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Menstrual Variation in Salivary Testosterone among Regularly Cycling Women

Key Words
Testosterone
Menstrual cycle
Salivary steroids

Abstract
To determine menstrual variation in salivary testosterone daily saliva samples were collected from 20 regularly cycling women. Results indicate that the menstrual profile of salivary testosterone for both ovulatory and anovulatory cycles exhibits local peaks during the follicular phase and at midcycle, as well as a luteal trough. However, the testosterone profile for anovulatory cycles exhibited a later midcycle peak than that for ovulatory cycles, as well as significantly higher average testosterone levels. These results extend the observation of a midcycle peak in serum testosterone to saliva and suggest the existence of a follicular peak in unbound testosterone coincident with the early androgen production of a cohort of developing follicles.

Introduction

Menstrual variation in testosterone is of interest both as it relates to ovarian events during the menstrual cycle [1, 2] and as a possible influence on changes in behavior over the menstrual cycle [3, 4]. Research has focused on the existence and significance of a midcycle peak in serum testosterone which represents peak ovarian production of testosterone [2], as well as predicting the frequency of intercourse among married couples [3]. Early studies based on daily measures reported conflicting results [5–7], but more recent investigations, using larger samples and better assays, support the existence of a midcycle peak of serum testosterone in ovulatory cycles [1, 8]. However, these studies still suffer from relatively small sample sizes and selected populations due to the difficulty of obtaining daily blood samples, making it difficult to draw conclusions about menstrual variation in testosterone among normal women.

Because of the noninvasive nature of sample collection, the use of salivary assays can alleviate the problem of obtaining daily samples, making it easier to study menstrual variation in testosterone among normal women. Salivary assays of testosterone have been shown to correlate well with serum free testosterone [9–11], despite questions about the uptake and metabolism of testosterone within the salivary gland [12]. The noninvasive sample collection and measurement of unbound testosterone make salivary assays especially attractive for the analysis of changes in testosterone when frequent and repeated samples must be collected.

Yet, despite the potential advantages of salivary assays, only a few studies have considered changes in salivary testosterone over the menstrual cycle. These studies have not unambiguously documented a midcycle peak in salivary testosterone. One study showing a midcycle peak in salivary testosterone in ovulatory cycles provided no statistical analysis of the pattern [13]. In two studies,

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Dabbs [4, 14] has found that salivary testosterone was highest at midcycle, though in only one of the two studies was the increase significant. However, he collected samples only once a week and did not distinguish ovulatory cycles from anovulatory cycles.

In this study, to characterize the pattern of salivary testosterone over the menstrual cycle and determine the existence of a midcycle peak, 20 regularly cycling, adult women were recruited from whom daily saliva samples were collected over the course of a menstrual cycle. Each saliva sample was assayed for testosterone. Luteal samples were assayed for progesterone to determine if cycles were ovulatory or not. Because of a large number of anovulatory cycles, it was also possible to determine if patterns of salivary testosterone differed among ovulatory and anovulatory cycles.

Materials and Methods

Subjects

Daily saliva samples were collected over the course of a single menstrual cycle from 20 adult women, recruited from the university community. To control for reported circadian variation in salivary testosterone, saliva samples were collected within an hour of rising each day [4, 14]. All subjects reported a history of regular menstrual cycles and no known gynecologic disorders. Subjects were selected if they had not been pregnant or lactating within the past 6 months, and were not on oral contraception or taking any medication known to effect steroid hormone metabolism. All subjects reported only minimal participation in exercise activities. One cycle was not used in the analysis because the saliva samples were not sufficient in volume to determine progesterone levels. Another cycle was not used because of extremely high salivary testosterone levels. The average age of the remaining 18 subjects ranged from 24 to 42 years with an average of 29.5 ± 4.4 years; the average cycle length was 27.8 ± 3.2 days, and subjects had an average weight of 56.8 ± 0.72 kg and height of 167 ± 4.6 cm. Their average percent of ideal body weight was 92 ± 13% with a range from 72% to 125%.

Assay

All daily saliva samples were assayed for testosterone by radioimmunoassay, as described previously [15], with modifications. Testosterone was extracted from 2.4 ml of saliva. Chromatographic separation resulted in significantly lower values, so a purification step using a Celite column [16] was introduced between the extraction and assay. Average recovery over columns was approximately 85% with only an occasional sample below 75%. Antiserum (G250) used was supplied by Gordon Niswender. Interassay variability ranged from 23 to 32%. Intra-assay variability was 8.3%. The limit of detection, the point at which values could be distinguished from 0 with 95% confidence, was calculated on the basis of the standard curve for each run of the assay, and values falling below the limit were replaced by the limit value for that assay. The limit of detection ranged from 3.0 to 13.5 pmol/l and averaged 9.3 pmol/l. All samples from a single cycle were included in the same assay to minimize the effects of interassay variability.

Every other daily saliva sample from the luteal phase (days −2 to −16) was assayed for progesterone by radioimmunoassay as described previously [17]. Interassay variability ranged from 13.7 to 18.0%. Intra-assay variability was 6.4%. Average limit of detection ranged from 3.3 to 11.4 pmol/l and averaged 7.3 pmol/l.

Analysis

Cycles were classified as ovulatory or anovulatory on the basis of the highest daily luteal progesterone value. Cycles with maximal salivary progesterone values during the luteal phase > 300 pmol/l were classified as ovulatory, while those with maximal luteal progesterone values < 300 pmol/l were classified as anovulatory [18, 19]. Since the use of at least 1 luteal salivary progesterone value > 300 pmol/l as the criterion of an ovulatory cycle was originally based on daily sampling of luteal progesterone, the proportion of anovulatory cycles reported above may be overstated. However, other work from this lab indicates that sampling every second day results in a misclassification rate of only 5% [unpubl. data].

To minimize the effects of individual variation in salivary testosterone levels and to normalize their distribution, the daily testosterone value for each woman were converted to z scores based on the average value for her cycle [8]. All values > 3 SD from the mean for an individual woman (total = 4) were removed before averaging across all cycles to create an average profile, based on a reverse cycle alignment. Anova with repeated measures was used to determine the significance of variation across the menstrual cycle [20]. An autoregressive correlation matrix was chosen for the Anova to account for the time series nature of the data.

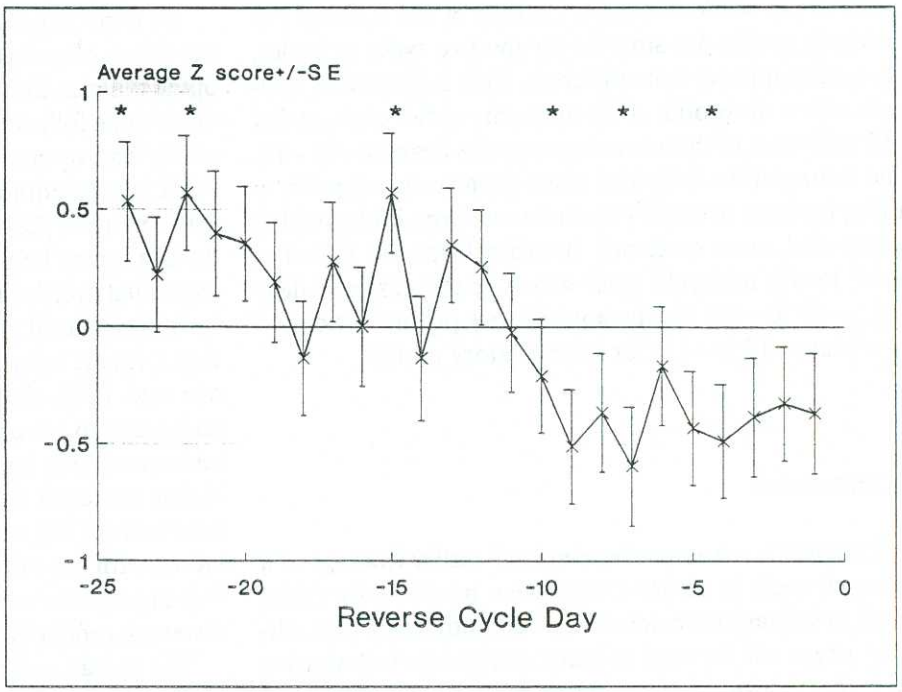
Anova was chosen over time series because it provides a better test of changes in testosterone within the menstrual cycle. Time series assumes a regular periodicity in cyclic changes making it appropriate for analyzing two or more cycles [21]. However, within a single cycle, changes in testosterone are not expected to have a regular period (e.g. 7 or 14 days, shorter than that of the cycle as a whole).

Results

Salivary testosterone values ranged from 5.5 to 155.2 pmol/l. These values are within the range reported by various labs [10, 14, 22].

11 cycles were classified as ovulatory while 7 were classified as anovulatory on the basis of luteal progesterone. Because of the comparable number of cycles, the testosterone profiles of anovulatory and ovulatory cycles were analyzed separately. Average salivary testosterone, by cycle, was 42.3 ± 14.0 pmol/l for the 11 luteal cycles, and 68.4 ± 23.6 pmol/l for the 7 anovulatory cycles ($t = 2.65$, $d.f. = 16$, $p < 0.02$).

The menstrual profile of normalized salivary testosterone among ovulatory cycles exihibits three main features: a luteal trough, a midcycle peak and an early follicular peak (fig. 1). Anova with repeated measures indicates that within each of these features there are individual days for



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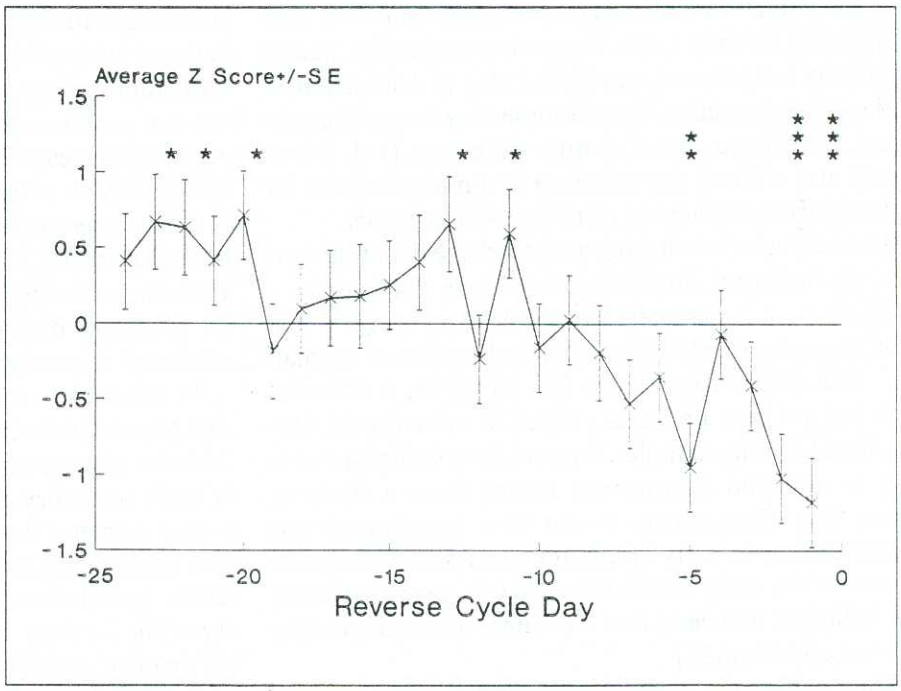

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Fig. 1, 2. Menstrual profile of normalized salivary testosterone in ovulatory (**1**) and anovulatory (**2**) cycles. * Indicates daily values identified by Anova as significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

which the normalized testosterone values achieve statistical significance. For luteal cycles, these are days −4, −7 and −9 during the luteal trough, day −15 at midcycle, and days −22 and −24 during the follicular increase.

The menstrual profile on testosterone for the anovulatory cycles is similar to that of the ovulatory cycles, with a luteal trough, midcycle peak and early follicular peak (fig. 2). For anovulatory cycles, normalized testosterone values on days −1, −2 and −5 during the luteal depression, −11 and −13 at midcycle, and −20, −22 and −23 during the follicular peak achieve statistical significance.

However, while the main features of the salivary testosterone profile are similiar for the two types of cycles, the timing appears to be different. This is clearest at midcycle where the profile of the ovulatory cycles peaks at day −15 while that of the anovulatory cycles peaks at day −13. The timing of the follicular phase pattern also appears to differ, between ovulatory and nonovulatory cycles, with a longer and more sustained increase from the follicular nadir to the midcycle peak which encompasses 7 days (days −19 to −13) for the anovulatory profile but only 4 days (days −18 to −15) for the ovulatory profile.

Discussion

The results presented here extend earlier findings of a midcycle peak in serum testosterone in ovulatory cycles [1, 8] to salivary testosterone as well, indicating that salivary assays can be used to track changes in testosterone over the menstrual cycle. However, the magnitude of changes is small, and testosterone is significantly elevated at midcycle for only 1 day, suggesting that earlier studies of salivary testosterone may have failed to demonstrate a midcycle peak in salivary testosterone because of the relatively low frequency of sample collection [14]. These results also indicate the existence of a midcycle peak for anovulatory cycles among regularly cycling women.

In addition to confirming a midcycle peak in testosterone, the menstrual profile presented here also exhibits a follicular peak and a luteal trough. A luteal trough is evident in previously reported menstrual profiles of normalized total serum testosterone [8]. However, a follicular peak has not been previously reported in menstrual profiles based on daily samples. Support for a follicular elevation in unbound testosterone comes from a study of serum 'free' testosterone. Serum 'free' testosterone was highest during the early follicular phase and declined over the rest of the cycle, based on samples from the early and late follicular, and early and late luteal phases aligned by forward cycle day [23].

Since neither the current study nor that of serum 'free' testosterone [23] are aligned by LH peak, the method used by other studies of menstrual variation in testosterone, it is possible that elevated salivary testosterone levels during the early follicular phase are an artifact of the method of cycle alignment. However, regardless of the method of cycle alignment (reverse cycle day, forward cycle day, or midcycle nadir in testosterone) the follicular phase increase is evident, though it varies in its extent [24].

An early follicular peak of testosterone may represent the effects of testosterone production by a cohort of developing follicles. During the first half of the follicular phase, developing follicles, stimulated by LH, produce predominantly androgens, including testosterone [25]. However, in all but the dominant follicles, high levels of androgens lead to atresia [26], and levels of testosterone secreted by the ovary may be expected to decline, as suggested by the menstrual profile presented here.

It is not until the second half of the follicular phase that, with the induction of aromatase activity by testosterone and FSH, the dominant follicle begins to convert androgens to estrogen at an increasing rate [27]. Testosterone secretion increases as a by-product, as suggested by higher androgen levels in the ovary containing the dominant follicle [28], reaching its peak at midcycle. After ovulation, with the shift from estrogen production by the follicle to progesterone production by the corpus luteum, testosterone production decreases.

The overall menstrual profile of salivary testosterone is similar for ovulatory and nonovulatory cycles, indicating that testosterone levels, though higher, are not sufficiently elevated among the nonovulatory cycles, represented here to disrupt cycling. However, differences in the timing of the two profiles may reflect more subtle changes associated with increased testosterone levels. The facts that the midcycle peak of testosterone in the nonovulatory cycles is 2 days later and that it takes 7 days to reach after the follicular nadir, compared to 4 days for ovulatory cycles, are both consistent with evidence that testosterone inhibits follicular development, lengthening the follicular phase and shortening the luteal phase [29].

In conclusion, salivary testosterone shows significant changes across the menstrual cycle with peaks during the follicular phase and at midcycle as well as a luteal trough in both ovulatory and anovulatory cycles of regularly cycling women. The presence of a follicular peak in salivary testosterone, though not previously reported for total serum testosterone, is consistent with what we know about the ovarian steroid hormone metabolism during the first half of the follicular phase [25]. Differences in the timing of salivary testosterone profiles of ovulatory and anovulatory cycles are consistent with the inhibition of follicular maturation by elevated testosterone levels [30].

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