**Short Report**

Menstrual Cycle Irregularities Are Associated With Testosterone Levels in Healthy Premenopausal Women

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**ABSTRACT**  
High androgen levels have been associated with menstrual irregularities in clinical populations, but not in healthy women. We examined the association between testosterone and menstrual irregularities in a nonclinical population of 194 healthy premenopausal women, none of whom reported chronic health conditions. Women provided saliva samples for assay of salivary testosterone, and responded to questions about length of menstrual cycles, variability in menses, and retrospective history of menstrual irregularity. Results showed significant correlations between testosterone and menstrual irregularities, even when women with the most irregular cycles were excluded from analyses. This pattern was also apparent for a subgroup of 27 women using hormonal contraceptives. Based on our findings, it appears that even in healthy women reporting no health concerns, menstrual irregularities are associated with higher levels of circulating androgens. Am. J. Hum. Biol. 18:841–844, 2006.

**Menstrual cycles can be important indicators of health and fertility because they reflect basic physiology, and irregular cycles may be indicative of underlying endocrine disorders. For example, elevated androgens have been associated with dysmenorrhea. Polycystic ovary syndrome (PCOS) is one well-known disorder of ovarian dysfunction with dysmenorrhea and high levels of androgens, as well as obesity and hirsutism (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).**

Despite the well-established link of high levels of androgens to conditions affecting menstrual cycles, little research has examined how androgens and the menstrual cycle may be associated in healthy women. One study found that menstrual irregularity was linked with higher levels of androgens (also stress and lower insulin sensitivity) in healthy adolescents with no known clinical diagnosis (Rodrigues Fernandez et al., 2005; van Hooff et al., 1998); whether this is true in healthy adult women remains uninvestigated. Here, we report on associations between salivary testosterone (T) and menstrual irregularity in healthy women.

**METHODS**

**Participants**

Participants were recruited as part of other studies (van Anders and Watson, 2006), approved by the Simon Fraser University (SFU) Research Ethics Office, from the SFU participant pool and the Vancouver community. Women (n = 4) who were pregnant, lactating, or using noncontraceptive medications were excluded. Healthy participants were purposively recruited, and none reported health conditions, including PCOS. There were 194 women (mean (M) age, 24.48 years; SD, 7.89 years), of whom 27 were using hormonal contraceptives (HC; this was exclusionary criteria at most sites). Some participants did not respond completely, resulting in a slightly reduced n for some items. There were 124 heterosexual women and 68 nonheterosexual women (two did not respond), determined via the Kinsey measures (Kinsey et al., 1948). Nonheterosexual women were overrepresented because recruiting partly occurred at the Vancouver Pride Parade.

**Materials and procedure**

All samples were collected between 13:00–20:00 hr to control for diurnal rhythms in T (Rose et al., 1972). Participants completed...
health and background questionnaires. Participants indicated the length of their menstrual cycles by selecting either: 23 days or less, 24–26 days, 27–30 days, 31–34 days, or 35 or more days. Participants indicated the regularity of their menses by selecting either: perfectly regular, varies by 1–2 days, varies by 3–4 days, varies by 5–6 days, varies by 7 or more days, or completely unpredictable. Participants were asked whether they ever went through long periods of time without having their menstrual period (for reasons other than pregnancy), and whether this had happened in the last 12 months.

Saliva samples were collected in polystyrene tubes pretreated with sodium azide, and frozen afterwards at −20°C until assay. An inert chewing gum was used to stimulate saliva flow. Samples were assayed for T in four batches at the Endocrine Core Laboratory at Yerkes National Primate Research Center, Emory University, in duplicate, using a modified kit from Diagnostic Systems Laboratories (Webster, TX). Sensitivity was 2 pg/ml per 200-μl dose, and the interassay coefficient of variation was 8.77% at 0.65 ng/ml and 6.88% at 5.06 ng/ml. The intra-assay coefficient of variation was 6.54% at 98.82 pg/ml.

Analysis

All analyses were performed with SPSS, version 10.0.1. Age was controlled in all analyses because of associations with increased T and altered menstrual cycles. Means were examined with analyses of covariance (ANOVA), and associations with partial correlations. All analyses excluded HC users, unless otherwise indicated. Analyses did not control for sexual orientation, since this did not alter the pattern of results.

RESULTS

Salivary T and menstrual cycle length were significantly correlated: partial r(158) = 0.19, P = 0.015. We examined the means to address whether high T might be present in women with short as well as long cycles, as both can be anomalous. However, higher T was associated with increased cycle length, and T was highest in women with reported cycles of 35 days or more: F(1,158) = 6.78, P = 0.010 (Fig. 1).

T and irregularity of menstrual cycle onset were significantly correlated: partial r(157) = 0.21, P = 0.010. ANCOVA revealed that T was significantly higher in those whose menstrual onsets varied by 7 days or more, or were completely unpredictable: F(1,157) = 6.69, P = 0.011 (Fig. 2).

We also conducted an ANCOVA to see whether T was associated with amenorrhea. Women with a history of missing menstrual periods (M = 26.69 pg/ml, SD = 13.59, n = 36) had significantly higher T than other women (M = 20.79, SD = 9.47, n = 124): F(1,157) = 8.23, P = 0.005. This phenomenon was most pronounced in women who had missed periods recently. Those who reported missed periods in the last 12 months had significantly higher salivary T (M = 29.25 pg/ml, SD = 16.18, n = 22) than other women (M = 20.86 pg/ml, SD = 9.18, n = 141): F(1,160) = 11.54, P = 0.001.

To address the possibility that the overall correlation between T and cycle length might be reducible to the data on women with missed periods, we excluded women who reported missed periods in the past 12 months and reran the correlational analyses. Salivary T and menstrual cycle length remained significantly correlated: partial r(135) = 0.18, P = 0.033. The partial correlation between T and menstrual cycle irregularity approached significance: partial r(134) = 0.15, P = 0.076. Thus, the association between high T and menstrual irregularities exists even in women with regularly occurring periods.

In HC users, T was significantly and positively correlated with irregularity of menstrual onset, at r(21) = 0.71, P < 0.001, but not with menstrual cycle length: partial r(21) = −0.08, P = 0.704. HC users who had ever missed periods (M = 18.69 pg/ml, SD = 8.77, n = 5) had higher T than other HC users (M = 12.82 pg/ml, SD = 4.63, n = 19), but not significantly so: F(1,21) = 3.01, P = 0.097. Two women had had long periods of time without

Fig. 1. Mean salivary testosterone (pg/ml) and standard error bars by self-reported regularity of menstrual cycle onset. *Significant difference at z < 0.05.
menstruating in the past year, and their mean T was higher (25.79 pg/ml, SD = 11.67) than the other women (M = 12.55 pg/ml, SD = 4.46). Thus, HC users demonstrated the same pattern of high T-menstrual irregularities as non-HC users.

To see how HC use was associated with T levels, we conducted an ANCOVA. Nonusers (M = 22.06 pg/ml, SD = 10.68) had significantly higher T than HC-users (M = 13.57, SD = 6.05): F(1,188) = 15.25, P < 0.001, presumably secondary to HC-induced suppression of gonadotropin secretion.

**DISCUSSION**

The present study examined the association between androgen levels (salivary T) and menstrual irregularities in a sample of healthy women. Women with longer cycles and more irregular menstrual onset had higher T, even when analyses included only regularly cycling women. Additionally, women with missed periods ever and in the past 12 months had higher circulating T. The data from this healthy sample accord with previous studies of clinical populations of adult women (Ayala et al., 1999), as well as nonclinical adolescent populations (Rodrigues Fernandez et al., 2005), but this is the first study (to our knowledge) to extend this to healthy adult women.

Surprisingly, high T was associated with menstrual irregularities, even in HC users. Since modern HCs are “low dose,” and Spellacy et al. (1980) found that lower estrogen did not strongly suppress gonadotropins, it may be that an extant high T-menstrual irregularity pattern is not greatly altered by HC use. One limitation of the present study is the use of self-report of past menstruation; the results could be strengthened through the use of longitudinal designs.

High levels of androgens are associated with a decreased likelihood of conception (Fukuda et al., 2003) and with increased risk of breast cancer in women (Micheli et al., 2004; Sturgeon et al., 2004; Kaaks et al., 2005). Thus, menstrual irregularity may be an important and easily detected indicator of underlying or potential physiological health problems. However, it is unlikely that many of the women participating in this study had undiagnosed neuroendocrine disorders (such as sub- or preclinical PCOS) that would explain the results, as participants self-reported good health, and there were no other potential markers of neuroendocrine disorders (e.g., higher weight). The functional significance of the association between T and menstrual cyclicity in normal healthy women thus awaits further study.

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**LITERATURE CITED**


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