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Characterization of Reproductive Hormonal Dynamics in the Perimenopause*

NANETTE SANTORO, JESSICA ROSENBERG BROWN†, TOVAGHGOL ADEL, AND JOAN H. SKURNICK

Departments of Obstetrics and Gynecology (N.S., J.R.B., T.A.) and Epidemiology and Biostatistics (J.H.S.), University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey 07103-2757

ABSTRACT

Medical therapy for women in the perimenopausal period is controversial, in part due to varying degrees of ovarian hormone secretion characteristic of this time of life. To extend our understanding of the reproductive endocrine milieu of perimenopausal women, we studied 6 cycling women, aged 47 yr and older, for 6 months with daily collections of first morning voided urine. Five additional older reproductive aged (43–47 yr old) women were studied with daily urine and serum sampling for a single menstrual cycle; their urinary hormone data were combined with the former group for menstrual cycle comparisons. Urine was assayed for LH, FSH, estrone conjugates, and pregnanediol glucuronide and normalized for creatinine (Cr). Eleven midreproductive aged (19–38 yr old) normally cycling women, 5 women with well defined premature ovarian failure, and 5 women aged 54 yr and older who were at least 1 yr postmenopausal were used for comparison.

Perimenopausal women had shorter follicular phases (11 ± 2 days *vs.* 14 ± 1 days; $P = 0.031$) and, hence, shorter menstrual cycles than midreproductive aged controls. FSH excretion in perimenopausal women was greater than that in younger women (range of means, 4–32 *vs.* 3–7 IU/g Cr; $P = 0.0005$). LH secretion was overall greater than that in younger normal subjects (range of means, 1.4–6.8 *vs.* 1.1–4.2 IU/g Cr; $P < 0.026$). Overall mean estrone conjugate excretion was greater in the perimenopausal women compared to that in the younger women [76.9 ng/mg Cr (range, 13.1–135) *vs.* 40.7 ng/mg Cr (range, 22.8–60.3); $P = 0.023$] and was similarly elevated in both follicular and luteal phases. Luteal phase pregnanediol excretion was diminished in the perimenopausal women compared to that in younger normal subjects (range for integrated pregnanediol, 1.0–8.4 *vs.* 1.6–12.7 μg/mg Cr/luteal phase; $P = 0.015$). Compared to postmenopausal women, perimenopausal women had more overall estrone excretion (2.5–6.2 ng/mg Cr in postmenopausal women; $P = 0.02$) and lower mean FSH (range of means for postmenopause, 24–85 IU/g Cr; $P = 0.017$) and LH (range for postmenopause, 4.3–14.8 IU/g Cr; $P = 0.041$). Compared to women with premature menopause, perimenopausal women again had lower FSH (range of means for premature menopause, 36–82 IU/g Cr; $P = 0.0022$), lower LH (range of means for premature menopause, 5.5–23.8 IU/g Cr; $P = 0.0092$), borderline higher mean estrone conjugates (range of means for premature menopause, 4–44 ng/mg Cr; $P = 0.064$), and far longer periods of ovarian activity (one to two cycles in prematurely menopausal women *vs.* three to six cycles in perimenopausal women). We conclude that altered ovarian function in the perimenopause can be observed as early as age 43 yr and include hyperestrogenism, hypergonadotropism, and decreased luteal phase progesterone excretion. These hormonal alterations may well be responsible for the increased gynecological morbidity that characterizes this period of life. (*J Clin Endocrinol Metab* **81:** 1495–1501, 1996)

THE YEARS immediately preceding menopause constitute a period of protean ovarian activity. The exact nature and the clinical sequelae of these changes in the reproductive axis are not well understood. It has been implied that prolonged and persistent elevations in circulating FSH (1) and loss of ovarian inhibin secretion (2) characterize this period of life. Intermittent anovulation and irregular menstrual bleeding have been observed in small cohorts of women (1, 3–7). Dysregulation of the hypothalamic-pituitary-gonadal axis, with altered frequency of pulsatile GnRH release (8, 9) and failure to mount a LH surge in the face of adequate estrogen priming (10, 11) suggest that the reproductive axis may undergo central perturbations during this period of life. Gynecological symptoms, such as growth of uterine leiomyomata and dysfunctional uterine bleeding, often result in hysterectomy for short term control of benign self-remitting conditions.

To characterize longitudinally the nature of reproductive hormone secretion during the menopausal transition, we performed daily urinary sampling for 6 months on six cycling women, aged 47 yr or older, and daily sampling for a single menstrual cycle on an additional five women, aged 43–47 yr. Their menstrual and hormonal patterns were compared to those in midreproductive aged women, postmenopausal women, and a younger group of prematurely menopausal women.

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Address all correspondence and requests for reprints to: Dr. Nanette Santoro, Department of Reproductive Endocrinology, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, 185 South Orange Avenue, MSB E-506, University Heights, Newark, New Jersey 07103-2757.

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† Current address: Maimonides Medical Center, Department of Obstetrics and Gynecology, 4802 10th Avenue, Brooklyn, New York 11219.

Materials and Methods

Participants

Women were selected through university-wide advertising and word of mouth. The protocol was approved by the local institutional review board. All subjects gave informed consent before participation. Perimenopausal women enrolled in the longitudinal study (collection of daily urine for 6 months) met the following criteria 1) age 47 yr or older; 2) a history of regular menstrual cycles 25–35 days in length; 3) nonsmoking status; 4) no excessive exercise (>1 h/day) or aggressive di-

eting (loss of >1 lb/week); 5) no diseases affecting gonadotropin or sex steroid secretion, clearance, or excretion; 6) no hormonal therapy within 3 months of the study; 6) at least 90% of normal weight for height; and 7) no period of amenorrhea exceeding 3 months in the past year. An additional five women, aged 43–47 yr, who underwent a single cycle of serum and urine sampling were combined with this perimenopausal group for certain analyses. These women met the same criteria as the original six subjects, except for age.

Controls

Three comparison groups were used for this study. Eleven normally cycling women, 19–38 yr, with regular ovulatory menstrual cycles and absence of hyperprolactinemia or hyper- or hypothyroidism whose data have been previously reported (12) constituted the standard for midreproductive aged menstrual cyclicity. Five additional women, aged 40 yr or younger, who were diagnosed with premature ovarian failure before age 35 yr were also used for comparison. They served both as younger aged controls for the postmenopausal women and as a comparison group for the perimenopausal women. Women with premature menopause have been hypothesized to remain in a persistent perimenopausal state characterized by hypergonadotropic hypogonadism and unpredictable menstrual cyclicity. These prematurely menopausal women were additionally required to be 1) karyotypically normal, without a prior history of ablative ovarian surgery, chemotherapy, or radiation; 2) amenorrheic for at least 6 months before study; and 3) free of exogenous hormones for at least 3 months before study. Finally, five women, aged 54–79 yr, with age-appropriate menopause, whose last menstrual period was at least 1 yr previously, were used as the standard for end-stage (*i.e.* complete and persistent) hypergonadotropic hypogonadal amenorrhea. These women met the same criteria as the perimenopausal women, except for age and menstrual status.

Specimen collection

Participants were given polypropylene tubes prefilled with glycerol in sufficient quantity to result in a final concentration of 7% glycerol in the urine sample when the tube was filled to the premarked fill line (13). Subjects were asked to collect a daily first morning voided urine specimen upon awakening, transfer a portion of the urine to the prepared tube, and immediately freeze the specimen at −20 C. Specimens were delivered to the laboratory approximately quarterly; no subject collecting over 6 months missed more than 2 consecutive weeks of collection. No subject collecting a monthly sample missed more than 2 consecutive days. Participants were also asked to keep a menstrual diary noting the onset of bleeding episodes.

Urinary assays

Urine specimens were assayed for LH, FSH, estrone conjugates, pregnanediol glucuronide (PDG), and creatinine (Cr) (14). The methods for the urinary assays have been described previously (12, 13). Gonadotropins were measured using a solid phase, two-site fluoroimmunometric assay (DELFIA, Pharmacia, Gaithersburg, MD) adapted for urine specimens and validated in this laboratory (13). Glycerol preservation of the urine was essential for the maintenance of gonadotropin immunoactivity (4–6, 13). The preservative had no effect on estrone or pregnanediol measurements. The interassay coefficient of variation (CV) for LH was 18%, and the intraassay CV was 8%. Corresponding CVs for the FSH assay were 21% and 12%, respectively. Urinary estrone conjugates and PDG were measured in duplicate by enzyme-linked immunosorbent assay using antisera and conjugate tracers provided by Dr. Bill Lasley (University of California-Davis). The estrone conjugate antiserum reacts equally with both estrone-3-sulfate and estrone-3-glucuronide. The EIA-read program (provided by Dr. Dennis Stewart, Davis, CA) was used to transform the data.

For estrone conjugates at B/Bo of approximately 15%, 50%, and 75%, interassay CVs were 16%, 19%, and 20%, respectively; corresponding intraassay CVs were 5%, 6%, and 8%. For PDG at bound/free ratios of approximately 15%, 40%, and 60%, interassay CVs were 14%, 15%, and 18%; corresponding intraassay CVs were 8%, 9%, and 7%.

For both steroid assays, most specimens required considerable dilution and were read at or below the ED_{50} on the standard curve. Whenever possible, all specimens from a single subject were run in the same assay. Urinary Cr was assayed using a direct colorimetric reaction (14). All urinary hormones were normalized for Cr and corrected for glycerol and are expressed per mg Cr for sex steroids and as international units per g Cr for gonadotropins.

Serum assays

Validation of urinary data in this study was carried out in five women. LH and FSH were measured using the same reagents as those for urine (DELFIA). Interassay CVs for the serum LH and FSH assays were 6.9% and 4.3%, respectively. Corresponding intraassay CVs were 4.5% and 1.9%. Estradiol was measured using a direct ^{125}I RIA (Pantex, Santa Monica, CA), and progesterone was measured by RIA (Coat-a-Count, Diagnostics Products Corp., Los Angeles, CA). Interassay CVs were 8.2% and 5.2%, respectively, and intraassay CVs were 2.3% and 5.1%.

Data analysis

Based upon previous analysis of the normal cycles in midreproductive aged women, algorithms were developed to determine the presence of a midcycle gonadotropin surge, ovulation, and estrogen peaks (12). A cycle was considered ovulatory if an estrone peak was followed by a clear-cut surge of LH and subsequent pregnanediol excretion to a minimum of at least 3 μg/mg Cr for at least 4 days. In previous work, a significant increase from a 5-day, lagged, moving average was required to consider an increase in PDG significant (12). In normally cycling, midreproductive aged women, this increase exceeded 5 μg/mg Cr in all 11 cycles studied to date. In the perimenopausal women, clear-cut increases in PDG were observed that lasted for 10–14 days and resulted in a menstrual period, but did not exceed 5 μg/mg Cr. We included these cycles as having evidence of ovulatory activity and used a consistent (4-day) threshold of 3 μg/mg Cr PDG as indicative of ovulation. Essentially identical criteria have been independently suggested by others with widespread experience using these assays in large field studies (15). Similar criteria (peak PDG, 4 μg/mg Cr) have been reported previously (16).

Day 0, or the day of ovulation, was determined for each cycle based upon the following criteria: 1) day of or day after the LH and FSH peak, 2) day of or up to 2 days after the peak level of estrone conjugates, and 3) PDG less than 2 μg/mg Cr. Peak preovulatory estrone conjugates were determined for each cycle. Mean gonadotropin concentrations were also determined and compared across groups. In women who were cycling, midcycle gonadotropin surges detected by previously described algorithms (12) were deleted from calculations of mean gonadotropin concentrations. Menstrual cycles in data sets from women collecting samples from a single cycle used data from the onset of one menstrual period to the next, when the participant stopped collecting urine. In the longitudinal data series, we considered menses a less reliable marker of ovarian activity than hormones. We used data standardized to day 0 (as described above), then derived luteal phase length as lasting from day 1 until PDG concentrations fell to or below the follicular phase mean for that subject. The next day was taken as day 1 of the subsequent cycle. There was no difference in results regardless of whether menses or hormone concentrations were used to calculate cycle length.

Estrone and pregnanediol excretion were integrated using the trapezoidal rule, and total cycle estrone conjugates and luteal pregnanediol were estimated. The average pregnanediol level during ovulatory cycles was computed for 2 phases of the cycle. Peak or postovulatory levels were computed as the average of pregnanediol levels on days −2 to +16 (referenced to day 0, as defined above). Levels on days with missing measurements were interpolated linearly. The 11 midreproductive aged women each had 1 cycle of data. The 11 perimenopausal women had from 1–6 ovulatory cycles. A separate average was computed for each cycle. Because perimenopausal luteal phase lengths varied, some windows were truncated on day 15. The mean of cycle averages was used to represent women with more than 1 ovulatory cycle. Basal (follicular phase) pregnanediol was computed as the average pregnanediol level on all remaining days of any ovulatory cycle (days before day −2 and after day +16, but not days of anovulatory cycles or days of noncyclic activity). Measurements missing on those days were not imputed by

interpolation. The mean excess (cap mean) during the peak was then computed for each cycle as the peak average minus the basal level.

Before performing comparisons with the 43- to 47-yr-old women, each of whom contributed data from only a single cycle, the variability of each perimenopausal woman was estimated to determine the degree to which selection of a single cycle was valid. When women were experiencing ovulatory cycles, all cycles were comparable, allowing us to compare single cycles as representative of women from both groups. There were no significant differences in any of the hormone measures between the 47 yr and older group and the slightly younger group of 43–47 yr olds; these 2 groups were, therefore, combined for a total of 11 women for the analysis of cycle characteristics (Fig. 1).

Comparisons were achieved by reducing the variable for a cycle (randomly selected if more than one cycle was collected, except for PDG as noted above) to a single number, *e.g.* integrated luteal phase pregnanediol, whole cycle mean estrone conjugates, *etc.* These numbers were then compared between groups to characterize ovulatory and anovulatory cycles. The luteal phase was considered day 1 to the end of the cycle. All cycles were standardized to day 0, as previously defined.

Groups were compared using Wilcoxon rank sum testing and Kruskal-Wallis testing for multiple group comparisons, as appropriate. $P < 0.05$ was considered statistically significant. Data are presented as the ranges of means for women in the longitudinal study to illustrate the within-group variability.

Results

Group comparisons are shown in Table 1.

Perimenopausal vs. younger cycling women

Perimenopausal women had shorter cycles than midreproductive aged women (19–38 yr old; Fig. 1). This finding was due to an attenuated follicular phase. Overall estrone conjugate excretion was greater in the perimenopausal women than in the midreproductive aged women [Figs. 1 and 2; 77 ng/mg Cr (range, 13–135) *vs.* 41 ng/mg Cr (range, 23–60); $P = 0.023$]. Follicular phase estrone excretion was elevated in perimenopausal women (65 ng/mg Cr; range, 25–126) compared to that in midreproductive aged women (36 ng/mg Cr; range, 17–53; $P = 0.03$). Similarly, mean luteal phase estrone excretion was elevated in perimenopausal women (87 ng/mg Cr; range, 11–144) compared to that in midreproductive women (45 ng/mg Cr; range; 26–65; $P = 0.03$). Midcycle peak estrone concentrations did not differ between the two groups [peak estrone, 151 ng/mg Cr (range, 47–278) in perimenopausal women; mean peak, 116 ng/mg Cr (range, 58–237) in the younger women; $P = 0.29$].

To examine the possibility that accelerated and/or exaggerated folliculogenesis was occurring in the perimenopausal women, estrone concentrations were transformed to a logarithmic scale. The slope of the estrone rise was computed for each cycle (on the logarithmic scale), and the intercept was derived, fitting simple linear regressions of the logarithm of estrone for cycle days −10 to −1. Where women had more than one cycle, the mean slope and the mean intercept were used to represent her cycle. The rate of rise in estrone did not differ between perimenopausal and midreproductive controls (mean slope, 0.152 *vs.* 0.133, respectively); however, the intercept was significantly greater in the perimenopausal women (mean intercept, 12 ng/mg Cr; range, 11–12) compared to that in the younger women (mean intercept, 11 ng/mg Cr; range, 10–12; $P = 0.034$). Integrated cap pregnanediol ranged from 1.0–8.4 μg/mg Cr in the perimenopausal women *vs.* 1.6–12.7 μg/mg Cr in the midreproductive aged controls ($P = 0.015$; Fig. 1). Basal (follicular phase) pregnanediol excretion did not differ between perimenopausal women (range, 0.4–2.2 μg/mg Cr) and midreproductive aged women (range, 0.4–4.2; $P = 0.95$).

Gonadotropin elevations, particularly those in FSH, were evident in the perimenopausal women and most pronounced in the early follicular phase of the cycle (Fig. 2). Mean follicular phase FSH was elevated in perimenopausal women (median, 14 IU/g Cr; range, 4–32) compared to that in midreproductive aged women (median, 4 IU/g Cr; range, 3–7; $P = 0.0005$). Although less apparent, LH was also elevated in perimenopausal women (median, 3.9; range, 1.4–6.8 IU/g Cr) compared to that in midreproductive aged women (median, 2.3; range, 1.1–4.2 IU/g Cr; $P = 0.026$).

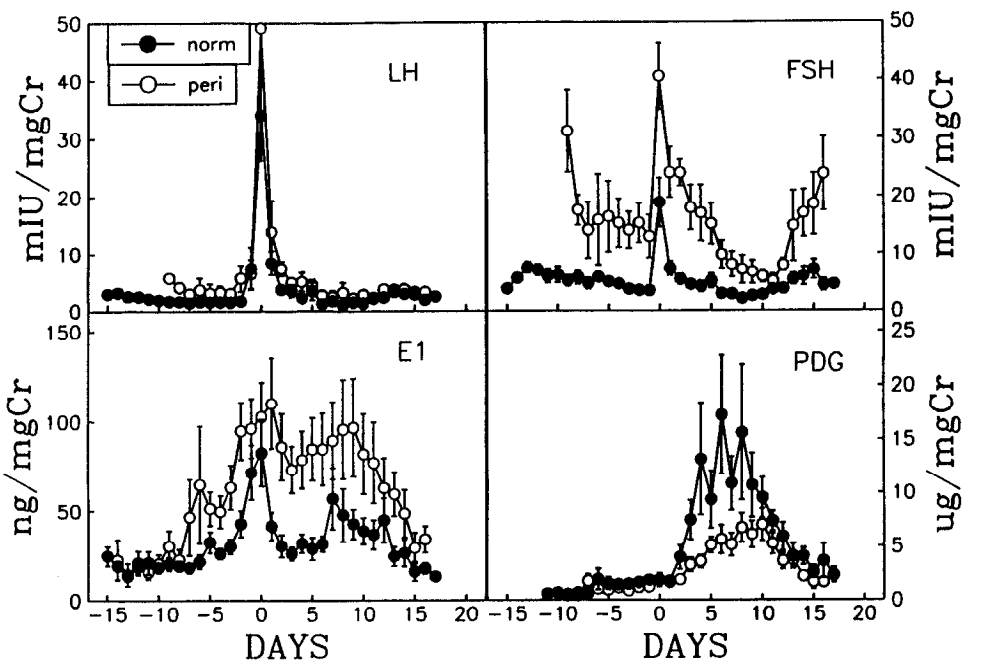

FIG. 1. Mean ± SEM daily urinary gonadotropin and sex steroid excretion patterns in 11 perimenopausal women, aged 43–52 yr (○) compared to those in 11 midreproductive aged women (●). Data are standardized to day 0, the putative day of ovulation, as described in the text. E_1, Estrone conjugates.

TABLE 1. Comparison of hormonal parameters by group

	PERI	Young	Premature	Menopause
Mean estrone (ng/mg Cr)	13–135[a]	23–60	4–44	3–6
Peak estrone (ng/mg Cr)	47–278	58–237	—[b]	—
Integrated pregnanediol (μg/mg Cr · luteal phase)	1.0–8.4[a]	1.6–12.7	—	—
Mean FSH (IU/g Cr)	4–32[c]	3–7	36–82	24–85
Mean LH (IU/g Cr)	1.4–6.8[d]	1.1–4.2	5.5–23.8	4.3–14.8

Ranges of hormonal values are shown. PERI, Perimenopausal women (n = 11); Young, youthful controls, aged 19–38 yr (n = 11); Premature, women with premature menopause (n = 5); Menopause, age-appropriately postmenopausal women (n = 5).

[a] $P < 0.02$ *vs.* younger women.

[b] A *line* in lieu of data indicates that insufficient observations were available for a comparison.

[c] $P < 0.02$ *vs.* young, premature, and menopausal women.

[d] $P < 0.05$ *vs.* young, premature, and menopausal women.

To rule out age-related alterations in hormone excretion accounting for these findings, correlation of serum and urinary gonadotropin and sex steroid levels were carried out for the older women, as previously reported for midreproductive aged controls (12, 13). Identical correlation coefficients were observed, indicating that serum to urine relationships were preserved (Fig. 3) regardless of reproductive age.

Perimenopause vs. premature menopause (Table 1)

Prematurely menopausal women had less frequent evidence of folliculogenesis and cyclic ovarian function (Fig. 4). Only one or two contiguous cycles of ovarian activity sufficient to result in menses were observed in two of five prematurely menopausal women studied (Fig. 4) (12). When ovulatory, women with premature menopause had follicular phase dynamics that more closely resembled those in the midreproductive aged women, without an acceleration and with normal pregnanediol excretion. Persistent elevations in FSH were seen in the prematurely menopausal women and were greater than those in perimenopausal women (range of means, 36–82 IU/g Cr for premature menopause *vs.* 4–32 IU/g Cr for perimenopause; $P = 0.0022$). The elevated estrone excretion noted in the perimenopausal women was not evident in the prematurely menopausal women (range, 4–44 ng/mg Cr for premature menopause; $P = 0.064$ *vs.* perimenopause).

Perimenopause vs. menopause (Table 1)

Age-appropriate postmenopausal women demonstrated little variation in their urinary hormone excretion patterns, with tonically elevated LH and FSH and persistently low estrone conjugate excretion (range of means, 2.5–6.2 ng/mg Cr; $P < 0.02$ *vs.* premature menopause, perimenopause, and midreproductive aged women; Fig. 5). Estrone excretion in postmenopausal women demonstrated few algorithmic increases, none of which exceeded 5.9 ng/mg Cr. In contrast, most perimenopausal cycles we studied were ovulatory, with clear-cut estrone rises and LH surges. However, periods of tonically elevated gonadotropin levels and persistently low estrone excretion were noted in the perimenopausal women and became more common with proximity to menopause. Anovulatory cycles were noted in four of six women who collected serial cycles of urine. One subject (data not included in the analysis) became amenorrheic within 1 month of beginning her collection and did not menstruate again. In this woman, a typical postmenopausal hormone pattern was observed. In two other women who became amenorrheic within 2 yr of completing the study, periods of up to 3 months of amenorrhea were observed in which hormone excretion appeared identical to that in postmenopausal women. Both of these women subsequently had menses

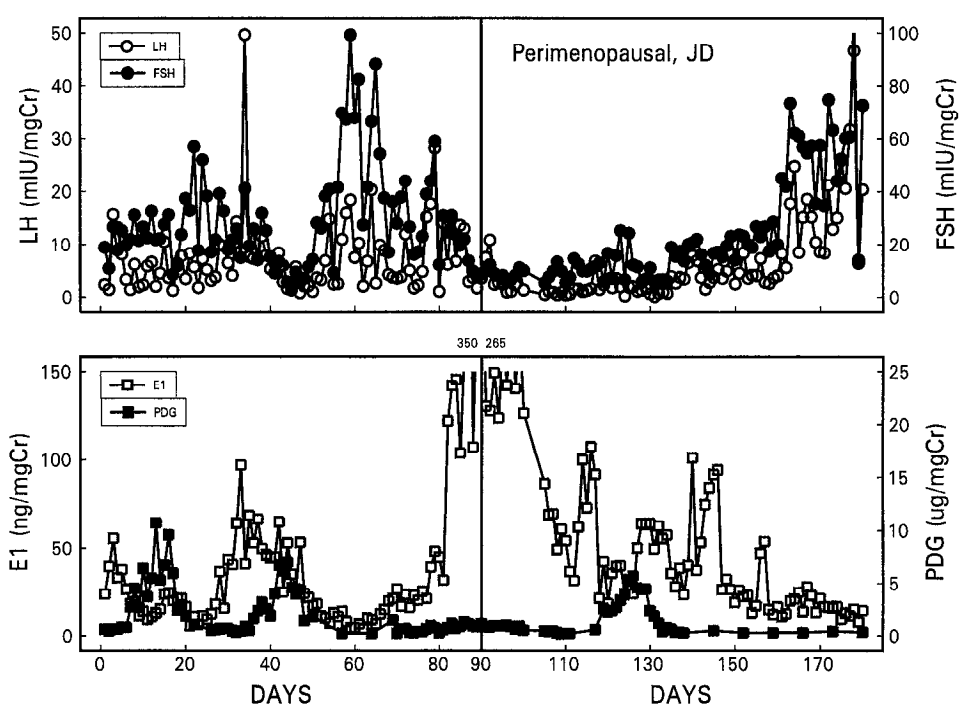

FIG. 2. Representative pattern of hormone excretion in a perimenopausal woman. A prolonged episode of hypergonadotropic hypoestrogenism is followed by hyperestrogenic anovulatory ovarian function.

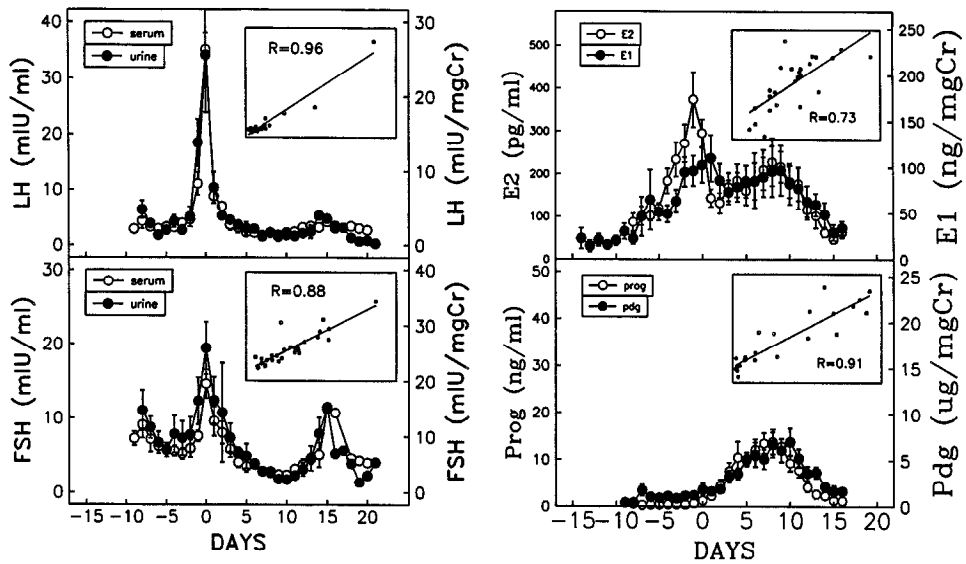

FIG. 3. Mean ± SEM serum and urine gonadotropin, estradiol/estrone conjugate, and progesterone/PDG levels in five perimenopausal women, aged 43–47 yr, who collected daily urine and serum for one menstrual cycle. r valves are displayed in the *upper right corner* of each graph. E_2, Estradiol; Prog, progesterone.

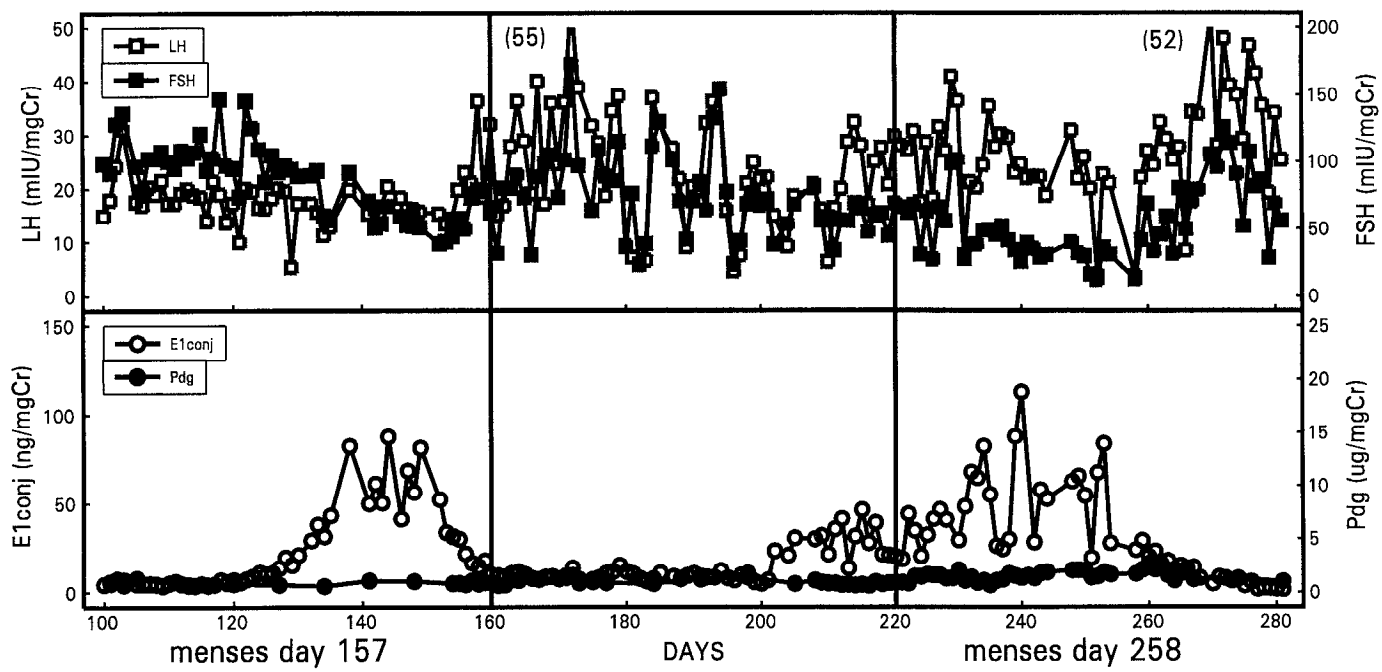

FIG. 4. Representative hormone excretion pattern of a woman with premature menopause. Note the absence of hyperestrogenic episodes.

along with increased estrone excretion, with or without ovulation.

Discussion

In this study, we followed daily urinary sex steroid and gonadotropin concentrations for up to 6 months in a longitudinal fashion. This relatively lengthy window of observation allowed the assessment of long term patterns and cycle to cycle variability. Thus, representative cycles could be used to demonstrate the characteristics of both ovulatory and anovulatory cycles in the perimenopause.

We have confirmed and extended previous comparative observations and included postmenopausal and prematurely menopausal women for comparison. The sensitive and specific gonadotropin fluoroimmunoassays used detected an abidingly elevated FSH and LH excretion throughout the cycles of perimenopausal women compared to those in younger controls. Elevated LH excretion has been noted by others (5, 17) in perimenopausal women. Elevated LH excretion may play a role in the alteration of folliculogenesis and corpus luteum function. We confirm the previously reported observation of diminished pregnanediol excretion in the luteal phase of perimenopausal women (18) 2nd shortened follicular phase length (19). Moreover, we add a significant and clinically meaningful finding of increased estrone excretion in perimenopausal women compared to that in midreproductive aged controls. Our finding of elevated estrone conjugate excretion in perimenopausal women

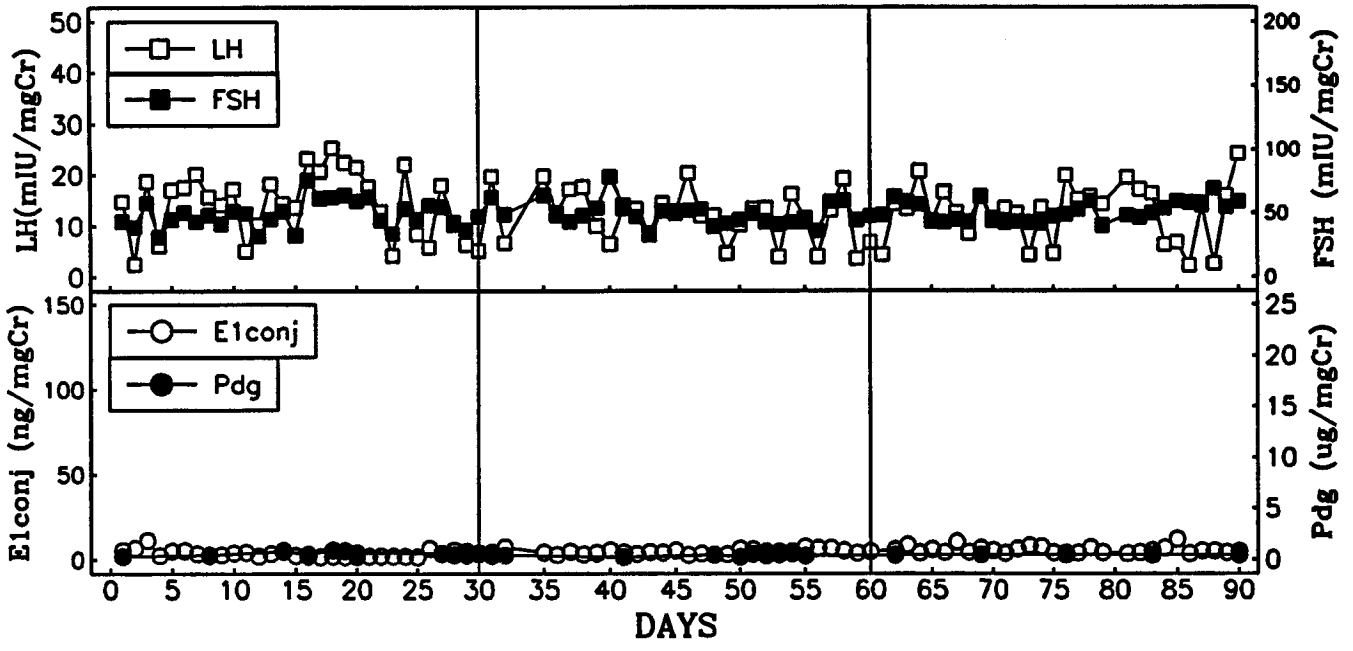

FIG. 5. Representative hormone excretion pattern of a postmenopausal woman, demonstrating consistent hypergonadotropic hypoestrogenism.

suggests that although follicle numbers are dwindling, the ability of the ovary to produce estrogen is more than adequate in women before menopause. The elevated estrone excretion we observed persisted throughout the menstrual cycle. This observation is consistent with the clinical findings of increased endometrial hyperplasia, growth of uterine myomata, and dysfunctional uterine bleeding that characterize this period of life. Whether the increased estrone excretion we observed was due to increased estradiol secretion from single or multiple follicles cannot be determined from these data; however, studies on twinning have noted that women in their forties who conceive have a higher incidence of twinning (20).

Anecdotal observations of increased estrogen secretion and excretion have been reported previously in women in their forties. Shideler *et al.* (7) observed increased estrone conjugates excretion in a small number of perimenopausal women; however, statistical comparisons to younger cycling women were not presented. Other researchers observed anovulatory cycles with elevated estrogen excretion in perimenopausal women and women with endometrial hyperplasia (11, 21).

Age-related alterations in the excretion or conjugation of estradiol are unlikely to have contributed to our findings. In previous work from our group, young women with premature menopause and women aged 54 yr and older with age-appropriate menopause excreted similar basal amounts of conjugated estrogen (12), suggesting that estrone conjugate excretion does not differ with age. Secondly, the five perimenopausal women who underwent concomitant urine and serum sampling demonstrated excellent agreement between urinary and serum values and regression curves similar to those previously reported in younger women, indicating that urine sampling accurately reflects the circulating hormone.

The common perimenopausal syndromes of endometrial hyperplasia and dysfunctional uterine bleeding have been attributed to episodes of anovulation (7, 10, 11). However, our data suggest that a consistent degree of hyperestrogenism is present even during ovulatory cycles. This relative hyperestrogenism may well contribute to the gynecological morbidity that characterizes the menopausal transition in women and leads to hysterectomy for over 20% of the American female population (22). The decline in luteal phase progesterone excretion noted by us and others (18) in the perimenopause may aggravate the relatively unopposed estrogen state, further predisposing perimenopausal women to endometrial hyperplasia, and uterine and perhaps even breast cancer. This is the first study, to our knowledge, that has demonstrated statistically significant hyperestrogenemia in ovulatory perimenopausal cycles.

Based upon these data, clinical management of perimenopausal women should take into account the possibility of a hyperestrogenic hypoprogestagenic state. Women with perimenopausal abnormalities of menstrual bleeding might be best served by measures designed to restore adequate progestational maturation of the endometrium. Low dose oral contraceptive pills or combined hormone replacement therapy regimens have been suggested as interventions that can be used before menopause.

Research by several investigators implies that the perimenopause may be a period of life during which cardiac risk factors increase (23), and bone loss accelerates (24). However, these sequelae are primarily thought to be due to decreased estrogen exposure. Our data suggest that concern about perimenopausal bone loss or increases in cardiovascular disease may be unfounded, as estrogen levels are higher, rather than lower, than those experienced by midreproductive aged women.

We also observed key differences between perimeno-

pausal women and prematurely menopausal women. Of particular interest was the greater FSH excretion in the prematurely menopausal group. As FSH secretion appears to be controlled by both hypothalamic (GnRH) and pituitary/ovarian peptide (inhibin/activin) secretion, it is possible that women with premature menopause have a younger central reproductive axis and are capable of greater pituitary responsiveness or greater GnRH stimulation. Alternatively, prematurely menopausal women may lack the restraint of FSH secretion imposed by inhibin. Qualitatively, menstrual cyclicity in prematurely menopausal women was far less frequent than that in perimenopausal women. The fact that women with premature menopause appear to remain in an intermittently cycling state for many years further attests to key differences in the pathophysiology of this condition compared to the perimenopause, which results in hypergonadotropic amenorrhea in all women.

Compared with their menopausal counterparts, perimenopausal women displayed occasional periods of hypergonadotropic hypoestrogenism interspersed with periods of cyclic ovulatory and anovulatory activity. The periods of hypoestrogenism appeared to become more common with proximity to menopause. Currently, three of the six originally studied women have become postmenopausal. The two women closest to menopause when they collected urine specimens demonstrated periods of hypoestrogenism of up to 2–3 months. An additional subject not included in the general data analysis had experienced minimal menstrual cycle irregularity before entry into the study; over the course of the urine collection she did not experience another menstrual period and was profoundly hypoestrogenic throughout a 1-yr observation window that defined her menopause. Only two of our six subjects 47 yr and older at the time of the study completed the menopausal transition (defined as 1 yr of amenorrhea) within 2 yr of completion of the study. The progression into menopause would, therefore, appear to be a discontinuous process at best, with varying types of ovulatory and anovulatory ovarian activity eventually resulting in permanent hypergonadotropic hypoestrogenic amenorrhea. Modification of this process by endocrine, genetic, and constitutional factors may serve to predict the age at cessation of ovarian activity and menses.

In summary, we have demonstrated that the constellation of reproductive hormonal excretion patterns in perimenopausal women favors a relatively hyperestrogenic, inadequate progestogenic milieu. Menstrual cycle shortening, with an accelerated follicular phase in ovulatory cycles, alternates with intermittent anovulation. These features conspire to render the perimenopausal woman more likely to suffer from abnormal uterine bleeding and place her at risk for endometrial hyperplasia. It is likely that the amount of time a woman spends in this transition is an individual phenomenon that dictates her specific level of risk for development of disease. The episodic nature of this transition, which can take several years to complete, suggests that much lengthier windows of longitudinal study will be needed to document the progress of a woman through perimenopause.

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References

1. **Sherman BM, West JH, Korenman SG.** 1976 The menopausal transition: analysis of LH, FSH, estradiol and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab. 42:629–636.
2. **Buckler HM, Evans CA, Mantora H, Burger HG, Anderson DC.** 1991 Gonadotropin, steroid and inhibin levels in women with incipient ovarian failure during anovulatory and ovulatory rebound cycles. J Clin Endocrinol Metab. 72:116–124.
3. **Metcalf MG, Donald RA, Livesey JH.** 1981 Classification of menstrual cycles in pre- and perimenopausal women. J Endocrinol. 91:1–10.
4. **Metcalf MG, Donald RA, Livesey JH.** 1981 Pituitary ovarian function in normal women during the menopausal transition. Clin Endocrinol (Oxf). 14: 245–255.
5. **Metcalf MG, Donald RA, Livesey JH.** 1982 Pituitary ovarian function before, during and after the menopause: a longitudinal study. Clin Endocrinol (Oxf). 17:489–494.
6. **Metcalf MG, Livesey JH.** 1985 Gonadotropin excretion in fertile women: effect of age and the onset of the menopausal transition. J Endocrinol. 105:357–362.
7. **Shideler SE, DeVane GW, Kalra PS, Benirschke K, Lasley BL.** 1989 Ovarian-pituitary hormone interactions during the perimenopause. Maturitas. 11:331–339.
8. **Reame N, Kelch RP, Beitins IZ.** Pulsatile LH secretion during ovulatory menstrual cycles in perimenopausal women [Abstract 910]. Proc of the 74th Annual Meet of The Endocrine Soc. 1991.
9. **Soules MR, Bremner WJ, Dahl KD, Woodruff TK, Clifton DK.** Aging effects on the hypothalamic-pituitary-ovarian (HPO) axis in normal premenopausal women [Abstract 460]. Proc of the 39th Annual Meet of the Soc for Gynecol Invest. 1992.
10. **Fraser IS, Baird DT.** 1974 Blood production and ovarian secretion rates of estradiol 17-beta and estrone in women with dysfunctional uterine bleeding. J Clin Endocrinol Metab. 39:564–570.
11. **van Look PFA, Lothian H, Hunter WM, Michie EA, Baird DT.** 1977 Hypothalamic-pituitary ovarian function in perimenopausal women. Clin Endocrinol (Oxf). 7:13–31.
12. **Brown JR, Skurnick J, Sharma N, Adel T, Santoro N.** 1993 Frequent intermittent ovarian function in women with premature menopause: a longitudinal study. Endocr J. 1:467–474.
13. **Saketos M, Sharma N, Adel T, Raguwanshi M, Santoro, N.** 1994 Utility of time resolved fluorescent immunoassay technique for urinary gonadotropin measurement. Clin Chem. 40:749–753.
14. **Taussky HH.** 1954 A microcolorimetric determination of creatine in urine by the Jaffe reaction. J Biol Chem. 208:853–861.
15. **Lasley BL, Lohstroh P, Kuo A, et al.** 1985 Laboratory methods for evaluating early pregnancy loss in an industry-based population. Am J Ind Med. In press.
16. **Gray RH, Campbell OM, Zacur HA, Labbock MH, MacRae SL.** 1987 Postpartum return of ovarian activity in nonbreastfeeding women monitored by urinary assays. J Clin Endocrinol Metab. 64:645–650.
17. **Longcope C, Franz C, Morello C, Baker R, Johnston Jr CC.** 1986 Steroid and gonadotropin levels in women during the perimenopausal years. Maturitas. 8:189–196.
18. **Reyes FI, Winter JSD, Faiman C.** 1977 Pituitary-ovarian relationships preceding menopause. Am J Obstet Gynecol. 129:557–564.
19. **Lenton EA, Landgren BR, Sexton L, Harper R.** 1984 Normal variation in the length of the follicular phase of the menstrual cycle: effect of chronological age. Br J Obstet Gynaecol. 91:681–684.
20. **MacGillivray I.** 1986 Epidemiology of twin pregnancy. Semin Perinatol. 10: 4–8.
21. **Brown JB, Kellar R, Matthew GD.** 1959 Preliminary observations on urinary oestrogen excretion in certain gynaecological disorders. J Obstet Gynaecol Br Commonw. 66:177–211.
22. **Dicker RC, Greenspan JR, Strauss LT, et al.** 1982 Complications of abdominal and vaginal hysterectomy among women of reproductive age in the United States. Am J Obstet Gynecol. 144:841–848.
23. **Matthews KA, Wing RR, Kuller LH, Meilahn EN, Plantinga P.** 1994 Influence of the perimenopause on cardiovascular risk factors and symptoms of middle-aged healthy women. Arch Intern Med. 154:2349–2355.
24. **Slemenda C, Hui SL, Longcope C, Johnston CC.** 1987 Sex steroids and bone mass: a study of changes about the time of menopause. J Clin Invest. 80:1261–1269.