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Clinical Research Article

Postnatal Dysregulation of Androgens in Extremely Preterm Male Infants

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Abstract

Context: Neurodevelopmental impairments are common among survivors of extremely preterm birth, particularly in males. Hyperactivation of the hypothalamic–pituitary–gonadal (HPG) axis has been suggested as an underlying cause, but this has been poorly investigated.

Objective: Establish levels and temporal changes in circulating androgens in extremely preterm infant males.

Methods: Observational cohort study analyzing cord blood serum (n = 25) and postnatal plasma (n = 13) collected from day 0 until week 11 from infant males born at 22.8-27.9 weeks gestational age. Testosterone and dihydrotestosterone (DHT) were determined using gas chromatography mass spectrometry, sex hormone–binding globulin (SHBG) with an enzyme-linked immunosorbent assay, and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) with the Luminex xMAP multiplex assay.

Results: Testosterone and DHT levels were higher on day 0 (median 4.27 and 0.30 ng/mL) than in cord blood (0.15 and 0.01 ng/mL) ($P < .001$ for both). Levels of the hormones then declined rapidly until day 5 (median 0.16 and 0.12 ng/mL), then remained relatively constant throughout the study period. Median levels of testosterone and DHT across the whole study period were approximately 6-fold higher than reported in utero levels. FSH and LH showed similar postnatal patterns as the androgens. SHBG steadily increased over time, and, as a result, the fraction of bioavailable testosterone declined with infant postnatal age.

Conclusion: The HPG axis is activated immediately after birth in extremely preterm infant males, resulting in an androgen pulse occurring several months earlier than during a normal pregnancy. The long-term implications of high androgen exposure during a sensitive neurodevelopmental period warrant further studies.

Key Words: androgens, dihydrotestosterone, extremely preterm, follicle-stimulating hormone, luteinizing hormone, minipuberty, sex hormone–binding globulin, testosterone

Abbreviations: DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; GA, gestational age; HPG, hypothalamic–pituitary–gonadal; LH, luteinizing hormone; LLOQ, lower limit of quantification; SHBG, sex hormone–binding globulin.

Children born extremely preterm (less than 28 weeks' gestational age [GA]) are at increased risk of neurodevelopmental impairments [1-3]. Additionally, there are sex-specific effects of prematurity on brain structure and function [4-7]. Preterm males appear to be particularly vulnerable to impairment of brain development, thereby increasing the prevalence

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of neurodevelopmental disorders in males compared with females [8-11]. Elevated postnatal androgen levels have been postulated as a contributing factor of preterm male altered brain maturation and later adverse neurodevelopmental outcomes [12].

Testosterone secretion by the fetal testis is apparent at around gestational week 8 [13, 14]. During the first trimester, fetal testosterone production is driven by placental human chorionic gonadotropin and later in gestation also by pituitary luteinizing hormone (LH) [15]. Circulating testosterone concentration in the male fetus peaks around week 16 [13, 14] and then gradually decreases until term as the hypothalamic–pituitary–gonadal (HPG) axis is silenced [14, 16]. After birth, the HPG axis is temporarily reactivated following the loss of placental suppression [17-19]. This causes a rise in testosterone production in male infants starting around 1 week of age, peaking at 1-3 months of age, and is followed by deactivation of the HPG axis again, resulting in low testosterone levels until the onset of puberty [20]. The transient postnatal activation of the HPG axis is also known as the minipuberty [21]. In parallel to the postnatal rise in testosterone in the first months, the circulating concentration of sex hormone–binding globulin (SHBG) increases [22]. Testosterone and dihydrotestosterone (DHT) bind tightly to SHBG and only the nonbound androgen fraction is biologically active. Although the reactivation of the HPG axis in the neonatal period appears to influence growth velocity [23], development of male genitalia [20, 24], and behavioral programming [25, 26], the role of this androgen surge has not been fully elucidated.

Preterm birth causes untimely disruption of the fetoplacental unit. The subsequent early activation of the HPG axis in preterm compared with term infant males has been shown to cause excess production of androgens during minipuberty [27-29] resulting in faster postnatal penile and testicular growth and potentially contributing to long-term diminished reproduction [28, 30]. It was thus suggested that an immature HPG axis, lack of feedback regulation, and the persistence of fetal adrenal steroids cause the increased androgen production following preterm birth.

Masculinization of the fetal male brain occurs at relatively late gestation. Androgen exposure during the third trimester in utero can affect the developing brain and result in permanent anatomical and functional changes [31]. Longitudinal postnatal levels of circulating androgens and associated gonadotropins are poorly described in extremely preterm infant males, and their potential long-term role in brain imprinting and effect on other organs remains unknown.

In this study, we followed the levels of androgens, gonadotropins, and SHBG longitudinally in the blood of extremely preterm male infants aiming to further understand hormonal changes taking place during a critical developmental window.

Materials and Methods

Participants and Blood Sampling

To obtain sufficient material to study birth (umbilical cord blood) and postnatal androgen levels with high temporal resolution, we used samples collected from 3 cohorts of extremely preterm infants. Cord blood samples were from the Donna Mega trial (ClinicalTrials.gov ID NCT02760472) [32] and the Mega Donna Mega trial (ClinicalTrials.gov ID NCT03201588) [33]. The 2 trials were designed to study the effect of supplementation of long-chain polyunsaturated fatty acids on retinopathy of prematurity, other morbidities, and growth. In both trials, intervention therapies started after cord blood collection. The inclusion criterion was <28 weeks GA at birth, and the exclusion criterion was major congenital malformations. Recruitment took place at the Queen Silvia Children's Hospital, Gothenburg, Sweden, during 2013-2015 (Donna Mega) and 2016-2019 (Mega Donna Mega). No discrimination between arterial and venous blood was made upon cord blood sampling. Blood was collected in serum-separating tubes, kept refrigerated for 0.5 to 2 hours after the blood draw, and then centrifuged at 1500*g* for 10 minutes at room temperature. The serum was aliquoted into cryo-vials and temporarily stored at −20 °C before long-term storage at −80 °C.

Serial blood samples were retrieved from the EPITOP study, a study aimed at saving residual blood from routine clinical samples for research purposes at the Queen Silvia Children's Hospital, Gothenburg. Eligible for inclusion were all infants <28 weeks GA at birth. The present study used heparinized blood collected for arterial blood gas analyses. The blood was collected in blood gas syringes from an umbilical catheter, a peripheral arterial line, or by venipuncture. After blood gas analysis, syringes were kept at room temperature until the following weekday morning (median [min-max] storage time 20 hours [1 hour-5 days], 78% of the samples were frozen within 2 days after sampling), whereafter the blood was transferred to false-bottom tubes and centrifugated at 2000*g* for 10 minutes, and the plasma was separated and frozen at −80 °C. Sample collection was between March 2020 and March 2021. We aimed to have individual samples for hormone analysis from the first day of life, days 1, 2, 3, 5, 7, and 10, and then weekly for as long as available. The first sample was registered as day 0 if it was taken on the same date as the infant was born.

Ethics

This study was approved by the Regional Ethical Board at the University of Gothenburg (reference numbers 303-11 and T570-15) and by the Swedish Ethical Review Authority (EPITOP study, 2019-03110). Written informed consent was retrieved from the parents/guardians of all participants.

Clinical and Anthropometric Data

Infant clinical and anthropometric data were retrospectively collected from medical charts or electronic case report forms. Morbidities were defined as previously described [33]. Standardized weight calculations were based on the growth charts by Fenton and Kim [34].

Sex Steroid, SHBG, FSH, and LH Analysis

Testosterone and DHT were analyzed by gas chromatography-tandem mass spectrometry as described previously [35, 36]. Fifty microliters of serum or plasma was used for the analysis. Steroid measurements below the lower limit of quantitation (LLOQ) were set to LLOQ/2. The performance of the method has been described previously [36]. The fractions of free testosterone and bioavailable testosterone were calculated using the equation proposed by Vermeulen et al [37]. In these calculations, samples missing SHBG measurements (n = 6) were imputed with the mean value at the time point, and an albumin concentration of 22.6 g/L was assumed, corresponding to the

mean albumin concentration at birth for infants born in GA weeks 25-26 [38]. For calculating bioavailable testosterone in the full-term reference [39], an albumin concentration of 34.3 g/L was used [38].

Serum and plasma SHBG were determined using enzyme-linked immunosorbent assay (Human SHBG Quantikine ELISA, R&D Systems Cat# DSHBG0B, RRID:AB_3101841) as previously described [35]. Briefly, a 3-µL sample (prepared in duplicates) was diluted in kit diluent (1:75), and absorbance was determined at 450 nm. From the longitudinal sample set, 6 samples had insufficient plasma volumes for SHBG analysis, and 8 samples had concentrations below the LLOQ at 4.7 nmol/L and were set to half of the LLOQ. The interassay coefficient of variation was 5.8% at a mean of 62.5 nmol/L.

Follicle-stimulating hormone (FSH) and LH were analyzed in a multiplex assay (Millipore Cat# HPTP1MAG-66 K, RRID:AB_3423929) using the Luminex xMAP platform (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Samples were analyzed as singlets using 25 µL of undiluted plasma. Some low values were extrapolated beyond the standard range when they were still within the limits of the fitted logistic curve. Out of range samples below the LLOQ were set to the lowest calibration point divided by 8, corresponding to 0.006 mIU/mL for FSH and 0.0003 mIU/mL for LH. The interassay coefficient of variation was 0.73% at mean 0.5 mIU/mL FSH and 2.8% at mean 1.1 mIU/mL LH.

Unless stated otherwise, the concentrations of all analytes are reported as medians (25th-75th percentiles).

Statistical Analyses and Data Visualization

Statistical analyses were performed in IBM SPSS Statistics version 29 (IBM Corp, Armonk, NY, USA) and SAS version 9.4 (SAS Institute Inc, Cary, NC, USA). Group comparisons were made using the nonparametric Mann–Whitney U test and cross-sectional correlation analysis was performed with Spearman's rank test. Random coefficient models were applied for the longitudinal description of testosterone, DHT, FSH, and LH as a function of postnatal age and GA categories. All variables were modeled by lognormal distribution due to skewness in data. An unstructured covariance pattern was used, and the time was modeled using natural cubic splines. Additionally, testosterone levels were expressed as a function of the levels of DHT, FSH, and LH separately including GA and postnatal age using the same methodology. The change in testosterone over increasing DHT, FSH, and LH was described by relative risk and 95% CI and associated *P* values. Diagnostic plots were visually reviewed for goodness of fit and appropriateness of the methods used. Data were visualized using R studio [40] and the ggplot2 package [41] or SAS. All tests were 2-sided, and the significance level was .05.

Results

Description of Cohorts

Cord blood androgens were analyzed in 25 males born <28 weeks GA, and postnatal blood androgens were analyzed in a separate cohort of 13 males born <28 weeks GA. Birth and perinatal characteristics of infants are presented in Table 1 and graphically illustrated for the postnatal cohort in Fig. 1A and 1B. Baseline characteristics were similar between the 2 cohorts, with a mean GA (SD) at birth of 25.0 (1.6) and 25.0 (1.2) weeks, and birth weights of 773 (244) and 669 (158) g, in the cord blood and postnatal cohort respectively.

Table 1. Perinatal characteristics of infants analyzed for cord blood and postnatal hormones

Variable	Cord blood cohort (n = 25)	Postnatal cohort (n = 13)
Gestational age, weeks		
Mean (SD)	25.0 (1.6)	25.0 (1.2)
Median (min-max)	24.7 (22.8-27.9)	25.3 (22.9-27.0)
Birth weight, g		
Mean (SD)	773 (244)	669 (158)
Median (min-max)	695 (470-1255)	618 (490-950)
z birth weight		
Mean (SD)	0.04 (0.95)	−0.53 (1.03)
Median (min-max)	−0.15 (−1.41-1.98)	−0.51 (−1.22-0.31)
Vaginal delivery, n (%)	12 (48)	4/13 (31)
Retinopathy of prematurity		
Any, n (%)	18/23 (78)	12/13 (92)
Severe (stage 3 or treated), n (%)	10/23 (43)	9/13 (69)
Bronchopulmonary dysplasia, n (%)	11/23 (48)	11/13 (85)
Patent ductus arteriosus, n (%)	17/25 (68)	9/13 (69)
Intraventricular hemorrhage	13/25 (52)	5/13 (38)
Any, n (%)	13/25 (52)	5/13 (38)
Severe (grade 3 or 4), n (%)	4/25 (16)	1/13 (8)
Sepsis, n (%)	11/25 (44)	5/13 (38)
Cryptorchidism at discharge[a], n (%)		1/12 (8)

[a]Data missing for 1 infant.

For the determination of postnatal androgens, a total of 175 plasma samples were collected from the first day of life until postnatal week 11, as illustrated in Fig. 1C. The time of the first sample (day 0, n = 10), ranged from 0 to 8 hours after birth. The last serial samples were collected at postnatal weeks 8-11, corresponding to a postmenstrual age of 32-38 weeks (Fig. 1C).

Preterm Infants Show Elevated Androgens in the Neonatal Period

In cord blood, the median (25th-75th percentiles) concentration of testosterone was 0.15 (0.06-0.32) ng/mL (Fig. 2A), and DHT was 0.014 (0.001-0.035) ng/mL (Fig. 2B). However, a large number of the DHT measurements (40%) were below the LLOQ of the analytical method.

Temporal changes in androgen concentrations in the infants were found to follow postnatal age rather than postmenstrual age (not shown). We thus chose to analyze the results according to postnatal age (Fig. 2). Although there was a large between-infant variability, both testosterone and DHT displayed clear temporal changes in concentration during the first week of life. Concentrations on the first day after birth of both testosterone (4.27 [2.17-6.66] ng/mL) and DHT (0.299 [0.155-0.557] ng/mL) were significantly higher than those in cord blood ($P < .001$ for both). The postnatal

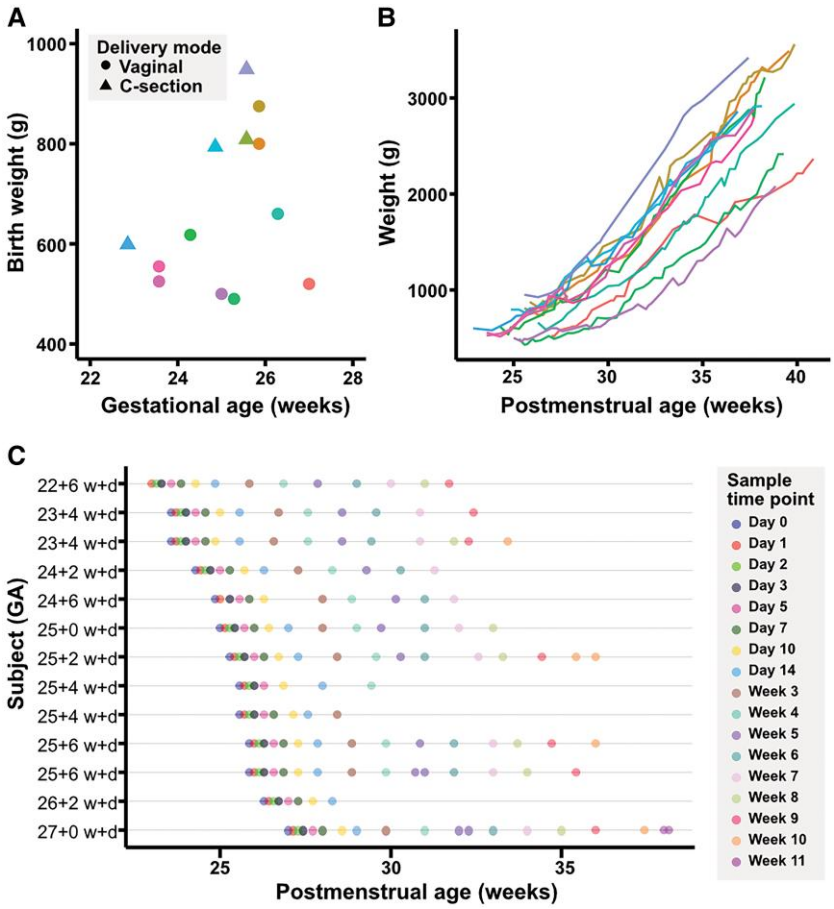

Figure 1. Anthropometrics and plasma sampling scheme. (A) Infant gestational age at birth plotted against birth weight and labeled according to delivery mode. (B) Infant weight gain over postmenstrual age. The same color coding of subjects is used in A and B. (C) Time of collection of plasma samples used for steroid profiling from each subject according to postmenstrual age and color-coded accordingly to postnatal sampling time point as indicated in the legend. Infants are ordered by gestational age at birth as labeled on the y-axis.

testosterone concentration declined rapidly during the first days of life to the lowest concentration of 0.52 (0.31-1.37) ng/mL on day 5. Thereafter, testosterone levels remained relatively constant with a tendency to increase towards the end of the study period. A similar temporal pattern was observed for DHT, with a concentration of 0.117 (0.043-0.211) ng/mL on day 5. Levels of testosterone were on average 7-fold higher than DHT throughout the study period and the 2 androgens were strongly correlated (Figs. S1 and S2 [42]). Furthermore, GA was positively correlated with the levels of both testosterone and DHT on the first day after birth (rho = 0.63, $P = .028$ and rho = 0.61, $P = .036$), respectively. There was also a significant interaction between GA and the association between testosterone and DHT (Figs. S1B, S1C, and S2 [42]), where the more mature infants (>25 weeks GA) compared with the more immature infants (22-24 weeks GA) showed a higher testosterone to DHT ratio. However, overall, there were similar patterns in postnatal levels of testosterone and DHT levels in the 2 GA groups (Fig. S3 [42]).

Postnatal Gonadotropins Follow Similar Patterns as the Androgens

To further study androgen regulation and pituitary gland function in preterm infants, we determined levels of FSH and LH. Serial samples for this analysis were available from 12 out of 13 infants in the postnatal cohort. Similar postnatal patterns were observed for the gonadotropins and the androgens: FSH levels declined in the first days after birth, from 2.0 (1.3-4.6) IU/L to 0.7 (0.5-0.9) IU/L between day 0 and 5, then maintained at low levels, although there was a large individual variation (Fig. 2C). LH levels also declined after birth, from 2.1 (0.9-24.2) IU/L to 0.2 (0.1-0.3) IU/L between days 0 and 5 (Fig. 2D). LH levels then remained low throughout the maintaining study period with many measurements close to the LLOQ of the assay. Levels of both FSH and LH were significantly positively related to testosterone across the study period (Fig. S3 [42]).

SHBG Increases With Postnatal Age and Lowers Bioavailable Testosterone

To assess androgen bioavailability, we determined SHBG levels. The concentration of SHBG was 13.6 (9.8-17.9) nmol/L in cord blood (Fig. 3) compared with 11.3 (7.5-20.1) nmol/L on the first day after birth, with no significant difference between the 2 ($P = .86$). After birth, the SHBG concentration steadily increased and was about 10-fold higher at week 10 (104.0 [46.0-205.9] nmol/L) than on day 0. Compared with SHBG in cord blood of term infants, preterm SHBG levels were similar at birth but higher during later postnatal ages as concentrations increased over time.

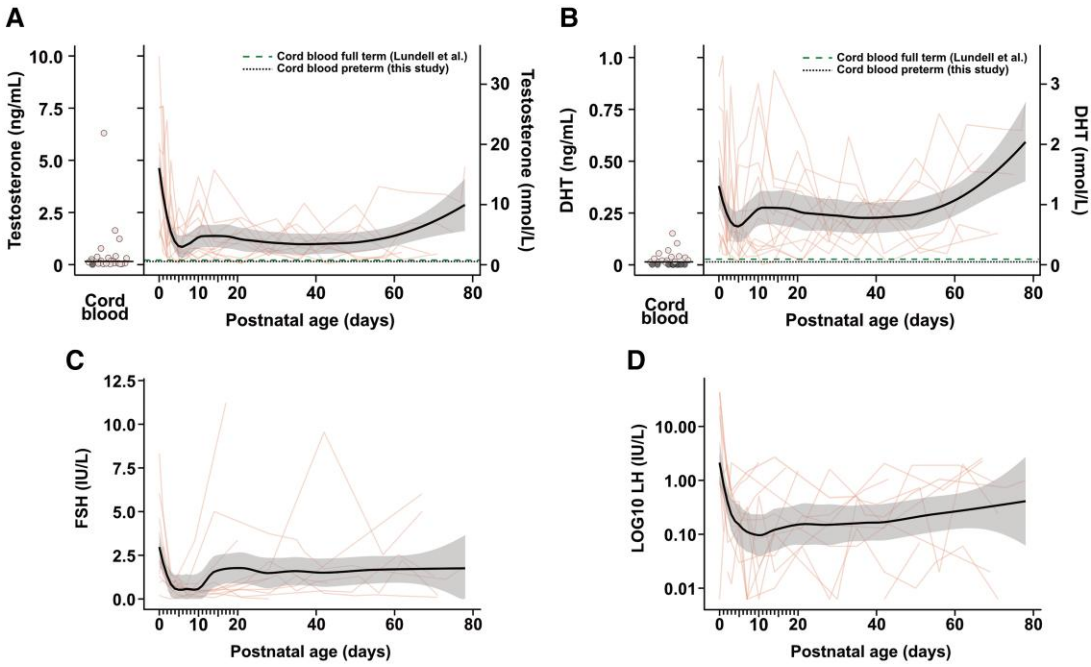

Figure 2. Cord blood (n = 25) and postnatal (n = 12-13) concentrations of androgens and gonadotropins in preterm male infants. (A) testosterone; (B) dihydrotestosterone (DHT); (C) follicle-stimulating hormone (FSH); and (D) luteinizing hormone (LH). In cord blood, circles represent individual measurements, with gray-filled circles indicating samples with concentrations below the lower limit of quantification (LLOQ), which are set to half of the LLOQ. The lines in cord blood samples show medians. For postnatal levels, the thick black lines show locally estimated scatterplot smoothing with 95% CI (shaded area). Longitudinal individual levels are shown in red lines. Cord blood medians at mean 25 weeks of gestational age from this study are indicated with dotted black lines and cord blood medians from term infants at mean 40 weeks of gestational age are indicated with dashed green lines (testosterone = 216 pg/mL and DHT = 28 pg/mL). The term references are from Lundell et al [39], measured in the same lab using the same method as for the preterm samples. These reference lines represent expected in utero concentrations during the corresponding time of a normal pregnancy.

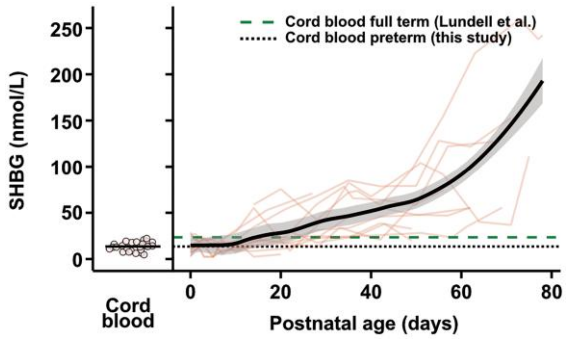

Figure 3. Cord blood (n = 25) and postnatal (n = 13) concentrations of SHBG in preterm male infants. In cord blood, circles represent individual measurements, and the line shows the median concentration. For postnatal levels, the thick black line shows locally estimated scatterplot smoothing with 95% CI (shaded area). Longitudinal individual levels are shown in red lines. The cord blood median at mean 25 weeks of gestational age from this study are indicated with dotted black lines and the cord blood median from term infants at mean 40 weeks of gestational age are indicated with dashed green lines (23.4 nmol/L). The term reference is from Lundell et al [39].

The calculated fraction of bioavailable testosterone decreased over postnatal age with increasing levels of SHBG (Fig. 4A). On the first day, around 65% of the total plasma testosterone was biologically active but decreased to around 15% at postnatal week 10, corresponding to median 0.27 ng/mL bioavailable testosterone on the first day vs 0.13 ng/mL at week 10 (ie, a 20-fold reduction) (Fig. 4B). Therefore, although total testosterone tended to increase with postnatal age (see Fig. 2A), this did not translate to more bioactive testosterone, as a larger fraction was inhibited by SHBG binding.

Discussion

In this study, we followed the changes in the levels of testosterone and DHT as well as their associated gonadotropins during the first months of life in extremely preterm male infants. Androgen levels were higher throughout the postnatal period than those in cord blood. Importantly, in preterm infants, the loss of the maternal control of the HPG axis after birth results in an androgen pulse that is otherwise biologically programmed to take place at a much later developmental stage.

In adults, paired measurements of androgens in plasma and cerebrospinal fluid have shown a high correlation between the 2 compartments [43], indicating that blood levels are a good proxy for brain exposure. This suggests that androgen levels in preterm infant serum, as measured in this study, reflect the exposure to the immature brain.

We found significantly higher testosterone on the first day of life than in cord blood, with values after birth in the range of adult males. This agrees with observations from preterm and term males, but not females, showing a dramatic increase in blood testosterone immediately after birth [19, 44, 45]. This rapid rise in testosterone is preceded by pulsatile secretion of LH in both preterm and term newborn males [18]. A testosterone surge in the first few hours after birth is evident in many mammalian species [19]. In rodents, this has been shown to contribute to brain masculinization with permanent effects on behavior and physiology [46-48]. Our data show that the postnatal testosterone peak occurs approximately 15 weeks earlier for extremely preterm infants than for an

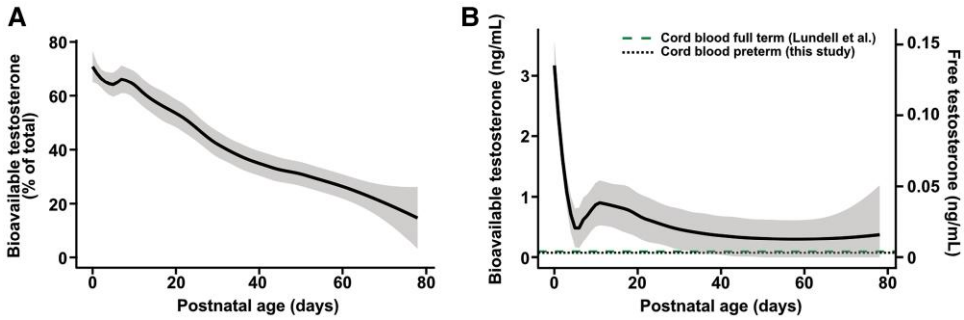

Figure 4. Postnatal plasma bioavailable testosterone in preterm male infants (n = 13). (A) fraction of bioavailable testosterone of total testosterone; (B) bioavailable testosterone (left y-axis) and free testosterone (right y-axis). Bioavailable testosterone and free testosterone concentrations were calculated from total quantified testosterone as described elsewhere [37]. The thick black lines show locally estimated scatterplot smoothing with 95% CI (shaded area). Reference lines in B are calculated from the concentrations of testosterone in cord blood and SHBG on day 0, and cord blood levels of testosterone and SHBG from term infants reported by Lundell et al [39] (97 pg/mL).

infant following a normal pregnancy (ie, at a stage when the immature brain undergoes rapid growth and development). Whether this has lasting effects on brain circuitry and neurodevelopment in the extremely preterm male warrants further studies.

Over the whole study period, the median postnatal levels of testosterone and DHT were approximately 6-fold higher than previously reported in cord blood of term infants using the same analytical method [31]. Thus, not only is there a peak in androgens just after birth, but levels appear to be elevated for several months compared with the corresponding in utero period.

Androgen levels indicative of a minipuberty have been observed in moderately/late preterm males [29, 30, 49], and some studies found this androgen peak to be more pronounced and prolonged in very/moderately preterm compared with term infants [27, 28, 50]. We did not observe any increase in the androgens reflecting a minipuberty, although there was a trend towards elevated androgen levels beyond 60 days. Thus, our results suggest that the postnatal androgen surge is delayed and/or less pronounced in extremely preterm males when it comes to postnatal age. Analysis over an extended time is needed to determine the timing and extent of the minipuberty in the extremely preterm population.

Blood SHBG followed infant postnatal age with increasing levels throughout the study period. This translates into a reduced fraction of bioavailable testosterone over time. In the second and third months of infant life, bioavailable testosterone was in the same range as would be expected in utero. The increase in SHBG may thereby, to some extent, dampen the effect of an early activation of the HPG axis. A postnatal increase in SHBG as reported here in preterm infants is also present in term infants males and has been suggested to render the peak testosterone activity in the first month of life biologically less significant [22].

Testosterone, LH, and FSH show similar postnatal patterns in male infants [20] and data from animal studies support that LH contributes to the neonatal testosterone surge (reviewed in [46]). As for testosterone, very/moderately preterm infants have been found to display higher FSH and LH at 1 to 3 months when compared with term infants [28, 30, 50]. Reference ranges for FSH and LH in extremely preterm males in the first 6 weeks of life [51] are similar to concentrations reported here. We found a rapid decline and a nadir of FSH and LH at around 1 week of age, and then there was a small trend towards increased levels, although levels were below those at day 0 throughout the study period. Levels of these hormones thus mirror the androgens and support the data indicating that there are no clear signs of a minipuberty in the first 10 weeks of life in this population.

Baker et al compared neonatal plasma testosterone levels between preterm infants diagnosed with cryptorchidism at 18 months' corrected age and control infants with normal testicular descent [52]. They found that infants with cryptorchidism failed to exhibit a normal rise in testosterone after birth and displayed a diminished testosterone surge in the second month of life. In our study, only 1 infant was diagnosed with cryptorchidism, limiting our ability to explore relationships between this condition and androgens.

Extremely preterm infants may also receive plasma transfusions in the neonatal period. Only plasma from adult males is used for neonatal transfusions. Thus, the androgen concentration in donor plasma is often higher than that in infant plasma, thereby further increasing the androgen load on the recipient preterm infant [35].

Speculatively, strategies to limit the impact of the high androgen exposure in preterm infants could include androgen receptor antagonist treatment in the first week of life or estradiol replacement therapy to recreate this aspect of the in utero environment. The latter has been investigated in a randomized controlled pilot study providing postnatal estradiol together with progesterone to extremely preterm infants, but only in females [53]. In a rabbit model of preterm birth, estrogen treatment promoted normal neuronal maturation [54, 55]. However, in baboons born preterm, no differences in investigated brain parameters were found between estradiol-treated and control animals [56].

Strengths and Limitations

Among the strengths of this study are repeated measurements of blood samples from the same subjects, steroid quantification using mass spectrometry with high sensitivity and specificity, and determination of SHBG to evaluate the fraction of bioavailable androgens. Many previous studies of postnatal androgens in moderately or very preterm infants are based on immunoassays, which may overestimate concentrations [57]. This study also has limitations. First, the study population was small, and infants included for the assessment of longitudinal hormone levels were selected based on the

availability of routine clinical samples collected in long series. Thus, these infants represent critically ill children with long stays in the neonatal intensive care unit and may not be representative of the whole extremely preterm population. Secondly, these samples were kept for up to 5 days at room temperature before further processing, although most samples were retrieved within 2 days. However, storage of whole blood samples at room temperature for up to 1 week before analysis was reported not to affect testosterone concentrations [58], and plasma LH and FSH were reported stable for at least 8 days at 20 °C [59]. Finally, in our measurement of cord blood androgens, we did not discriminate between venous and arterial blood, although testosterone concentrations are reported to be higher in the cord artery, both in preterm and term infants [44]. Although preanalytical errors are potential shortcomings of our study, it is indeed difficult to obtain the corresponding data from samples collected under ideal conditions. In preterm infants, sampling-related blood loss is associated with increased risks for morbidity [60, 61], making repeating blood draws for research purposes unethical. Likewise, extensive blood sampling from healthy term infants is not ethically justifiable, and we could thus only relate our results to cord blood from term infants.

Conclusions

Taken together, our results reinforce the view that preterm infant males, particularly those born extremely preterm, display an untimely reactivation of the HPG axis during a critical developmental period. It is plausible that the resulting testosterone surge immediately after birth has lasting effects on neurodevelopment. Further, the data suggest that the postnatal androgen peak during minipuberty is delayed or quiescent in this population and that increasing SHBG lowers the fraction of bioavailable testosterone, thereby potentially counteracting some of the effects of elevated androgens. Future studies should be aimed at determining the development of androgens over extended time in extremely preterm infants and seek potential impacts on the developing immature brain.

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Author Contributions

A.K.N., C.O., and A.H. developed the concept and design of the study. A.L. performed the steroid analysis and US analyzed gonadotropins and SHBG. A.K.N. performed primary analyses of the data and drafted the manuscript. A.P. contributed to statistical analyses. All authors contributed to data interpretation, critically revised, and approved the final manuscript.

Disclosures

The authors have nothing to disclose.

Data Availability

The datasets generated and/or analyzed during the current study are not publicly available due to ethical permits and The General Data Protection Regulation (GDPR) Regulation (EU) 2016/679 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data law regulates the availability of personal data, but de-identified data are available from the corresponding author on reasonable request.

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