2020-08-27

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Gail A. Greendale
University of California, Los Angeles

Et al.

Journal of the Endocrine Society, 2020, Vol. 4, No. 11, 1–10
doi:10.1210/jendso/bvaa115
Clinical Research Article

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Melatonin Patterns and Levels During the Human Menstrual Cycle and After Menopause

Gail A. Greendale,[1] Paula Witt-Enderby,[2] Arun S. Karlamangla,[3] Fahima Munmun,[4] Sybil Crawford,[5] MeiHua Huang,[1] and Nanette Santoro[6]

[1]Department of Medicine, Division of Geriatrics, University of California, Los Angeles, Los Angeles, California, 90095; [2]Division of Pharmaceutical, Administrative and Social Sciences, Duquesne University School of Pharmacy, Pittsburgh, Pennsylvania 15282; [3]Department of Medicine, Division of Geriatrics, University of California, Los Angeles, Los Angeles, California 90095; [4]Division of Pharmaceutical, Administrative and Social Sciences, Duquesne University School of Pharmacy, Pittsburgh, Pennsylvania 15282; [5]University of Massachusetts Medical School, Graduate School of Nursing, Worcester, Massachusetts 01655; [6]Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Aurora, Colorado 80045.

ORCiD number: 0000-0003-1054-1081 (G. A. Greendale).

Abbreviations: aMT6s, 6-sulfatoxymelatonin; CL, corpus luteum; DHS, Daily Hormone Study; DLT, day of luteal transition; E1c, estrone conjugates; ELA, evidence of luteal activity; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MT, menopause transition; NELA, no evidence of luteal activity; PdG, pregnanediol glucuronide; SWAN, Study of Women's Health Across the Nation.

Received: 15 June 2020; Accepted: 26 August 2020; First Published Online: 27 August 2020; Corrected and Typeset: 14 October 2020.

Abstract

Context: Melatonin may play a role in the regulation of the human menstrual cycle and may decline with menopause and/or aging.

Objective: The objective of this work is to investigate the relations between melatonin and the menstrual cycle, menopause, and aging.

Methods: This was a cross-sectional and longitudinal analysis of 20 participants from the Study of Women's Health Across the Nation (SWAN) Daily Hormone Study (DHS). The outcome measure was first-morning urine assay of 6-sulfatoxymelatonin (aMT6s), a gauge of melatonin. For each participant, aMT6s was measured daily during one premenopausal cycle with evidence of luteal activity (ELA) and one postmenopausal collection with no evidence of luteal activity (NELA).

Results: In addition to the organized patterns of hormone metabolites (estrone conjugates [E1c], and pregnanediol glucuronide [PdG]) and gonadotropins that characterized ovulatory menstrual cycles, there was a late luteal rise in aMT6s. In NELA collections, there was no periodicity of E1c, PdG, gonadotropins, or aMT6s. The strongest predictors of aMT6s levels were PdG values 11 to 12 days prior to aMT6s ($\beta = 1.46$, $P = .001$ and $\beta = 1.44$, $P = .001$, respectively). E1c and gonadotropins were not statistically significantly associated with aMT6s. Mean aMT6s in premenopause was 53.5 ng/mL, greater than the mean of 37.4 ng/mL in postmenopausal samples from the same women ($P = .0002$).

ISSN 2472-1972

Conclusions: This study confirms a late luteal melatonin rise, likely signaled by progesterone, which may influence menstrual cycle pacemaker control. Melatonin declined from premenopause to postmenopause. A high correlation between menopause transition stage and age precludes distinction between the influences of ovarian and chronological aging.

Key Words: melatonin, menstrual cycle, menopause, aging

Melatonin, a hormone released from the pineal gland in response to darkness, is the biochemical signal of sleep; it governs the daily periodicity of the endogenous circadian system [1]. However, the physiological influence of melatonin may extend beyond that of circadian pacemaker, ranging from control of reproductive function to bone balance [2-4].

A pioneering study of melatonin patterns during the human menstrual cycle uncovered an organized melatonin rise in the late luteal phase [5]. Sampling serum once every 3 days for a full menstrual cycle in 5 women, Wetterberg et al reported that melatonin levels were 4.5 times higher in the luteal phase than in the follicular phase and that the melatonin rise followed the postovulatory progesterone elevation. In another study of 10 women, Webley and Leidenberger assayed serum melatonin at 4-hourly intervals on 1 day during the follicular phase and 1 day during the luteal phase and reported that melatonin levels were significantly higher during the latter [6]. To our knowledge, melatonin levels in the normal menstrual cycle have not been examined since this early work.

Melatonin may also play a role in the development of conditions related to aging and/or menopause. Cross-sectional reports find that melatonin secretion declines with age, raising the possibility that this decrease may, in part, underlie the increase in age-related disorders such as poor sleep [7, 8]. A qualitative summary of 20 cross-sectional studies estimated that the nocturnal melatonin secretion in older adults (age ~ 60-90 years) is about half that of younger individuals (age ~ 20-30 years). This large, cross-sectional effect size encourages more definitive, longitudinal studies of melatonin in relation to aging. The question of whether menopause, apart from aging, also influences melatonin secretion is inspired by the concordance between menopause transition (MT) symptoms and systems related to melatonin. Examples of the potential menopause symptom-melatonin intersections include sleep disorders, dysphoric mood, and bone loss [9-14].

Our exploratory study, therefore, investigated the relations between melatonin and the menstrual cycle, menopause, and chronological aging using data and specimens from the Study of Women's Health Across the Nation (SWAN) Daily Hormone Study (DHS). SWAN DHS participants, who were initially premenopausal, collected first-morning urine samples daily for one menstrual cycle or, if no bleeding occurred, for 50 days. The SWAN DHS assayed metabolites of estrone (estrone conjugates [E1c]), progesterone (pregnanediol glucuronide ([PdG]), and gonadotropins and applied standard criteria to classify each collection as having evidence of luteal activity (ELA) or no evidence of luteal activity (NELA) [15, 16]. This project newly measured 6-sulfatoxymelatonin (aMT6s), a well-established gauge of melatonin, in stored DHS samples [17]. We analyzed daily aMT6s in one premenopausal ELA cycle and one postmenopausal NELA cycle from 20 DHS participants.

Study aims were to 1) describe within-collection excretion patterns of aMT6s, E1c, PdG, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), stratified by ELA or NELA classification; 2) describe and quantify, if present, relations between menstrual cycle excretion patterns of aMT6s and PdG, E1c, and gonadotropins; and 3) assess longitudinal, within-woman whole-collection excretion of aMT6s in premenopausal ELA compared to postmenopausal NELA collections. We proposed 4 hypotheses: 1) in ELA collections, temporal patterns of aMT6s and PdG would be related; 2) in NELA collections, which manifest no organized patterns of PdG, E1c, and gonadotropin excretion, aMT6s excretion would also be disorganized; 3) in ELA cycles, the quantity of aMT6s would be directly related to the amount of PdG; and 4) whole-collection amount of aMT6 would be less in postmenopausal NELA than in premenopausal ELA collections.

1. Materials and Methods

A. Participants

SWAN is the parent study from which the DHS sample was derived [18]. In brief, during 1995 to 1997, a community-based sample of 16 065 women residing near the 7 SWAN clinical sites was screened to yield an inception cohort of 3302 women. All sites enrolled White women and each site enrolled one minority population (Black, Chinese, Hispanic, or Japanese). Women were eligible for the longitudinal SWAN study if they were age 42 to 52 years, had an intact uterus and at least one ovary, were not using exogenous hormones that affect ovarian function,

had 1 or more menstrual periods in the last 3 months, were not pregnant/lactating, and self-identified with one of the designated races/ethnicities. Standardized, uniform questionnaires and interviews ascertained participant characteristics. SWAN defined premenopause as having menstrual cycles that were unchanged from the participant's usual pattern and early perimenopause as having cycles that were less predictable, but with no overt gaps of 3 or more months. Experiencing a cycle gap of 3 or more months defined late perimenopause and 12 months of amenorrhea were the criteria for natural menopause.

The SWAN DHS totaled 848 participants [19]. Eligibility criteria were the same as those for SWAN. At each SWAN visit, DHS volunteers collected a first-morning urine sample, starting on their first day of menstrual bleeding through the first day of bleeding in the next cycle, or, if no menses occurred, for a maximum of 50 days. Collections continued for 2 years after their last menstrual period [16]. The urinary hormone metabolites and gonadotropin results used by the current project were assayed during the DHS. The DHS categorized urinary collections as having ELA or NELA based on validated criteria [15, 16].

We identified 20 SWAN DHS participants who did not report nocturia more than once per week (because we sought to estimate overnight aMT6) and who submitted at least one ELA cycle prior to menopause (either premenopausal or early postmenopausal cycles qualified) and one NELA cycle after menopause. More than 20 women met these criteria. Therefore, using the already available DHS data, we identified collections that best met ELA and NELA criteria (to get the strongest hormone and gonadotropin signals with which to compare the aMT6 patterns). We also prioritized the ELA-NELA within-woman cycle pairs that were farthest apart in calendar time.

B. Laboratory Methods

Existing DHS data included measures of LH, FSH, E1c, and PdG, each run by a single, central laboratory on daily morning urine samples, using adapted chemiluminescent assays, and normalized to creatinine [20]. FSH and LH were measured with 2-site chemiluminescent immunoassays (Bayer Diagnostics). The range for the urine FSH assay is 0.3 to 136 mIU/mL, the minimum detectable concentration is 0.3 mIU/mL, and the interassay and intra-assay coefficients of variations (CVs) of the assay are 11.4. The range for the LH assay is 0.1 to 55.2 mIU/mL, minimum detectable concentration is 0.1 mIU/mL, and interassay and intra-assay CVs are 10.9% and 4.6%, respectively. Measures of E1c and PdG were performed with competitive immunoassays, adapted to chemiluminometric technology (Lasley Center for Health and the Environment, 441-437 CHE Labs). The reporting range for the E1c assay is 5.10 to 408.0 ng/mL, minimum detectable concentration is 0.1 ng/mL, and interassay and intra-assay CVs for the E1c assay are 11.5% and 8.1%, respectively. The reporting range for the urine PdG assay is 0.005 to 25.5 μg/mL, the minimum detectable concentration is 0.0001 μg/mL, and the interassay and intra-assay CVs are 17.8% and 7.7%, respectively. The present study performed aMT6S assays using urine samples from the selected DHS collections (which were stored at –80°C) measured by enzyme-linked immunosorbent assay (IBL International) [21]. Cross-reactivity with other substances tested is less than 0.0001% (% cross-reactivity: melatonin sulfate 100; melatonin 0.002; 6-OH-melatonin 0.001; N-acetyl-L-OH-tryptamine 0.0005; N-acetyl-L-tryptophan < 0.0001; 5-methoxy-tryptamine < 0.0001; tryptamine < 0.0001; 5-HIAA < 0.0001). The limit of detection is 1.0 ng/mL, range is 5.8 to 204 ng/mL, and CV is 5.2% to 12.2%. Interassay measurements are 12.4 to 220 ng/mL with CV of 5.1% to 14.9%. aMT6s values were normalized to urinary creatinine measured in the same urine sample (Sigma-Aldrich Corp; creatinine assay results available from the DHS).

C. Data Analysis

To examine day-to-day variation, we plotted the daily mean of each analyte (aMT6s, E1c, PdG, FSH, and LH) by menstrual cycle day over all ELA collections, and by collection day over all NELA cycles. Menstrual cycle day 0 designates the day of luteal transition (DLT), days prior to the DLT (follicular phase) are designated with negative values, and days after the DLT (luteal phase) are given positive values [19].

To quantify relations of aMT6s with PdG, E1c, and gonadotropins, we took a 3-step approach. In step 1, we inspected the daily mean analyte plots in ELA cycles, to identify the peak day of each analyte's excretion. In step 2, we separately regressed the aMT6s value (outcome) against lagged values of each of the sex-steroid hormones and gonadotropins (predictor) in ELA collections, with lag times set at the number of days between the peak days estimated (in step 1) of predictor and aMT6s (± 1 day to allow for errors in estimation). For example, peak FSH excretion occurred on cycle day 0 and peak aMT6s on cycle day 15, thus the lag time between FSH and aMt6s was set at 15 ± 1 days (Fig. 1A and 1D. We used individual fixed-effects models for the regressions, thereby eliminating between-woman differences in all characteristics and isolating relationships between within-woman changes in predictor and outcome [22]. If we found no evidence of an association between aMT6 and predictor in the step 2 regressions ($P > .05$), we ceased analysis for that exposure. In step 3, we regressed aMT6 on the candidate exposures and lag times identified in step 2 in the combined ELA and NELA data. Again, we used individual fixed-effects regression, treating ELA and

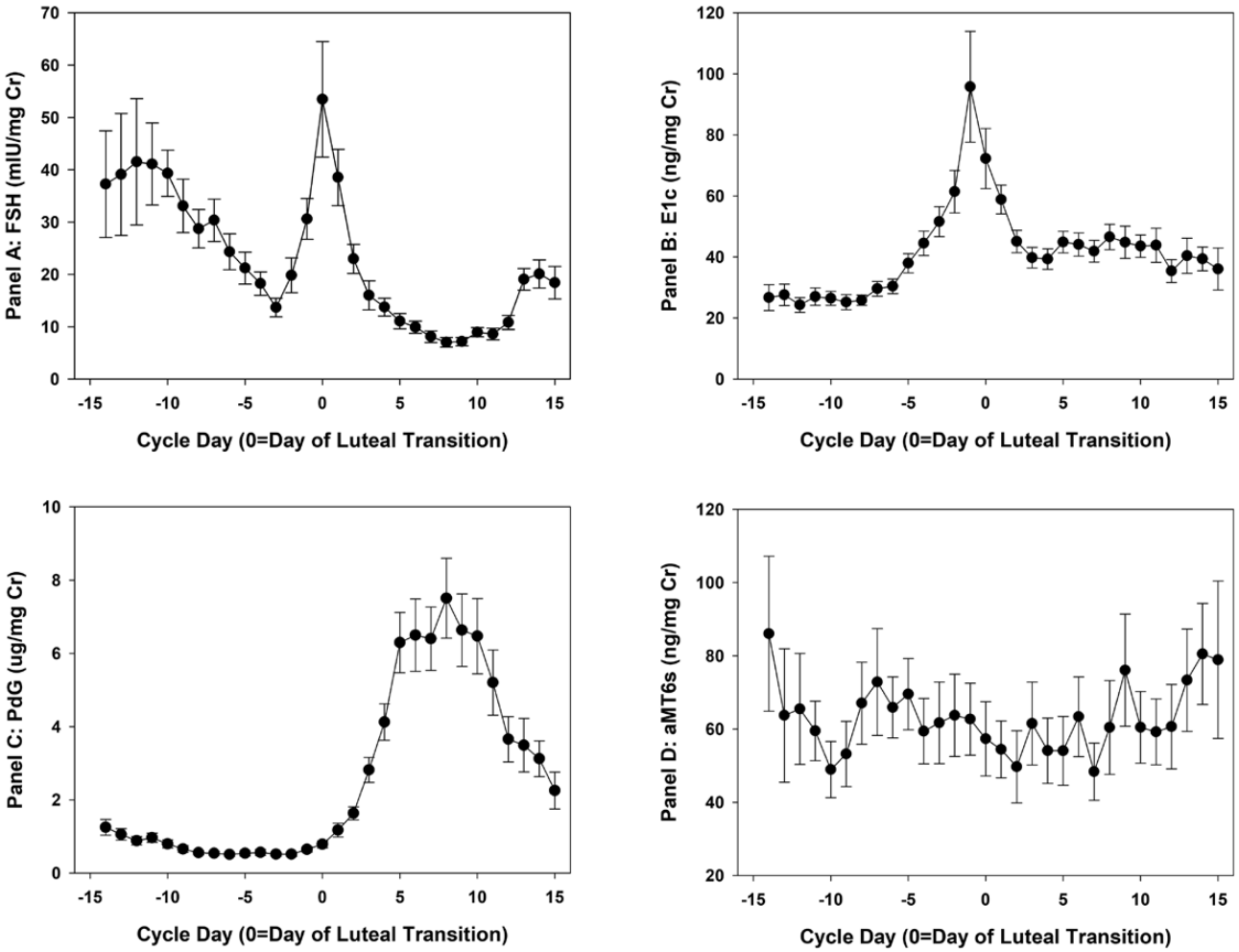

Figure 1. Study participants collected a first-morning urine sample, starting on their first day of menstrual bleeding through the first day of bleeding in the next cycle, or, if no menses occurred, for a maximum of 50 days. Fig. 1 graphs daily mean urinary levels of follicle-stimulating hormone (FSH), estrone conjugates (E1C), pregnanediol glucuronide (PdG), and 6-sulfatoxymelatonin (aMT6s) over all urine collections that had evidence of luteal activity (ELA) [15, 16]. These cycles come from the 20 participants in the current study sample when they were premenopausal. Mean values of each analyte are plotted by menstrual cycle day, where day 0 designates the day of luteal transition (DLT), days prior to the DLT (the follicular phase) are designated with negative values, and days after the DLT (the luteal phase) are shown with positive values.

NELA collections from the same woman as data from distinct individuals, to allow for variability in the ELA-NELA difference in average hormone levels. If we did find an association, we expanded the duration of the lag in both directions until the association between aMT6 and candidate predictor was no longer statistically significant.

To allow for the possibility that aMT6 drives the other hormones, we repeated the 3-step analysis with aMT6 as predictor and using as dependent variables the sex-steroid hormones and gonadotropins that were confirmed to have a relationship with aMT6 in the third step of the main analysis. Because the aMT6 peak in ELA cycles occurs in the luteal phase, the number of days to lag aMT6 for these analyses was determined by assuming the ELA cycle repeats itself and counting the number of days between the aMT6 peak and the hormone/gonadotropin peak in the next cycle.

To compare the level of excretion of aMT6s in premenopausal ELA vs postmenopausal NELA collections, we calculated the geometric means of aMT6 over each collection after interpolating values for single missing days [20]. To statistically test for a difference between ELA and NELA means within women, we used linear mixed-effects regression of the log-transformed geometric means on ELA/NELA status, with a random intercept at the participant level [19, 23].

2. Results

A. Participant Characteristics

The study sample (N = 20) consisted of 5 Black (25%), 11 Japanese (55%), and 4 White (20%) women (Table 1). Mean age at the first urine collection was 46 years. At the second collection, women were an average of 6 years older. By design, all were premenopausal at the first collection

and postmenopausal at the second. Table 1 also summarizes participants' anthropometric characteristics at the time of each collection.

B. Evidence of Luteal Activity and No Evidence of Luteal Activity Patterns of Sex-Steroid Metabolites, Gonadotropins, and 6-Sulfatoxymelatonins

Plots of daily mean values of sex-steroid metabolites and gonadotropins in ELA cycles according to menstrual cycle day (where 0 marks the DLT, negative values indicate days prior to DLT, and positive values designate days after the DLT) show the expected contours of a cycle that is likely ovulatory (Fig. 1A-1C). Unequivocal midcycle surges of E1c, FSH, and LH, followed by a luteal phase rise in PdG characterized all ELA cycles. The graphs also suggest that average daily aMT6s rises in the luteal phase (Fig. 1D). In NELA collections, the periodicity of sex-steroid hormones and gonadotropins is absent (Fig. 2A-2C). Similarly, aMT6s excretion also appears to be unsystematic (Fig. 2D).

C. Follicle-Stimulating Hormone, Luteinizing Hormone, Estrone Conjugates, and Pregnanediol Glucuronide as Predictors of 6-Sulfatoxymelatonin in Evidence of Luteal Activity Cycles

To test the hypothesis that the excretion of aMT6s was driven by FSH, LH, E1c, and/or PdG, with a lag of several days, we first identified peak cycle days (maximum values) of each of these analytes, in ELA cycle patterns (see Fig. 1).

Table 1. Characteristics of the Study of Women's Health Across the Nation Daily Hormone Study (SWAN DHS) subsample at the time of each urine collection (N = 20)[a]

Participant characteristics	Premenopausal cycle with evidence of luteal activity (ELA)[b]	Postmenopausal cycle with no evidence of luteal activity (NELA)[b]
Race/Ethnicity		
White	4 (20.0%)	
Black	5 (25.0%)	
Japanese	11 (55.0%)	
Age, y	46.00 ± 1.56	52.25 ± 1.80
Body mass index, kg/m^2	25.91 ± 4.73	27.20 ± 5.09
Weight, kg	64.56 ± 12.80	67.70 ± 14.29

[a]SWAN DHS participants who did not report nocturia more than once per week, submitted at least one ELA cycle during premenopause, and one NELA cycle during postmenopause.
[b]DHS categorized urinary collections as having ELA with an algorithm that uses the degree of rise in progesterone glucuronide excretion as an indicator of likely ovulation. Collections that did not meet the ELA standard were classified as having NELA.

Proposed peak days by visual inspection were cycle day 15 for aMT6s, cycle day 0 for FSH and LH, cycle day –1 for E1c, and cycle day 8 for PdG. We postulated lag times between each potential predictor (FSH, LH, E1c, or PdG) and aMT6s (outcome) corresponding to the differences between the predictor peaks and outcome peak, plus or minus 1 day. Thus, initially tested lag times were 15 ± 1 days for the gonadotropins, 16 ± 1 days for E1c, and 7 ± 1 days for PdG. This first round of models did not disclose an association between LH or E1c levels and aMT6s (Table 2). FSH measured 14, 15, or 16 days prior to aMT6s was negatively associated with aMT6s (β coefficients = –0.164 to –0.149; P = .030-.061). In addition, aMT6s was positively related to PdG values from 6, 7, or 8 days prior to aMT6s measurement (β coefficients = 0.115-0.142, ; P = .011-.015).

D. Relations Between 6-Sulfatoxymelatonin, Follicle-Stimulating Hormone, and Pregnanediol Glucuronide in Evidence of Luteal Activity and No Evidence of Luteal Activity Cycles Combined

Building on the lag time hypotheses generated from the ELA cycles, we conducted further analysis of the relations between the statistically significant predictors of aMT6s, which were FSH (lagged by 14-16 days) and PdG (lagged by 6-8 days) (see Table 2). The next set of models examined a wider span of lag times to discern the lag at which the association between candidate predictor and aMT6s was no longer statistically significant and included data from both the ELA and NELA cycles. PdG was strongly associated with aMT6s at lags ranging from 6 to 13 days prior to aMT6s level (Table 3). The magnitude of the association between PdG and aMT6s became larger as the number of lag days increased, reaching maximal effect size at 11 to 12 days. PdG values that lagged by 11 days (β coefficient = 1.46, P = .001) or 12 days (β coefficient = 1.44, P = .001) were the strongest predictors of aMT6s. On day 11, the peak day of the association, for each 1-µg/mL rise in PdG, aMT6s level goes up by 0.46 ng/m. The magnitude of the association between PdG and aMT6s diminished when lagged by 13 days and was no longer statistically significant when a 14-day span was tested. The amount of within-cycle variance in aMT6s accounted for by PdG in each of the regressions was high: Each R^2 was greater than 0.52. In regressions using data from both ELA and NELA cycles combined, FSH and aMT6s levels were no longer related (see Table 3).

E. Examining 6-Sulfatoxymelatonin as a Predictor of Pregnanediol Glucuronide

In ELA and NELA collections combined, PdG was the sole significant predictor of aMT6s—however, the biological relation could be in the reverse direction. We

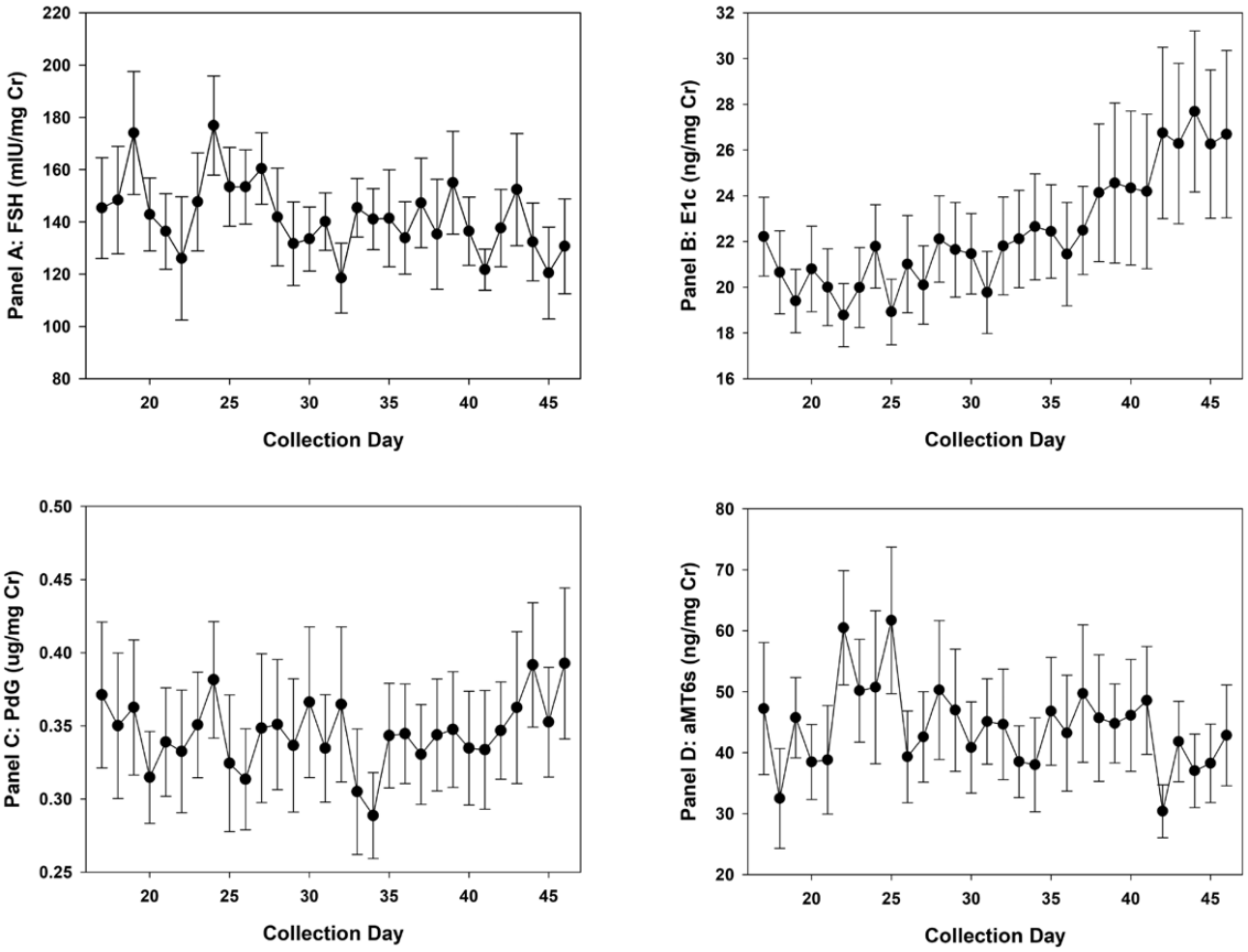

Figure 2. Study participants collected a first-morning urine sample, starting on their first day of menstrual bleeding through the first day of bleeding in the next cycle, or, if no menses occurred, for a maximum of 50 days. Fig. 2 graphs daily mean urinary levels of follicle-stimulating hormone (FSH), estrone conjugates (E1C), pregnanediol glucuronide (PdG), and 6-sulfatoxymelatonin (aMT6s) over all urine collections without evidence of luteal activity (NELA) [15, 16]. These cycles come from the 20 participants in the current study sample when they were postmenopausal. Mean values of each analyte are plotted by day of collection (1-50).

therefore modeled aMT6s as the predictor of PdG. As the first estimate of the lag between predictor and outcome, we used the number of days between the aMT6 peak and the PdG peak in the next cycle, assuming the cycle repeats. (The lag was based on a visual inspection of the hormone graphs, then expanded around the first estimate, as described in "Materials and Methods.") We examined the R^2 values of each model to gauge which direction of prediction had the stronger association (ie, PdG predicting aMT6 vs aMT6 predicting PdG). Shown in Table 3, the R^2 values for models of PdG predicting aMT6s, with number of days prior to aMT6 ranging from 11 to 16, are between 0.52 and 0.53. We expanded the lag times to include PdG 6 days prior to 13 days prior to aMT6s; this range was selected based on the β coefficient for PdG remaining statistically significant in each model. R^2 values for these additional lag times were also between 0.52 and 0.53. For the reverse direction, aMT6s predicting PdG, we examined lag times of 21 days before through 14 days before PdG. (This range was chosen based on the β coefficient for aMT6s remaining statistically significant in each model.) The R^2 values of the aMT6 predicting PdG models were between 0.33 and 0.34 (data not shown).

F. Longitudinal Change in 6-Sulfatoxymelatonin

The geometric mean aMT6s value in premenopausal ELA samples was 53.5 ng/mL (95% CI, 41.19-69.56 ng/mL), statistically significantly greater than the mean of 37.4 ng/mL (95% CI, 28.80-48.65 ng/mL) in postmenopausal NELA samples from the same women; (P = .0002 for ELA-NELA difference) (data not tabulated). Mean age during ELA collections was 46.0 years (range, 43.2-49.7 years), whereas in NELA visits it was 52.2 years (range, 49.2-56.1 years).

Table 2. Associations between urinary gonadotropins or sex-steroid hormone conjugates and urinary melatonin sulfate (aMT6) levels across a range of lag times for each candidate predictor in cycles with evidence of luteal activity only

Candidate predictors: urinary gonadotropins and sex-steroid hormone conjugates	Lag between each candidate predictor and aMT6, d[a]	Association of each candidate predictor with aMT6 level, ng/mL	
		β coefficient	*P*
Follicle-stimulating hormone (FSH), mIU/mL	14	–0.164	.03
	15	–0.146	.06
	16	–0.149	.05
Luteinizing hormone, mIU/mL	14	–0.020	.95
	15	0.117	.68
	16	0.040	.89
Estrone conjugates, ng/mL	15	0.088	.13
	16	0.092	.12
	17	0.046	.44
Pregnanediol glucuronide, µg/mL	6	1.115	.02
	7	1.142	.01
	8	1.125	.01

[a]The lag time between each proposed predictor and aMT6 outcome was based on the peak day of each analyte's excretion relative to the peak day of aMT6s, ± 1 day. For example, peak FSH excretion occurred on cycle day 0 and peak aMT6 on cycle day 15, for a postulated lag time of 15 ± 1 days.

Table 3. Associations between follicle-stimulating hormone (FSH) or pregnanediol glucuronide (PdG) and urinary melatonin sulfate (aMT6) levels across a range of lag times for each candidate predictor in all cycles

Candidate predictors: urinary FSH or PdG[a]	Lag between each candidate predictor and aMT6, d[b]	Association of each candidate predictor with aMT6 level, ng/mL		
		β coefficient	*P*	R^2
FSH, mIU/mL	11	0.005	.83	0.524
	12	–0.003	.89	0.520
	13	–0.011	.63	0.519
	14	0.037	.10	0.523
	15	–0.007	.76	0.524
	16	0.002	.91	0.523
PdG, µg/mL	4	0.338	.42	0.522
	5	0.687	.10	0.525
	6	1.116	.01	0.526
	7	1.122	.01	0.522
	8	1.120	.01	0.529
	9	1.274	.00	0.526
	10	1.299	.00	0.529
	11	1.461	.001	0.530
	12	1.437	.001	0.525
	13	1.168	.00	0.523
	14	0.645	.12	0.523

[a]Based on associations observed in the evidence of luteal activity (ELA) cycles (Table 2), we pursued additional analysis of the relations between aMT6s and FSH and PdG, using data both from ELA and no evidence of luteal activity cycles.
[b]Candidate predictor level precedes aMT6s level by the number of days shown.

3. Discussion

Using existing data and new urine aMT6s assays from the SWAN DHS, this study identified a cyclic rise in aMT6s, a melatonin metabolite, that occurred in the late luteal phase of the menstrual cycle. Moreover, the luteal rise in PdG, a metabolite of progesterone, predicted the aMT6s rise. A within-woman, longitudinal analysis of aMT6s excretion patterns, first during premenopausal cycles and then during postmenopausal collections, generated 2 findings. There was no organized pattern of aMT6s excretion in postmenopausal cycles, each of which had no evidence of luteal activity, and the total amount of aMT6s excretion declined by 30% in the postmenopausal collections compared to the premenopausal ones.

Prior studies of melatonin patterns during the human menstrual cycle are sparse but concordant with our results. In a study of 10 women, Webley and Leidenberger assayed serum melatonin at 4-hourly intervals for 1 day during the follicular phase and 1 day during the luteal phase and reported that melatonin levels were significantly higher during the luteal phase [6]. Measuring morning serum melatonin and progesterone levels every 3 days for one menstrual cycle in a sample of 5 women, Wetterberg and colleagues described that a melatonin rise occurred specifically in the late luteal phase, and that it followed the midluteal increase in PdG [5, 24]. The present study adds a larger sample size, a daily, overnight urinary estimate of aMT6s, and concomitant measures of gonadotropins and metabolites of estrogen and progesterone, permitting the analysis of daily aMT6s excretion relative to the daily hormonal contours of a full menstrual cycle. The longitudinal component, which measured these same analytes in a subsequent, postmenopausal, nonovulatory cycle, can be viewed as a negative control—the aMT6s rise is absent as are the organized patterns of gonadotropins and sex-steroid hormones.

What is the function of the melatonin rise in the luteal phase of the menstrual cycle? One thesis is that melatonin, either directly or indirectly through its antioxidant properties, stimulates progesterone production and stabilizes the corpus luteum (CL) [25]. While our

regression analyses disclosed an association between progesterone and melatonin, the magnitude of that association was stronger when we modeled progesterone *predicting* melatonin, rather than the reverse. The direction of the stronger association is at odds with the idea that melatonin stabilizes the CL. However, melatonin levels in preovulatory follicular fluid are approximately 3-fold higher than are serum levels [26]. It is possible that the level of *intrafollicular* melatonin (not captured by the present study) is what supports the subsequent production of progesterone and stabilizes the CL. Another distinct function of melatonin may be to govern the menstrual cycle through its reciprocal relation with gonadotropin-releasing hormone pulse frequency [27]. The gonadotropin-releasing hormone pulse generator is slowest (approximately every 4-6 hours) in the late luteal phase, when melatonin is high, and is fastest (approximately circhoral) at midcycle, when melatonin is low [28]. Therefore, the late-luteal melatonin increase originally described by Wetterberg and confirmed here may be part of the coordinated pacemaker system that regulates the human menstrual cycle [24].

Longitudinal analysis revealed that the whole cycle excretion of aMT6s was 30% lower when participants were postmenopausal compared to when they were premenopausal. However, because of collinearity between age and menopause stage in this small study, we are unable to separate their effects. It is plausible that chronological aging and reproductive aging (menopause) have independent influences on melatonin levels across the lifespan. Besides its preeminent function as a circadian pacemaker, some have hypothesized that melatonin is etiologic in the development of age-related diseases [7]. However, support for this concept comes mainly from cross-sectional studies that report lower melatonin secretion with increasing age, corresponding to the rise of potentially melatonin-related conditions. Similarly, the overlap between MT symptoms and those that may be influenced by melatonin (eg, poor sleep quality, dysphoric mood) raises the question of whether melatonin declines in relation to menopause and whether such a decline underlies some menopause-associated conditions [10, 11, 29-31]. One cross-sectional study of 17 cycling women and 18 postmenopausal women found substantially lower melatonin levels in postmenopause (but was unable to adjust for age because of collinearity with menopause) [32]. To our knowledge, no longitudinal studies have had measures of both melatonin and outcomes of interest in relation to aging or menopause.

The principal limitations of this study are its small size, a design that created confounding between MT stage and age, and potential misestimation of absolute aMT6s levels due to long-term storage. This confounding resulted from our selection of premenopausal and postmenopausal observations that maximized the amount of chronologic time between ELA and NELA collections. Therefore, there was complete separation of chronological age between the premenopausal and postmenopausal collections. Thus, although the study was able to describe patterns of hormones and their interrelations during the menstrual cycle, it was unable to discern whether menopause and aging independently influenced the observed, longitudinal drop in melatonin excretion. The long-term stability of aMT6s is robust [33], but urine samples may have concentrated in the freezers, which could result in falsely inflated absolute levels of aMT6s. However, it is likely that all repository samples would be affected similarly by such concentration. Therefore, the patterns of aMT6s within each collection and the differences between premenopausal and postmenopausal levels should not be affected. Study strengths include daily sampling and an integrated, overnight assessment of melatonin, gonadotropin, and sex-steroid hormone excretion. Moreover, although the sample size is modest, it is substantially greater than those of prior reports and the first sample in which aMT6s was assessed longitudinally in premenopause and postmenopause.

In summary, this study contributes to our understanding of the biology of the human menstrual cycle by confirming a late-luteal melatonin peak that follows the progesterone rise. This melatonin increase appears to be signaled by progesterone; thus, circulating levels of late-luteal melatonin are more likely to be involved in cycle pacemaker control rather than influencing progesterone production. Finally, a definitive, 30% diminution of melatonin occurred in the postmenopausal collections, but whether this is an aging and/or menopause effect cannot be determined within the present design. Larger, longitudinal studies of melatonin in relation to aging and menopause are warranted.

Acknowledgments

We thank the study staff at each site and all the women who participated in SWAN.

Financial Support: The Study of Women's Health Across the Nation (SWAN), which includes the SWAN Daily Hormone Study, was supported by the National Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National Institute of Nursing Research (NINR), and the NIH Office of Research on Women's Health (ORWH) (Grants U01NR004061, U01AG012505, U01AG012535, U01AG012531, U01AG012539, U01AG012546, U01AG012553, U01AG012554, U01AG012495, and 5R01AG026463), Marie-Clement Rodier, C.S.Sp. Endowed Chair funds (PWE) and the SWAN Repository, 5U01AG017719). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIA, NINR, ORWH, or the NIH.

Clinical centers: University of Michigan, Ann Arbor, Michigan—Siobán Harlow, principal investigator (PI) 2011 to present, MaryFran Sowers, PI 1994 to 2011; Massachusetts General Hospital, Boston, Massachusetts—Joel Finkelstein, PI 1999 to present; Robert Neer, PI 1994 to 1999; Rush University, Rush University Medical Center, Chicago, Illinois—Howard Kravitz, PI 2009 to present; Lynda Powell, PI 1994 to 2009; University of California, Davis/Kaiser, California—Ellen Gold, PI; University of California, Los Angeles, California—Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, New York—Carol Derby, PI 2011 to present, Rachel Wildman, PI 2010 to 2011; Nanette Santoro, PI 2004 to 2010; University of Medicine and Dentistry–New Jersey Medical School, Newark, New Jersey—Gerson Weiss, PI 1994 to 2004; and the University of Pittsburgh, Pittsburgh, Pennsylvania—Karen Matthews, PI. NIH Program Office: National Institute on Aging, Bethesda, Maryland—Chhanda Dutta 2016 to present; Winifred Rossi 2012 to 2016; Sherry Sherman 1994 to 2012; Marcia Ory 1994 to 2001; National Institute of Nursing Research, Bethesda, Maryland—program officers. Central laboratory: University of Michigan, Ann Arbor—Daniel McConnell (Central Ligand Assay Satellite Services). Coordinating Center: University of Pittsburgh, Pittsburgh, Pennsylvania—Maria Mori Brooks, PI 2012 to present; Kim Sutton-Tyrrell, PI 2001 to 2012; New England Research Institutes, Watertown, Massachusetts—Sonja McKinlay, PI 1995 to 2001. Steering committee: Susan Johnson, current chair, Chris Gallagher, former chair.

Author Contributions: Obtaining funding for the current pilot project: G.A.G., N.S., and P.W.E.; SWAN Daily Hormone Study participant recruitment and enrollment: G.A.G., M.H.H., and N.S.; conducting aMT6s assays: F.M. and P.W.E.; data management and cleaning: S.C., M.H.H., and A.S.K.; study design: G.A.G., S.C., N.S., P.W.E., and A.S.K.; statistical analysis: S.C., G.A.G., M.H.H., and A.S.K.; primary manuscript drafting: G.A.G.; and critical review and revision of manuscript: all.

Additional Information

Correspondence: Gail A. Greendale, MD, Department of Medicine, Division of Geriatrics, UCLA, Los Angeles, CA, 10945 Le Conte Ave, Suite 2339, Los Angeles, CA 90095, USA. E-mail: ggreenda@mednet.ucla.edu.

Disclosure Summary: The authors have nothing to disclose.

Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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