Index-to-Ring Finger Length Ratio (2D:4D) Predicts Levels of Salivary Estradiol, But Not Progesterone, Over the Menstrual Cycle

MATTHEW H. MCINTYRE,* JUDITH FLYNN CHAPMAN, SUSAN F. LIPSON, AND PETER T. ELLISON
Department of Anthropology, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT We tested the association between the index-to-ring finger length ratio (2D:4D) and ovarian steroid hormone concentrations measured over the course of a menstrual cycle in the saliva of 38 young women. Estradiol levels were positively associated with right-hand, but not left-hand, 2D:4D, and also with the difference between right- and left-hand 2D:4D. None of these measures predicted progesterone level.

A prior study found a relationship between serum estradiol concentration in adults and the ratio of the second-to-fourth finger lengths (2D:4D), especially on the right hand (Manning et al., 1998). Estradiol concentrations in single serum samples from 58 men and 40 women were positively correlated with 2D:4D, which is also higher in women than in men.

In a related finding among 29 boys and girls, (Lutchmaya et al., 2004) found nonsignificant trends for amniotic fluid testosterone and estradiol to be related to right-hand 2D:4D, such that testosterone was associated with lower 2D:4D and estradiol with higher 2D:4D. They also found that the ratio of testosterone to estradiol in amniotic fluid significantly predicts right-hand 2D:4D (Lutchmaya et al., 2004).

On the basis of these and other findings, it has been proposed that 2D:4D, or perhaps only right-hand 2D:4D, is influenced by prenatal sex differentiation, especially the effects of androgens (Manning, 2002; Manning et al., 1998; McIntyre, 2006). A number of researchers have also proposed that the difference between right-hand and left-hand 2D:4D is an independent marker of prenatal sex differentiation (Benderlioglu and Nelson, 2004; Manning, 2002; Manning et al., 2000).

We studied the relationship of right- and left-hand 2D:4D, and the difference between them, with estradiol and progesterone concentrations in young women using first morning saliva samples collected over the course of one menstrual cycle.

METHODS

Participants and measures

The 38 women included in this study were part of a study of ovarian function, stress, and nutrition in young women. All participants were college students recruited in various on-campus settings. Their ages ranged from 18 to 23 (median = 20). None of the women had used oral contraceptives and all reported not having been pregnant about 6 months later. The project was approved by Harvard University’s committee on the use of human subjects.

Subjects were asked to collect saliva samples upon waking, for one complete menstrual cycle. Samples were collected directly into tubes pre-treated with sodium azide, a preservative, and were stored at room temperature until being returned to the laboratory (Lipson and Ellison, 1989). We assayed estradiol only in samples collected over the last 20 days of the menstrual cycle, to ensure the presence of detectable levels. For the same reason, we also assayed progesterone in samples collected only over the last 14 days of the cycle.

Saliva samples were assayed for estradiol and progesterone using previously described methods (Jasienska et al., 2004). Each saliva sample and standard was run in duplicate. The empirical sensitivity limits were 4 pmol/L for estradiol and 13 pmol/L for progesterone. For estradiol, inter-assay coefficients of variation were 16% for high-hormone-concentration pooled saliva controls, 22% for low-hormone-concentration pooled saliva controls, and 17% and 11% for the two manufacturer-supplied
controls (diluted 1:3). The intra-assay coefficient of variation was 10%. For progesterone, inter-assay coefficients of variation were 14% for high pools, 33% for low pools, and 23% and 8% for the two manufacturer-supplied controls (diluted 1:25 and 1:50, respectively). The intra-assay coefficient of variation was 9%.

We made photocopies of the right and left hands of 38 women about 6 months after the studied menstrual cycle. Finger lengths were measured from the photocopies (to a resolution of 0.5 mm), following established protocols (Manning, 2002), from proximal flexion crease to tip.

**Statistical methods**

To evaluate overall associations between estradiol and progesterone and digit ratios, we first used average measures of hormone levels. We took the averages of raw and logged, nonmissing values of estradiol and progesterone because the distributions of each were right skewed. To control for cycle phase effects we further analyzed the data using linear mixed modeling. Mixed modeling allows for separate regression modeling of random (in this case, within-subjects) and fixed (in this case, between-subjects) effects. In all linear mixed models reported below, the dependent variable is the natural log of the hormone level. We modeled the effect of cycle phase by grouping reverse cycle days into four groups (n0 through n16, n15 through n11, n10 through n6, and n5 through n1). Models included three indicator variables (1 if the hormone sample comes from that part of the cycle, or 0 if it does not) for the last three parts of the cycle, with the first part serving as the reference group. Estimates for these terms reflect within-woman differences in hormone level between that group and the reference group (i.e., n20 through n16).

**RESULTS**

Mean right-hand 2D:4D was 0.971 (SD = 0.032) and mean left-hand 2D:4D was 0.972 (SD = 0.032). Right- and left-hand 2D:4D were correlated (r = 0.65, P < 0.01). Correlations of digit ratios with measures of average steroid levels are shown in Table 1. We observed significant positive correlations of both right-hand 2D:4D and the difference between right- and left-hand 2D:4D with average estradiol and average log estradiol levels.

Simple linear mixed models showed similar effects. Fixed effects models including only right-hand 2D:4D also showed a significant positive association with estradiol (P = 0.03), but not with progesterone concentration (P = 0.98). Models with only left-hand 2D:4D showed no association with either estradiol (P = 0.91) or progesterone concentration (P = 0.36). The difference between right- and left-hand 2D:4D were also positively associated with estradiol (P < 0.01), but not with progesterone concentration (P = 0.26).

A model with indicator variables for reverse cycle day and interactions with 2D:4D suggested that the effect of right-hand 2D:4D on estradiol level is strongest early and late in the cycle, i.e., in trough rather than peak periods of estradiol production. However, the heterogeneity in effects by period was not significant (P for interaction = 0.06). Figure 1 compares the mean estradiol concentration (transformed from the log scale) predicted by 2D:4D values of 0.9 and 1.0, for each reverse cycle day group.

**DISCUSSION**

We found a positive relationship between right-hand, but not left-hand, 2D:4D and levels

---

**TABLE 1. Correlations between average ovarian steroid measures over one menstrual cycle and measures of finger length ratio**

<table>
<thead>
<tr>
<th></th>
<th>Right-hand 2D:4D</th>
<th>Left-hand 2D:4D</th>
<th>Right-left 2D:4D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average estradiol</td>
<td>r (P)</td>
<td>0.33 (0.04)</td>
<td>−0.03 (0.88)</td>
</tr>
<tr>
<td>Average Ln(estradiol)</td>
<td>r (P)</td>
<td>0.36 (0.02)</td>
<td>−0.02 (0.91)</td>
</tr>
<tr>
<td>Average progesterone</td>
<td>r (P)</td>
<td>0.03 (0.84)</td>
<td>−0.13 (0.43)</td>
</tr>
<tr>
<td>Average Ln(progesterone)</td>
<td>r (P)</td>
<td>&lt;0.01 (&gt;0.99)</td>
<td>−0.15 (0.36)</td>
</tr>
</tbody>
</table>

---

Fig. 1. Salivary estradiol concentrations predicted at two values of 2D:4D.
of salivary estradiol measured serially over the course of one menstrual cycle. Effects were stable over the course of the cycle. These findings support previous results based on estradiol levels obtained from single blood samples (Manning et al., 1998). We also found a strong positive association between estradiol levels and the difference between right-hand and left-hand 2D:4D. We failed to detect an association between 2D:4D and salivary progesterone levels.

The association we observed between estradiol levels and the difference between the right and left sides encourages further study of the role of body side in the interpretation of digit ratios as markers. In particular, we found a similar pattern of association of digit ratios with estradiol levels in women as was previously demonstrated with androgen receptor CAG repeat length. Shorter CAG repeats in the androgen receptor are associated with greater androgen receptor activity, and also with lower right-hand 2D:4D, but not left-hand 2D:4D (Manning et al., 2003). Moreover, the difference between right and left-hand 2D:4D is even more strongly associated shorter CAG repeat than right-hand 2D:4D alone (Manning et al., 2003). Many masculine traits appear to be expressed more strongly on the right side of the body (Manning, 2002; Mittwoch, 2001; Tanner, 1990). However, the causes of this phenomenon remain poorly understood.

Sex-specific physiology of the sexual endocrine system does not allow for generalization of our results beyond women. Furthermore, we did not independently assess ovulation during the menstrual cycles under study and cannot distinguish between gross effects on ovulatory failure and finer effects on estradiol production in ovulatory cycles. That said, these results contribute to an increasingly consistent literature showing important associations of both right-hand 2D:4D, and lateral differences in 2D:4D, with reproductive endocrine parameters in both men and women. These results further encourage the use of right-hand 2D:4D as a marker for perinatal androgen exposure and help to clarify the importance of body side.

**LITERATURE CITED**


