Early Human Development 117 (2018) 68–73

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Digit ratio (2D:4D) and circulating testosterone, oestradiol, and progesterone levels across the menstrual cycle

Gareth Richards[a,*], Magdalena Klimek[b], Grazyna Jasienska[b], Urszula M. Marcinkowska[b]

[a] Autism Research Centre, Department of Psychiatry, University of Cambridge, UK
[b] Department of Environmental Health, Faculty of Health Sciences, Jagiellonian University Medical College, Poland

ARTICLE INFO

Keywords:
2D:4D
Digit ratio
Estradiol
Menstrual cycle
Progesterone
Testosterone

ABSTRACT

Background: Digit ratio (2D:4D) is used by researchers as an indicator of prenatal sex hormone exposure. Two previous studies have examined associations between 2D:4D and circulating sex steroid concentrations across the menstrual cycle in adult females. One reported that digit ratio correlated positively with oestradiol levels, whereas the other found no such effect; neither observed significant associations with progesterone.
Aims: To examine associations between 2D:4D, as well as asymmetry (i.e. right minus left 2D:4D), and circulating sex steroids across the menstrual cycle.
Study design: Correlational.
Subjects: 32 naturally cycling adult females from rural southern Poland.
Outcome measures: Salivary oestradiol, progesterone, testosterone, and testosterone to oestradiol ratio (T:O) measured during the follicular, peri-ovulatory, and luteal phases. Average levels across the cycle were also examined.
Results and conclusions: Asymmetry in digit ratio correlated positively with oestradiol at each phase, as well as with average levels across the cycle. Each association, other than that relating to average levels, remained statistically significant after a range of covariates had been controlled for. No other significant correlations were observed between digit ratio variables and circulating hormone levels. Our results might suggest that low exposure to androgens and/or high exposure to oestrogens during gestation is a predictor of high oestradiol levels in naturally cycling females of reproductive age. However, considering that it was asymmetry in digit ratio, and not either right or left 2D:4D, that was a significant predictor, it is also possible that these effects reflect more general associations between bilateral asymmetry and circulating oestradiol levels.

1. Introduction

Manning et al. [1] reported significant correlations between circulating sex steroids and 2D:4D in a sample of 131 (69 men, 62 women) participants attending an infertility clinic. Oestrogen was positively related to 2D:4D, and the relationship was independent of sex, age, height and weight. Although testosterone was not measured in women, it was negatively related to 2D:4D in men. All relationships were stronger for the right hand (R2D:4D) relative to the left (L2D:4D). Manning et al. [1] suggested that these correlations between 2D:4D and circulating sex steroids reflected echoes of causal associations between prenatal sex steroids and 2D:4D, and that R2D:4D was more sensitive to the effects of prenatal sex hormones. Later, the variable $D_{[R-L]}$ (R2D:4D–L2D:4D) was introduced to reflect this right-sided effect in the expression of 2D:4D (see Discussion of $D_{[R-L]}$ by Manning [2], p. 21–22). Manning [2] (p. 37–38, Fig. 2.8) found that high $D_{[R-L]}$ (i.e. relatively high L2D:4D compared to R2D:4D) was associated with high oestrogen levels in the same sample of men and women attending an infertility clinic reported on by Manning et al. [1], although the effect was no longer significant after controlling for sex.

The links between 2D:4D, prenatal testosterone and oestrogen and their receptors were confirmed in mice by Zheng & Cohn [3] (see also Manning [4]). Mice show a similar sex difference in 2D:4D as humans (males < females), and in their development the sexual dimorphism of R2D:4D appears earlier than the sex difference in L2D:4D [3]. In humans, the effect size for the sex difference in R2D:4D has been reported to be larger than that of L2D:4D [5]. The side difference in sensitivity to sex steroids has also been shown in studies of 46XY individuals with a female phenotype (i.e. complete androgen insensitivity syndrome [CAIS], a condition characterised by non-functional or absent androgen receptors due to genetic mutations in the androgen receptor gene). Such individuals have high “female-type” digit ratios in comparison to male

* Corresponding author at: Autism Research Centre, University of Cambridge, Douglas House, 18b Trumpington Road, Cambridge CB2 8AH, UK.
E-mail address: gvr22@cam.ac.uk (G. Richards).

https://doi.org/10.1016/j.earlhumdev.2017.12.006
Received 25 October 2017; Received in revised form 4 December 2017; Accepted 5 December 2017

norms, and the effect size for this is larger for R2D:4D than L2D:4D [6,7].

There is conflicting evidence for correlations between circulating sex steroids and 2D:4D. The original reports of links between circulating sex steroids and digit ratio variables [1,2,8] considered an unusual population (i.e. men and women attending infertility clinics), and so the findings may not be generalisable. Regarding normative samples of men and women, some studies have observed significant correlations between 2D:4D and circulating levels of sex steroids [9,10], whereas others have not [8,11] (see Hönekopp et al. [11] for a review). However, the position regarding $D_{[R-L]}$ and testosterone and oestrogen is different. Men who are subjected to challenge (e.g. physical exercise and/or aggressive encounters) show marked spikes in sex steroid levels. In such situations, $D_{[R-L]}$ has been reported to be a correlate of circulating levels of testosterone and oestrogen in men but not women [12,13] (see also, Manning et al. [14]).

In premenopausal women who have regular cycles, there may be correlations between $D_{[R-L]}$ and oestrogen levels across their cycle. McIntyre et al. [15] reported positive correlations between oestradiol and R2D:4D and $D_{[R-L]}$ in a sample of normally cycling women. Klimek et al. [16] did not replicate this finding for R2D:4D in a larger sample (and did not examine $D_{[R-L]}$). Thus, there remains the possibility that $D_{[R-L]}$ may be positively correlated with oestrogen across the cycle. The present study addresses this possibility and considers the relationship between $D_{[R-L]}$ (and 2D:4D) and salivary oestradiol, progesterone, testosterone, and testosterone to oestradiol ratio (T:O) measured during the follicular, peri-ovulatory, and luteal phases of the cycle.

2. Materials and methods

2.1. Participants

Thirty-two females aged 22–37 (M = 30.3, SD = 4.83) from the Mogielica Human Ecology Study Site in rural southern Poland [17] took part in the current research, none of whom had been pregnant, breastfeeding, or taking hormonal contraception for at least three months prior to the study. The majority (30, 93.8%) reported that they were in a relationship, and most (23, 71.9%) had been pregnant before. The number of pregnancies reported ranged from 0 to 4 (M = 1.38, SD = 1.1), and the women had between 0 and 3 children (M = 1.28, SD = 0.958). Age of menarche ranged from 10 to 17 (M = 13.31, SD = 1.6), and only 5 (15.6%) reported experiencing differences in the length of consecutive cycles larger than ± 5 days (range = 25–40 days, M = 29, SD = 3.47). The study was granted approval by the Jagiellonian University Bioethical Committee. All procedures were undertaken with the understanding and written consent of each subject, and in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Associations (Declaration of Helsinki).

2.2. Hormone measurements

Phases of the menstrual cycle were initially determined via self-reported ovulation strip tests, which were based on luteinizing hormone (LH) levels. These tests were performed from the 10th until the 20th days of the cycle or until a positive result was obtained. LH tests have been shown to be a highly accurate means of determining timing of ovulation [18]. Twenty-one (65.6%) participants had a positive LH test, indicating that ovulation had been successfully detected, although it is noted that one participant did not have a strong positive result (i.e. lines visible were not intense red, but pale red). For the other 10 participants, occurrence of ovulation was suggested by 17-β-oestradiol drop.

Per each participant, hormonal profiles of 17-β-oestradiol (O), progesterone (P) and testosterone (T) were created based on 15 daily measurements centred around the ovulation for O, the last 14 days of the cycle for P, and 20 days centred around ovulation for T. Participants individually collected saliva at least 30 min after eating, drinking or smoking and froze all samples immediately after collection. Hormonal measurements were conducted using commercially available hormonal assays of DRG International Incl.: Elisa plates SLV4188 for 17-β-oestradiol (sensitivity: 0.4 pg/ml, standard range: 1–100 pg/ml), SLV3140 for 17-α-hydroxy-progesterone (sensitivity: 2.5 pg/ml, standard range: 10–5000 pg/ml) and SLV3013 for testosterone (sensitivity: 1.9 pg/ml, standard range: 10–5000 pg/ml). To ensure high quality of the measurements, all hormonal assays were conducted in duplicates. Quality of measurements was then controlled for each plate separately by including (in duplicates) samples of known concentrations ("pools") of O, P, and T (in total these control measurements consisted of 19 pools per plate). Inter- and intra-assay coefficients of variability (CVs) were computed: for oestradiol, inter-assay CV was 10.01% and intra-assay was 7.5%; for progesterone, inter-assay CV was 14.1% and intra-assay was 4.9%; for testosterone, inter-assay CV was 6.2% and intra-assay CV was 1.3% [19].

The hormonal measurements reported here for the follicular, peri-ovulatory, and luteal phases are those collected during the meetings held for each of the three phases (i.e. on the same days on which hand scans were made). Averaged hormonal levels were counted based on all daily measurements made: for O it was ± 7 days around ovulation plus the ovulation day; for P it was the last 14 days of the cycle; for T it was ± 10 days around ovulation.

2.3. Digit ratio measurements

Hand scans were made at three times during one menstrual cycle (one each phase of the cycle). The visits were scheduled for the early follicular phase (i.e. between day 2 and day 8), at the peri-ovulatory phase (not later than 72 h after a positive LH test, or, if no positive test result was obtained, on day 20), and in the mid-luteal phase (approximately one week post-ovulation). The second and fourth digits for each hand were measured from the hand scans separately by two researchers who were blinded to the identity of the participants. Each researcher made two sets of measurements, several weeks apart. Inter- and intra-observer reliability of measurement were examined using intraclass correlation coefficients (*ICC*) with two-way mixed-effects and absolute agreement definition. *ICC* for Observer 1 varied between 0.94 and 0.99; *ICC* for Observer 2 varied between 0.90 and 0.98. The *ICC* between observers varied between 0.89 and 1.00.

Klimek et al. [20] have already reported the R2D:4D, L2D:4D, and $D_{[R-L]}$ values for this sample that were measured during the follicular, peri-ovulatory, and mid-luteal phases. As these values did not differ significantly between phases, average R2D:4D, L2D:4D, and $D_{[R-L]}$ values were created from these separate sets of measurements (note that hand scans were not made during the follicular phase for three participants; in these cases, average digit ratio values were computed from the measurements taken during the peri-ovulatory and luteal phases only). This resulted in mean values of 0.977 (SD = 0.022) for R2D:4D, 0.97 (SD = 0.026) for L2D:4D, and 0.007 (SD = 0.02) for $D_{[R-L]}$.

2.4. Statistical procedures

Of 16 hormone measures (i.e. follicular, peri-ovulatory, luteal, and average levels of oestradiol, progesterone, testosterone, and T:O), all but three (follicular oestradiol, peri-ovulatory testosterone, and average testosterone) were non-normally distributed, as index by Shapiro-Wilk test. To address this, and for ease of comparison, all hormone measures were transformed using natural logarithm (ln). R2D:4D, L2D:4D, and $D_{[R-L]}$ were all normally distributed, and were not transformed. However, one outlier was identified for $D_{[R-L]}$. This was a particularly low (i.e. masculinised) value. On closer inspection, the participant to which this score belonged also recorded outlying (high) values for