

# Urinary oestrogen patterns in long follicular phases

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**Long menstrual cycles have been associated with reduced risk of breast cancer and increased risk of osteoporosis. These observations have led to assumptions about the endogenous oestrogen exposure of women with long cycles. However, daily oestrogen profiles in long menstrual cycles have not been described. This paper examines daily urinary oestrogen profiles during the follicular phases of 416 conception and non-conception cycles. Women were aged 21–42 years, had no history of infertility and were not under treatment. Twenty-eight cycles were defined as long, with a follicular phase that lasted 24 days or more. Five patterns were observed among these long cycles, the most common being a pattern consistent with delayed emergence of a dominant follicle. Other patterns were a pattern consistent with demise and replacement of a dominant follicle, one consistent with delayed follicular recruitment, one showing a prolonged initial drop in oestrogen and one with an extended oestrogen peak. Average follicular phase oestrogen concentrations were highest in cycles with short follicular phases (7–11 days). Oestrogen concentrations from long follicular phases (24–59 days) did not differ substantially from follicular phases of usual length (12–17 days). The oestrogen profiles in long follicular phases are heterogeneous and not necessarily hypo-oestrogenic.**

*Key words:* follicular phase/menstrual cycle/oligomenorrhoea/urinary oestrone-3-glucuronide

## Introduction

Associations have been described between long menstrual cycles and decreased risk of breast cancer and increased risk of osteoporosis (reviewed in Harlow and Ephross, 1995). Menstrual cycle length, in turn, is determined primarily by the rate and quality of follicular growth (Speroff *et al.*, 1994). Follicular phase length has also been associated with other reproductive endpoints including sex of offspring (Weinberg *et al.*, 1995). Although population variability in follicular phase length has been described (Matsumoto *et al.*, 1962; Vollman, 1977; Landgren *et al.*, 1980; WHO, 1983; Lenton

*et al.*, 1984; Baird *et al.*, 1995), the question of how the ovary functions in long menstrual cycles has received only cursory attention. Oestrogen profiles in long follicular phases, not associated with lactation, have not been previously described.

Harlow and Zeger (1991) proposed that long cycles in non-lactating women may result from delayed folliculogenesis (a lag between demise of the corpus luteum from the previous cycle and the start of gonadotrophin-dependent folliculogenesis). This process would be similar to the follicular suppression observed in many lactating women, who experience a delay in the onset of gonadotrophin-dependent folliculogenesis and recruitment of follicles. Faundes *et al.* (1996) more recently reported on the functional life span of the dominant follicle in cycles that were anovulatory due to administration of continuous low dose progesterone (Norplant). In this study, the lifespan of a follicle was relatively fixed once it emerged as dominant. This lifespan was unrelated to menstrual cycle length. They concluded that variation in the length of the menstrual cycle must therefore be due to variation in the duration of time to follicular recruitment (consistent with an hypothesis of follicular suppression) or to variation in the time to emergence of the dominant follicle once recruitment has occurred. Alternatively, long cycles may result from early death of a dominant follicle and its replacement (Hirschfield, 1997).

In order to explore the relationship between ovarian function and menstrual cycle length in non-lactating women, oestrogen profiles were examined for follicular phases of various lengths using daily urinary hormone data. We were specifically interested in whether the oestrogen patterns in long cycles are most consistent with (i) delayed follicular recruitment (ovarian suppression); (ii) prolonged time between recruitment and emergence of the dominant follicle; or (iii) repeated folliculogenesis due to early death of a dominant follicle.

## Materials and methods

This paper is based on urinary hormone data collected as part of the North Carolina Early Pregnancy Study (NCEPS) (Wilcox *et al.*, 1988). Details of the study design have been reported elsewhere (Wilcox *et al.*, 1988). A total of 221 women were enrolled when they discontinued contraception to become pregnant. Participants collected and froze a first-morning urine specimen each day, at which time they recorded whether they had experienced any vaginal bleeding during the prior 24 h. Participation lasted until women were clinically pregnant or 6 months had passed with no clinically apparent pregnancy. The first day of a new menstrual cycle was defined as the first day of recorded bleeding in a sequence of at least 2 consecutive days of bleeding.

Oestrogen and progesterone metabolites [oestrone-3-glucuronide (E<sub>1</sub>G) and pregnanediol-3-glucuronide] were measured using radio-

immunoassay (Samarajeeva *et al.*, 1979; Wilcox *et al.*, 1987). Oestrogen metabolite data were available for 724 cycles from 217 women. Seven cycles were unambiguously anovulatory and these cycles have been excluded (cycle lengths of 28–53 days). A day of ovulation could be identified for 696 cycles. For a subset of about 40% of the cycles, assays were performed only for a mid-cycle window, so the full follicular phase oestrogen profile was not available. Follicular phases with no defined start day for the menstrual cycle, with no defined day of ovulation or with more than 5 days missing were excluded. This left 416 follicular phases, contributed by 167 women (one to eight cycles per woman). Conception occurred in 144 (35%) of these cycles, including subclinical conceptions that ended prior to clinical recognition.

Day of ovulation was estimated from the relative concentrations of urinary oestrogen and progesterone metabolites. This ratio drops rapidly around the time of ovulation, when oestrogen production falls and progesterone production increases as the follicle luteinizes. This marker has been validated against the urinary luteinizing hormone (LH) peak (Baird *et al.*, 1991) and appears to be as precise a marker of ovulation as estimates based on serum LH (Baird *et al.*, 1995). The follicular phase was defined as the first day of the cycle to the day before the estimated day of ovulation. Thus, in a cycle with ovulation on day 14, the follicular phase is 13 days.

### Analysis

A long follicular phase was defined as a follicular phase of 24 days or longer, which is equal to the mean +1 SD ( $17.2 \pm 6.7$  days) of the follicular phase length distribution in the NCEPS study (Baird *et al.*, 1995). [As reviewed (Harlow and Ephross, 1995), the mean in this data set is consistent with the mean in other studies that included long cycles but longer than that reported by studies that exclude long cycles and/or report a geometric mean. It is the latter studies that report the mean follicular phase length to be 13–15 days] This definition is consistent with previous literature on the length of extreme follicular phases (reviewed in Harlow and Ephross, 1995). If a luteal phase of 13 days is assumed (the mean luteal phase length observed in the NCEPS; Baird *et al.*, 1991), plus the day of ovulation, this definition of a long follicular phase is roughly equivalent to having a menstrual cycle length of 38 days or more.

In order to evaluate how oestrogen patterns might vary across cycles with different follicular phase lengths, the oestrogen profiles of individual cycles were visually examined for selected follicular phases of various lengths. A random sample was selected of 30 follicular phases of 13–15 days, all 24 follicular phases of 19–20 days, and all 28 long follicular phases (24 days or longer). The  $E_1G$  pattern in each case was smoothed using a 3 day moving average. All cycles were also categorized by follicular phase length (7–11 days, 12–17 days, 18–23 days and 24–59 days with  $n = 77, 230, 81$  and 28 respectively) and the daily geometric mean  $E_1G$  values were graphed, with reference to the day of ovulation.

The oestrogen pattern during a typical follicular phase of length approximately 14 days is a slow rise followed by a more rapid rise just before ovulation (van Santbrink *et al.*, 1995). The oestrogen profile of each follicular phase was statistically summarized using a bent stick regression model (Neter and Wasserman, 1974). Essentially, this linear two phase regression model estimates one slope for a portion of the time scale and a second slope beyond some change point, with this change point being estimated from the data. The first segment typically captured the early-to-mid-follicular slow oestrogen rise. The second segment typically bent upward to capture the late-follicular rapid rise prior to ovulation. The fit is optimized by minimizing the sum of squared differences between the observations and the bent line. The first few days of a cycle often show a pattern

**Table I.** Sociodemographic characteristics of the 167 women from the North Carolina early pregnancy study included in this analysis<sup>a</sup>

	Subset of 167 women	
	<i>n</i>	percent
Age (years)		
21–25	24	14
26–30	92	55
31–35	41	25
36–42	10	6
Education		
high school	12	7
some college	37	22
college	65	39
postgraduate	53	32
Income		
<\$20 000	51	31
\$20 000–\$29 999	51	31
≥\$30 000	64	39
Missing	1	
Age at menarche (years)		
9–10	7	4
11	19	11
12	52	31
13	50	30
14	23	14
15–17	16	10
Gravidity		
0	56	34
1	60	36
2	30	18
>2	20	12
Missing	1	

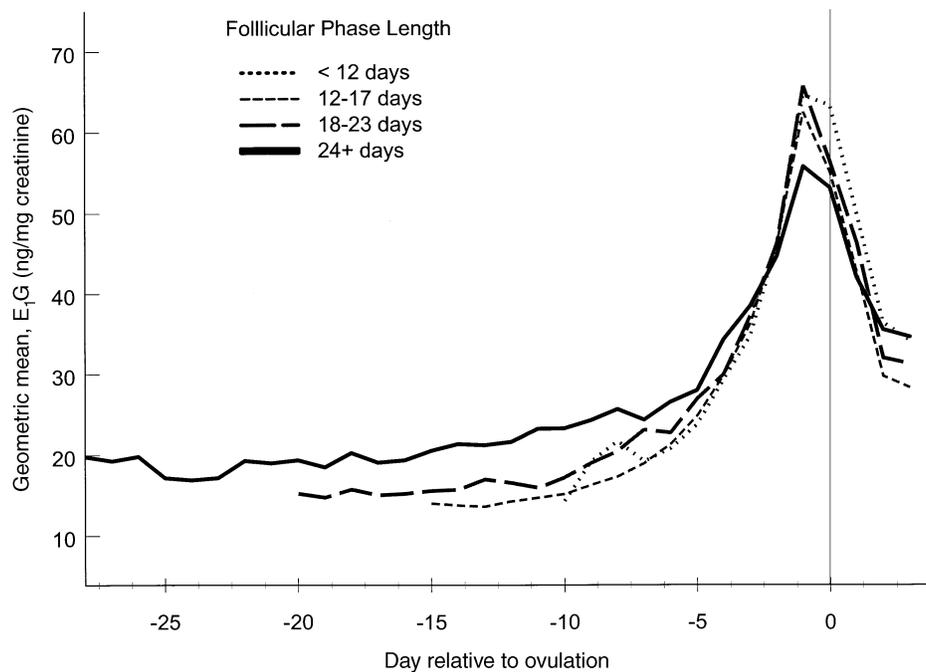
<sup>a</sup>Because of rounding the percentages for each characteristic may not equal 100.

of declining oestrogen from the end of the previous luteal phase. These days were excluded by beginning the regression analysis with the day after the early drop in urinary oestrogen. The regression analysis was concluded at the mid-cycle peak of urinary oestrogen. As these slopes were calculated for each individual cycle, this analysis procedure was applied only to follicular phases with no more than 10% missing data ( $n = 316$ ). The average early slope for follicular phases of various lengths was then calculated and these were compared using a generalized linear mixed models approach (SAS procedure PROC MIXED) in order to account for repeated observations within the same woman. Averages of the later slopes were compared similarly across the four categories of follicular phase length as were average number of days from the start of the rapid rise until the day of ovulation.

It is commonly assumed that women with long cycles have reduced exposure to endogenous oestrogen. The amount of oestrogen exposure was evaluated during the follicular phase, stratifying by follicular phase length. The mean daily  $E_1G$  concentration was calculated for each cycle for the entire follicular phase and also for the period of the slow rise (to estimate average oestrogen concentrations with and without the oestrogen peak). The distributions of the  $E_1G$  concentrations were plotted for the four phase length categories (7–11 days, 12–17 days, 18–23 days and 24–59 days).

### Results

Table I shows the sociodemographic characteristics of the 167 women included in this analysis. The distributions of age, gravidity, education and income for this subset of women were similar to that of the total study cohort. Follicular phase length



**Figure 1.** Geometric mean oestrone-3-glucuronide ( $E_1G$ ) values by day centred on the day of ovulation (shown as a vertical line) by follicular phase length for 416 follicular phases. Respective numbers in each category from short to long are 77, 230, 81 and 28.

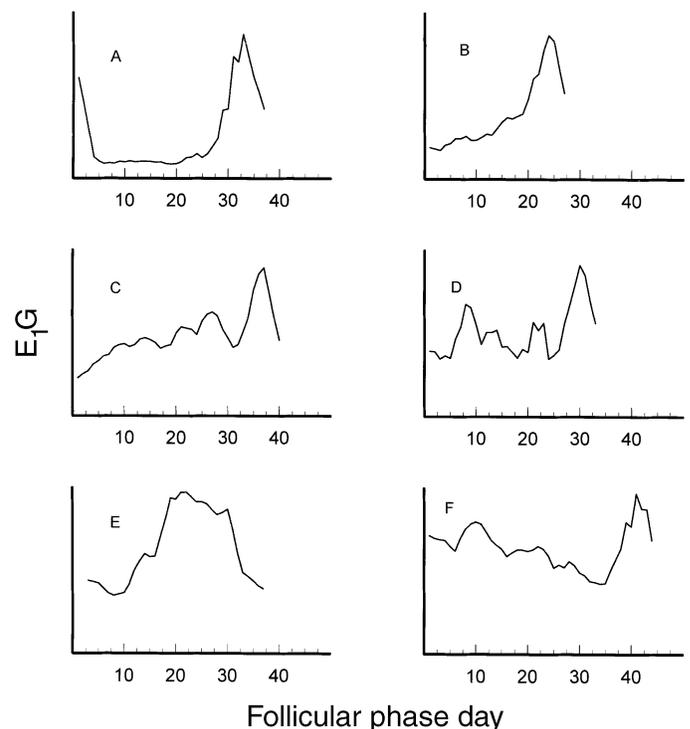
ranged from 7–59 days. Twenty-eight cycles (7%) had follicular phases of 24 days or longer compared to 8% in the total study cohort.

Figure 1 shows the daily geometric-mean  $E_1G$  values with reference to the day of ovulation, for each category of follicular phase length. No differences by phase length were apparent in the rate or duration of the rapid rise in oestrogen prior to ovulation. The long follicular phases appeared to have slightly higher oestrogen concentrations prior to the start of the acute oestrogen rise.

When smoothed daily oestrogen profiles were examined for individual follicular phases of length 13–15 days, the oestrogen pattern generally followed the expected profile. In every case, urinary oestrogen concentrations either dropped slightly or were flat for the first 2–4 days, rose slowly for subsequent 6–10 days, and then rose sharply for 2–5 days, after which they peaked and dropped. Cycles with long follicular phases followed a similar overall pattern but one or more of the various components were lengthened. The patterns were categorized as follows: low-flat: oestrogen concentrations remained low and flat for an extended interval; extended slow rise: the initial slow rise in oestrogen occurred over an extended interval and the pattern of rise was either fairly linear or somewhat erratic with slight rises and falls in oestrogen concentrations; multiple peaks: a series of rapid rises followed by falls; extended peak: the  $E_1G$  peak was elevated for more than 10 days; extended initial drop: the decline in oestrogen at the start of the cycle occurred over an extended interval of time (7 days or longer).

Figure 2 presents typical examples of each type of long follicular phase. The most common pattern among the long cycles was the extended slow rise which occurred in more than half of the observed follicular phases. The other four long patterns were infrequent.

Women who contributed more than one long follicular



**Figure 2.** Smoothed graphs of urinary oestrogen profiles for long follicular phases including examples of (A) the low-flat pattern, (B and C) the extended slow rise, (D) multiple peaks, (E) an extended peak and (F) a prolonged initial drop in  $E_1G$  values. As the absolute  $E_1G$  concentrations vary considerably a scale for the y-axis is not provided.

phase did not necessarily repeat the same hormone pattern. Of the eight women who contributed two long cycles to this analysis, six women had both cycles classified as an extended slow rise. One woman had an extended slow rise and a low-

**Table II.** Mean slope and SE of the slow and rapid oestrone-3-glucuronide (E<sub>1</sub>G) rise, by follicular phase length for 315 follicular phases<sup>a</sup>

	<i>n</i>	Slow rise slope		Rapid rise slope	
		mean	SE	mean	SE
Follicular phase length (days)					
7–11	41	3.81	0.38	18.01	5.66
12–17	176	2.19	0.21	23.37	3.76
18–23	73	1.25	0.28	26.21	4.51
24–59 days	25	0.68	0.48	25.56	6.98
<i>P</i> value <sup>b</sup>	0.0001	0.27			

<sup>a</sup>One follicular phase had no estimable breakpoint and is excluded from this analysis.  
<sup>b</sup>Test for trend.

flat pattern. One woman had an extended slow rise and an extended peak.

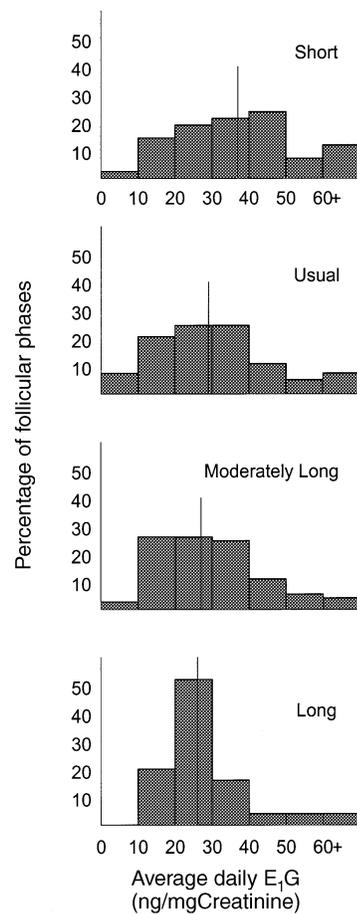
Table II shows the mean slope and SE for the slow and acute rise in E<sub>1</sub>G values by the four categories of follicular phase length. The slope for the slow rise in the shortest follicular phases (7–11 days) was considerably steeper on average and more variable than the slope of the slow rise in 12–17 day or longer follicular phases. The slope of the slow rise declined with length of the follicular phase (*P* value = 0.0001). In contrast, the slopes during the rapid rise were not significantly different by length of the follicular phase (*P* value = 0.27). The time from the start of the rapid rise until the day of ovulation increased slightly with length of the follicular phase with the mean (SE) being 2.98 (0.34), 3.91 (0.17), 4.21 (0.25) and 4.95 (0.42) days for follicular phases of 7–11, 12–17, 18–23 and 24–59 days respectively.

Oestrogen concentrations in long cycles with an extended slow rise or with multiple peaks were often substantial. When the distributions of the average daily concentrations of E<sub>1</sub>G were evaluated by follicular phase length (Figure 3), the histograms illustrate both the heterogeneity in E<sub>1</sub>G concentrations within any given category of follicular phase length and the extent to which the values overlap across categories. The median average daily E<sub>1</sub>G value tended to decrease as follicular phase length increased, with the medians being 37, 29, 27, and 26 ng/mg creatinine for short, usual, moderately long and long follicular phase lengths respectively. When the average oestrogen concentration was based only on the daily E<sub>1</sub>G values during the period of the slow rise (Figure 4), median oestrogen values were 26 ng/mg creatinine for short phases and 19, 20 and 23 ng/mg creatinine for usual, moderately long and long phase lengths respectively.

**Discussion**

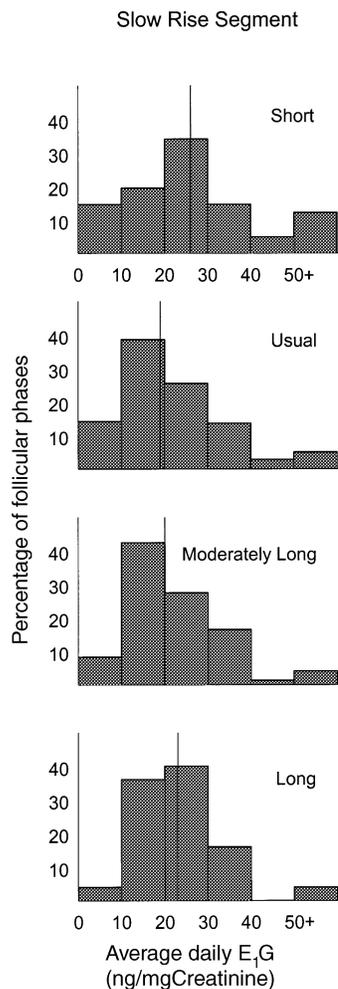
Menstrual cycle length is easily measured, but the hormonal patterns that underlie cycle length are much more difficult to assess. The few long series of daily hormonal data that are available come from women with lactational amenorrhoea. Those data demonstrate heterogeneity, but the majority of the women appear to experience extended periods of low oestrogen production (Shaaban *et al.*, 1987). Earlier studies of individual women’s oestrogen profiles in non-lactating women did not include examples of long cycles (Burger *et al.*, 1968). In this

Complete Follicular Phase



**Figure 3.** Distributions of mean E<sub>1</sub>G concentration for the entire follicular phase by follicular phase length for short (7–11 days), usual (12–17 days), moderately long (18–23 days) and long (24–59) phases. Median values are shown by vertical lines.

study, follicular oestrogen patterns in the cycles of 167 healthy women who collected daily first morning urine samples are described. The heterogeneous patterns observed suggest that long cycles can arise from several different variations in ovarian folliculogenesis. One pattern showed prolonged low and flat oestrogen concentrations. This pattern is consistent with follicular suppression as seen in lactation associated



**Figure 4.** Distributions of mean E<sub>1</sub>G concentration for the period of the slow rise by follicular phase length for short (7–11 days), usual (12–17 days), moderately long (18–23 days) and long (24–59) phases. The period of the slow rise was identified by the first segment of a best-fit broken stick regression. Median values are shown by vertical lines.

amenorrhoea (Shaaben *et al.*, 1987). A second pattern showed distinct multiple oestrogen peaks, consistent with emergence of a dominant follicle, its subsequent demise, and emergence of a replacement follicle. While both of these patterns are discussed in the clinical and toxicological literature as causes of long cycles, they were infrequent in the data presented here.

Most of the long follicular phases in this study showed a prolonged initial slow rise in oestrogen. The initial slow rise began early in the cycle, as also seen in follicular phases of 13–15 days, but this slow rise continued well beyond the usual 5–10 days. It is postulated that this prolonged slow rise may reflect delay in emergence of a dominant follicle. Concentrations of the oestrogen metabolite in this pattern were often relatively high.

Two other patterns were also observed, although infrequently: an extended oestrogen peak and a prolonged initial drop. An extended peak may be due to the presence of an ovarian cyst. The prolonged initial drop followed an anovulatory cycle in some instances. Follicular phases whose lengths were only moderately long (i.e., 18–23 days), exhibited the

same heterogeneity observed in the long cycles. Consistent with this heterogeneity of patterns, concentrations of the excreted oestrogen metabolite also varied in both long and moderately long follicular phases.

It was concluded previously that most of the variation in menstrual cycle length reflects differences in the duration of follicle recruitment and selection (Faundes *et al.*, 1996), which is consistent with the findings of this study. Their observations of the lifespan of the dominant follicle were among cycles of women under continuous low-dose progesterone administration. The data presented here are also consistent with human ultrasound data (Gore *et al.*, 1995). They observed multiple large follicles ( $\geq 8$  mm in diameter) present during any given ovulatory cycle, several of which may have the potential to attain dominance. The follicle that ovulated was often not the first large follicle to be identified. They also found that once a follicle became dominant, it grew rapidly. If a dominant follicle showed arrested growth, it became atretic and was replaced by another. They concluded that successful emergence from the pool of large follicles requires precise conditions and timing. They postulated that when larger numbers of follicles are recruited, more time will elapse before a single follicle emerges as dominant.

One of the important clinically-recognized causes of oligomenorrhoea is polycystic ovaries (PCO) (Adams *et al.*, 1986; Botsis *et al.*, 1995). It was demonstrated (Van der Meer *et al.*, 1998) that women with polycystic ovaries have larger cohorts of follicles sensitive to follicle stimulating hormone (FSH) and thus any individual follicle may have a more difficult time establishing dominance (Gore *et al.*, 1997). There appear to be no studies reporting daily oestrogen profiles in women with PCO. None of the women in this study was diagnosed with PCO, but undiagnosed PCO could nonetheless have contributed to some of the patterns observed.

Several studies have examined menstrual cycle length in relation to breast cancer (Henderson *et al.*, 1985; Garland *et al.*, 1998; Whelan *et al.*, 1994) with the assumption that cycle length is a surrogate for hormonal exposure. Other studies have demonstrated associations between follicular phase length and reproductive endpoints such as sex ratio (James, 1995; Weinberg *et al.*, 1995), contralateral ovulation and pre-embryo development (Fukada *et al.*, 1998). When the average amount of exposure to endogenous oestrogen in the follicular phase was examined, as measured by the major urinary metabolite E<sub>1</sub>G, women with the shortest follicular phases had the highest mean follicular oestrogen concentration. Follicular phases that were usual in length, moderately long or long were similar in their mean follicular E<sub>1</sub>G concentrations. It appears that women with short cycles may have higher oestrogen exposure both because mid-cycle oestrogen peaks occur more frequently and because the early and mid-follicular phase concentrations are higher in short cycles.

When information about menstrual cycles are obtained only by self report of bleeding using menstrual diaries, other hormonal patterns would also occur among the long cycles. For example, some anovulatory cycles may also be included. Three of the seven anovulatory cycles in the NCEPS were  $>38$  days in length. Two of these anovulatory cycles had low oestrogen throughout the cycle, while the third had moderately

high concentrations. Another possibility is the occurrence of what are referred to as 'double cycles'. In the data reported here, four diary-reported menstrual cycles of >38 days actually showed hormonal patterns of two ovulatory cycles with no reported menstruation in between. Whether this phenomenon reflects reporting error or suppression of menstrual flow (Strassman, 1996) is unknown; however, such 'double' cycles do not show reduced follicular oestrogen excretion.

This study has limitations. Observations were drawn from a group of self-selected volunteers, although none had any fertility problems. Daily oestrogen profiles were characterized by a single urinary oestrogen metabolite that varies among women in the accuracy with which it reflects total oestrogen production and in how well it corresponds to circulatory concentrations. Data on daily FSH concentrations is also lacking. As many of these cycles resulted in pregnancy, this data set does not lend itself to examining the relationship between follicular phase oestrogen patterns and luteal-phase length. Finally, this is an analysis of cycles and not of women. It is not known how these results might compare to long cycles in women with clinically diagnosed oligomenorrhoea. While these limitations should be kept in mind, there appears to be no other description of oestrogen patterns in long follicular phases of healthy, untreated, non-lactating women.

In summary, the daily oestrogen profile in long cycles is heterogeneous. Although some cycles show a pattern consistent with follicular suppression, the most common pattern underlying the long follicular phases in women in the NCEPS appears to be prolongation of the time from recruitment to emergence of a dominant follicle. Given the apparent heterogeneity of oestrogen patterns associated with long follicular phases, caution is advised in assuming that long cycles reduce a woman's average amount of endogenous exposure to unopposed oestrogens. Whether this conclusion also applies to women with clinically diagnosed oligomenorrhoea requires further investigation. Additional studies of daily oestrogen profiles in women with oligomenorrhoea are warranted, as are studies in women with amenorrhoea and women with polycystic ovaries. Subsequent studies would benefit by inclusion of a more ethnically diverse sample, daily measures of gonadotrophins, concurrent sonographic data, and a sample size adequate to evaluate age effects more precisely.

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