The androgen metabolome of preterm infants reflects fetal adrenal gland involution

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Abstract

Context. The human adrenal cortex changes with fetal-neonatal transition from the fetal to the adult organ, accompanied by changes in the steroid metabolome.

Objective. As it is unclear how the observed developmental changes differ between preterm and full-term neonates, we investigated whether the involution of the fetal adrenals is following a fixed time course related to postmenstrual age or whether it is triggered by birth. Furthermore, the fetal and postnatal androgen metabolome of preterm infants was characterized in comparison to term babies.

Design. Prospective, longitudinal, two centre study collecting spot urines of preterm and term infants during the first 12-18 months of life.

Methods. Steroid metabolites were measured from spot urines by gas chromatography-mass spectrometry. Data relating were modelled according to established pre- and postnatal pathways.

Results. Fetal adrenal involution occurs around term-equivalent age in preterm infants and is not triggered by premature birth. Testosterone levels are higher in preterm infants at birth and decline slower till term compared to full-term babies. Dihydrotestosterone levels and the activity of the classic androgen biosynthesis pathway are lower in premature infants as is 5α-reductase activity. No difference was found in the activity of the alternate backdoor pathway for androgen synthesis.

Conclusions. Human adrenal involution follows a strict timing that is not affected by premature birth. By contrast, prematurity is associated with an altered androgen metabolome after birth. Whether this reflects altered androgen biosynthesis *in utero* remains to be investigated.

Key words: fetal adrenal, steroid metabolome, premature neonates, fetal-neonatal transition

Introduction

Proper function of the human adrenal cortex is important for normal fetal development and indispensable for postnatal life (1). However, the adult and fetal adrenals differ markedly in structure and function. The human adult adrenal cortex produces mineralocorticoids, glucocorticoids and adrenal C19 steroids (also known as adrenal androgens) in three distinct layers. By contrast, the fetal adrenals produce almost exclusively C19 steroids. Abnormal fetal adrenal androgen production and metabolism during pregnancy affects fetal sexual development as seen in females with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency (1, 2). Instead, lack of cortisol may not affect a fetus in utero, but it becomes life-threatening for a newborn soon after birth (1). Thus the development of the fetal adrenals, their adaptation to birth and postnatal involution to allow for the formation of the adult organs are crucial events in normal human biology. These events are still not understood in great detail (3).

The human fetal adrenal cortex is derived from the mesenchymal adrenogonadal anlage. Subsequently, cells derived from the neural crest invade the adrenal primordium and will ultimately develop into the adrenal medulla(3-6). During the first trimester of pregnancy, cells from the adrenal primordium give rise to the fetal adrenal cortex comprising of a capsule, a small outer definitive zone (DZ) and a large inner fetal zone (FZ), which surround the medulla with the catecholamine-producing chromaffin cells. The FZ is responsible for producing the adrenal C19 steroid dehydroepiandrosterone (DHEA) in large amounts that is converted to estriol by the fetal-placental unit (1, 7). The DZ produces small amounts of cortisol throughout pregnancy with a substantial increase in production towards term (7, 8). Between the DZ and FZ an additional transitional zone (TZ) develops during the second trimester. By this time, the fetal adrenals have grown to a relative size of more than 10 times of that of the mature adult adrenals and are as large as the fetal kidneys (6). The development and function of the fetal adrenals is tightly regulated principally by ACTH produced within the developing hypothalamic-pituitary-adrenal axis from 14 weeks gestation onwards (6-10). Towards term, the DZ and TZ of the human fetal adrenals begin to resemble the zona glomerulosa (zG) and the zona fasciculata (zF) of the adult adrenal cortex (6). Meanwhile, the FZ involutes rapidly after birth with the consequence that DHEA levels drop and the organ size decreases by half (6). The zG and zF of the adult adrenal cortex are structurally and functionally existing at birth, but the installment of the diurnal circadian rhythm of cortisol production varies greatly between individuals (both in term and preterm born children) in the first 6 months of life concerning its age at appearance and stability (11, 12). The innermost zona reticularis (zR) is not established after birth. This third layer of the

adult cortex only forms gradually over time and becomes active in the production of adrenal C19 steroids at the age of around 8 years in children of both sexes (13).

The molecular regulation underlying the transformation of the fetal to the adult adrenal cortex remains a conundrum. Current literature offers both duration of gestation and parturition as possible triggers of this transformation (6, 14). Therefore, in this study we used prospective urine steroid profiling of preterm and term infants to address the question whether the involution of the fetal adrenals is following a fixed time course related to postmenstrual age (PMA) or whether it is triggered by birth. Furthermore, we characterized the fetal and postnatal androgen metabolome of preterm infants in comparison to term babies longitudinally. By measurements of steroids from spot urines using gas chromatography-mass spectrometry and modelling of the data, we show that fetal adrenal involution occurs around term-equivalent age in preterm infants while preterm birth seems not to affect the timing. Nevertheless, the androgen metabolome of premature and term babies shows both similarities and differences at term and beyond.

Material and Methods

Cohort

Written informed consent was obtained from all study participants' parents. The ethics committee of the Northern Savo hospital district, Finland, approved the study (permission numbers 11/2008 and 417/2015).

As part of the Finnish Prebaby study of metabolism, serial morning urine samples were collected from 16 very preterm infants (8 boys) born at <30 gestation weeks (Table 1 and Suppl Figure 1) (15). The first sample was collected at around one week of age (range, 4 to 9 days) and then at two-week intervals (at the calendar age of 3, 5, 7, 9, 11, 13 and 15 weeks, depending on the degree of prematurity at birth) until discharge from the hospital. Thereafter, samples were collected at serial follow-up visits during the first 12 months of life. Altogether 147 urine samples of very preterm infants were obtained between the calendar age of 1 week and 61 weeks (median, 9 weeks) corresponding to PM age of 24 to 86 weeks (median, 36 weeks). The number of samples ranged from 4 to 15 per child (median, 9). The calendar and postmenstrual ages at sampling in preterm infants are depicted in Supplementary Figure 1 (15). Antenatal glucocorticoid treatment was given to all mothers prior to delivery. The median time gap between the antenatal glucocorticoid administration (the first dose) and delivery was 8 days in boys (range, 0-10 days) and 13 days in girls (range, 2-41 days). All girls and 5 boys were born by caesarean section. All but two boys

had respiratory distress syndrome (RDS) requiring surfactant treatment. Three boys and five girls received postnatal glucocorticoids (Table 1).

Detailed data of serial spot urine samples of term babies were available from a previous longitudinal study (the Bern Baby Urine study), in which 43 healthy infants (22 females) born at 37 gestational weeks or later were investigated during the first year of life at 13 time points (from the age of 1 week to 49 weeks). For details of methods of this study we refer to (16-18). In essence, the study design was very similar to the Finnish Prebaby study. Most importantly, all samples were processed and analysed by the same steroid profiling method in the same laboratory.

Steroid measurements and data analysis

Reference steroid compounds were obtained from Steraloids, USA. All other chemicals were obtained from Sigma-Aldrich (Switzerland) and were of analytical grade. A list of all steroids analysed in this study, including abbreviation, trivial name and systematic name, and all raw data, is given as Supplementary Material Table 1 to be found at https://doi.org/10.6084/m9.figshare.19213746.v1 (15).

Steroids were quantified using gas chromatography-mass spectrometry (GC-MS) using an established method (19). In brief, 1.5 mL of urine was spiked with a mixture isotopically labelled internal standards comprising all steroid classes, followed by solid phase extraction, enzymatic hydrolysis, derivatisation (methoxamine and N-trimethylsilyl-imidazole) and purification using liquid-liquid extraction with using cyclohexane and water. All measurements were performed on a 7890A gas chromatograph coupled to a mass selective detector (5977; both Agilent Technologies, USA). All steroid concentrations were normalized with the creatinine level of the corresponding sample as measured by the QuantiChrom Creatinine Assay (DICT-500; BioAssay Systems, USA). Steroid data for the term reference group were re-used from a previously published study which was performed in the same laboratory using the same GC-MS method (16-18).

Statistical analysis was performed in R (Version 4.1.1). Calculation of fitted curves and confidence intervals was based on local polynomial regression.

Results

The temporal changes in concentrations of steroids involved in the fetal zone steroid pathways were compared between very preterm infants born between 23.4 and 29.7 gestational weeks (Table 1) (20) and full-term infants (born after 37 gestational weeks) (16-18).

The initial analyses did not reveal sex differences in steroid metabolites involved in the fetal zone pathways in either very preterm or full-term infants (Supplemental Figure 2) (15).

Further, no effect of antenatal and/or postnatal steroid administration on these steroid metabolites was seen in the preterm group (Supplementary Figure 2) (15). Therefore, sex or exposure to glucocorticoids was not taken into account in the final analyses. This is in agreement with our previously published data on the steroid metabolome of term babies (16-18).

The human fetal adrenals produce predominantly high levels of dehydroepiandrosterone (DHEA) and sulphated DHEA (DHEA-S) from cholesterol, and these are then peripherally metabolized to yield estriol or androstenetriol (1, 3, 6). Figure 1 shows the analysed steroids of this human fetal pathway for the synthesis of estriol (21) in relation to the postmenstrual (PM) age of the infants. We chose to display data rather in relation to the PM age than the calendar (i.e. postnatal) age in order to better depict the changes in steroid levels between preterm birth and term-equivalent age.

In the preterm group, no immediate reduction of the corresponding steroids upon birth occurs as was observed for the full-term infants. In comparison to full-term infants, highly elevated levels for all steroids of the fetal pathway were observed right after birth in preterm infants. A gradual decrease of the fetal pathway steroid levels was seen during the first weeks of life (Figure 1). At the term-equivalent age and thereafter (PM age of 40 weeks or more), the steroid concentrations in the preterm infants and the full-term control infants did not show any significant differences (Figure 1).

We also investigated whether prematurity has an impact on steroid production of the classic androgen pathway starting from 17-OH-pregnanolone (17HP; urinary metabolite of 17OH-pregnenolone) and resulting in the production of testosterone and dihydrotestosterone (DHT). After birth highly elevated steroid metabolite concentrations, including testosterone concentration, were observed in the preterm infants in comparison to the full-term infants. However, by the term-equivalent age, testosterone concentrations of the preterm infants decreased to the same level as in full-term infants (Figure 2). Interestingly, markedly higher DHT concentrations were observed in full-term babies after birth and throughout the follow-up period than in preterm infants, indicating higher 5α-reductase activity in full-term babies. No significant differences were seen for estradiol concentrations, which were very low in comparison to all other steroid metabolites in both groups. Low levels of estradiol also confirm that the elevated levels of estriol observed in preterm infants from birth to term-equivalent age are not produced through conversion of estradiol.

For a better illustration of the differences in activities of the fetal zone pathway and the classic androgen pathway in preterm and full-term infants, we also compared the product-substrate ratios of the corresponding pathways (Figure 3). We chose 17HP as the precursor

for both pathways, estriol as the final product of the fetal zone pathway and testosterone of the classic androgen pathway. This comparison revealed that the fetal zone pathway remains active after birth for preterm infants and overlays with the full-term infants only after 40 weeks of PM age, with a similar decline thereafter (Figure 3 A and B). On the contrary, we found that at term-equivalent age, the classic androgen pathway is more active in full-term infants than in preterm infants (Figure 3 C). In term infants, the period from birth to 60 PMA (around the age of 5 months) corresponds to the postnatal time window of minipuberty (16, 22).

When analysing the classic androgen pathway testosterone/17HP ratio in relation to the calendar age starting from birth (i.e. shifting the starting point of the plot for the preterm infants right to starting point of the full-term infants), the two groups overlay (Figure 3 D). This indicates that in contrast to the fetal zone pathway, the decline in activity of the classic androgen biosynthesis pathway is rather related to parturition. Thus, the measured activity of the classic androgen pathway might also reflect the postnatal gonadal steroid activity in addition to the adrenal steroidogenesis.

Next, we investigated the activity of the alternative ("backdoor") androgen pathway that results in production of DHT without classical precursors such as DHEA, androstenedione and testosterone (16, 23). To this end, we calculated the apparent 17,20-lyase activity essential for androgen production. This conversion can follow either the Δ5- or Δ4-steroid pathway: the Δ5-pathway leads from pregnenolone to DHEA via 17OH-pregnenolone and thus directly to classic androgen biosynthesis, whereas the Δ4-pathway feeds into the alternative pathway via production of 17-OH progesterone. In the backdoor pathway 17-OH progesterone is 5α and 3α reduced to yield metabolites such as androsterone and androstanediol.

We did not find any difference in the Δ4-pathway activity between the preterm and full-term infants, and a slightly increased activity for the Δ5-pathway for full-term babies (Figure 4). In addition, androsterone levels, which serve as an indicator for the activity of the backdoor pathway barely differed between the two groups. The ratio androsterone/etiocholanolone has also been described as an indicator for backdoor androgen production (24). In our cohort, the preterm infants showed lower values for this ratio compared to the full-term infants. Yet, this calculation might be misleading because the androsterone/etiocholanolone ratio can also serve as an indicator for 5α-reductase activity (25), which, based on the DHT/testosterone ratio, seems to be lower in the preterm babies. Therefore, the difference in androsterone/etiocholanolone ratio may just reflect 5α-reductase activity, which in turn would indicate that there is no difference in backdoor androgen production in the two groups.

Discussion

In this paper, we studied the involution of the fetal adrenal glands by investigating the adrenal FZ steroid metabolome through longitudinal comparison of urinary steroid profiles of preterm and full-term infants. Our data clearly show that in very preterm infants (<30 weeks gestational age), the fetal adrenal pathways remain active after preterm birth until term. Thereafter, a similar decline in activity of fetal adrenal steroidogenesis as in term-born infants is observed. These findings indicate that rather maturation by the increasing postmenstrual age than the event of birth triggers the involution of the fetal adrenals.

So far there has been controversial views on the timing of involution of the fetal adrenals after birth in preterm infants. Similar to our study, a few longitudinal studies of preterm infants have reported that the urinary excretion of FZ steroids (3β-OH-5ene steroids) persists until term and then decreases, as observed in full-term infants (9, 26, 27). Most recently, this finding has been confirmed in healthy early (<30 weeks gestation) and late preterm infants (30-36 weeks gestation) (14). Moreover, the same pattern has also been described for plasma and urinary DHEA-S levels in preterm and full-term infants (28, 29); and in the same line higher DHEA-S levels were reported for extremely preterm infants (23-26 weeks gestation) compared to very preterms (27-29 weeks gestation) in the first week of life (30). Morphometric studies using prenatal and neonatal ultrasound imaging or autopsy methods revealed that the fetal adrenals shrink faster in full-term compared to preterm infants (31, 32). On the other hand, a study including infants of 26-35 weeks gestation suggested that parturition is the trigger for the involution of the fetal adrenals (33). They found that the size of the adrenal glands decreased to its normal infantile size within the first 2 weeks after birth, and serum DHEA-S decreased rapidly regardless of gestational age at birth. Overall, studies using comprehensive urinary steroid profiling point towards adrenal regression being rather an event related to postmenstrual age than birth, while studies using a single steroid in plasma (e.g. DHEA-S) resulted in ambiguous results. Even though the reasons for these differing results are not entirely clear, the fact that steroid metabolism is not equally reflected in serum and urine may account for these discrepancies; note that urinary steroids provide a more comprehensive representation of steroid biosynthesis and metabolism (34). Also, opposite to the studies that focused only on preterm infants during the first four weeks of life, our study provides comparative data in very preterm infants and full-term infants throughout the first year of life.

Looking at the androgen metabolome of preterm and full-term infants besides the adrenal fetal zone pathway, we found differences in the classic androgen biosynthesis from 17HP to testosterone and DHT. For this pathway preterm infants had higher urinary levels of

testosterone at birth but comparable levels at term in relation to PM age of full-term infants. However, when looking at the activity of the classic pathway in relation to the calendar age, this difference essentially disappears, indicating that the adrenal regression may not be alone responsible for this decline after parturition. In contrast to testosterone, DHT levels were lower at both birth and term-equivalent age in preterm infants, suggesting that prematurity causes a relative 5α-reductase deficiency (inefficient conversion of testosterone to DHT). Although there is a clear sex difference for intrauterine testosterone production between males and females (especially between 12-20 weeks gestation when the male gonads reach their peak production (35)), at birth male and female infants have similar serum and urinary testosterone levels that decline rapidly within the first week of life (17, 35, 36). Studies on DHT levels show inconsistently a sexual dimorphism at birth in full-term as well as in preterm neonates (17, 36, 37). To the best of our knowledge, no data are available in the literature concerning the relationship between DHT production, 5α-reductase activity and gestational age.

DHT is an important androgen for the formation of the normal male external genitalia (38). Its synthesis may occur through the classic androgen pathway using DHEA, androstenedione and testosterone as intermediates, or through the alternative, backdoor pathway which uses 17-OH progesterone as precursor for the production of DHT through several intermediates including androsterone (23, 38). Both pathways depend on 5α-reductase activity for the production of DHT. Our study revealed no difference between preterm and full-term infants for the activity of the alternative androgen pathway at birth. Importantly, no difference was found for androsterone, which has been recently identified as the predominant androgen of the backdoor pathway in the fetus, and is synthesized across several tissues in the human fetus including the placenta, liver, adrenal, and testis (39).Yet, for androgen synthesis through the classic pathway, we found a slightly higher activity in full-term infants. Overall, our data indicate that changes or differences observed in testosterone and DHT levels after birth in both preterm and full-term infants are less related to adrenal involution, but rather reflect postnatal changes of gonadal steroidogenesis. In our previous studies, we have shown that the postnatal activity of the hypothalamic-pituitary-gonadal axis is higher in preterm than in full-term infants as witnessed by their higher gonadotropin levels (40, 41); however, the differences disappear around term. In preterm boys, high LH levels were closely related to high urinary testosterone levels and rapid penile growth (40). On the other hand, despite their higher testosterone levels, urinary PSA levels were lower in preterm than in full-term boys during the first weeks of life and increased only later (40), which could be explained by the lower 5α-reductase activity in preterm infants reported in the present study. Moreover, lower and delayed 5α-reductase activity could explain why acne in infancy is less

common in preterm than in full-term infants despite of their higher DHEA and testosterone levels (29). Nevertheless lower DHT levels and activity of the classic androgen pathway (e.g. due to a relative 5α-reductase deficiency) in preterm infants at birth and beyond may represent important findings that should be further explored. If this relative 5α-reductase deficiency preexists *in utero*, it may explain why mild syndromes of undermasculinization (e.g. cryptorchidism and hypospadias) are more often seen in prematurely born boys (42-45).

In our study, estriol levels of preterm infants were high at birth and decreased to low levels observed in term infants by term-equivalent age. However, estradiol levels were similarly low in preterm and term infants independent of the gestational age. During pregnancy, the fetal-placental unit is the principal source of estrogen production, and estriol is produced abundantly (21). For estriol production fetal adrenal DHEA(-S) is 16α-hydroxylated in the fetal liver and then stepwise converted to estriol in the placenta (21). At parturition, the fetal-placental unit is destroyed. Therefore, persistent estriol production from fetal zone DHEA in preterm infants then requires the involvement of other peripheral tissues besides the liver; but this is not yet known in further details. Overall, the physiologic role of estrogens for the fetus remains unclear. Recent studies suggest that estradiol as well as fetal zone steroids may have neuroprotective effects (14, 46, 47). In fact the fetal brain expresses estrogen receptors (48) and aromatase for the production of estrogens (49).

Our study has several limitations. First, due to the difficulty of collecting 24h urine in infants, we were only able to analyze spot urines. Even though urine concentrations were normalized with creatinine concentration, certain circadian fluctuations in steroid levels cannot be excluded, even though infants do not display a diurnal rhythm during the first four months of life (50). Second, similar to other studies the sample size of our cohort is limited. However, the longitudinal setting with the total number of nearly 150 samples provides us reliable data on postnatal maturation of steroid metabolism. Further, steroid profiling by GC-MS has the great advantage of delivering a rather complete snapshot of the steroid metabolome from a single measurement, thus allowing the reconstruction and activity assessment of entire steroid pathways, turning it into an invaluable tool for system biology. Third, and as a consequence of the aforementioned, the urinary steroid metabolome as such is not able to discriminate for steroidogenesis between organs. However, when knowing the specific metabolites of active steroid pathways of organs, patterns reflecting an organ may be recognized. Fourth, prematurity is by definition an abnormal condition which is the result of various pregnancy complications that may be caused by the mother, the fetus, or both. In addition, prematurity comes with a higher risk of neonatal complications. Therefore, a study

group of premature infants will always be much more heterogeneous than a group of term infants. This was also the reason why from our small sample size of preterm data no analyses for minipuberty and sex differences compared to controls were possible.

In conclusion, we show that fetal adrenal androgen production of the FZ remains active until term equivalent age in very preterm infants. Thus, our data confirm that involution of the fetal adrenal is guided by maturation with increasing postmenstrual age and not by birth itself. Testosterone production at birth is high despite prematurity, but DHT levels and the activity of the classic androgen biosynthesis pathway are lower in premature infants. Whether these findings reflect the androgen biosynthesis in utero and might explain the higher prevalence of undescended testis and hypospadias in preterm boys remains to be studied.

Data Availability

Supplementary Material listing all steroids analyzed in this study, including abbreviation, trivial name and systematic name (Suppl Table 1), and all raw data (Suppl Table 2) can be found free of charge at https://doi.org/10.6084/m9.figshare.19213746.v1. We also provide in Suppl Figure 1 the sampling scheme of all preterm study subjects comprised in Suppl Table 2, and in Suppl Figure 2 data for the effects of pre- and postnatal glucocorticoid treatments (A) and sex (B) on investigated steroids.

Declaration of interest

None declared.

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Table and Figure Captions

Table 1. Characteristics of the preterm infants. Data are presented as median and range or number of infants.

Figure 1. Profile of the main fetal steroid pathway in preterm infants born at < 30 gestational weeks (red lines) in comparison to full-term infants (blue lines). Serial urine samples were analysed using GC-MS for the depicted fetal androgen pathway steroid metabolites. Note that the reported level of DHEA represents the sum of both DHEA and DHEA-S, since GC-MS analysis requires enzymatic hydrolysis of sulphated steroids, and therefore we cannot report on the levels of sulphated and non-sulphated steroids, e.g. of DHEA and DHEA-S, separately. Shaded areas indicate 95% confidence intervals. Data of full-term infants originate from (16-18). PM, postmenstrual; DHEA, dehydroepiandrosterone; 17HP, 17-OH-pregnanolone. Metabolizing enzymes are indicated in the blue boxes.

Figure 2. Profile of the classic pathway of dihydrotestosterone (DHT) and estradiol biosynthesis in preterm and term infants. In this pathway DHEA is predominantly converted to testosterone and DHT. Measured steroid levels in preterm (red) and full-term (blue) infants are shown. Shaded areas indicate 95% confidence intervals. Metabolizing enzymes are indicated in the blue boxes.

Figure 3. Apparent activities of the fetal zone pathway (Estriol/17HP; **A** and **B**) and classic androgen pathway (Testosterone/17HP; **C** and **D**) for preterm (red) and full-term (blue) infants. Top panels use the postmenstrual (PM) age as the x-axis, bottom panels the calendar (Cal) age. Shaded areas indicate 95% confidence intervals. 17HP, 17-hydroxypregnanolone.

Figure 4. Apparent enzyme activities for evaluation of the activity of the alternative backdoor androgen pathway for preterm (red) and full-term (blue) infants (16). **A** Apparent CYP17A1 activity for the Δ4 pathway: (DHEA + 16a-OH-DHEA+androstenediol)/pregnenetriol. **B** Apparent CYP17A1 activity for the Δ5 pathway: 11b-OH-androsterone/pregnanetriol. **C** Androsterone level. **D** Ratio of backdoor to classical androgen pathway:

Androsterone/Etiocholanolone. **E** Apparent 5α-Reductase activity: Dihydrotestosterone/Testosterone. Shaded areas indicate 95% confidence intervals.

Table 1. Characteristics of the preterm infants. Data are presented as median and range or number of infants.

	Boys n=8		Girls n=8	
Gestational age (weeks)	27.0	23.4 – 29.7	27.0	24.0 – 29.4
Caesarean section	3		8	
Birth weight (grams)	1100	540 – 1630	710	475 – 1360
Birth weight (SDS)[1]	0.97	-0.96 – 2.77	-1.27	-3.69 – 0.50
Apgar 5 min	7	2 – 9	7	2 – 9
NEC[2]	1		1	
RDS[3]	6		8	
BPD[4]	3		4	
IVH grade III-IV[5]	0		2	
ROP[6]	1		3	

[1] Standard deviation score, conversion using the Finnish population-based birth weight reference (Ref 19).

[2] Necrotizing enterocolitis Bell stage 2 or 3

[3] Respiratory distress syndrome

[4] Bronchopulmonary dysplasia diagnosed at 36 postmenstrual weeks

[5] Intraventricular hemorrhage

[6] Retinopathy of prematurity

Figure 1

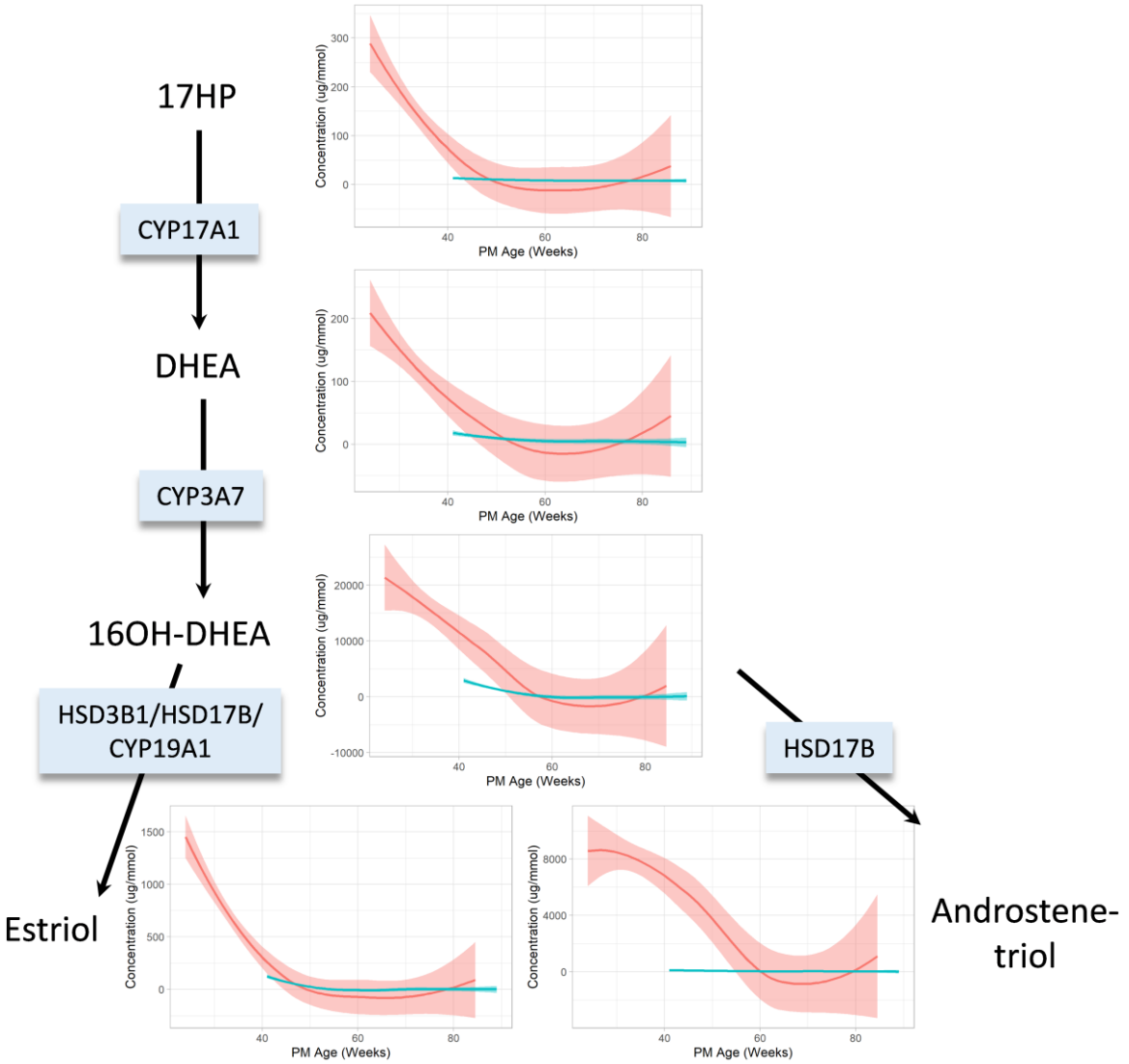

Figure 2

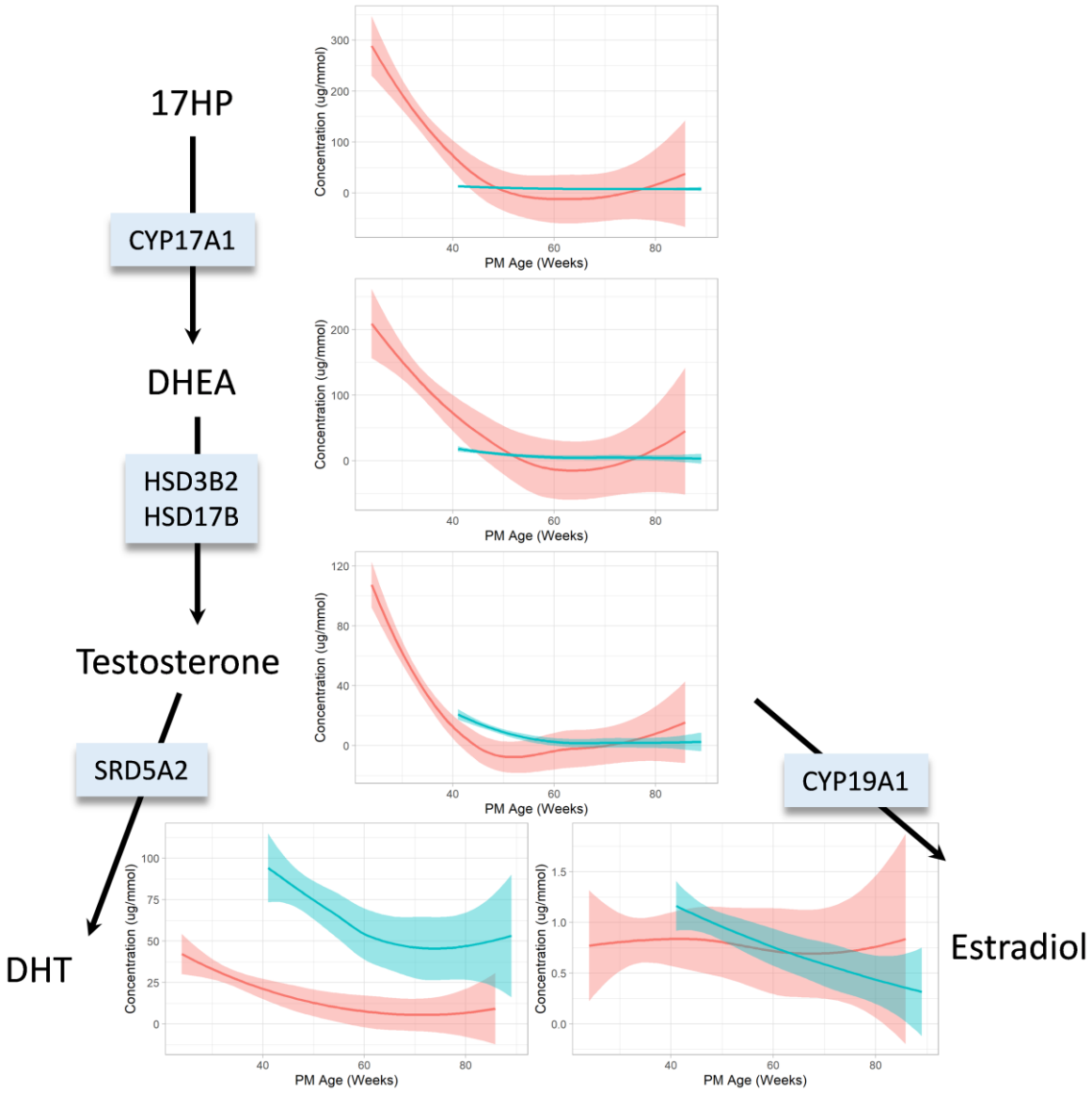

Figure 3

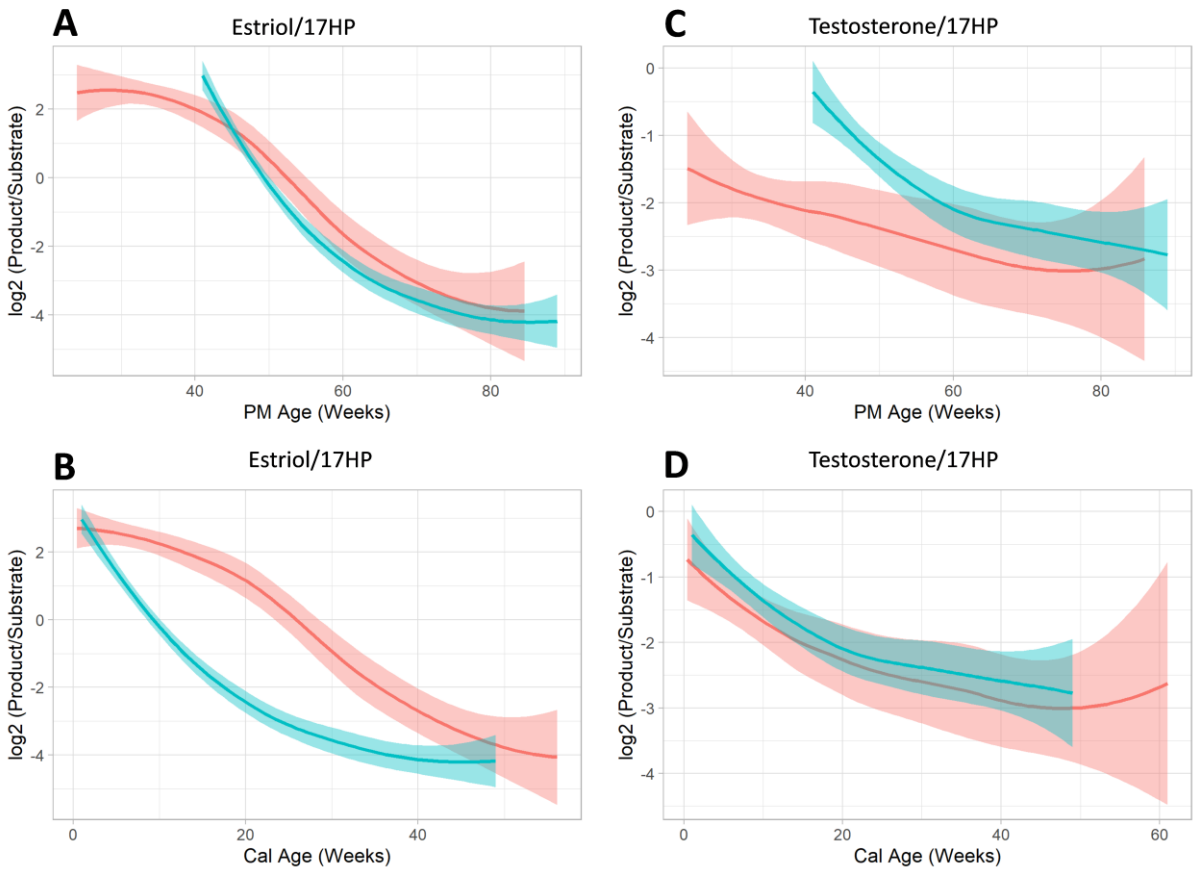

Figure 4