Original Research Article

Digit Ratio (2D:4D) Does Not Correlate with Daily 17β-Estradiol and Progesterone Concentrations in Healthy Women of Reproductive Age

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Objectives: Second-to-fourth digit ratio (2D:4D) is proposed as a proxy for the prenatal balance of sex hormones, is related to hormone-dependent characteristics in adult life, and is a possible predictor of disease later in life. Here, we studied the relationship between 2D:4D and ovarian steroid hormones (17β-estradiol and progesterone) among women of reproductive age.

Methods: From 186 healthy premenopausal women, aged 24–37 years, we collected saliva samples daily during the entire menstrual cycle. Data on reproductive and lifestyle characteristics were collected via questionnaires, and anthropometric measurements were performed.

Results: No statistically significant relationships were detected between adult women's sex hormone concentrations (17β-estradiol and progesterone) during the menstrual cycle and 2D:4D, in either left or right hand, when controlling for size at birth, body mass index, and physical activity.

Conclusions: This study shows, for the first time in a large sample of women of reproductive age, that 2D:4D is not a predictor of adult women's sex hormone concentration. The lack of relationship may be because 2D:4D might be genetically determined and is not related to maternal nutritional environment during fetal development. These results support the hypothesis that, in contrast to the nutritional quality of the fetal environment, the fetal hormonal environment (reflected by 2D:4D) does not determine reproductive physiology in later life. Am. J. Hum. Biol. 00:000–000, 2015.

The prenatal balance of sex hormones, reflected in the second-to-fourth digit ratio (2D:4D), might have a multilateral long-term effect on the human biological condition and might affect adult disease risk (Manning et al., 2014; Muller et al., 2011). It has also been suggested that 2D:4D is related to sex hormone concentrations during adulthood (Manning et al., 1998, 2014) and can be considered a marker of the relationship between prenatal and postnatal circulating sex hormone levels. We have previously shown that low (more maculine) right-hand 2D:4D is related to a higher testosterone concentration among healthy Polish men (Klimek et al., 2014). A similar relationship was reported for men referred for prostate biopsy and for patients of infertility clinics (Garcia-Cruz et al., 2012; Manning et al., 2004).

Among women, a majority of studies have found a link between digit ratio and testosterone levels (Kempel et al., 2005; van Anders and Hampson, 2005), whereas only a few studies have investigated estrogen and/or progesterone (P) levels among premenopausal woman (Hönekopp et al., 2007; Manning et al., 1998; McIntyre et al., 2007). However, only one study has so far measured hormone concentrations in samples collected throughout a full menstrual cycle among women of reproductive age (McIntyre et al., 2007). In a small group of 38 women, McIntyre et al. documented a positive association between right-hand 2D:4D and estradiol, but no association with P. In all other studies of premenopausal women, hormone levels were measured in blood or saliva samples that were collected only once during the menstrual cycle (Hönekopp et al., 2007; Manning et al., 1998). Similarly, only two of the aforementioned studies analyzed P (Hönekopp et al., 2007; McIntyre et al., 2007) and did not confirm any relationship between 2D:4D and P concentration in premenopausal healthy women. Other studies did not find a significant relationship between 2D:4D and adult levels of steroid hormones (Muller et al., 2011), including a large meta-analytic review of 332 female and 850 male participants (Hönekopp et al., 2007).

Hormonal studies in premenopausal women are methodologically challenging because of intracycle variation in hormone levels (Jasienska and Jasienski, 2008). A single sample does not provide a reliable estimate of cycle hormone levels: daily collection is preferable, or at least several samples taken throughout the follicular and luteal phases of the cycle, in order to precisely characterize average hormone levels during the cycle (Jasienska and Jasienski, 2008).

Our previous studies among Polish women have shown that ponderal index (PI; calculated as birth weight/birth length^3), an indicator of fetal nutritional status and thus

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TABLE 1. Characteristics of study participants

Characteristics	Mean	SD	Median	Range	*n*
Age (years)	29.5	3.31	29.0	24.0–37.0	185
Ponderal index (kg/m^3)	21.2	3.41	21.1	9.9–31.9	144
Body mass index (kg/m^2)	22.9	3.56	22.5	17.2–38.0	185
Mean physical activity (metabolic equivalent of task (hour/day))	5.4	3.64	4.6	0.4–20.2	154
Right 2D:4D	1.00	0.035	1.00	0.92–1.11	183
Left 2D:4D	0.99	0.035	0.98	0.91–1.11	183
Estrogen					
Mean E2 (pmol/l)	18.8	9.67	17.1	5.6–65.4	174
Mean follicular E2 (pmol/l)	19.3	10.84	16.9	5.6–92.1	172
Mean midcycle E2 (pmol/l)	22.3	11.76	19.6	6.2–74.4	174
Mean luteal E2 (pmol/l)	18.3	9.59	17.1	4.1–59.9	174
Progesterone					
Mean luteal P (pmol/l)	128.2	61.19	117.3	20.1–368.6	185
Mean midluteal P (pmol/l)	162.9	83.44	148.3	22.6–410.0	185

a marker of prenatal environment, was positively related to 17β-estradiol (E2) levels, measured daily for the entire menstrual cycle (Jasienska et al., 2006c). Women with the highest values of PI had the highest average concentration of E2. It was also documented that girls born small for gestational age had a reduced rate of ovulation and small ovaries (Ibanez et al., 2000, 2002). Furthermore, we investigated the relationship between fluctuating asymmetry (a marker of developmental stability) and concentration of E2 (Jasienska et al., 2006a). Women with a high degree of symmetry had a higher average concentration of E2 during the entire menstrual cycle. These results suggest that prenatal environment may influence adult female reproductive function. It is not clear, however, whether there are traits that can be measured during postnatal life that are reliable markers of prenatal conditions. Further, it is not clear whether these markers of prenatal conditions can predict adult health and the reproductive characteristics of an individual.

2D:4D is established around the 13th–14th week of gestation (Putz et al., 2004) and is stable during the postnatal period (Manning et al., 1998). 2D:4D has been shown to correlate with sex hormone concentrations among both men and women in different age groups (Lutchmaya et al., 2004; Manning et al., 1998) and several hormone-related traits, for example, risk of breast cancer (Muller et al., 2012), prostate cancer (Garcia-Cruz et al., 2012; Manning et al., 2004; Rahman et al., 2011), cervical intraepithelial neoplasia (associated with higher risk of cervical cancer) (Brabin et al., 2008), autism (for a review see: Hönekopp, 2013) and congenital adrenal hyperplasia (Brown et al., 2002; Okten et al., 2002).

In this study, we examined the relationship between left- and right-hand 2D:4D and adult concentrations of E2 and P during an entire menstrual cycle among 186 healthy women of reproductive age. This is the first study to investigate relationships between a marker of prenatal concentrations of estrogens and androgens and reliably measured ovarian hormones in a large sample of women of reproductive age. This study replicates the results of previous studies, but in a more comprehensive way, as we analyzed a large group of women and their hormone levels during the entire menstrual cycle.

MATERIALS AND METHODS

Participants and study design

The study was conducted between 2001 and 2003 in Southern Poland. The participants were 186 women, aged 24–37 years (mean = 29.5 years; standard deviation (SD) 3.31). According to criteria for inclusion to the study, none of the women had gynecological problems, hormonal disorders, or had used oral contraceptives or steroid medication for at least 6 months before participating. All women had regular menstrual cycles (between 21 and 36 days) for at least 6 months before participating in the study, had their last pregnancy at least a year before and breastfed at least 6 months before participating in the study. Descriptive statistics of the study group is presented in Table 1. The research protocol was approved by the Jagiellonian University Bioethical Committee.

Salivary steroid measures

Morning saliva samples were individually collected by participants beginning on the first day of menstruation and then for each day throughout the entire menstrual cycle. Packages containing plastic vials and laboratory-tested chewing gum were distributed to women before the beginning of their menstrual cycle. During one complete menstrual cycle, every day in the morning after waking up, women collected saliva samples in plastic tubes pretreated with sodium azide following published protocols (Lipson and Ellison, 1989). After collection, samples were stored in a refrigerator until the date of shipment to the Laboratory of Reproductive Ecology, Harvard University. At the laboratory, all samples were frozen in −80°C and thawed at the time of analysis. Only 5.3% of daily samples were missing because of incomplete collection or loss during laboratory procedures (Jasienska et al., 2006d). Radioimmunoassay analysis of E2 and P concentrations in saliva samples were conducted according to standardized methodology (described in Jasienska et al., 2004, 2006d). For analysis of E2 concentrations, an I-125-based RIA kit (#39100, Diagnostic Systems Laboratories, Webster, TX) was used, with published modifications to the manufacturer's protocol. Saliva samples from 20 days (−5 to −24 reverse cycle days) were analyzed for E2 concentration. Average intra-assay variability was 9%, and interassay variability varied from 23% for lower (15 pmol/l) to 13% for higher (50 pmol/l) values. The assay sensitivity was 4 pmol/l (Jasienska et al., 2004). For radioimmunoassay analysis of P concentration, saliva samples from 14 days (reverse cycle day −1 to −14) of each cycle were analyzed. P measurements were conducted using an I-125 based radioimmunoassay kit (#3400, Diagnostic Systems Laboratories) with the following modifications: standards were prepared in assay buffer and run at six concentrations

TABLE 2. Relationships between digit ratio 2D:4D and hormone indices tested by multiple regression analyses (with selected covariates included in the models)

	Right 2D:4D			Left 2D:4D		
	β	95% CI	P	β	95% CI	P
Mean E2	0.17	−1.98 to 2.33	0.88	−0.54	−2.77 to 1.68	0.63
Mean follicular E2	−0.21	−2.39 to 1.96	0.85	−0.42	−2.67 to 1.83	0.72
Mean midcycle E2	0.60	−1.66 to 2.87	0.60	0.17	−2.18 to 2.51	0.89
Mean luteal E2	0.61	−1.70 to 2.92	0.61	−0.47	−2.86 to 1.92	0.70
Mean luteal P	−0.29	−2.53 to 1.94	0.80	−0.27	−2.52 to 1.99	0.82
Mean midluteal P	−0.44	−2.86 to 1.98	0.72	−0.77	−3.20 to 1.67	0.54
Controlled for ponderal index						
Mean E2	−0.49	−2.93 to 1.95	0.70	−1.10	−3.53 to 1.32	0.37
Mean follicular E2	−1.08	−3.61 to 1.45	0.41	−1.06	−3.59 to 1.47	0.41
Mean midcycle E2	−0.47	−3.00 to 2.06	0.72	−0.61	−3.13 to 1.91	0.64
Mean luteal E2	0.23	−2.30 to 2.77	0.86	−0.82	−3.35 to 1.70	0.52
Mean luteal P	0.16	−2.50 to 2.81	0.91	−0.73	−3.33 to 1.87	0.58
Mean midluteal P	<0.01	−2.89 to 2.89	1.00	−1.18	−4.00 to 1.63	0.41
Controlled for body mass index						
Mean E2	0.18	−1.98 to 2.34	0.87	−0.54	−2.77 to 1.70	0.64
Mean follicular E2	−0.21	−2.39 to 1.97	0.85	−0.40	−2.67 to 1.86	0.73
Mean midcycle E2	0.59	−1.68 to 2.86	0.61	0.13	−2.22 to 2.48	0.91
Mean luteal E2	0.61	−1.71 to 2.93	0.61	−0.48	−2.88 to 1.92	0.70
Mean luteal P	−0.36	−2.58 to 1.86	0.75	−0.37	−2.60 to 1.87	0.75
Mean midluteal P	−0.51	−2.91 to 1.90	0.68	−0.87	−3.29 to 1.56	0.48
Controlled for physical activity						
Mean E2	−0.01	−0.03 to 0.02	0.57	−0.49	−3.14 to 2.16	0.72
Mean follicular E2	0.29	−2.44 to 3.03	0.83	−0.32	−2.98 to 2.35	0.82
Mean midcycle E2	1.08	−1.74 to 3.91	0.45	0.52	−2.23 to 3.26	0.71
Mean luteal E2	−0.01	−0.04 to 0.02	0.45	−0.48	−3.34 to 2.38	0.74
Mean luteal P	−0.83	−3.46 to 1.79	0.53	−0.67	−3.16 to 1.83	0.60
Mean midluteal P	−1.12	−4.00 to 1.76	0.45	−1.22	−3.95 to 1.51	0.38
Controlled for physical activity and ponderal index						
Mean E2	0.10	−3.16 to 3.37	0.95	−0.99	−3.95 to 1.96	0.51
Mean follicular E2	−0.40	−3.77 to 2.97	0.82	−0.97	−4.05 to 2.10	0.54
Mean midcycle E2	0.21	−3.11 to 3.53	0.90	−0.18	−3.19 to 2.83	0.91
Mean luteal E2	0.75	−2.67 to 4.17	0.67	−0.67	−3.77 to 2.44	0.67
Mean luteal P	0.15	−3.14 to 3.44	0.93	−0.87	−3.82 to 2.08	0.57
Mean midluteal P	−0.33	−3.97 to 3.30	0.86	−1.47	−4.72 to 1.79	0.38

from 2 to 200 pg/ml. Samples were added in 100 μl amounts together with 100 μl of assay buffer. Antibody was diluted in the ratio of 1:4. Antibody and labeled steroid were added to each tube in 100 μl amounts to yield a total reaction volume of 400 μl per tube. After overnight incubation at 4°C, 500 μl of second antibody was added to each reaction tube. Reaction tubes were subsequently centrifuged for 45 min; after aspiration of the supernatant, tubes were counted in a gamma counter for 2 min. Prior to statistical analyses, cycles were aligned on the basis of identification of the midcycle drop day (Day 0), which provides a reasonable estimate of the day of ovulation (Lipson and Ellison, 1996). Reliable identification of the day of the midcycle E2 drop could not be performed for 12 participants, and these women were excluded from E2 analyses. Values of E2 concentration from 18 consecutive days were used in the analysis, and the following E2 indices were calculated: (i) "mean E2" (mean of all 18 days); (ii) "mean midcycle E2" (mean of days −2 to +2); (iii) "mean follicular E2" (mean of days −9 to −1); and (iv) "mean luteal E2" (mean of days 0 to +8) (Jasienska et al., 2006d). P concentrations were measured in the luteal phase, defined as the last 14 days (reverse cycle days −1 to −14) of each cycle. Based on the daily values, two P indices were calculated: (v) "mean luteal P" (mean of last 14 days of the luteal phase); and (vi) "mean midluteal P" (mean of reverse cycle days −5 to −9, representing days with the highest P concentration). One woman was excluded from the analysis because of a missing sample.

General and physical activity questionnaires and anthropometric measurements

A general questionnaire and medical health records were used to collect data on participants' age, size at birth, and menstrual cycle characteristics. Women were asked to write down each day the number of hours spent sleeping, wake-up time, and time spent during the day on physical activities in five defined categories during the entire menstrual cycle. Metabolic equivalent of task was calculated for each participant. A detailed description of the methods for the assessment of physical activity has been published previously (Jasienska et al., 2006d).

Birth weight and length were used to calculate PI (birth weight/length^3). We measured body mass (to the nearest 0.1 kg) and body height (to the nearest 0.01 cm) for all women, and calculated their body mass index (BMI). Finger lengths were measured directly on the ventral surface of the palm using a manual caliper by a trained assistant. The measurements of second and fourth finger length in both hands were taken from the proximal finger crease to the distal tip of the finger, according to previously published procedures (i.e., Manning et al., 1998), but to the nearest 0.1 cm. The length of index finger was divided by length of ring finger in both hands to calculate right- and left-hand 2D:4D. All anthropometric measurements were performed by the same trained anthropologist. Three women with finger injuries were excluded from the study.

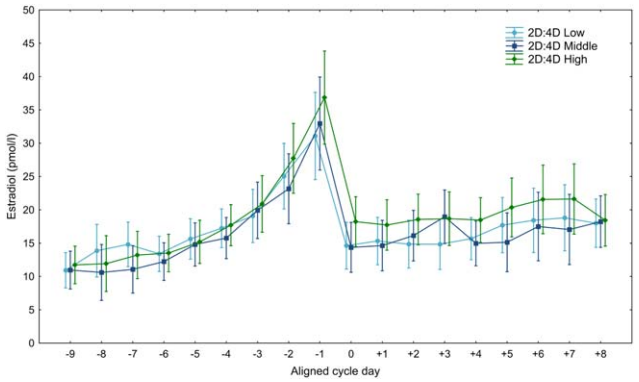

Fig. 1. Profiles of estradiol (pmol/l) during cycle days −9 to +8 for women that differ in digit ratio (group division based on tertiles of right-hand 2D:4D).

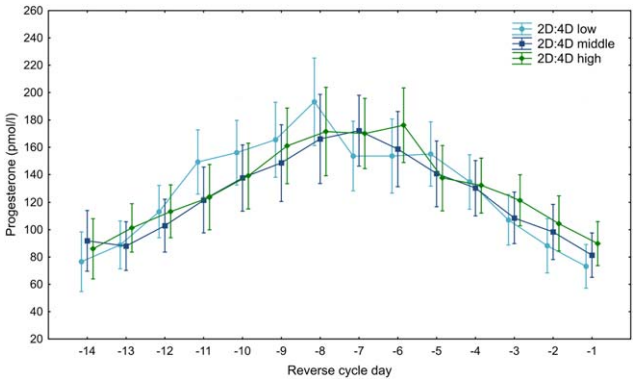

Fig. 2. Profiles of progesterone (pmol/l) during the luteal phase of the cycle for women that differ in digit ratio (group division based on tertiles of right-hand 2D:4D).

Statistical analyses

Hormonal data from 171 women were included in the analyses of E2 indices, and data from 182 women in the analyses of P indices. Firstly, the main analyses were performed with 2D:4D as a continuous variable (following published methods, i.e., Hönekopp et al., 2007; Manning et al., 1998; McIntrye et al., 2007). Relationships between 2D:4D and mean E2 and P indices were tested using a univariate linear regression model and repeated in multivariate linear regression models with PI, BMI, and physical activity included as potential confounders.

Secondly, 2D:4D ratios were examined as categorized variables as has been done in many previous studies (i.e., Hussain et al., 2014; Klimek et al., 2014; Tamiya et al., 2012). This was done in order to allow for comparison with previous results, even though this categorization of 2D:4D has previously been criticized by a number of researchers (i.e., Fitzsimons, 2008; MacCallum et al., 2002). Women were divided into three groups based on hand-specific tertiles of 2D:4D, in which the first tertile had the lowest 2D:4D ($\leq$0.983 for the right hand and $\leq$0.970 for the left hand) and the third tertile had the highest 2D:4D ($>$1.013 for the right hand and $>$1.000 for the left hand). Differences among tertiles of 2D:4D in E2 levels for aligned cycle days −9 to +8 and P levels in reverse cycle days −1 to −14 were analyzed by repeated-measures two-way analysis of variance (ANOVA). Classification as low 2D:4D, middle 2D:4D, or high 2D:4D was used as one factor, and day of the menstrual cycle was used as the second factor (with 18 levels for E2 and 14 levels for P). Additionally, differences among groups of women with low 2D:4D, middle 2D:4D, and high 2D:4D in E2 and P indices were analyzed by a one-way ANOVA. Moreover, trend analyses (considering linear component) were performed. In the next step, PI, BMI, and physical activity separately, and PI and physical activity simultaneously in a single model, were included as covariates in covariance analyses. No analysis of the association between 2D:4D and hormonal indices, when controlling for PI, BMI, and physical activity simultaneously, were performed because of a strong, significant relationship between BMI and physical activity ($\beta = 0.22$, $t = 2.71$, $P = 0.006$). Mean right-hand 2D:4D was 0.996 (SD 0.035) and mean left-hand 2D:4D was 0.985 (SD 0.035; see Table 1).

All statistical analyses were performed separately for the left- and right-hand digit ratio. Positive skewness was observed among all hormonal indices: as a result, they were log-transformed in order to normalize the distribution. Statistical analyses were conducted in Statistica package version 9.0.

RESULTS

No statistically significant relationships were observed between either left- or right-hand 2D:4D digit ratio and hormonal indices of E2 (mean E2, mean midcycle E2, mean follicular E2, and mean luteal E2) and P (mean luteal P and mean midluteal P), either in univariate linear regressions (*P*-values ranging from 0.54 to 0.88 for different hormonal indices) or in multivariate regression, when PI, BMI, and mean physical activity (metabolic equivalent of task (hour/day)) were included in the models separately, or in a model where PI was included together with mean physical activity (*P*-values ranging from 0.38 to 0.99 for different hormonal indices; Table 2).

The lack of any significant relationship between 2D:4D digit ratios and E2 concentrations was further confirmed by the repeated measures two-way ANOVA that did not show any significant differences in E2 concentrations among the tertiles of 2D:4D ratios ($F_{(34,\ 1,547)} = 0.85$, $P = 0.72$ for the right hand and $F_{(34,\ 1,547)} = 1.37$, $P = 0.08$ for the left hand; Fig. 1). Moreover, no statistically significant relationships were observed in the analysis on P concentrations ($F_{(26,\ 2,301)} = 1.18$, $P = 0.25$ for the right hand and $F_{(26,\ 2,301)} = 1.04$, $P = 0.41$ for the left hand; Fig. 2). There were no significant differences in E2 and P indices between groups of women with low, moderate, and high 2D:4D (Table 3). These results remained statistically nonsignificant when PI, BMI, and mean physical activity were included separately as covariates in covariance analyses (*P*-values ranging from 0.17 to 0.97 for different hormonal indices; Table 4).

DISCUSSION

2D:4D has been previously reported to correlate with sex hormone concentrations (Hönekopp et al., 2007; Manning et al., 1998; McIntyre et al., 2007), but in our study, we did not observe any statistically significant relationship between adult women's sex hormone concentrations

TABLE 3. Hormone indices among groups of women divided into tertiles of 2D:4D

	Right 2D:4D							Left 2D:4D						
	Low 2D:4D (n = 61), mean	Middle 2D:4D (n = 60), mean	High 2D:4D (n = 61), mean	F	P	F^a	P^a	Low 2D:4D (n = 61), mean	Middle 2D:4D (n = 76), mean	High 2D:4D (n = 45), mean	F	P	F^a	P^a
Mean E2	16.1	17.2	17.0	0.33	0.72	0.36	0.55	16.8	16.5	17.1	0.08	0.93	0.04	0.84
Mean follicular E2	16.6	17.6	17.0	0.19	0.83	0.07	0.79	17.1	16.9	17.3	0.04	0.97	0.03	0.87
Mean midcycle E2	18.2	20.6	20.3	0.97	0.38	1.27	0.26	18.9	20.1	20.0	0.24	0.79	0.28	0.60
Mean luteal E2	15.1	16.5	16.6	0.59	0.55	0.94	0.33	16.0	15.7	16.8	0.20	0.82	0.19	0.66
Mean luteal P	112.5	111.1	113.8	0.03	0.97	0.01	0.91	107.2	113.2	118.7	0.46	0.63	0.91	0.34
Mean midluteal P	139.7	139.4	140.5	<0.01	0.99	<0.01	0.96	137.2	138.7	145.6	0.14	0.87	0.26	0.61

Means were derived from calculations on log-transformed values and then back-transformed by taking the antilog.
[a]For trend.

TABLE 4. Analysis of covariance of digit ratio and hormone indices when selected covariates are included in the models

	Right 2D:4D		Left 2D:4D	
	F	P	F	P
Controlled for ponderal index				
Mean E2	0.15	0.85	0.21	0.81
Mean follicular E2	0.28	0.76	0.18	0.84
Mean midcycle E2	0.17	0.84	0.17	0.84
Mean luteal E2	0.06	0.95	0.17	0.85
Mean luteal P	1.07	0.34	0.10	0.91
Mean midluteal P	0.90	0.41	0.17	0.85
Controlled for body mass index				
Mean E2	0.64	0.53	0.20	0.82
Mean follicular E2	1.79	0.17	0.72	0.49
Mean midcycle E2	0.02	0.36	0.04	0.97
Mean luteal E2	0.54	0.59	0.04	0.96
Mean luteal P	0.49	0.62	0.95	0.39
Mean midluteal P	1.19	0.31	1.25	0.29
Controlled for physical activity				
Mean E2	0.42	0.66	0.19	0.82
Mean follicular E2	0.64	0.53	0.19	0.83
Mean midcycle E2	0.37	0.69	0.77	0.46
Mean luteal E2	0.24	0.79	0.20	0.82
Mean luteal P	0.37	0.69	0.25	0.78
Mean midluteal P	0.34	0.71	0.16	0.85
Controlled for physical activity and ponderal index				
Mean E2	0.38	0.69	0.71	0.50
Mean follicular E2	0.74	0.48	0.85	0.43
Mean midcycle E2	0.24	0.79	1.43	0.25
Mean luteal E2	0.27	0.77	0.55	0.58
Mean luteal P	0.71	0.49	0.65	0.52
Mean midluteal P	1.68	0.19	0.30	0.75

during the menstrual cycle and digit ratio 2D:4D in either left or right hand. Although other indicators of fetal environment, such as size at birth and fluctuating asymmetry, have been shown to predict levels of reproductive steroid hormones in adult women (Jasienska et al., 2006a,c), there is a fundamental difference between these indicators and 2D:4D. Size at birth is an indicator of maternal condition during fetal development, mostly driven by availability of energy (Bateson et al., 2004). Fluctuating asymmetry is an indicator of developmental stability (Møller and Swaddle, 1997) and, thus, also to a significant degree, reflects the quality of the fetal environment that, in turn, depends on maternal condition. Fetal developmental conditions are known to influence the future condition of individuals, because the quality of maternal environment exerts permanent changes to fetal physiology. According to the predictive adaptive response hypothesis, a fetus adjusts its developmental trajectory in response to in utero conditions, modifying its physiology in a way that better prepares it for postnatal life (Gluckman et al., 2005; Jasienska, 2009, 2013). Female fetuses that develop in good nutritional conditions should have, as adults, reproductive functions operating at high levels (Jasienska, 2013). Prenatal conditions that are beneficial for fetal development indicate that the postnatal environment is likely to be rich in energy as well, especially if the maternal environment reflects information about environmental quality integrated over the few last generations (Jasienska et al., 2006d; Kuzawa et al., 2005; Wells, 2007). By contrast, female fetuses that develop in energy-poor fetal environments (as indicated by small size at birth or a high degree of fluctuating asymmetry) tend to exhibit a reproductive function better adjusted to postnatal life in an energy poor environment (Jasienska, 2013). For example, girls who are smaller at birth have smaller ovaries (Ibanez et al., 2000), and we have shown that women with low PI at birth have a higher sensitivity of ovarian response to the suppressing effects of physical activity (Jasienska et al., 2006b). Furthermore, women with a higher degree of fluctuating asymmetry have reduced levels of ovarian hormones in comparison with more symmetrical women, which also indicates that fetal developmental conditions may permanently influence reproductive physiology in women (Jasienska et al., 2006a).

However, it is unlikely that 2D:4D reflects maternal nutritional condition. For example, the Dutch Hunger Winter Families Study showed that 2D:4D was not affected even by extreme prenatal undernutrition during any period of gestation (Stein et al., 2010). Moreover, there is no direct association between mother's and fetus's testosterone serum levels (van de Beek et al., 2004), which suggests that maternal hormonal environment during pregnancy is not associated with fetus's 2D:4D (related to developmental androgen concentration) (but see Manning et al., 1999). 2D:4D's sensitivity to maternal testosterone was also previously suggested to be related to the X-linked androgen receptor gene (Manning et al., 2003), but this hypothesis has been criticized (Voracek and Dressler, 2009) and is not supported by a meta-analytic reviews (Hönekopp, 2013; Voracek, 2014). Voracek (2014) suggested that the 2D:4D value is not related to variants of the X-linked androgen receptor gene and proposed several others explanations, i.e., a lack of family correlation patterns indicating X-linked inheritance or a lack of greater intrasex 2D:4D variability among men. Moreover, two large genome-wide association studies also did not confirm any links between the androgen receptor gene and

the formation of 2D:4D (Lawrance-Owen et al., 2013; Medland et al., 2010). Other potential genetic influences on 2D:4D values are proposed in the literature, i.e., a relationship between 2D:4D and a single variant in the LIN28B gene in a large group of children among whom a genome-wide single nucleotide polymorphism test was performed (Medland et al., 2010). Another possible gene suggested to play a significant role in determining 2D:4D value is SMOC1, which is crucial in limb development (Lawrance-Owen et al., 2013).

Going further, it is likely that very high or very low levels of steroid hormones during fetal life may have an impact on the development and reproductive physiology of the individual. However, such an effect should be considered pathological rather than adaptive, and should be unlikely to occur in response to normal variation in steroid hormones during the prenatal period. Indeed, some previous studies have shown that 2D:4D was negatively related to several pathological hormone-dependent features, i.e., polycystic ovary syndrome (Cattrall et al., 2005) and congenital adrenal hyperplasia (Hönekopp and Watson, 2010). We argue that relationships between 2D:4D and hormonal-dependent disorders should not be generalized to healthy women in the wider population.

Links between prenatal and postnatal hormonal levels are more often observed among men than among women (Manning et al., 2014). For example, we have shown that more masculine 2D:4D is related to higher adult testosterone levels among adult men (Klimek et al., 2014), which is consistent with observations that 2D:4D might be more sensitive to fetal testosterone levels than to estrogen levels (Lutchmaya et al., 2004). It has also been suggested that 2D:4D is strongly heritable through male lines (Voracek and Dressler, 2009) and that the fetal hormonal environment may result from the action of sexually antagonistic genes (which exert their effects prenatally) (Manning et al., 2000). Fetal androgen concentration (reflected in 2D:4D) is associated with adult testosterone levels (i.e., Klimek et al., 2014; Manning et al., 2004) and several reproductive parameters, including sex drive, level of sexual excitement (Manning and Fink, 2008), number of sexual partners per individual (Hönekopp et al., 2006), and age at first marriage (Sorokowski et al., 2012). These traits, related to 2D:4D, might lead to higher reproductive success.

Additionally, developmental androgen exposure may be related to brain development (Geschwind and Galaburda, 1985; Kimura, 1999; Manning et al., 1998) and cause a permanent organizational effect on reproduction-related characteristics of adults (i.e., mating choice and sex drive) (Fisher et al., 2006), which may be beneficial for reproduction. Indeed, several studies (i.e., Manning et al., 2000, 2003; Manning and Fink, 2008), including ours (Klimek et al., 2014), have shown that masculine 2D:4D is related to having a higher number of children among men.

Our findings do not confirm results from previously published studies. However, these previous studies had substantial limitations because of single hormone measurements (Hönekopp et al., 2007; Manning et al., 1998) or small sample sizes (McIntrye et al., 2007). The main advantages of our study are reliable measurements of hormone levels, a relatively large number of participants, and the incorporation of additional factors that are known to influence hormone levels (PI, BMI, and physical activity) as potential confounders in the analyses. In our study, hormones were measured in samples collected during an entire menstrual cycle, taking into account intracycle variation, which has constituted a major methodological problem in many other hormonal studies. This approach allowed us to calculate several hormonal indices and to follow hormonal changes during the cycle. Moreover, the analysis of hormonal concentration and 2D:4D were performed by two different statistical methods, with 2D:4D as a continuous variable and a categorized (into tertiles) variable, following two different approaches used separately in the literature (i.e., Hussain et al., 2014; Manning et al., 1998; McIntrye et al., 2007; Tamiya et al., 2012).

Our study has some limitations. The study was based (in contrast to some other 2D:4D studies) on a single finger length measurement, performed by only one assistant. Our one measurement (performed by one person) did not allow us to calculate intraobserver or interobserver errors. Moreover, our measurements were collected directly from the fingers, which was found by some studies to give less accurate results than other methods, i.e., computer-assisted analysis (Allaway et al., 2009). Further studies should conduct multiple finger length measurements with use of computer-based techniques, and lead to development of standardized and validated methods of measurement (Allaway et al., 2009; Voracek et al., 2007). Our study, because of its methodology (home visits), did not allow us to use more accurate methods of measurement. It is also important to mention that finger lengths (and, in consequence, 2D:4D value) measured among premenopausal women might be influenced by the changes in hormone concentration across the menstrual cycle (Mayhew et al., 2007). However, Mayhew et al. based their observation on a very small sample, and so their study should be replicated to confirm these observations.

To summarize, we find that 2D:4D does not predict daily salivary E2 or P concentration among healthy, premenopausal women, even after controlling for other factors that are known to influence hormone levels. These results support the hypothesis that, in contrast to the quality of the nutritional fetal environment, the fetal hormonal environment (reflected by 2D:4D) does not determine women's reproductive physiology in later life.

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