

Total and Unopposed Estrogen Exposure across Stages of the Transition to Menopause

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Abstract

Detailed characterization of estrogen dynamics during the transition to menopause is an important step toward understanding its potential implications for reproductive cancers developing in the transition years. We conducted a 5-year prospective study of endogenous levels of total and unopposed estrogen. Participants ($n = 108$; ages 25–58 years) collected daily urine specimens for 6 months in each of 5 consecutive years. Specimens were assayed for estrone-3-glucuronide (E1G) and pregnanediol-3-glucuronide. Linear mixed-effects models were used to estimate exposure to total and unopposed estrogen by age and reproductive stage. Reproductive stage was estimated using menstrual cycle length variance. E1G mean area under the curve and mean E1G 5th and 95th percentiles represented total estrogen exposure. An algorithm identifying days of above-baseline E1G that coincided with the days of

baseline pregnanediol-3-glucuronide was used to identify days of unopposed estrogen. Mean E1G area under the curve increased with age in the pretransition and early transition and decreased in the late transition. Ninety-fifth percentile E1G levels did not decline until after menopause, whereas 5th percentile levels declined from the early transition to the postmenopause. The number of days of unopposed estrogen was significantly higher during the transition compared with the pretransition. Given the length of time women spend in the transition, they are exposed to more total and unopposed estrogen than has been previously appreciated. Coupled with epidemiologic evidence on lifetime exposure to estrogen, these results suggest that variation in the amount of time spent in the transition may be an important risk factor for reproductive cancers. (Cancer Epidemiol Biomarkers Prev 2009;18(3):828–36)

Introduction

Recent data show that, among both White and Black women, age-specific incidence rates of malignant cancers of the breast, ovaries, and endometrium rise through the perimenopausal years up to at least age 60 years (1). Substantial evidence supports an association of endogenous reproductive hormone exposure with increased risk of reproductive cancers (2–4). Greater estrogen exposure, assessed via indirect indicators such as number of years spent having menstrual cycles (e.g., ref. 5) or direct indicators such as hormone measures (e.g., ref. 6), is associated with increased risk for cancers of the breast and ovary (3, 7, 8). Similarly, exposure to estrogen unopposed by progesterone is a risk factor for endometrial and ovarian cancers (8). The precise mechanism by which estrogen contributes to reproductive cancers is not known, but etiologic theories highlight the role of estrogen in cell

proliferation in the endometrium and breast and epithelial repair of the ovary following ovulation (3, 7–10).

Erratic estrogen secretion is characteristic of the perimenopausal years (11) and may be an important source of risk for reproductive cancers. However, the perimenopause, which may last several years (12), has not been explicitly considered in studies of hormones and reproductive cancers. Hale et al. (13) recently emphasized Pike's (14) suggestion that the perimenopausal years constitute an important "window of risk" for endometrial cancer. These years may also be an important window of risk for other estrogen-related cancers, such as breast and ovarian cancers (15), as well as for the increased likelihood of uterine fibroids, endometrial hyperplasia, dysfunctional uterine bleeding, and progression of endometriosis symptoms observed in the perimenopause (16). Thus, detailed characterization of estrogen dynamics during the perimenopause is important for understanding its potential implications for reproductive cancers and other health outcomes.

Menstrual cycle length changes as women make the transition to menopause. On average, cycles become longer and more variable with increasing proximity to menopause (17). Compared with the hormonal patterns of the prime reproductive years, the transition to menopause is associated with increased variability in estrogen and progesterone patterns (11, 18, 19). Previous

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studies have documented higher (20-24), lower (25-30), or unchanged (31-35) estrogen levels in older or perimenopausal women compared with younger or premenopausal women. In recent work, we found that individual-level urinary estrone-3-glucuronide (E1G) increased from 25 to 45 years and then declined in the late 40s for most women (36). These disparate results arise because of (a) differences among studies in the timing of sampling, sample selection or sample size, and study design, (b) erratic fluctuations in hormone levels confounding comparisons during the perimenopause, and (c) use of age as an anchor for comparisons among women: aggregate estrogen patterns can mask hormonal trajectories that individual women actually experience as they age (36).

Thus, it remains unclear whether women are exposed to more or less overall estrogen during the perimenopause compared with the prime reproductive years. Here, we examine estrogen trajectories for individual women across reproductive stages (37). We estimate total and unopposed estrogen exposure by stage while controlling for age and body mass index (BMI).

Materials and Methods

Participants. Data were collected as part of the Biodemographic Models of Reproductive Aging (BIMORA) project (36). Participants were recruited from the Tremin Research Program on Women's Health (TREM; ref. 38). Participants included women aged between 25 and 60 years, not using prescription reproductive hormones, and who had at least one intact ovary. Pregnant or breastfeeding women and women receiving cancer treatment were not eligible. Monetary compensation was provided for participation. All subjects provided written informed consent, and all procedures were approved by the institutional review boards of the University of Utah, Pennsylvania State University, Georgetown University, and University of Washington.

Data Collection. First morning urine specimens and information on menstrual bleeding were collected daily from January 15 to July 14 of each year from 1998 to 2002. Daily information was collected on major medical conditions and treatments and over-the-counter and prescription medication. Most participants continued to record menstrual bleed data on calendar cards for TREM. We combined the TREM bleed data for July 15 to January 14 with the BIMORA data for January 15 to July 14 for each project year from 1998 to 2001; for 2002, we have only BIMORA bleed data.

Height and weight for BMI come from a 2000 self-administered health survey, at the midpoint of the study. BMI was available for the year 2000 for 90 of the 108 women included in the analyses. For the remaining 18 women, we used all available BMI data from previous years for each woman in a linear mixed-effects model of BMI by year and used the estimated model fits to impute a 2000 value. The mean (SD) and median BMI for the sample of 108 women was 24.2 (5.1) and 23.2 kg/m², respectively. Seventy percent of the BMIs were <25 (normal), 18% fell between 25 and 29 (overweight), and 12% were ≥30 kg/m² (obese).

Laboratory Methods. Urine specimens were assayed with enzyme immunoassays for E1G, a metabolite of estradiol, and pregnanediol-3-glucuronide (PDG), a metabolite of progesterone. Interassay and intra-assay coefficients of variation (CV) were 9.2% and 10.3% for the PDG enzyme immunoassay and 4% and 3.6% for the E1G enzyme immunoassay (39, 40). The metabolites and significant cross-reactants closely parallel the serum levels of estradiol and progesterone (39, 40).

Hormone concentrations were estimated from absorbance (Biolinx 1.0 software; Dynex Laboratories). Urinary hormone concentrations, assayed in duplicate, were adjusted by specimen specific gravity using a population mean specific gravity of 1.020 (41). E1G concentrations were statistically corrected for slight assay nonparallelism using a 1:5 dilution as the standard to which all values were corrected (40).

Reproductive Stage. A reproductive stage was assigned to each menstrual cycle in the study using a four-category scale (Table 1) derived from the Staging Reproductive Aging Workshop (STRAW) recommendations (37). Based on the criteria of variability in cycle length described in STRAW, we used the CV of menstrual cycle length to assign stage.

Cycle length was calculated as the number of days from the first day of a menstrual bleed to the last day before the next bleed. A menstrual bleed was defined as a segment with at least 2 days of bleeding in 6 consecutive days, which had to be preceded by at least 5 consecutive days of no bleeding.

A rolling cycle length CV (the SD of cycle length divided by the mean cycle length) was calculated for each cycle using the length of the current cycle and the five previous cycles; this CV was used to assign stage to each cycle. When fewer than five previous cycles were available, all cycle lengths observed before the current cycle were used.

Cutoff values for the rolling cycle length CV were chosen to represent reproductive stages as close as

Table 1. Criteria for assigning reproductive stage

| Stage no. | Description | Defining criteria |
|-----------|--------------------------------------|--------------------------------------------------------------------------------|
| Stage -3 | Premenopausal | CV of length of current cycle + previous 5 (or fewer) cycles = <20% |
| Stage -2 | Early stage of menopausal transition | CV of length of current cycle + previous 5 (or fewer) cycles = 20-40% |
| Stage -1 | Late stage of menopausal transition | CV of length of current cycle + previous 5 (or fewer) cycles = >40% |
| Stage +1 | Postmenopausal | OR presence of a cycle length ≥ 60 d No menstruation for the previous 12 mo |

Table 2. Age and E1G (mean \pm SD) for 6-month intervals by stage

| | Stage -3 (n = 103)* | Stage -2 (n = 17) | Mixed stage (n = 34) | Stage -1 (n = 71) | Stage +1 (n = 134) |
|------------------------------------|------------------------|----------------------|-------------------------|----------------------|-----------------------|
| Age at beginning of interval | 41.8 \pm 6.5 | 47.5 \pm 4.2 | 47.9 \pm 6.7 | 51 \pm 4.2 | 56.5 \pm 3 |
| E1G AUC [(pg/L) \times no. days] | 6,495 \pm 1,846 | 7,332 \pm 2,413 | 5,376 \pm 1,713 | 4,362 \pm 2,274 | 1,479 \pm 680 |
| 5th percentile (pg/L) | 13.7 \pm 4.6 | 13.9 \pm 6.4 | 9.5 \pm 4.8 | 7.0 \pm 3.4 | 4.6 \pm 1.7 |
| 95th percentile (pg/L) | 71.2 \pm 22.9 | 83 \pm 28.3 | 64.2 \pm 21.4 | 58.6 \pm 32.2 | 14.2 \pm 10.3 |

*Number of 6-mo intervals in the stage.

possible to the STRAW system (ref. 37; Table 1). Because CV values of <20% represent <7 days deviation from a 34-day cycle, we used it to indicate premenopausal cycling (stage -3). A 20% to 40% CV was chosen to represent the STRAW criterion of cycle length variance >7 days (stage -2). A CV >40% or the presence of a cycle >60 days in length was used for the STRAW criterion of two or more skipped cycles (stage -1). Postmenopause (stage +1) was defined as 1 year with no menstrual bleeding.

Cycles that were right or left censored at <60 days within a 6-month interval were assigned a stage using the predominant stage (the stage occurring \geq 60% of the time within the 6-month segment) in the interval. Cycles censored at \geq 60 days in length were assigned either stage -1 or +1; in some censored cycles, it was not possible to reliably differentiate between stages -1 and +1, but this occurred in only 11 of 3,303 menstrual segments. Intervals where the most prevalent stage occurred <60% of the time were coded as mixed stage. This designation was assigned to 39 of the 359 six-month intervals. Forty-four percent of the 39 cases included stages -3, -2 and -1 within an interval, 8% were cases of one reproductive stage and a substantial censored segment, and the remainder were other combinations of the stages -3, -2, and -1 and censored segments.

Quantification of Total and Unopposed Estrogen. To quantify total estrogen levels, we examined mean E1G area under the curve (AUC). We also examined the 5th and 95th percentiles of E1G as indicators of baseline and peak levels of E1G, respectively. We assessed how these varied by age and stage while controlling for BMI.

To quantify unopposed estrogen, we used the total number of days per cycle where PDG was at a per-cycle baseline level and E1G was above a per-cycle baseline level. A daily running 5-day PDG average was used to create a daily PDG ratio (PR): the PDG of the current day divided by the average PDG. A PR < 3.0 was considered baseline PDG; when the PR exceeded 3.0 for at least 3 days in a 5-day sequence (the criterion used in ovulation detection algorithms to identify a sustained rise in PDG; e.g., ref. 42), then all days from the first day the PR exceeded 3.0 to the end of the cycle were scored as having sufficient progesterone to oppose estrogen. To identify days where E1G was above baseline level, we began with a day of estrogen takeoff (43). All subsequent days were scored as above E1G baseline until the end of the cycle or a clear decline in E1G occurred. E1G data for menstrual cycles were first smoothed using the function: smoothed E1G = (previous day's E1G value) (0.25) + (current day's E1G value) (0.50) + (next day's E1G value) (0.25). Inactive (baseline estrogen) periods were identified by one of us (R.J.F.) from graphs showing menstrual

bleeds and smoothed E1G data. The end of an inactive period was indicated by a sharp and sustained increase in E1G and was scored as the first day of E1G above baseline.

All days between the day of estrogen takeoff and the day of progesterone takeoff were designated as unopposed; all other days in a cycle were designated as opposed. Additional criteria were needed in \sim 20% of cycles. In such cases, we used a steroid ratio (SR = E1G/PDG) to indicate days when estrogen was elevated while progesterone was at baseline (SR \geq 20). We developed a hierarchical set of rules to accommodate all possible cases and applied these to all cycles; the rules are presented in the appendix. To test implementation of the rules, a sample of 70 six-month segments of data was scored independently by two investigators (K.A.O. and R.J.F.). Inter-rater agreement based on the κ statistic was 95% for 12,030 observations (κ = 0.8533; SD = 0.0091). The discrepancies between raters were small and not systematic. One investigator then assigned days of unopposed estrogen to all data.

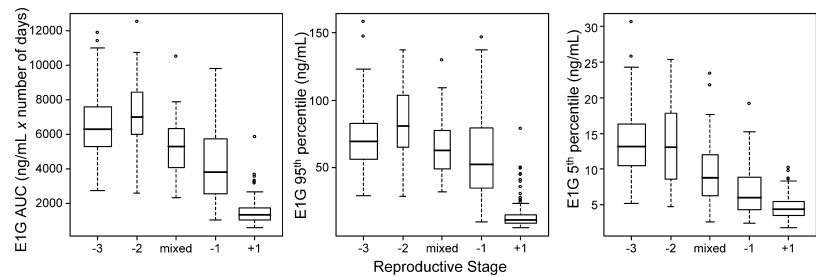
Statistical Analyses of Total and Unopposed Estrogen by Age and Reproductive Stage. The unit of analysis was a 6-month interval. Total estrogen was calculated as the AUC for daily E1G over the interval from January 15 to July 14 of each year. If >30 days were missing data, the interval was excluded. If <7 consecutive days were missing, AUC was interpolated. Where >7 consecutive days were missing or there were missing data at either end, AUC was computed without that segment, and the final result was weighted upward to represent the full 181-day span. We also used daily E1G data to estimate baseline (5th percentile) and peak (95th percentile) E1G levels for the 6-month intervals. The total number of days of unopposed estrogen for each interval was estimated by summing the individual days of unopposed E1G across the interval. For intervals with <181 days of complete data, the sums were weighted upward to represent counts over 181 days.

We used linear mixed-effects models to assess whether AUC, baseline, peak, and unopposed estrogen differed by stage. Models had two levels: individual and within-individual. Individual was considered a random effect; fixed effects comprised stage, age at study entry, and within-subject longitudinal aging (difference between age at study entry and age at beginning of 6-month interval). Analyses were done on logged values of E1G.

Results

One hundred fifty-six women ages 26 to 58 years participated in BIMORA. Fifty-three women participated

Figure 1. Boxplot of 6-month E1G AUC (left), E1G 95th percentile (middle), and E1G 5th percentile (right) by stage. Box width is proportional to the number of intervals in a stage.



for the full 30 months (five 6-month study intervals); the average length of participation was 21 months (36). For this article, data were excluded for (a) participants with no uterus, (b) 3 months following exogenous hormone use, pregnancy, breastfeeding, miscarriage, major surgery, chemotherapy, or use of any medications known to affect reproductive hormone or menstrual bleed patterns, and (c) ambiguous bleed or cycle day information. The resulting sample included 108 women, 64,671 woman-days of observation, and 359 six-month intervals.

Reproductive Stage. The CV method for assigning stage is very similar to the SD method used by Lisabeth et al. (44) for classifying women into stages; comparison of the two methods using our data yielded 92% agreement in assigning the same discrete stages based on cycle length variation.

Although mean age increased across stages, each stage had a broad and similar range of ages represented (Table 2).

Total, Baseline, and Peak Estrogen. Participant age, mean \pm SD values for E1G AUC, and 5th and 95th percentiles are shown for each stage in Table 2. Total (AUC), baseline (5th percentile), and peak (95th percentile) E1G varied by stage adjusting for age ($P < 0.0001$ for all three models; Fig. 1). E1G AUC was higher for stage -3 than for stages -1 ($P = 0.0013$) and +1 ($P < 0.0001$); there were no differences in AUC among stages -3 and -2 and mixed stages (Fig. 1). There were differences ($P < 0.0001$) in 5th and 95th E1G percentiles by stage while adjusting for cross-sectional age (at beginning of interval) and longitudinal age (time in study). Mean peak E1G was higher in stage -3 than stage +1 ($P < 0.0001$), and mean baseline E1G was higher in stage -3 than in stages -1 and +1 ($P < 0.0001$ for each). There were no significant differences in mean peak or total E1G between stage -3 and the transition stages combined (-2, mixed, and -1). Baseline E1G was higher in stage -3 than the transition stages combined ($P < 0.001$; Fig. 1).

There was a significant interaction between subject age and stage for total and peak E1G ($P < 0.0001$), with a

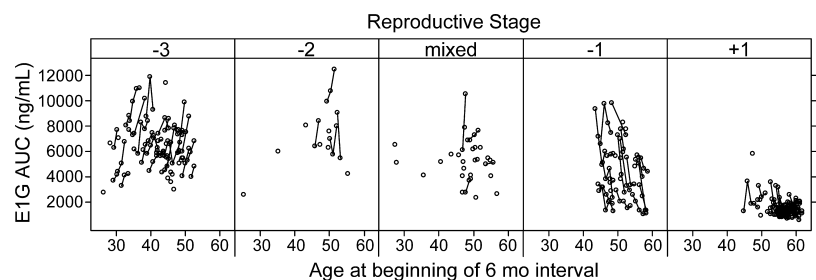
much weaker result for baseline E1G ($P = 0.032$). In general, even after adjusting for age at study entry, E1G tended to increase with age in stage -3 but decrease with age in stage -1 (Fig. 2).

The above findings that average E1G AUC and average baseline E1G did not decline until late in the transition (stage -3), whereas average peak E1G did not decline until the postmenopause (stage +1), were unchanged with the addition of BMI to the models.

Unopposed Estrogen. Figure 3 shows typical examples of 6 months of E1G, PDG, and menses for participants in stage -3 (A), -2 (B), -1 (C), and +1 (D). In premenopausal women, the days of unopposed estrogen cluster tightly in the mid to late follicular phase. Anovulatory cycles and prolonged periods of follicular development contribute to days of unopposed estrogen in stage -2 women. In stage -1, prolonged follicular growth and estrogen secretion, with much of it unopposed by progesterone, is typical. Fig. 3D is from a recently (within the 2 previous years) menopausal participant with low E1G and PDG but a clear period of follicular development not followed by ovulation or a menses.

Two of the 359 intervals were excluded because >30 days of data could not be scored for unopposed estrogen. Eighty-two percent (110) of intervals in stage +1 had no days of unopposed estrogen, whereas other stages always had days of unopposed estrogen. We therefore focused analysis on the other stages, comprising 223 intervals from 78 women. We found no differences in mean total days of unopposed estrogen (TDUE) among these stages ($P = 0.10$). However, mean TDUE for stage -2 and the mixed stage were higher than mean TDUE for stage -3 (Fig. 4). There was no significant difference between mean TDUE for stages -3 and -1, which may be partially a result of the large between- and within-subject variability of TDUE in stage -1 (Fig. 5). When the transitional stages (-2, mixed, and -1) were combined into a single category, TDUE was higher in the transition compared with stage -3 ($P = 0.027$; Fig. 4). In each of these models, there was significant interaction between stage

Figure 2. Trellis plots of 6-month means of E1G AUC by age for stages -3, -2, -1, +1, and mixed. Each line represents a single participant.



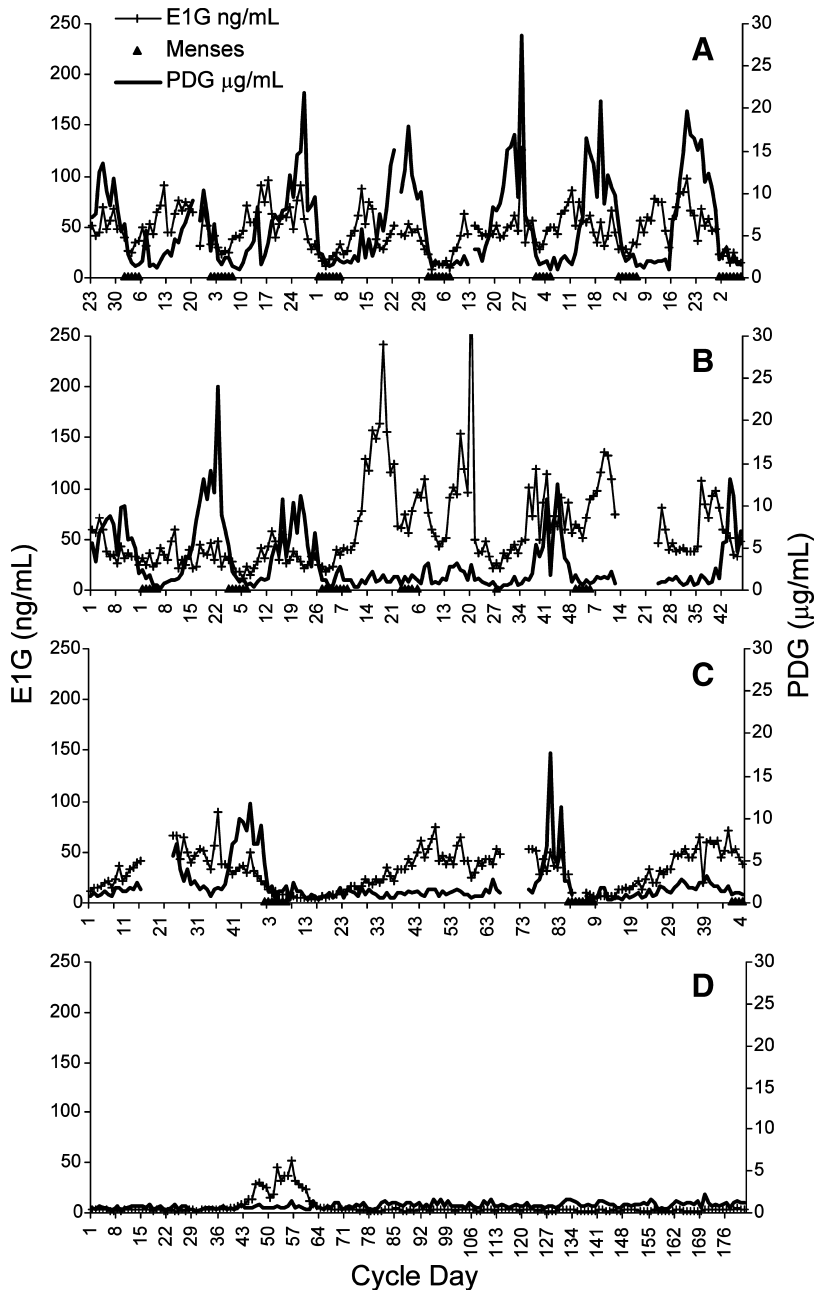


Figure 3. Illustrative examples of 6 mo of E1G, PDG, and menses for four participants in reproductive stages -3 (A; age 45 y), -2 (B; age 47 y), -1 (C; age 51 y), and +1 (D; age 53 y).

and longitudinal aging ($P < 0.0001$), with TDUE tending to increase with age in stage -3 and decrease with age in stages -2 and -1 (Fig. 5). The results were unchanged by the addition of BMI to the models.

Discussion

Our results show that reproductive stage, as assessed by a running CV of menstrual cycle length, is an important correlate of a woman's total E1G: women of the same age can have quite different E1G profiles if they are in different stages. In previous work, we showed that E1G increased with age until the mid to late 40s (36); here, we show that this trend is characteristic of stage -3 and that

E1G decline with age is typical in stage -1 (Fig. 2). Total E1G levels as assessed by AUC were similar and at their highest in -3, -2, and mixed stages; they did not decline until the late transition, stage -1 (Fig. 1). The low E1G levels in stage -1 are largely attributable to long periods of no ovarian activity in the longer cycles characteristic of this stage (43). Peak E1G levels did not decline until the postmenopause (stage +1); baseline E1G levels declined across each stage from -3 or -2 to the mixed stage, to the -1 stage, and still further to stage +1 (Fig. 1).

Other studies have examined estrogen indicators by reproductive stage but used different stage definitions or hormone measures, rendering comparisons difficult. Overall, the results of our study and others (24) suggest that total and peak estrogen levels do not increase across

the transition to menopause, when the transition is defined by cycle length variability, and do not begin to decline until late in the menopausal transition. Thus, one would predict that cancer risk related to total or peak estrogen exposure would not change with the timing of onset of, or be directly related to the duration of, the menopausal transition. Hormone levels clearly vary by reproductive stage and cannot be predicted using age alone, so this relationship must be regarded as an important area for future research. Indeed, this was one of the reasons the STRAW system was developed (37).

Total level of estrogen is a risk factor for breast cancer in premenopausal (6) and postmenopausal (45, 46) women, with higher levels associated with higher risk. Similarly, long-term exposure to elevated estrogen is a risk factor for ovarian cancer (7, 47). Lifetime exposure to estrogen has long been of epidemiologic interest, and late menopause is considered a major risk factor for endometrial, ovarian, and breast cancers (47). The mechanism by which elevated or prolonged exposure to estrogens contribute to each of these cancers are not clear and may vary depending on the target organ (47).

In contrast to total and peak E1G, the number of days of unopposed estrogen increased during the transition to menopause and remained high even when total and peak E1G began to decline late in the transition (Figs. 1, 4, and 5). The number of days of unopposed estrogen exposure did not decline until the postmenopause (stage +1). Exposure to relatively high levels of unopposed estrogen even occurs, albeit at a greatly reduced frequency, in the postmenopausal years (Figs. 4 and 5). This evidence of follicular development occurred in seven participants who were tentatively classified as postmenopausal within the previous 1 to 2 years, and seven other participants whose last bleeds were known to have been 2 to 8 years prior. Metcalf et al. reported similar evidence for postmenopausal follicular development (48).

One other study (49) has characterized unopposed estrogen exposure in premenopausal (defined as regularly cycling and age <40 years) and perimenopausal (defined as having a spontaneous break in cycle regularity and age >40 years) women. That study used a ratio of estrogen to progesterone to estimate unopposed estrogen exposure (49). The percentage of time spent at high levels of urinary E1G (>50 nmol or 70 nmol/24 h) with reduced urinary PDG exposure was significantly higher in perimenopausal than premenopausal women. Unusually long cycles (>50 days) were common in perimenopausal women and were associated with prolonged episodes of unopposed estrogen secretion. The differences between the Metcalf et al. measure of unopposed estrogen and ours allows only a rough comparison, but the findings are similar: both reveal that there is significant exposure to unopposed estrogen during the perimenopause and that both longer and shorter cycles include long periods of unopposed estrogen (Fig. 3B and C). To obtain more comparable results, we also used a simple estrogen-to-progesterone ratio. Our results did not change significantly (data not shown). We have important concerns, however, about the ratio as applied to our data: interindividual variation in PDG and E1G levels (36) confounded the variation associated with stage and age. For some women, the ratio did not capture unopposed estrogen at all, because,

compared with other women, their PDG tended to be high relative to their E1G (data not shown). An advantage of our approach is that it is independent of absolute hormone levels.

The link between unopposed estrogen and endometrial cancer is well established (13, 50). Relatively low levels of either exogenous or endogenous estrogen trigger endometrial proliferation (50), with proliferation beginning as early as the second day of the cycle and continuing at significant levels until mitotic proliferation begins to decline shortly after ovulation (13). It is not clear at what point there is sufficient progesterone secretion to counteract the proliferative effects of estrogen, but the level and duration of elevated progesterone could be factors influencing endometrial cancer (51). High levels of progesterone may be needed to effectively oppose high levels of estrogens (13). Duration of progesterone exposure may also be important; a minimum of 12 to 16 days of progestins in oral contraceptives is required to prevent hyperplastic endometrial response (13). Our study did not make a distinction between high and low levels of E1G or PDG, nor did it measure duration of PDG elevation. Consequently, our approach may underestimate the number of days of unopposed estrogen. If this bias is important, and if ovulatory cycles also declined in frequency across the transition, we could be missing an overall decline in number of days of unopposed estrogen from stage -3 to transitional stages. However, even if we corrected for this possible bias, the number of days of unopposed E1G would remain high across the transition, especially late in the transition when total E1G declines.

Our results suggest that the perimenopausal years are an important period of exposure to both unopposed and high estrogen levels. This result, coupled with the epidemiologic evidence on lifetime exposure to estrogen, suggests that variation in the amount of time spent in the transition to menopause may be an important correlate or risk factor for ovarian, breast, and endometrial cancers. To the best of our knowledge, only one study has reported statistical information on the length of the perimenopause: McKinlay et al. give a median of 3.8 years based on a large prospective study (12). Given the findings presented here, quantifying the length of the perimenopause and how this may vary among women is an important area for future research.

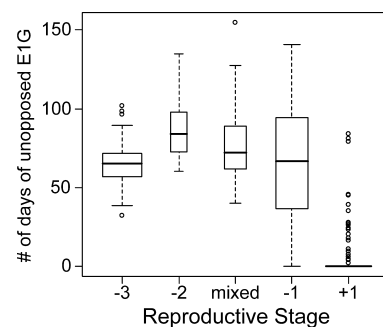


Figure 4. Boxplot of 6-month number of days of unopposed estrogen by stage. Box width is proportional to the number of intervals in each stage.

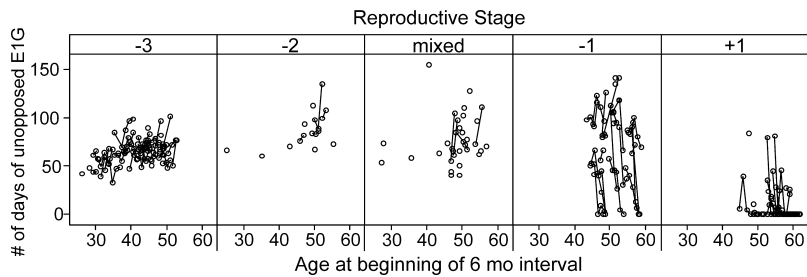


Figure 5. Trellis plot of total days of unopposed estrogen by age for stages -3, -2, -1, and mixed. Each line represents a single participant.

Our results may not be applicable to all women. Our sample is biased toward (a) women who chose not to use either hormone replacement therapy (HRT) as they went through the menopausal transition or oral contraceptives to prevent pregnancy and (b) White, middle class, and college educated women (36). Of particular concern is whether the women who did not participate in all 30 months of the study were in any way different from those for whom there are no missing data. The most common reason for withdrawal from our study was use of HRT, but there were no significant differences in mean age between women who withdrew from the study (for HRT or other reasons) and those who did not (36). There is evidence indicating that breast cancer type and risk may differ between HRT users and never-users (52); this suggests that cycle length and/or estrogen characteristics may differ between women who did and did not choose to go on HRT. Perimenopausal symptoms leading to HRT use have been associated with reduced estrogen secretion (53), which our and other data (43) show tends to be associated with elongated cycles. Our analyses here may thus be overestimating estrogen exposure across the transition to menopause for some women.

A limitation of our study is that we have only one measure of BMI, taken at the midpoint of the study, and these data were available for only 90 of the 108 women in our analyses. We were able to impute BMI for the year 2000 using data from previous years for the remaining 18 women. BMI is associated with estrogen levels, and it is this link that is believed to explain the association between body weight and cancer risk (8, 54). Studies that have examined BMI change across the transition to menopause report an average annual increase in BMI of 0.2 to 0.4 kg/m²/y (55, 56). Thus, although adding BMI did not alter the results of our analyses, our midpoint estimate may not be an accurate representation of BMI across the study period.

The cycle length variance we chose as cutoffs for each stage could have been determined in a different manner. Our goal was to operationalize the STRAW system for use with a large collection of cycle data. The advantage of a CV-based approach is that it is sensitive to the mean. Like the method used by Lisabeth et al. (44), our approach replicates the spirit of the original STRAW system, designed to be used by women, clinicians, and researchers alike. That reproductive stage is broadly predictive of estrogen exposure is promising for future work examining how other, more nuanced, serial indicators of menstrual cycle length may be associated with hormone levels. This study focused on “variability” in menstrual cycle length as an indicator of

reproductive status. This can be expanded to include an assessment of menstrual cycle length “regularity” using an indicator of serial irregularity in time-series data—approximate entropy. In previous work using approximate entropy with TREMIN data on cycles from women ages >40 years, we found that both increased variability and greater serial regularity were significant predictors of the onset of menopause (17). A woman’s lifetime history of menstrual cycle variability and stability may also be an important predictor of hormone status across the transition to menopause; in recent work on a cohort of TREMIN women, we identified five categories of women’s menstrual histories based on variability and stability and found that taxonomic category was associated with age at menarche, number of births, and age at menopause (57). For the BIMORA women, we have up to 30 years of reproductive histories, which we are linking with the hormone data to explore how hormone levels across the transition may be associated with a woman’s lifetime history of more stable or more erratic patterns of menstrual cycle lengths.

Conclusions

We found that (a) E1G levels increased with age in stage -3 women up until the mid to late 40s; (b) E1G AUC did not decline significantly until later in the menopausal transition (stage -1); (c) whereas baseline (5th percentile) E1G levels declined across the transition, peak (95th percentile) levels did not decline until the postmenopause; and (d) the total number of days of unopposed estrogen exposure were higher in the transition than before it and remained high until the postmenopause. Given the length of time women spend in the transition to menopause, they are exposed to more total and unopposed estrogen than has been previously appreciated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Appendix A. Scoring Cycles for Days of Unopposed Estrogen

Definitions: FD, follicular development; C, close of inactive phase (start of E1G secretion).

Rules:

Summary: mark all days of SR \geq 20 as unopposed EXCEPT the standard case, where we go strictly from (C + 1) to (PR \geq 3.0-1). The standard case constitutes

much of the data, and the other captures most of the exceptions.

1. Look at profile
 - a. If yes menopausal (no bleeds)
 - i. If no evidence of FD ($FD \geq 5$ days of increase and decline in E1G), mark all days as opposed
 - ii. If yes evidence of FD
 1. If no evidence of ovulation, mark all days as unopposed within period of FD with $SR \geq 20$
 2. If yes evidence of ovulation, mark all days as unopposed from first day within period of FD where $SR \geq 20$ up to the day before the first $PR \geq 3.0$ where ovulation is occurring
 - b. If not menopausal proceed to no. 2
2. If not menopausal, find first day of $PR \geq 3.0$
 - a. If yes $PR \geq 3.0$
 - i. If first day $PR \geq 3.0$ is within the first 5 days of the cycle, ignore and find next time $PR \geq 3.0$.
 - ii. If there is run of $PR \geq 3.0$ (at least 3 days within a 5-day period), it is not within the first 5 days of the cycle, and it is not within a very long cycle (long ≥ 40 days), this is ovulation. Mark the first day where $PR \geq 3.0$ and go to no. 3.
 1. If a run of $PR \geq 3.0$ occurs within a very long cycle, consult profile to identify if these are anomalous, anovulatory, or ovulatory. Mark all days to end of cycle with $SR \geq 20$ as unopposed.
 2. If there are multiple days scattered through cycle where $PR \geq 3.0$ (statistical artifact of long cycles with inactive phases), visually assess where ovulation is occurring and identify the closest $PR \geq 3.0$ to close the period of unopposed estrogen. If cycle is anovulatory ($ovul2 = -1$), mark all days up to end of cycle with $SR \geq 20$ as unopposed.
 - b. If no $PR \geq 3.0$
 - i. If there is only one or no days of $PR \geq 3.0$ outside of the first 5 days of the cycle, cycle is anovulatory ($ovul2 = -1$), and $SR \geq 20$, mark all days from (C +1) on as unopposed as long as $SR \geq 20$.
 1. If no C use all days where $SR > 20$.
 2. For right censored cases, mark to end of cycle if $SR \geq 20$.
 3. If ovulation is present in graph but PR does not pick it up, use all days where $SR \geq 20$ up to day before ovulation on graph.
3. If yes C has a value, mark all days unopposed from (C + 1) to (PR marked day -1) regardless of SR values.
 - a. If C is a negative number, assign first cycle day as beginning of unopposed estrogen

- b. C cannot come after $PR > -3.0$. Visually assess and use alternative criteria (SR)
- c. Check graph re C \rightarrow

- i. Left censored cycles are a place where this commonly occurs. If $SR \geq 20$, record as unopposed E.
- ii. This will catch unopposed estrogen that is from a previous cycle but is not linked to the current cycle's C. If $SR \geq 20$, record as unopposed E.

4. If no C value mark all days $SR \geq 20$ as unopposed up to day before $PR \geq 3.0$. If no $PR \geq 3.0$ see 2b.
5. Ignore C if no hormone data for a day. Do not score cycle; it probably has no unopposed E days.
6. If cycle is missing >10 days of data, with most missing days consecutive, do not score.
7. Right and left censored cycles can be scored using above rules in conjunction with viewing the profile.
8. Check graphs when scoring cycles. For cases that do not fit into above, score visually and/or use SR criteria.

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