MiDL) which aimed to ascertain the relationship of daily moods to the menstrual cycle over 24-weeks in a non-help-seeking, community sample of women aged 18–40, living in the Greater Toronto Area in Ontario, Canada. Participants were recruited using a random digit dialing service that called each phone number up to seven times. Interested respondents were asked to attend an initial face-to-face interview, in which research staff collected relevant demographic and health information. Participants were then asked to commit to collecting mood and lifestyle information daily for 24-weeks using smart phone technology. Participants were blinded to the purpose of the study: understanding the relationship between mood and the menstrual cycle. All procedures were approved by the Sunnybrook and Women's College Hospital Research Ethics Board. Participants provided written informed consent prior to participation and were compensated for their time.

Daily Life Questionnaire (DLQ)

Each participant was issued a Palm Inc. Treo 650 HHC smart phone loaded with VADIS mental health telemetry software, which allowed participants to provide their responses on a visual analog scale (Kreindler et al., 2003). Each day, at a participant's preferred time, VADIS presented the Daily Life Questionnaire (DLQ). The DLQ consisted of eight positive mood items (Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things, Motivation, Enough Time for Self, and Getting Along with Others; adapted from Woods, 1987; Metcalf and Livesey, 1995), four negative mood items (Irritability, Sadness, Anxiety and Difficulty Coping; from the Short Premenstrual Assessment Form, Allen et al., 1991), one overall binary summary of mood (Current Mood) and two additional health and daily activity items (Stress and Physical Health) (Table 1). In addition, on a weekly basis, participants responded to three items assessing social support.

Mood and ovarian hormone study

Once enrolled in the MiDL study, participants were informed of the option to participate in a six-week (42 day) study for which they would collect their first morning voided urine. Participants were told that data from urine collection would be used to determine other physiological markers related to their daily lives. After collection, urine samples were frozen: first in the participant's home freezer and after collection by a research assistant, in lab freezers at the University of Toronto. Freezing is an effective means of preserving hormones in urine (Fuhrman et al., 2010).

Hormone assays

Hormone analysis was conducted using a competitive enzyme immunoassay (EIA; Munro et al., 1991) at the Women's Exercise and Bone Health Laboratory and Laboratory of Cognitive Neuroscience and Women's Health, both at the University of Toronto. EIAs to determine levels of estrone glucuronide (E1G, measured in ng/mL; the primary urinary metabolite of estradiol) and pregnanediol-3 glucuronide (PdG, measured in μg/mL; the primary urinary metabolite of progesterone) were carried out using polyclonal antibodies for E1G and PdG obtained from Coralie Munro at the University of California

Table 1
Daily Life Questionnaire and social support items.

Daily Life Questionnaire item	Anchor points	
In the past day, how much have you enjoyed things? (Enjoyment)	Not at all	Very much
In the past day, how was your overall physical health (Physical Health)	Worst ever	Best ever
In the past day, how confident have you felt? (Confidence)	Not at all	Very much
In the past day, how much have you felt that you just "couldn't cope" or were overwhelmed by ordinary demands? (Diff. Coping)	Not at all	Very much
In the past day, how anxious and worried have you felt? (Anxious)	Not at all	Very much
In the past day, how much have you felt under stress? (Stress)	Not at all	Very much
In the past day, how happy have you felt? (Happy)	Not at all	Very much
In the past day, how sad or blue have you felt? (Sad)	Not at all	Very much
In the past day, did you have enough time for yourself? (Time for Self)	Not at all	Very much
In the past day, how motivated have you felt? (Motivated)	Not at all	Very much
How would you describe your current mood? (Current Mood)	Worst ever	Best ever
In the past day, how much have you felt on top of things? (Felt on Top)	Not at all	Very much
In the past day, how well did you got along with people? (Get Along)	Worst ever	Best ever
In the past day, how energetic have you felt? (Energetic)	Not at all	Very much
In the past day, how irritable have you felt? (Irritable)	Not at all	Very much
Social support item	**Anchor points**	
In the last 7 days, were you happy with the amount of social support received at home?	Not at all	Very much
In the last 7 days, were you happy with the amount of social support received at work?	Not at all	Very much
In the last 7 days, were you happy with the amount of social support received from female friends and relatives?	Not at all	Very much

Davis (E1G R522-2, PdG R13904). Competitors for these assays were E1G and PdG conjugated to horseradish peroxidase. Inter and intra-assay coefficients of variation of low and high controls for the E1G assay were 10.94 and 9.64 (inter) and 10.82 and 9.95 (intra). For the PdG assay, inter and intra coefficients of variation for low and high controls were 14.11 and 15.18 (inter) and 11.02 and 6.82 (intra). The sensitivity of E1G and PdG assays was 0.08 ng/mL and 1.9 ng/mL. All urine samples were adjusted for specific gravity to account for variations in urine concentration.

Statistical analyses

All statistical analyses were conducted using SPSS 20.0. Positive mood items (Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation) were averaged to create a composite positive mood measure indexing internal positive mood. Enough Time for Self and Getting Along with Others were not included in composite positive mood because they are interpersonal in nature and depend on social context. Similarly, negative mood items (Irritability, Sadness, Anxiety and Difficulty Coping) were averaged to create a composite negative mood measure indexing internal negative mood. In addition, a composite social support measure was calculated by averaging the amount of support received over the previous 7 days from 3 domains: work, home, and female friends/relatives.

All hormone variables were log transformed to normalize their distributions, as well as group mean centered to account for baseline differences in physiological measures (Wilder, 1967). Social Support, Stress and Physical Health were grand mean centered to account for an individual's variance from the population mean. For each DLQ item, as well as for composite negative and positive measures (14 outcomes in total), a linear mixed model (LMM) with a variance components covariance matrix and Satterthwaite method of estimating degrees of freedom, was used to assess the direct contribution of daily E1G and PdG levels to daily mood. A two-level LMM was used to account for daily data nested within a participant by estimating the random intercept for each participant. Subjective measures of Stress, Physical Health, and composite Social Support, were included in the model to assess the role of additional factors that might also affect daily mood. LMMs were chosen for their power to handle repeated measures from the same participant, and missing data occurring randomly. Hormone levels from the same day as mood reports were used in the first set of models. Hormone levels from both 1 and 2 days prior to mood report were analyzed in two additional sets of models, in order to assess whether there was a lag between hormonal and mood changes. Models for hormone levels from 3, 4 and 5 days prior to mood report can be found in the supplementary materials.

For all models, proportional reduction in error of the model (R^2) was calculated according to Snijders and Bosker (1994).

Mood and menstrual cycle phases

Hormone data from each participant were used to determine four menstrual cycle phases for that individual. Criterion for phase divisions was: early follicular (EF), E1G and PdG below baseline—35 ng/mL and 2 μg/mL respectively; late follicular (LF), E1G above baseline and PdG below baseline; early luteal (EL), E1G below baseline, PdG above baseline and rising; and late luteal (LL), E1G below baseline, PdG descending from peak value. A cycle was deemed anovulatory if there was a failure to meet the PdG ratio criterion of three consecutive days of PdG levels threefold the moving baseline (Kassam et al., 1996) or peak PdG level below 2.49 μg/mL (De Souza et al., 2008). Participants with anovulatory cycles were excluded from the following analyses.

For each DLQ item, as well as for composite negative and positive mood measures, a repeated measures (RM) ANOVA was performed to assess the effect of menstrual cycle phase on mood. Where sphericity could not be assumed, a Huynh–Felt correction was used. Within subject contrasts comparing EF, LF and EL to LL were included, in order to assess differences in mood relative to the premenstrual period specifically.

Results

Participants

Twenty-one women participated in the mood and ovarian hormone study. Two participants taking oral contraceptives (assessed via self-report in the initial interview and confirmed via hormone assays) were excluded from all analyses. Thus, 19 participants were included in the LMM analyses assessing the relationship between daily hormones and daily mood. These women ranged in age from 18 to 41 (M ± SD = 33.68 ± 5.50).

From the 19 in whom we assessed the relationship between daily hormones and mood, 5 participants were excluded from the subsequent RM ANOVA analyses ($N = 14$) assessing the relationship between mood and menstrual cycle phase, because their hormone levels indicated they were anovulatory or they had missed urine collections, preventing accurate phase divisions.

While each participant chose the time of day they would fill out the DLQ, they maintained their chosen time throughout the study. The majority (13/19) chose to complete the DLQ in the evening, between 6 and 10 PM. A further five participants chose to complete the DLQ in the afternoon, between 12 and 6 PM. Only one participant chose to complete the DLQ in the morning, around 8 AM.

Hormones and mood: Same day

Contributions to positive mood

There was no significant contribution from either E1G or PdG measured the same day as mood to the models of any positive mood DLQ items (Table 2) or to composite positive mood (Fig. 1A). On the other hand, Social Support made a significant positive contribution to Getting Along with Others ($t(445.370) = 2.840$, $p = .0047$), and Physical Health made a significant positive contribution to all models of positive mood ($p < .001$ for all models). Stress made a significant negative contribution to all models of positive mood ($p < .001$ for all models).

Table 2
LMM same day models.

Mood items	R^2	Model variables				
		E1G	PdG	Social support	Stress	Physical health
	Positive mood items					
Composite [a]	.285	−1.457	1.044	−.914	−9.597◆	9.455◆
Happiness	.233	−.992	.523	−.763	−7.418◆	3.903***
Confidence	.315	−.519	−.288	1.505	−7.217◆	6.200◆
Enjoyment	.220	−1.898	1.066	−.136	−8.536◆	5.714◆
Energy	.174	−1.743	1.784	−.848	−3.969◆	8.841◆
Felt on Top	.125	.307	1.380	−.552	−3.401***	6.475◆
Motivation	.147	−1.126	−.395	.576	−4.387◆	8.172◆
Time for Self	.087	−.070	−1.076	−1.071	−3.979◆	3.323***
Get Along	.283	−1.442	−.104	2.840**	−4.243◆	5.334◆
	Negative mood items					
Composite [b]	.500	−1.012	.528	−.432	14.415◆	−5.147◆
Irritability	.313	−.668	2.008*	−2.063*	6.477◆	−3.159**
Sadness	.218	−.281	−1.847	−.526	6.175◆	−4.425◆
Anxiety	.337	−.351	.716	2.383*	11.064◆	−2.329*
Diff. Coping	.285	−1.016	.617	−.815	7.348◆	−4.996◆

R^2 and t-values for model variables (*$p<.05$, **$p<.01$, ***$p<.001$, ◆$p<.0001$).
[a] Composite positive mood is the average of Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation.
[b] Composite negative mood is the average of Irritability, Sadness, Anxiety and Difficulty Coping.

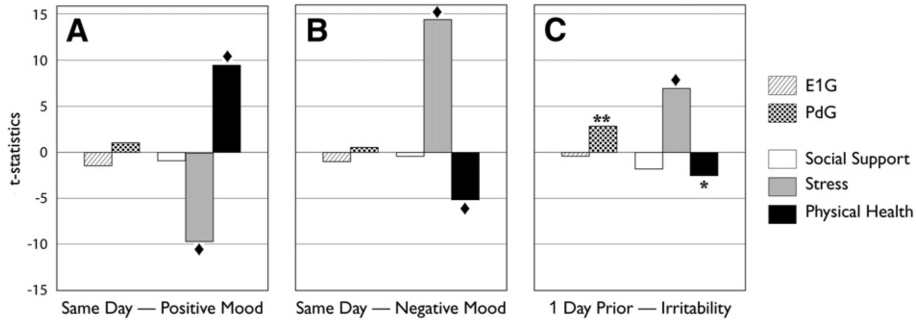

Fig. 1. Linear mixed models of mood items with *t*-statistics of model variables. All models include measures of E1G, PdG, weekly Social Support, and subjective Stress and Physical Health (*$p<.05$, **$p<.01$, ◆$p<.0001$). A. Model of composite positive mood with E1G and PdG measured the same day. Composite positive mood is the average of Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation. Only perceived Stress and Physical Health contributed significantly to the model ($p<.0001$). B. Model of composite negative mood with E1G and PdG measured the same day. Composite negative mood is the average of Irritability, Sadness, Anxiety and Difficulty Coping. Only perceived Stress and Physical Health contributed significantly to the model ($p<.0001$). C. Model of Irritability with E1G and PdG measured 1 day prior. PdG ($p=.0048$), Stress ($p<.0001$) and Physical Health ($p=.0120$) contributed significantly to the model.

Contributions to negative mood

There was no significant contribution from E1G measured the same day as mood to any models of negative mood DLQ items (Table 2) or composite negative mood (Fig. 1B). There was a significant positive contribution from PdG measured the same day to Irritability ($t(495.828)=2.008$, $p=.0452$), but not to any other models of negative mood. On the other hand, Social Support made a significant positive contribution to Anxiety ($t(494.224)=2.383$, $p=.0175$) and a significant negative contribution to Irritability ($t(409.378)=-2.063$, $p=.0398$). Stress made a significant positive contribution to all models of negative mood ($p<.0001$ for all) and Physical Health made a significant negative contribution to all models of negative mood ($p<.05$ for all models).

Hormones and mood: Hormones 1 day prior

Contributions to positive mood

There was no significant contribution from E1G or PdG measured 1 day prior to mood to any of the models of positive mood (Table 3). On the other hand, Social Support made a significant positive contribution to Confidence ($t(481.863)=2.217$, $p=.0271$) and Getting Along with Others ($t(412.286)=3.354$, $p=.0009$), and Physical Health made a significant positive contribution to all models of positive mood ($p<.01$ for all models). Stress made a significant negative contribution to all models of positive mood ($p<.01$ for all models).

Contributions to negative mood

There was no significant contribution from E1G measured 1 day prior to mood to any of the models of negative mood (Table 3). There was a significant positive contribution from PdG to Irritability ($t(473.239)=2.836$, $p=.0048$), but not to any other models of negative mood (Fig. 1C). On the other hand, Social Support made a significant positive contribution to Anxiety ($t(464.114)=1.982$, $p=.0481$). Stress made a significant positive contribution to all models of negative mood ($p<.0001$ for all models) and Physical Health made a significant negative contribution to all models of negative mood, except Anxiety ($p=.1434$).

Hormones and mood: Hormones 2 days prior

Contributions to positive mood

There was a significant contribution from E1G measured 2 days prior to mood to Motivation ($t(457.707)=-2.106$, $p=.0358$), but not to any other models of positive mood (Table 4). There was no significant contribution from PdG measured 2 days prior to any models of positive mood. Social Support made a significant positive contribution to Confidence ($t(466.627)=2.333$, $p=.0201$) and Getting Along with Others ($t(390.270)=3.037$, $p=.0026$). Physical Health made a significant positive contribution to all models of positive mood ($p<.01$ for all models). Stress made a significant negative contribution to all models of positive mood ($p<.01$ for all models).

Contributions to negative mood

There was no significant contribution from E1G or PdG measured 2 days prior to mood to any of the models of negative mood (Table 4). Social Support made a significant positive contribution to Anxiety ($t(446.037)=1.994$, $p=.0468$) and a significant negative contribution to Irritability ($t(362.234)=-2.258$, $p=.0245$). Stress made a significant positive contribution to all models of negative mood ($p<.0001$ for all models) and Physical Health made a significant negative contribution to all models of negative mood ($p<.05$ for all models).

Hormones and mood: Hormones 3, 4 and 5 days prior

Contributions to positive mood

There was a significant negative contribution from E1G measured 3 days prior to mood to Getting Along with Others ($t(446.282)=-2.333$, $p=.0201$, Supplementary Table 1). There was no significant

Table 3
LMM 1 day prior models.

Mood items	R^2	Model variables				
		E1G	PdG	Social support	Stress	Physical health
	Positive mood items					
Composite [a]	.292	−1.232	1.353	−.645	−9.373◆	9.073◆
Happiness	.230	−1.146	.463	−.857	−7.429◆	3.403***
Confidence	.340	−.478	.345	2.217*	−7.039◆	5.825◆
Enjoyment	.220	−1.385	.609	.021	−8.624◆	5.072◆
Energy	.174	−1.273	1.580	−.736	−3.377***	8.451◆
Felt on Top	.129	1.357	.903	−.563	−3.223**	6.378◆
Motivation	.166	−1.775	1.383	1.472	−4.083◆	7.913◆
Time for Self	.101	−.007	−.728	−.181	−3.734***	2.808**
Get Along	.283	−.905	−.913	3.354***	−4.122◆	4.674◆
	Negative mood items					
Composite [b]	.511	−.952	1.582	−.121	15.363◆	−4.334◆
Irritability	.328	−.409	2.836**	−1.806	6.932◆	−2.522*
Sadness	.223	−1.422	−.705	−.251	6.621◆	−4.010◆
Anxiety	.371	−.473	1.481	1.982*	11.938◆	−1.466
Diff. Coping	.300	−.055	.844	−.814	7.792◆	−4.309◆

R^2 and *t*-values for model variables (*$p<.05$, **$p<.01$, ***$p<.001$, ◆$p<.0001$).
[a] Composite positive mood is the average of Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation.
[b] Composite negative mood is the average of Irritability, Sadness, Anxiety and Difficulty Coping.

Table 4
LMM 2 days prior models.

Mood items	R^2	Model variables				
		E1G	PdG	Social support	Stress	Physical health
	Positive mood items					
Composite [a]	.304	−.721	.690	−.167	−9.337◆	8.895◆
Happiness	.241	−.638	−.033	−.241	−7.575◆	3.502***
Confidence	.348	−.068	−.117	2.333*	−7.230◆	5.295◆
Enjoyment	.223	−.527	.060	.183	−8.167◆	5.275◆
Energy	.191	−.386	1.021	.028	−3.507***	8.251◆
Felt on Top	.111	1.563	.370	−.552	−2.887**	6.473◆
Motivation	.164	−2.106*	.687	1.584	−4.206◆	7.732◆
Time for Self	.124	.754	−.836	.408	−4.064◆	2.657**
Get Along	.305	−.729	−1.243	3.037**	−3.687***	5.637◆
	Negative mood items					
Composite [b]	.505	−.810	.938	−.122	14.680◆	−5.107◆
Irritability	.336	−.233	1.867	−2.258*	6.906◆	−2.757**
Sadness	.210	−1.068	−1.151	.079	6.161◆	−4.081◆
Anxiety	.374	−.569	.279	1.994*	11.602◆	−2.441*
Diff. Coping	.302	.011	1.843	−.762	7.174◆	−4.906◆

R^2 and t-values for model variables (*$p<.05$, **$p<.01$, ***$p<.001$, ◆$p<.0001$).
[a] Composite positive mood is the average of Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation.
[b] Composite negative mood is the average of Irritability, Sadness, Anxiety and Difficulty Coping.

contribution of E1G 3, 4 or 5 days prior, nor PdG 3, 4 or 5 days prior to any other models of positive mood (Supplementary Tables 1–3). The contributions of Social Support, Stress and Physical Health are consistent with previous models.

Contributions to negative mood

There was a significant negative contribution from E1G measured 4 days prior to mood to Anxiety ($t(432.281) = -2.014$, $p = .0447$, Supplementary Table 2), and a positive contribution from PdG measured 5 days prior to mood to Difficulty Coping ($t(420.686) = 2.010$, $p = .0450$, Supplementary Table 3). There was no significant contribution of E1G 3, 4 or 5 days prior, nor PdG 3, 4 or 5 days prior to any other models of negative mood (Supplementary Tables 1–3). On the other hand, Social Support, Stress and Physical Health continued to make significant contributions to mood, consistent with previous models.

Menstrual cycle phases and mood

RM ANOVAs showed no significant effect of menstrual cycle phase on any individual DLQ item or composite positive and negative mood measures (Table 5). In addition, within subject contrasts comparing EF, LF and EL to LL showed no significant differences between individual phases and the late luteal phase.

Discussion

We studied the effects of both ovarian hormone systems on daily mood over one and a half menstrual cycles in a random community sample of non-help-seeking women. We measured estrogen and progesterone metabolite levels daily and examined their contribution as well as that of perceived stress and physical health acquired daily over the same cycle, and social support acquired weekly, to both positive and negative mood items and their composite measures. Importantly, participants were blinded to the focus of the study. Our major finding is that with a few exceptions neither absolute hormone levels nor menstrual cycle phase as determined by hormone levels played a significant role in daily mood in this group of women. Rather, perceived stress and physical health were the strongest contributors to statistical models of daily positive and negative mood. This suggests

Table 5
Means and standard deviations of DLQ items across 4 phases of the menstrual cycle.

DLQ item	EF	LF	EL	LL	p-Value
	Positive mood items				
Composite [a]	51.19 (14.45)	51.78 (13.58)	50.54 (13.92)	51.41 (16.96)	.744
Happiness	54.64 (13.76)	53.66 (13.31)	51.33 (14.54)	50.83 (19.08)	.536
Confidence	54.16 (20.86)	54.76 (18.65)	53.67 (18.70)	55.50 (22.35)	.716
Enjoyment	55.69 (12.97)	53.89 (12.28)	53.71 (13.64)	50.81 (15.93)	.407
Energy	46.45 (12.69)	45.99 (12.86)	45.58 (12.40)	46.77 (16.55)	.923
Felt on Top	48.89 (17.64)	51.75 (17.48)	50.48 (17.50)	52.31 (19.20)	.378
Motivation	51.69 (16.87)	50.40 (15.87)	49.50 (16.39)	51.01 (18.83)	.598
Time for Self	43.63 (17.72)	43.88 (14.05)	43.49 (15.64)	39.68 (20.07)	.482
Get Along	65.27 (13.36)	61.45 (16.55)	62.66 (12.90)	59.07 (16.70)	.234
	Negative mood items				
Composite [b]	42.61 (18.08)	40.55 (16.99)	42.56 (16.80)	43.54 (18.78)	.476
Irritability	42.11 (16.26)	42.78 (19.66)	46.53 (19.18)	47.74 (21.88)	.251
Sadness	36.37 (21.34)	34.01 (17.31)	34.33 (19.15)	36.70 (20.56)	.387
Anxiety	49.32 (20.84)	48.79 (20.31)	46.76 (18.79)	49.40 (20.64)	.606
Diff. Coping	40.84 (20.85)	35.87 (21.73)	41.46 (21.53)	39.47 (22.40)	.156
	Other DLQ items				
Stress	51.10 (20.28)	48.88 (18.28)	48.92 (18.29)	49.89 (18.91)	.800
Physical Health	57.09 (7.70)	57.62 (9.73)	56.71 (9.66)	57.26 (11.65)	.845
Current Mood [c]	57.73 (12.29)	56.59 (13.23)	57.85 (10.74)	57.24 (14.19)	.925

p-Values for repeated measures ANOVA.
EF: early follicular; LF: late follicular; EL: early luteal; LL: late luteal.
[a] Composite positive mood is the average of Happiness, Confidence, Enjoyment, Energy, Felt on Top of Things and Motivation.
[b] Composite negative mood is the average of Irritability, Sadness, Anxiety and Difficulty Coping.
[c] Current mood is an overall binary summary item of mood.

that in randomly recruited, non-help-seeking women from the community, psychosocial factors have more of an impact on mood than ovarian steroid hormones.

Hormones contributed significantly to only five mood items: E1G to two positive mood items, and one negative mood item, and PdG to two negative mood items. LMMs showed that E1G measured 2 days prior to mood, contributed negatively to Motivation, and E1G measured 3 days prior contributed negatively to Getting Along with Others. Further, E1G measured 4 days prior contributed negatively to Anxiety. The direction of the relationships between E1G and Motivation and Getting Along with Others was surprising, given that exogenous estrogens have been used to effectively treat symptoms of perimenopausal and postmenopausal depression (Schmidt et al., 2000; Zweifel and O'Brien, 1997). On the other hand, Stoddard et al. (2007) found mean E1G levels to be positively correlated with loneliness and crying during the luteal phase. Kikuchi et al. (2010) found a positive correlation between estradiol levels and 5-hydroxytryptamine (5-HT, serotonin) in the premenstrual phase, which in turn was negatively correlated with tension-anxiety and fatigue, suggesting that estradiol may be associated with a decrease in some aspects of negative mood that are not necessarily linked with lack of motivation.

LMMs showed that PdG measured the same day and 1 day prior to mood, contributed positively to Irritability. We also found a five-day lag between progesterone and Difficulty Coping. This is not unlike

Redei and Freeman (1995) who found a five- to seven-day lag in their correlation between progesterone and irritability, albeit only in women with premenstrual syndrome (PMS), and not in women without PMS. Lentz et al. (2007) found a significant lagged relationship between progesterone and "emotional turmoil" when they compared daily measures of urinary hormone levels with scores on the Menstrual Symptom Severity List in women who identified as having PMS (or premenstrual magnification pattern) but less so in women with low-severity symptoms. Taken together these results suggest that when there are hormonal effects on mood they are lagged.

The particular timing of the lagged relationship between E1G and mood may be due to differences in the sensitivity requirements for measuring estrogens in serum as opposed to urine. Measures of urinary metabolites are a pooled value over time making their concentration in urine higher than in serum and making assay sensitivity less critical (Bellem et al., 2011). Time lags in our study may be particularly underestimated with respect to estrogen metabolites. Peak correlations occur between urine samples collected 1 day after serum samples, which suggests that urinary estrogen metabolites may be more indicative of serum estradiol from the previous day (O'Connor et al., 2003).

What is notable is that in spite of a fairly thorough search for hormonal relationships to different components of mood only E1G and Motivation, Getting Along with Others and Anxiety, PdG and Irritability, and Difficulty Coping show significance. With the exception of the relationship between PdG 1 day prior and Irritability, none of the associations are stronger than $p = .01$. In examining six sets of models (including supplementary materials), with the exception of the relationship between PdG and Irritability, each of the observed hormone–mood relationships (e.g. E1G and Motivation) occurred only once across the 6 sets of models. The number of analyses conducted taken together with the infrequency of significant relationships between ovarian steroid hormones and mood, contrasted with the frequent significant relationships between Stress, Physical Health and mood, suggests that the observed hormone–mood relationships may be spurious, underscoring that relative to other factors, the impact of hormones on mood over the menstrual cycle in non-help-seeking women is minimal.

Results from other studies that measured hormones directly and mood daily further support a minimal, at best, contribution of ovarian steroids to mood. Abplanalp et al. (1979) compared serum levels of ovarian hormones, obtained three times per week from healthy women, with scores on the Profile of Mood States (POMS). Hormone data were divided into "high" and "low" estradiol and progesterone days, based on absolute serum levels. They found scores on vigor to be significantly higher on high estradiol days, and depression to be significantly lower on low progesterone days.

Stoddard et al. (2007) measured daily urinary hormone levels for the last quarter of a menstrual cycle and mood daily using the Moos Menstrual Distress Questionnaire in physically active and sedentary (but otherwise healthy) women. Average mood scores were calculated for the premenstrual period and correlated with peak hormone values. Unlike Abplanalp et al. (1979), they found a negative relationship between estrogen and measures of negative mood: crying and loneliness. Similarly to Abplanalp et al. (1979), however, they found a positive correlation between peak progesterone levels and measures of negative mood: crying and loneliness. Fox et al. (2008) took daily measures of ovarian hormones using saliva samples, and weekly measures of mood using the POMS-bipolar form in healthy and cocaine addicted women. In the healthy women they found a negative correlation between negative mood and progesterone levels in the late luteal phase. Laessle et al. (1990) measured serum levels of ovarian hormones three times a week in healthy women, and correlated them with daily mood measures on a visual analog scale. They found no evidence of hormone–mood relationships. Further, Rapkin et al. (2011) found no correlations between serum hormone levels measured on a single follicular and single luteal day, and daily mood ratings in healthy control women. Our results, like those of these prior studies using somewhat different methods, suggest inconsistent, minimal or non-existent influences of menstrual cycle variations in estrogen and progestagens on daily mood in healthy women.

We found no changes in mood associated with menstrual cycle phase. While some previous studies have (Cohen et al., 1987; Freeman et al., 1996; Van Goozen et al., 1997), very few have determined menstrual cycle phases based on direct measurements of each woman's ovarian hormone levels across their own cycle. Direct examination of hormone levels across the menstrual cycle reveals large variation in phase lengths between women that is not captured using the count-back method or an idealized 28-day cycle. Further, by averaging ovarian hormone levels to create a summary statistic for each phase, subtle intra-individual changes in both hormones and mood within each menstrual cycle phase are lost. Thus, our findings support the idea that menstrual cycle phases are too coarse a means by which to assess hormone–mood relationships.

Most importantly, when psychosocial factors are also assessed, they bear a stronger relationship to both negative and positive mood than do ovarian steroids. Our findings demonstrate that the influence of factors such as perceived stress and physical health contribute to all components of positive and negative mood. This is consistent with the ample literature suggesting the importance of both perceived stress and physical health for mental health and well-being (Janisse et al., 2004; Kopp et al., 2008; McEwen, 2001). Laessle et al. (1990) also demonstrated a relationship between subjective stress and mood in the absence of a relationship between ovarian hormones and mood. These finding are consistent with the first phase of the MiDL study, in which 507 non-help-seeking women were surveyed about what factors they perceived to influence their daily moods. Results showed that 46–61.9% of women perceived stress, and 52.1–65.9% of women perceived physical health to influence their daily positive and negative moods "a lot" (Romans et al., 2009).

While a wealth of literature has shown increased social support to be associated with improved mood (Grav et al., 2012; Helliwell and Putnam, 2004; Janisse et al., 2004), our study demonstrated that social support is related to some, but not all, components of positive and negative mood. We found a positive contribution of social support to models of Confidence and Getting Along with Others, and a negative contribution to models of Irritability. We also found, somewhat surprisingly, that social support contributed positively to models of Anxiety. As our analysis does not allow for determination of causality, it may be the case that both anxiety and social support increased as a result of a third factor. Joiner et al. (1999) found that negative life events predicted increases in both anxiety and reassurance seeking, which could result in the observed positive relationship between social support and anxiety.

Limitations

This study has many methodological strengths including the following: random community sampling of non-help-seeking individuals, obscured focus on the menstrual cycle, inclusion of both positive and negative mood items, direct daily hormone measures, and use of linear mixed models. Nonetheless, there are some limitations that need to be considered. First, while the larger MiDL project obtained mood data from four to six menstrual cycles, due to increased demands on participants, this sub-study aimed to obtain data for only one and a half cycles (42 days). As women exhibit intra-individual variability across menstrual cycles, it would be helpful to have more than one cycle per participant in order to gain a more consistent picture. Further, while time of day for completion of the DLQ was left up to the discretion of individual participants (although each participant completed the DLQ at the same time each day), time of day for urine collection was consistent across all participants (first morning void). Matching of same day and lagged hormone levels to mood ratings may differ between participants who chose to complete the DLQ in the morning and those who chose to complete it later in the day. Finally, as sensitive as our analyses are, it is not possible to determine causality using LMM.

Conclusion

In sum, we found that ovarian hormones, as assessed by urinary metabolites E1G and PdG, contributed negligibly to positive and negative mood. In addition, we found no evidence of a relationship between menstrual cycle phase and mood. Rather, perceived stress and physical health were the strongest contributors to daily mood. Taken together, our findings suggest that natural fluctuations of ovarian hormones do not contribute significantly to variations in the daily moods of healthy women and that psychosocial factors may need to be controlled for in any study of mood.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yhbeh.2012.08.001.

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