Early Human Development 91 (2015) 111–117

Contents lists available at ScienceDirect

Early Human Development

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

Adult digit ratio (2D:4D) is not related to umbilical cord androgen or estrogen concentrations, their ratios or net bioactivity

Lauren P. Hollier [a,b,*], Jeffrey A. Keelan [c], Esha S.L. Jamnadass [a,b], Murray T. Maybery [b], Martha Hickey [d,1], Andrew J.O. Whitehouse [a,1]

[a] Telethon Kids Institute, University of Western Australia, Australia
[b] Neurocognitive Development Unit, School of Psychology, University of Western Australia, Australia
[c] School of Women's and Infant's Health, University of Western Australia, Australia
[d] Department of Obstetrics and Gynaecology, University of Melbourne and the Royal Women's Hospital, Victoria, Australia

ARTICLE INFO

Article history:
Received 3 October 2014
Received in revised form 17 December 2014
Accepted 28 December 2014

Keywords:
Androgens
Estrogens
Digit ratio
2D:4D
Cord blood
Raine study

ABSTRACT

Background: Ratio of second digit length to fourth digit length (2D:4D) has been extensively used in human and experimental research as a marker of fetal sex steroid exposure. However, very few human studies have measured the direct relationship between fetal androgen or estrogen concentrations and digit ratio.
Aims: We investigated the relationships between both androgen and estrogen concentrations in umbilical cord blood and digit ratio in young adulthood. In addition we calculated measures of total serum androgen and total estrogen bioactivity and investigated their relationship to digit ratio.
Study design: Prospective cohort study.
Subjects: An unselected subset of the Western Australian Pregnancy Cohort (Raine) Study (159 female; 182 male).
Outcome measures: Cord serum samples were collected immediately after delivery. Samples were assayed for androgen (testosterone, Δ4-androstenedione, dehydroepiandrosterone) and estrogen (estrone, estradiol, estriol, estetrol) concentrations using liquid-chromatography mass-spectrometry. Digit ratio measurements were taken from hand photocopies at age 19–22 years.
Results: For both males and females, there were no significant correlations between digit ratio and any androgen or estrogen concentrations considered individually, the testosterone to estradiol ratio, total androgen bioactivity measure or ratio of androgen to estrogen bioactivity (all $p > .05$). In males, but not females, total estrogen bioactivity was negatively correlated with left hand digit ratio ($r = -.172$, $p = .02$), but this relationship was no longer significant when adjusted for variables known to affect sex steroid concentrations in cord blood.
Conclusions: Our findings indicate that digit ratio is not related to fetal androgens or estrogens at late gestation.

1. Introduction

Prenatal exposure to sex steroids has long been posited to influence human development [7,11]. However, the direct examination of sex steroid exposure has been limited due to inherent difficulties in obtaining biological samples during prenatal life. Currently there is no 'gold standard' approach to the measurement of fetal sex steroid concentrations [50]. Obtaining samples of circulating human fetal sex steroids during early and mid gestation would require invasive fetal sampling (such as cordocentesis), which confers significant risks to the pregnancy. Amniotic fluid samples provide an approximation of circulating fetal hormones in mid-gestation by measuring the sex steroids that have entered the amniotic fluid via fetal urination or diffusion through fetal skin [36]. A significant limitation of this approach is that amniocenteses are performed only in high-risk pregnancies and therefore research samples are typically small and unlikely to be representative of the broader population. Alternatively, umbilical cord blood reflects fetal sex steroid concentrations at late gestation and can be easily collected from uncomplicated pregnancies following delivery. Studies have demonstrated consistent sex-differences in sex steroid concentrations in cord blood [13,24,29,48], suggesting that this approach can be used to examine the relationship between early-life sex steroid exposure and human development.

Previous studies by our group and others have demonstrated significant associations between umbilical cord testosterone concentrations and human development [18]. Higher concentrations of cord serum testosterone were associated with reduced vocabulary in males at 2 and 5 years of age [10,19], increased risk of language delay in early

* Corresponding author at: School of Psychology, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia. Tel.: +61 8 6488 1300; fax: +61 8 6488 1006.
E-mail address: lauren.hollier@graduate.uwa.edu.au (L.P. Hollier).
[1] Joint senior authors.

http://dx.doi.org/10.1016/j.earlhumdev.2014.12.011
0378-3782/

childhood [55], reduced spatial ability in 6 year old females [23], and left hemisphere lateralisation of language in adult males [20]. Sex differences in behaviour have also been associated with cord testosterone concentrations. Jacklin et al. [22] reported that males with low cord serum testosterone concentrations were more timid. We have recently reported that higher cord testosterone concentrations are associated with behavioural problems in early childhood. Attention difficulties in males and withdrawal symptoms in females were both negatively related to cord blood testosterone levels [39]. Together, these data suggest that umbilical cord blood is a valid method for examining early-life sex steroid exposure.

Characteristically, males have a longer fourth digit relative to their second digit, while women have comparable second and fourth digit lengths [32]. A recent meta-analysis illustrated that although the sex difference is small, it is consistent across the published literature [21]. Differences in digit ratios compared to controls have been partially supported in clinical populations exposed to atypical levels of prenatal hormones. There is some evidence that individuals with congenital adrenal hyperplasia (CAH), where the fetus is exposed to supraphysiological levels of androgens, have a lower 2D:4D ratio (male pattern) than typically developing individuals [4,37]. However, the relationship was not consistently observed in both hands. In addition, a study conducted by Buck et al. [5] using radiographs found no significant difference in digit ratio between females with CAH and typically developing females. These findings cast doubt as to whether the 2D:4D ratio is a reliable proxy measure of fetal testosterone levels [38].

To date, only three relatively small prospective studies have investigated the relationship between fetal sex steroid exposure and digit ratio. Lutchmaya et al. [28] examined the association between testosterone and estradiol levels in amniotic fluid collected mid-gestation with digit ratio recorded at 2 years of age in 33 children (18 males, 15 females). A low 2D:4D ratio in the right hand was associated with high testosterone relative to estradiol levels. No significant relationship was found between digit ratio and testosterone or estradiol concentrations individually. These observations suggest that the 2D:4D ratio reflects the relative levels of prenatal androgens and estrogens.

Ventura et al. [51] further investigated the relationship between amniotic testosterone concentrations sampled during mid-gestation and digit ratio measured at birth in a sample of 106 children (54 females, 52 males). For females, but not males, amniotic testosterone levels were negatively related to the digit ratio of both hands. This finding provides further evidence that digit ratio may be related to sex steroid concentrations in utero. However, Ventura and colleagues did not measure estrogen levels, so it was not possible to examine whether the ratio of testosterone to estradiol levels was related to the digit ratio.

Hickey et al. [14] provided an initial investigation into the relationship between cord testosterone concentrations and the 2D:4D ratio in a subset of females from the Western Australian Pregnancy Cohort (Raine) Study. No statistically significant relationship was found between umbilical cord blood testosterone concentrations and the 2D:4D ratio recorded for the females at 14 to 16 years of age ($n = 82$) or between maternal testosterone concentrations at 18 ($n = 118$) or 34 weeks ($n = 114$) of gestation and digit ratio. These findings suggest that variations in 2D:4D in females are not related to fetal testosterone concentrations late in gestation. However, the findings from Lutchmaya et al. [28] suggest that it may be the ratio of androgen to estrogen concentrations that is related to digit ratio. Furthermore, both Hickey et al. [14] and Lutchmaya et al. [28] utilised radioimmunoassay (RIA) to analyse sex steroid concentrations. Increasing awareness of the limitations of RIA for the measurement of umbilical cord sex steroids has led to the adoption of mass spectrometry as the preferred approach [24]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) can be more sensitive than RIA [46]. Further, sex steroid measurements with LC–MS/MS are consistently lower than those derived by RIA, reflecting the superior specificity of the LC–MS/MS technique [9,18,26,45,46,52]. Although, Ventura et al. [51] utilised LC–MS/MS to measure testosterone concentrations amniotic fluid, they examined only the relationship between testosterone and digit ratio.

1.1. Present study

The aim of the current study was to investigate whether androgen and estrogen concentrations in cord blood, measured using LC–MS/MS, are related to digit ratios recorded in early adulthood for samples of both males and females from the Raine study. Most published studies of fetal sex steroid exposure have measured only the most biologically active sex steroids: testosterone, and estradiol, both of which are bound to sex hormone binding globulin (SHBG) which greatly attenuates their bioactivity. However, the human fetus is exposed to a number of other androgens and estrogens in the prenatal environment, some of which are not bound by SHBG and so exert significant bioactivity despite a relative lack of potency. Accordingly, in the present study we measured the adrenal androgens, Δ4-androstenedione and dehydroepiandrosterone, as well as estrone, estriol and estetrol. Using these data, combined with data on protein binding and relative potency, we derived total composite measures of bioavailable androgen and estrogen exposure. We were then able to test whether these variables or their ratio correlated with the 2D:4D ratios.

We predicted that testosterone concentrations and the testosterone to estradiol ratio would be negatively related to digit ratio, while estradiol levels would be positively related. Similarly, it was predicted that the androgen composite and the androgen to estrogen composite ratio would be negatively associated with digit ratio, while the estrogen composite would be positively related.

2. Method

2.1. Participants

Participants were from the Western Australian Pregnancy Cohort (Raine) Study (www.rainestudy.org.au). Between May 1989 and November 1991, 2900 unselected pregnant women were recruited from the public antenatal clinic at King Edward Memorial Hospital in Perth, Western Australia, to study the effects of repeated ultrasound on fetal and postnatal growth, development and pregnancy outcomes. Among the 2834 women with singleton pregnancies, 1415 were randomised to the intensive ultrasound arm of the study, which included umbilical cord blood sampling, and formed the population for the present analysis. Immediately after delivery, mixed umbilical arterial–venous (UA:UV) cord blood was collected, allowed to clot and serum was frozen at −80 °C and stored without thawing until the initial investigation by Keelan et al. [24]. Eight hundred and sixty blood samples had sufficient serum for steroid analysis. Of these samples, there were 820 participants with complete androgen data (400 female; 420 male), and 853 participants with complete estrogen data (425 female; 428 male). Among these participants, 341 (159 female; 182 male) had digit ratio measured between 19 and 22 years of age.

2.2. Steroid analysis

Cord serum samples were thawed, aliquoted and shipped from Perth, Western Australia to Adelaide, South Australia for LC–MS/MS analysis (CPR Pharma Services Pty Ltd, Thebarton, SA); in total, samples were thawed and frozen less than three times following collection. Ten randomly selected cord blood samples confirmed the absence of detectable maternal contamination [55]. Assay performance was determined to be unaffected by up to three freeze–thaw cycles or 24 h at room temperature. Steroid analysis was performed blind to sample identity or characteristics.

Total testosterone (TT), Δ4-androstenedione (A4), and dehydroepiandrosterone (DHEA) were measured by liquid chromatography–