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Umbilical cord blood androgen levels in girls and boys assessed by gas chromatography–tandem mass spectrometry

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ABSTRACT

Androgen exposure of the fetus during gestation plays an important role in human physiology and pathophysiology, but assessment of androgens, in particular dihydrotestosterone (DHT), in human umbilical cord blood is technically challenging. The aim of this study was to assess umbilical cord androgen levels, including DHT, at birth by a highly sensitive assay, and study their association with sex of the infant, sex-hormone-binding globulin (SHBG) levels, and gestational age at delivery. Swedish infants (27 girls, 26 boys) were recruited at maternity care clinics in Southern Sweden. Umbilical cord blood levels of dehydroepiandrosterone (DHEA), androstenedione, testosterone and DHT at delivery were assessed by a gas chromatography–tandem mass spectrometry assay. Cord blood levels of DHT were 2.4-fold higher in boys (median 27.8 pg/mL) than in girls (11.5 pg/mL), while the sex difference was less pronounced for testosterone (1.3-fold higher in boys) and non-significant for DHEA and androstenedione. Gestational age at delivery associated inversely with DHT levels in boys and with DHEA levels in girls. There was a strong inverse correlation between SHBG and DHEA in both sexes, while there were no associations between SHBG and testosterone or DHT levels. In conclusion, using state of the art technology, we report that there is a pronounced sexual dimorphism in human umbilical cord blood DHT levels. The possibility to assess a complete androgen profile in human cord blood opens up for future increased understanding of the biological impact of the fetal androgen milieu.

1. Introduction

Androgen exposure of the fetus during gestation plays an important role in human physiology and development, such as the development of the male anatomic phenotype [1,2]. Emerging data also suggest that androgen exposure in utero affects other phenotypes, such as future behavior, psychiatric disorders, metabolism and cardiovascular disease [2], although understanding in this area is incomplete.

There are inherent obstacles for gathering human samples during pregnancy, but umbilical cord blood, which is a mixture of venous and arterial components in roughly equal proportions [1], is easily accessible after delivery. Cord blood provides a snapshot of the sex hormonal milieu at late gestation, although sex hormone levels may be additionally modulated by various obstetric and other factors [1,3]. Androgens assessed in cord blood are considered to reflect fetal production in adrenals and gonads, with a contribution from placental production and metabolism, while maternal steroid levels show weak associations with those in cord blood [1]. The main androgens and androgen precursors in humans are dehydroepiandrosterone (DHEA), androstenedione, testosterone and dihydrotestosterone (DHT). The precursors DHEA and androstenedione may be converted to active androgens, such as testosterone, in peripheral tissues and testosterone is further metabolized to DHT, a several times more potent agonist to the androgen receptor than testosterone itself [4].

Immunoassays, that have been used in most previous studies investigating umbilical cord blood androgens [1,3], have limited

Abbreviations: CV, coefficient of variation; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone; GC–MS/MS, gas chromatography–tandem mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; LOD, lower limit of detection; LOQ, lower limit of quantification; SHBG, sex hormone-binding globulin

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accuracy and specificity for the assessment of androgens, especially at lower concentrations [5]. Assessment of DHT in the lower ranges is challenging even using liquid chromatography-mass spectrometry (LC–MS) methods due to poor ionization of this steroid [6]. Thus, while consistent data suggest higher concentrations of testosterone in umbilical cord blood of male compared to female fetuses [1], there are few similar comparisons performed regarding DHT. In fact, reliable estimations of DHT have been performed in human cord blood in only one previous study [7] and it is currently unknown whether DHT levels at birth associates with gestational age, sex-hormone-binding globulin (SHBG) levels or other phenotypes.

In the present study, we have assessed an androgen profile in human umbilical cord blood using a high-sensitive gas chromatography–tandem mass spectrometry (GC–MS/MS) assay and studied the sexual dimorphism in androgen profile at birth. Further, we have studied its association with SHBG levels and gestational age at birth.

2. Materials and methods

2.1. Cohort and plasma samples

Families in rural areas in the Skaraborg region in South-West Sweden were enrolled at maternity care clinics, and healthy infants born at term (median gestational age at delivery 39 weeks, range 36–42 weeks) were included in the FARMFLORA study [8]. Information regarding maternal age, smoking, type of delivery (vaginal/planned section/emergency section), gestational age at delivery, weight and length at birth was gathered from a questionnaire to the parents and from birth records. The study protocol was approved by the Human Research Ethics Committee of the Medical Faculty, University of Gothenburg, Sweden.

Blood samples were obtained from the umbilical cord at the delivery; from these, heparin plasma was prepared and diluted 1:2 in PBS before storage in −80 °C. Out of the original 65 individuals included in the FARMFLORA cohort, plasma was available for sex hormone assay for 54 individuals. One sample was excluded due to technical failure of the GC–MS/MS analysis, leaving 53 samples for the present analysis (27 girls, 26 boys).

2.2. Androgen profile by GC–MS/MS

Plasma levels of testosterone, DHT, DHEA and androstenedione were measured in a single run by gas chromatography–tandem mass spectrometry (GC–MS/MS), as previously described [9]. Briefly, after the addition of isotope-labeled standards, steroids were extracted to chlorobutane, purified on a silica column, and derivatized using pentafluorobenzylhydroxylamine hydrochloride followed by pentafluorobenzoyl chloride. Steroids were analyzed in multiple reactions monitoring mode with ammonia as reagent gas using an Agilent 7000 triple quadrupole mass spectrometer equipped with a chemical ionization source. The assay for testosterone is validated by the Hormone Standardization Project at the Centers for Disease Control and Prevention (Atlanta, Georgia) using isotope-dilution LC-tandem MS [9,10]. Assay performance, including intra-assay and inter-assay coefficients of variations (CVs), has been published previously [9]. Lower limit of detection (LOD) for the assay is 50, 4, 1.6 and 4 pg/mL and lower limit of quantification (LOQ) 400, 12, 2.5 and 8 pg/mL for DHEA, androstenedione, DHT and testosterone, respectively [9].

2.3. Measurement of sex hormone-binding globulin (SHBG)

Human sex hormone-binding globulin (SHBG) was analyzed by a commercial sandwich ELISA (Catalog Number SHBG0B, R & D Systems, Inc.), following the instructions of the manufacturer. The mean minimum detectable dose of human SHBG of the assay is 0.006 nmol/L. At a SHBG level of 2.7 nmol/L, intra- and inter-assay CVs of the assay is 3.6% and 4.8%, respectively.

Table 1
Characteristics of the cohort.

	Boys	Girls	p^*
N	26	27	
Maternal age, years	34.3 ± 4.4	31.7 ± 4.5	0.037
Maternal smoking, n	0	1	ND
Vaginal delivery, n (%)	21 (84)	23 (92)	0.38
Planned sectio, n (%)	3 (12)	1 (4)	ND
Emergency sectio, n (%)	1 (4)	1 (4)	ND
Gestational age at delivery, d	278 ± 13	278 ± 9	0.94
Weight at birth, g	3634 ± 514	3484 ± 342	0.23
Length at birth, cm	51.2 ± 2.0	50.1 ± 1.4	0.045
SHBG, nmol/L	23.4 ± 18.7	16.7 ± 11.7	0.19

Values are mean ± SD, unless otherwise specified. ND, not determined.
* *p*-Values are from *t*-test, but SHBG levels were compared by Mann–Whitney *U* test and frequencies were compared by Chi-square test.

2.4. Statistical analysis

All variables were tested for normal distribution by Shapiro–Wilk normality test. For variables that were normally distributed with or without log-transformation, two-group comparisons were performed dby Student's *t*-test and multivariate associations by multiple linear regression models. Other (nonparametric) data were analyzed using a Mann–Whitney *U* test (two groups) or Spearman rank correlations (correlation coefficients). Frequencies were compared by chi-square test. $P < 0.05$ was considered statistically significant. Statistical evaluations were performed with SPSS (version 19; SPSS, Chicago, IL, USA).

3. Results

3.1. Characteristics of the cohort

Characteristics of the cohort are presented in Table 1. The mothers of boys were on average older than mothers of girls, and boys were taller at birth compared to girls. Only one mother was smoking. The majority of children had a vaginal delivery. There were no statistically significant differences in route of delivery, gestational age or birth weight between boys and girls.

3.2. Cord blood androgen levels by GC–MS/MS according to sex of the fetus

Of the 53 samples assayed, 4 female samples were below the LOQ for DHT; these were assigned values at the LOQ for statistical analysis. All samples were above LOQ for androstenedione, DHEA and testosterone.

Cord blood levels of DHT were significantly lower in girls compared to boys (Fig. 1A); the median level in boys (27.8 pg/mL) was 2.4-fold higher than in girls (11.5 pg/mL). Testosterone levels (Fig. 1B) were also significantly higher in boys (median 216 pg/mL) than in girls (171 pg/mL), but the sex difference was less pronounced for testosterone (median 1.3-fold higher in boys than in girls) than for DHT. In accordance, the ratio between DHT and testosterone, an indicator of 5α-reductase activity, was significantly higher in boys (0.13 ± 0.06) compared to girls (0.08 ± 0.04; P = 0.001). There were no statistically significant sex differences in DHEA or androstenedione levels (Fig. 1C and D).

Levels of SHBG, assayed by immunoassay, are shown in Table 1. SHBG levels were not statistically different between boys and girls.

3.3. Correlations among cord blood androgens and SHBG

Correlations among cord blood androgens in boys and girls,