Increased Activity of the Hypothalamic-Pituitary-Testicular Axis in Infancy Results in Increased Androgen Action in Premature Boys

Tanja Kuiri-Hänninen, Raija Seuri, Erja Tyrväinen, Ursula Turpeinen, Esa Hämäläinen, Ulf-Håkan Stenman, Leo Dunkel,* and Ulla Sankilampi*

Department of Pediatrics (T.K.-H., L.D., U.S.), University of Eastern Finland and Kuopio University Hospital, and Department of Clinical Radiology (R.S., E.T.), Kuopio University Hospital, FI-70211, Kuopio, Finland; and Department of Clinical Chemistry (U.T., E.H., U.-H.S.), Helsinki University Central Hospital, FI-00029, Helsinki, Finland

Context: Transient activation of the hypothalamic-pituitary-gonadal (HPG) axis is observed in boys during the first months of life. Previous research suggests increased HPG axis activation in premature infants, but the physiological significance of this has not been studied.

Objective: The objective of this study was to evaluate the differences in reproductive hormone levels and their biological effects between full-term (FT) and preterm (PT) infant boys.

Study Design and Participants: Twenty-five FT and 25 PT (gestational age 24.7–36.6 wk) boys were recruited at birth and followed up monthly from 1 wk to 6 months of age (d 7, months 1–6). Nineteen FT and 20 PT boys were reexamined at 14 months of age.

Main Outcome Measures: Urinary gonadotropins and testosterone were measured in serial urine samples and compared with testicular and penile growth. Urinary prostate-specific antigen was measured as an androgen biomarker.

Results: LH and testosterone levels were higher in PT boys ($P < 0.001$ for both) than FT boys. Compared with FT boys, FSH levels were lower at d 7 ($P = 0.002$) but higher from month 1 to month 3 ($P = 0.002–0.030$) in PT boys. This was associated with significantly faster testicular and penile growth in PT boys compared with FT boys. Transient increase in the prostate-specific antigen levels in both groups indicated androgen action in the prostate.

Conclusions: Postnatal HPG axis activation in infancy is increased in PT boys and associated with faster testicular and penile growth compared with FT boys. Possible long-term consequences of hyperandrogenism in PT infant boys warrant further research. (***J Clin Endocrinol Metab*** **96: 98–105, 2011)**

An important phase in normal male development takes place during the first postnatal months, when the hypothalamic-pituitary-gonadal (HPG) axis is transiently activated with elevated levels of gonadotropins and testosterone (T) (1–5). Although this activation was first described almost 40 yr ago, the mechanism and consequences of it are not properly understood. It seems that this event plays a role in gonadal development (6–12) and may be important in the establishment of future reproductive capacity. Furthermore, the activity may involve programming of social and sexual behavior (13). However, because SHBG levels rise concomitantly with T levels, leading to low levels of free T, it has been suggested that androgens secreted at this time are not biologically active (14).

Postnatal HPG axis activation is also observed in premature (PT) infants, and there is some evidence of even

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-1359 Received June 15, 2010. Accepted August 26, 2010.
First Published Online September 29, 2010
* L.D. and U.S. contributed equally to this work.

Abbreviations: AUC, Area under a curve; CV, coefficient of variation; FT, full term; HPG, hypothalamic-pituitary-gonadal; PSA, prostate-specific antigen; PT, preterm; SGA, small for gestational age; T, testosterone.

higher gonadotropin and T levels in them compared with full term (FT) infants (2, 15–17). The reason for this is unknown, but immaturity of the hypothalamic feedback mechanisms has been suggested. The possible significance and consequences of the stronger postnatal HPG axis activation in PT boys compared with FT boys have not been studied so far. Increased activation could be necessary to ensure the normal development of reproductive organs in PT boys, such as finalizing the testicular descent. However, in view of perinatal programming theory, hyperandrogenism during a critical developmental phase may lead to permanently altered programming of not only the HPG axis but also of other androgen sensitive organs as well.

The aims of this study were, first, to examine the differences of HPG axis activation longitudinally in infancy between FT and PT boys, and second, to evaluate its possible biological effects. Consequently, a monthly follow-up of 25 FT and 25 PT boys from birth to 6 months of age was conducted. Reproductive hormones in infancy were compared with measurements of penile and testicular growth. Urinary prostate-specific antigen (PSA) measurements were used for assessing androgen action.

Subjects and Methods

Subjects and study design

FT (n = 25) and PT (n = 25) newborn boys were recruited between August 2006 and March 2008 at the Kuopio University Hospital after informed consent was obtained from their parents. The first clinical examination was performed at 1 wk of age (d 7), and thereafter monthly until 6 months of age (months 1–6). The infants were reexamined at the corrected age of 14 months (month 14). The Ethics Committee of the Pohjois-Savo Health Care District approved the study.

The characteristics of the infants are shown in Table 1. Of the PT boys, 11 (44%) were born at less than 32 + 0 gestational weeks. Seven FT boys (28%) and 11 PT boys (44%) were born small for gestational age (SGA) [defined as birth weight and/or length ≤ -2 SD according to the Finnish birth size reference (18)]. Neither the number of SGA infants ($P = 0.377$) nor the mean birth weight ($P = 0.271$) or birth length in SD scores ($P = 1.0$) were significantly different between groups. Two sets of identical twins were included in the PT group.

Clinical measurements

At each examination from d 7 to month 14, length, weight, penile length, and testicular volume were measured and testicular position was determined.

The recumbent length was measured by infantometer (Holtain Ltd., Crymmych, Pembs, UK) to the nearest 0.1 cm. Recumbent weight was measured with a baby scale (model 727; Seca, Hamburg, Germany) to the nearest 0.005 kg. The testicular position was determined as previously described (19).

The flaccid, nonstreched penile length was measured with a ruler to the nearest 0.1 cm. In addition, the length of the corpus cavernosum was determined sonographically by using a 7.5-MHz linear transducer probe as previously described (20).

The length and width of the testes were measured sonographically in a single longitudinal plane. The epididymis was not included in the measurements. Testicular volume was counted by using the formula of an ellipsoid: length $\times$ width^2 $\times$ $\pi/6$.

All auxological measurements were repeated three times and the mean was used in the analyses. In the case of testicular volume, the mean of left and right testicle was used. All manual measurements were performed by a single observer (T.K.-H.), and three pediatric radiologists performed the sonographical measurements. All measurements were done without recollection of previous measurements of the infant.

Urine samples and assays

Spot urinary samples were collected with plastic urine collection bags or with clean catch at every follow-up visit. Gonadotropins and T were measured from d 7 to month 6. PSA levels were determined until month 14. Urine analysis was chosen instead of serum because urine can be obtained noninvasively enabling the frequent sampling in infants. The success rate in obtaining urine samples was 96.1%, so 374 urine samples were collected.

All urinary analytics were corrected for creatinine to adjust the results for urine concentration. Urinary creatinine was analyzed by an enzymatic method before the urine was stored in −70 C.

Urinary LH and FSH levels were quantitated with a sensitive time-resolved immunofluorometric assay (AutoDELFIA; Wallac, Turku, Finland) adapted for measurements of urinary samples (21). The detection limit of the LH assay is 0.05 IU/liter and the interassay coefficient of variation (CV) is less than 4% in the concentration range of 0.3–42 IU/liter. For FSH the detection

TABLE 1. Birth characteristics of FT and PT infants in the study

	Full term (n = 25)		Preterm (n = 25)	
	Median/n	Range	Median/n	Range
Maternal age (yr)	29.3	19.0–40.9	30.5	20.8–40.9
Mode of delivery				
Vaginal	21		8	
Elective cesarean section	3		7	
Emergency cesarean section	1		10	
Gestational age (wk)	39.9	37.3–42.1	32.4	24.7–36.6
Birth length (cm)	50.0	42.0–53.0	42.5	30.0–48.0
Birth weight (kg)	3.500	1.910–4.420	1.660	0.550–2.850
Apgar points at 5 min	9	9–10	9	7–10

limit is 0.05 IU/liter and the interassay CV less than 5% in the concentration range 2–78 IU/liter.

Urinary T assay

Urinary T was measured with HPLC-tandem mass spectrometry. Lyophilized β-glucuronidase type VII-A from *Escherichia coli* (25,000 U; Sigma, St. Louis, MO) was dissolved in 5 ml water. Urine, 250 μl, was diluted with 1 ml of 30.0 g/liter (0.25 mol/liter) sodium phosphate buffer (pH 6.9). Then 50 μl of enzyme (250 U) and 30 μl of internal standard containing 29.1 μg/liter (0.1 μmol/liter) deuterated T (D_2-T; RIVM, Bilthoven, The Netherlands) were added. Samples were incubated at 37 C for 22 h. Then 200 μl of 10% potassium carbonate was added to stop the enzymatic reaction, followed by 5 ml of diethyl ether. After mixing for 3 min and centrifugation, the upper layer was collected and evaporated to dryness under nitrogen. The residue was dissolved in 250 μl of 50% methanol. Calibrators containing 250 μl of 0.0576–28.81 ng/ml (0.2–100 nmol/liter) of T (Fluka, Buchs, Switzerland) and 30 μl of internal standard were prepared in 50% methanol. Twenty-five microliters of sample extracts and calibrators were analyzed on an HPLC-tandem mass spectrometry system equipped with an API 2000 or API 3000 triple quadrupole mass spectrometer (PE Sciex, Foster City, CA) as described earlier (22).

Urinary total and free PSA were analyzed by the Wallac prostatus PSA free/total Autodelfia assay (PerkinElmer-Wallac, Turku, Finland). The detection limit for total PSA was 0.1 and 0.01 μg/liter for free PSA. In the concentration range of 0.5–50 μg/liter, the intraassay CVs were 4.3–18.8% for total PSA and 4.1–7.8% for free PSA (23). The results of total and free urinary PSA were very similar ($n = 341$, $\rho = 0.834$, $P < 0.001$), but because the detection limit of the assay for free PSA was 10-fold lower than that for total PSA, data for free urinary PSA concentrations are used.

Statistical analyses

All results of urinary analytics that were under the detection limit of the assays were artificially set to zero because setting these results on the detection limit would have falsely elevated or decreased the values after creatinine correction. To avoid zero values from dropping out when data transformation was needed, the raw value plus one was used as a variable.

Between- and within-group comparisons were done by using mixed models analysis. The group, time point, mode of delivery, and birth weight and length in SD scores were included in the model as fixed effects and subject and twinness as random effects. Birth size was included in the model because being born SGA could affect hormone levels (2). In the case of sonographical measurements, the interobserver variation was taken into account by including the observer as a fixed factor in the analysis. In the analyses of the effect of the length of gestation on hormone levels, the length of gestation and birth weight and length in SD scores were included in the model as fixed effects and subject and twinness as random effects. The hormonal and PSA data were right skewed and transformed to achieve normality of the residuals in the mixed models analysis.

A summary measure to describe the average hormonal concentration from d 7 to month 6 was calculated to enable the use of correlation analysis between hormone levels and testicular and penile growth. Consequently, an average level for each hormone in each infant was defined by calculating the area under a curve (AUC) with trapezoid rule and dividing it by total time. Those for whom either the first or the last measurement, or the expected peak value at month 1 was missing, were excluded from AUC calculations. Consequently, average hormone levels were calculated for 24 FT and 21 PT boys. The associations of average hormone levels with each other and with testicular and penile growth percentages were tested using Spearman's correlation.

SPSS software version 17.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. *P* values less than 0.05 were considered statistically significant.

Results

Timing and magnitude of urinary gonadotropin and T levels

LH, FSH, and T levels in both groups from d 7 to month 6 are presented in Fig, 1. In PT boys, LH and T levels increased from d 7 to peak at month 1 ($P < 0.0001$). In FT

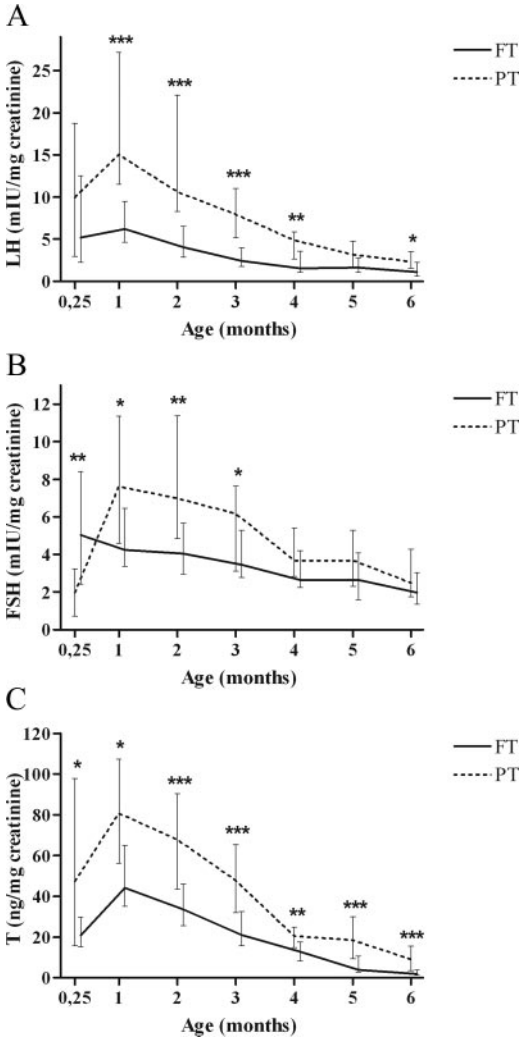

FIG. 1. Median levels with quartiles of urinary LH (A), FSH (B), and T (C) in FT and PT boys during the first 6 months of life. The *asterisks* indicate the statistical significance for the difference between groups in mixed model analysis after adjustment for birth length and birth weight in SD scores (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). To convert into SI units, the following conversion factors should be used: for gonadotropins 1 mIU/mg creatinine = 0.113 IU/mmol creatinine and for T 1 ng/mg creatinine = 0.393 nmol/mmol creatinine.

boys, there was no difference in LH levels between d 7 and month 1, but T levels increased to peak at month 1 ($P < 0.0001$). After month 1, LH and T levels declined significantly in both groups ($P < 0.0001$), but a significant difference between groups was still observed at month 6, with higher LH ($P = 0.017$) and T levels ($P < 0.0001$) in PT than FT boys. In FT boys, peak FSH levels were observed already at d 7, whereas in PT boys a significant increase occurred from d 7 to month 1 ($P < 0.0001$).

When the time period from d 7 to month 6 was evaluated as a whole, total LH and T levels were higher in PT boys ($P < 0.0001$ for both) than in FT boys. Total FSH levels from d 7 to month 6 did not differ between groups ($P = 0.12$), although the levels were significantly higher in PT boys than FT boys from month 1 to month 3 ($P = 0.001–0.021$). The peak median LH levels were 2.4-fold higher, FSH levels 1.5-fold higher, and T levels 1.8-fold higher in PT boys compared with FT boys.

Effect of developmental age on hormone levels

Hormone curves of each study subject are presented according to developmental age in Fig. 2, A–C. When hormone results were compared between FT and PT boys according to developmental (postterm) age, LH ($P = 0.0002$) and T ($P = 0.001$) levels were significantly higher in PT boys than FT boys at 0–30 d of postterm age. In FSH levels there was no difference between groups.

Because it was noted that the highest hormone levels were observed in the most premature boys, the effect of the length of gestation, *i.e.* the maturity of the child, on the magnitude of the postnatal HPG axis activation was evaluated in the whole group. In mixed-models analysis, length of gestation was significantly inversely associated with the levels of all three hormones ($P < 0.0001$ for LH and T and $P = 0.003$ for FSH). The estimated mean levels of LH, FSH, and T during the first 6 months of life for boys born at 24th, 28th, 32nd, and 36th gestational weeks are presented as multipliers of the levels of those born at term in Fig. 2D.

Testicular and penile growth and their association with urinary hormone levels

Undescended testis (uni- or bilateral) was observed at birth in one FT SGA boy and 11 (44%) PT boys, but all had spontaneous descent by month 2.

Testicular and penile growth in both groups from d 7 to month 14 is presented in Fig. 3.

Testicular volume increased significantly in both groups during the first 6 months of life ($P < 0.0001$ for both), and in both groups monthly increments were largest during the first months of life. From month 6 to month 14, a significant decrease in testicular volume was observed in both groups ($P = 0.001$ in FT and $P = 0.003$ in PT boys). Although the initial difference between groups was significant, testicular growth was faster in PT boys, resulting in similar testicular volumes in both groups before the age of 6 months.

A significant increase also was seen in penile and corpus cavernosum length between d 7 and month 6 ($P < 0.0001$ for both in both groups). Similarly as testicular growth, penile growth was faster in PT boys, and a significant difference between groups was not observed after month 2.

The maximal testicular and penile growth percentages correlated positively with the average levels of both gonadotropins and T (the Spearman correlation coefficients and significance levels are shown in Table 2).

Urinary PSA

Measurable PSA was detected in all but one of the 50 boys studied. There was considerable variation in the timing of the individual peak PSA levels in both groups, but the peak group median levels were observed at month 2 in FT boys and a month later in PT boys. In FT boys, the free PSA levels increased significantly from d 7 to peak at month 2 ($P = 0.001$) and decreased thereafter to very low/unmeasurable level

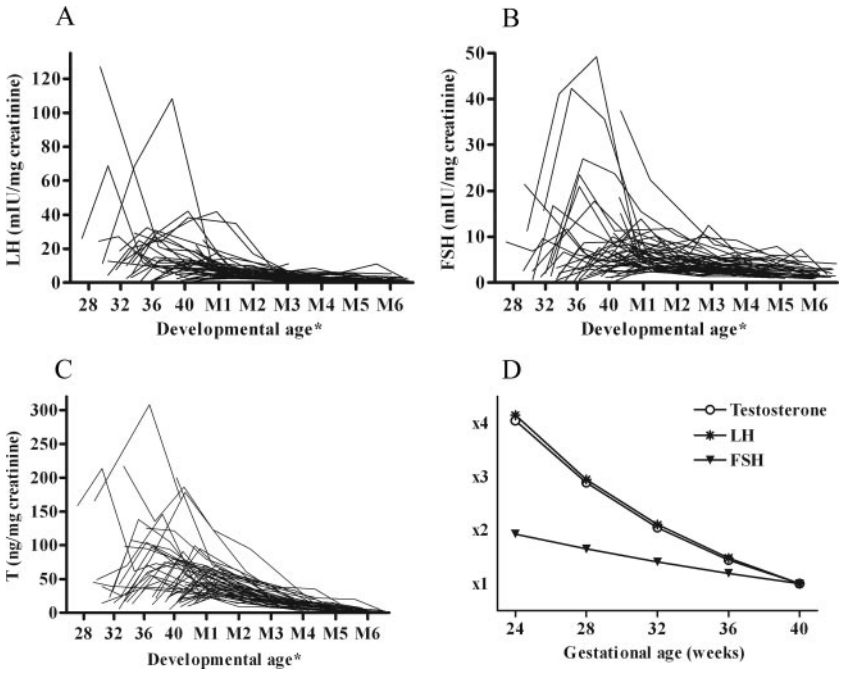

FIG. 2. A–C, Individual variation of LH, FSH, and T levels during the postnatal HPG axis activation according to developmental age. *, Gestational weeks until 40, thereafter postterm age in months. D, Mean T, LH, and FSH levels during the first 6 months of life according to gestational age at birth. The levels are expressed as multipliers of levels of infants born at 40th gestational week according to a linear mixed model after adjustment for birth length and weight in SD scores.

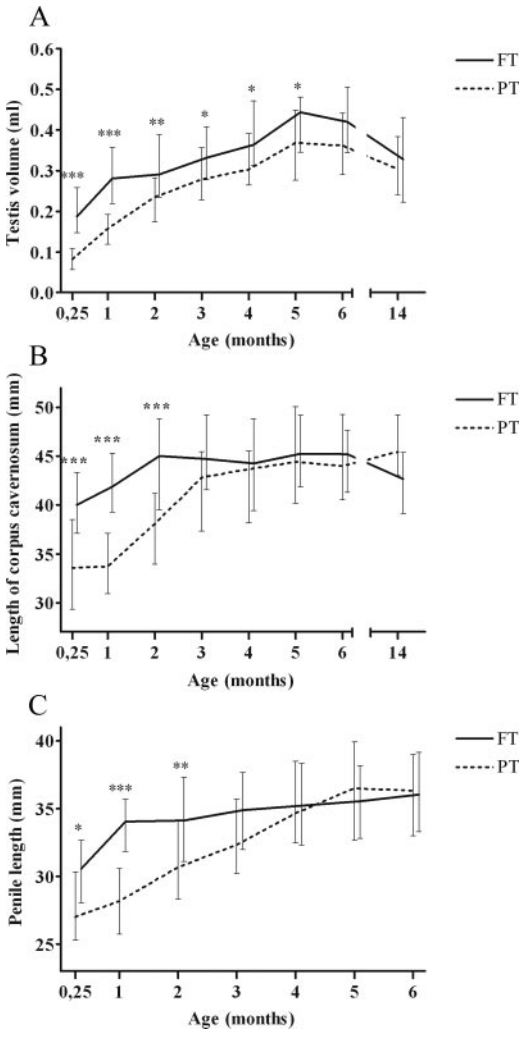

FIG. 3. Median values with quartiles for testicular (A, sonographical measurement), corpus cavernosum (B, sonographical measurement), and penile (C, manual, unstreched measurement with a ruler) growth in FT and PT boys during the first 6 months of life and for sonographical measurements at corrected age of 14 months. The *asterisks* indicate the statistical significance for the difference between groups in mixed model analysis after adjustment for birth length and birth weight in SD scores (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

by month 5 ($P = 0.00001$, Fig. 4). In PT boys, the PSA level began to increase after month 1 with a peak at month 3 ($P = 0.0002$). The total free PSA secretion from d 7 to month 6 did not differ between groups ($P = 0.3$). Whereas the PSA levels were very low from month 4 to month 14 in FT boys, in PT boys PSA levels at months 4–6 were still above the initial level but declined significantly from month 6 to month 14 ($P = 0.0002$). There was a weak, but statistically significant, positive correlation between the free PSA levels and the T levels ($\rho = 0.265$, $P < 0.00001$) in the same urine sample.

Discussion

This longitudinal study with frequent follow-ups allowed us to delineate the temporal relationships in hormone levels and their biological effects during the first 6 months of life in a unique way in FT and PT boys. We found significantly higher LH and T levels in PT boys compared with FT boys during the postnatal HPG axis activation. Previously, prematurity has been connected with significantly higher gonadotropin levels in girls but not boys (15–17). Although the initial FSH levels were higher in FT boys, from month 1 to month 3 PT boys had significantly higher FSH levels as well. Whereas a significant increase was observed from d 7 to month 1 in gonadotropin levels in PT boys, in FT infants the levels were high already at d 7 and no subsequent rise was observed in accordance with previous results in FT boys (24).

T levels increased significantly from the initial levels in both groups and peaked simultaneously at month 1. This peak is earlier than reported in previous studies on serum levels, which display the peak T levels at 1–3 months of age (16, 25, 26). This is probably explained by the longitudinal design of the present study because the significant interindividual variation in hormone levels and the timing of the hormone peak are aspects that cannot be properly taken into account in cross-sectional studies. Our present results confirm the previous observations in cross-sectional studies (2, 16) of higher T levels in PT boys compared with FT boys. Regardless of developmental age at birth, the HPG axis seems to activate soon after birth, and peak T levels are seen 1 month after birth. The magnitude of the HPG axis activation appears to depend on the developmental age, so that the highest activity is seen in the most premature ones. The negative association of all three hormones and developmental age at birth suggest maturation of the hypothalamic feedback mechanisms as term approaches.

We found a significant positive correlation between urinary T levels and penile growth, and between urinary FSH levels and testicular growth, probably reflecting the proliferation of Sertoli cells in seminiferous tubules (27). The faster growth of testes and penis in PT boys during the first months of life led to catch-up in testicular size and penile length earlier than catch-up in height or weight in comparison with FT boys (data for height and weight not shown). The effect of androgen action during postnatal gonadal activation was further demonstrated by the appearance of measurable PSA levels in urine after the T peak and later, disappearance of PSA along with waning T levels.

The measured total urinary T (including the glucuronidated and the free form) might not solely reflect the testicular T production. Although the testes are the main source of T during the first months of life, significant amounts of T are formed also by the adrenal glands in early infancy (28). T might also be synthesized by peripheral conversion of androgen precursors such as dehydroepi-

TABLE 2. Spearman correlation (95% confidence intervals in parentheses) between average hormone levels in urine (assigned as AUC values of monthly samples from 1 wk to 6 months of age and divided by total time) and maximum testicular and penile growth percentages during the first 6 months of life (n = 45)

	LH	FSH	T
LH		$\rho = 0.56$ (0.32–0.73) $P = 0.00006$	$\rho = 0.53$ (0.28–0.71) $P = 0.0002$
FSH			$\rho = 0.26$ (−0.04 to 0.51) $P = 0.089$
Maximum testicular growth percentage	$\rho = 0.48$ (0.22–0.68) $P = 0.001$	$\rho = 0.38$ (0.1–0.61) $P = 0.010$	$\rho = 0.31$ (0.02–0.56) $P = 0.036$
Maximum penile growth percentage	$\rho = 0.54$ (0.29−0.72) $P = 0.0001$	$\rho = 0.46$ (0.20–0.67) $P = 0.001$	$\rho = 0.47$ (0.21–0.67) $P = 0.001$

androsterone sulfate, which is secreted in significant amounts by the involuting fetal adrenal cortex during the immediate postpartum period in FT infants and possibly even for a longer period in PT infants (29). Therefore, the total urinary T reported here is rather an estimate of overall androgen production and exposure during the first months of life. However, the significant positive correlation between urinary LH and T levels in our data supports the notion that most of the T detected is of gonadal origin. Urinary gonadotropins reliably reflect the levels in serum (30).

Postnatal HPG axis activation has been suggested to play a role in completing the genital development because poor phallic growth and involution of the scrotum has been described in hypogonadal male infants (6). The descent of the testis is essential for its normal functioning, and the last, inguinoscrotal, phase of the descent is normally completed during the last trimester of pregnancy and is androgen dependent. Consequently, cryptorchidism is often observed in PT newborn boys (31). In this regard, higher LH and T levels observed in PT boys could be needed to complete the testicular descent. The pattern

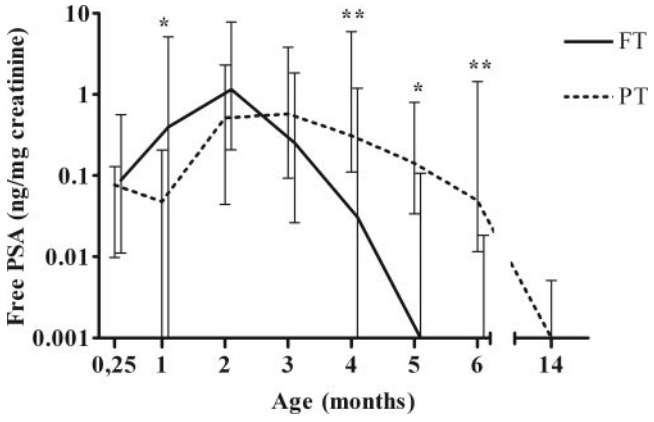

FIG. 4. Median levels with quartiles of urinary free PSA in FT and PT boys during the postnatal hypothalamic-pituitary-gonadal axis activation. The *asterisks* indicate the statistical significance for the difference between groups in mixed-model analysis after adjustment for birth length and birth weight in SD scores. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). To convert into SI units the following conversion factor should be used: 1 ng/mg creatinine = 113.1 ng/mmol creatinine.

of testicular growth in FT boys in our study was similar to that reported for FT infant boys by Kuijper *et al.* (32) with increase in volume until 5 months of age and decrease thereafter. In addition, the testicular volumes observed in FT boys in our study were very close to those reported by Kuijper *et al.* Compared with the testicular volumes in a large cohort of Finnish boys (33), the volumes in our study were in the same range at birth, but larger at 3 months of age. Whether this is due to our smaller sample size or an actual difference is not clear. In the same study, a positive correlation between testicular volume and inhibin B levels, but not FSH levels, was observed. In that study, the hormonal measurements were obtained at a single time point at 3 months of age, when, according to our present results, the levels of gonadotropins and T are already clearly declining. Our finding of the positive correlation of T levels and penile growth in infancy supports the results of a large cohort of FT boys (34) and extends this finding to PT boys as well.

PSA secretion in prostate epithelial cells is androgen dependent, and PSA levels begin to rise in puberty (35–37). Previous data on PSA secretion in infancy are scarce. Positive PSA staining in prostatic tissue has been reported in infants less than 6 months old (38). Sato *et al.* (37) analyzed serial urinary samples of six infants until the age of 18 wk and found measurable PSA in some samples. In our material, measurable PSA was detected at least once during the first 6 months of life in all but one of the 50 boys studied. Our findings show that PSA levels are transiently elevated during the postnatal HPG axis activation both in FT and PT boys, indicating that androgens exert their biological action in the prostate at this age. The reason for the later onset of urinary PSA secretion seen in PT infants is not clear but could be explained by developmentally regulated androgen responsiveness of prostatic tissue in PT boys. This might also explain why PSA levels in PT boys were not higher than in FT boys because the peak T levels had already passed before the onset of marked PSA secretion in PT infants. In addition, the smaller prostate

size in PT boys compared with FT boys might explain the lower PSA levels in the PT group.

The incidence of premature birth is rising worldwide, and owing to improved care, even extremely premature infants survive nowadays (39). The accumulating data show that the sequelae of prematurity, such as insulin resistance leading to risk of cardiovascular diseases, persist until adulthood (40). Less is known about the effects of prematurity on reproductive capacity. Recently the first large-scale longitudinal study on that issue showed lower reproductive rate in men and women born prematurely (41). The reason for this is not yet fully understood, and no difference has been found in gonadal function between young adults born PT or FT determined by the levels of anti-Mullerian hormone, inhibin B, gonadotropins, or T (42). Although the possible significance of stronger postnatal HPG axis activation of the PT boys for their future reproductive function can only be speculated about based on the present results, it could be the prerequisite for the normal gonadal function seen in PT boys as young adults (42).

Our present results of significantly higher androgen levels in PT boys compared with FT boys in early infancy raise a question about the possible organizational and programming effects of hyperandrogenism in PT infants. According to the perinatal programming theory, early life conditions might have long-lasting physiological effects (43), so the role of hyperandrogenism during such a critical developmental phase remains to be seen. Through programming, hyperandrogenism in infancy could influence the characteristics of growth, body composition, fat distribution, blood pressure, and lipid and glucose metabolism, thereby contributing to risk factors of many chronic diseases. In addition, androgens have organizational effects in the brain (44) and abnormally high T levels might affect neural and behavioral development in PT boys.

In conclusion, we found increased postnatal HPG axis activation in PT boys that was associated with faster testicular and penile growth than in FT boys. Urinary PSA was detected in both FT and PT infants, indicating androgen action in the prostate. Although stronger HPG axis activation could be beneficial in the reproductive development of PT boys, hyperandrogenism might have long-term disadvantageous effects, too. Further studies with long-term follow-up are needed to establish the exact significance of the stronger activation of the HPG axis seen in PT infants.

Acknowledgments

We are grateful for research nurse Anneli Paloranta for skillful assistance during the data collection and Olavi Kiekara, M.D., for expert sonographical work. Vesa Kiviniemi, Ph.D., is acknowledged for skillful statistical assistance.

Address all correspondence and requests for reprints to: Leo Dunkel, Department of Pediatrics, University of Eastern Finland and Kuopio University Hospital, P.O. Box 1777, FI-70211 Kuopio, Finland. E-mail: leo.dunkel@kuh.fi.

This work was supported by Kuopio University Hospital, the Pediatric Research Foundation, the National Graduate School of Clinical Investigation, the Emil Aaltonen Foundation, the Jalmari and Rauha Ahokas Foundation, the Sigrid Jusélius Foundation, and the Academy of Finland.

Disclosure Summary: The authors have no conflict of interests.

References

1. **Forest MG, Cathiard AM, Bertrand JA** 1973 Evidence of testicular activity in early infancy. J Clin Endocrinol Metab 37:148–151
2. **Forest MG, de Peretti E, Bertrand J** 1980 Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared with that of full-term infants. J Steroid Biochem 12:25–36
3. **Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI** 1975 Pituitary-gonadal relations in infancy. I. patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. J Clin Endocrinol Metab 40:545–551
4. **Winter JS, Hughes IA, Reyes FI, Faiman C** 1976 Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. J Clin Endocrinol Metab 42:679–686
5. **Schmidt H, Schwarz HP** 2000 Serum concentrations of LH and FSH in the healthy newborn. Eur J Endocrinol 143:213–215
6. **Main KM, Schmidt IM, Skakkebaek NE** 2000 A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. J Clin Endocrinol Metab 85:4905–4907
7. **Sharpe RM, Fraser HM, Brougham MF, McKinnell C, Morris KD, Kelnar CJ, Wallace WH, Walker M** 2003 Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. Hum Reprod 18:2110–2117
8. **Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE** 1998 Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metab 83:675–681
9. **Bay K, Virtanen HE, Hartung S, Ivell R, Main KM, Skakkebaek NE, Andersson AM, Toppari J, Nordic Cryptorchidism Study Group** 2007 Insulin-like factor 3 levels in cord blood and serum from children: Effects of age, postnatal hypothalamic-pituitary-gonadal axis activation, and cryptorchidism. J Clin Endocrinol Metab 92:4020–4027
10. **Müller J, Skakkebaek NE** 1984 Fluctuations in the number of germ cells during the late foetal and early postnatal periods in boys. Acta Endocrinol (Copenh) 105:271–274
11. **Cortes D, Müller J, Skakkebaek NE** 1987 Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. Int J Androl 10:589–596
12. **Codesal J, Regadera J, Nistal M, Regadera-Sejas J, Paniagua R** 1990 Involution of human fetal Leydig cells. an immunohistochemical, ultrastructural and quantitative study. J Anat 172:103–114
13. **Mann DR, Fraser HM** 1996 The neonatal period: a critical interval in male primate development. J Endocrinol 149:191–197
14. **Bolton NJ, Tapanainen J, Koivisto M, Vihko R** 1989 Circulating sex

hormone-binding globulin and testosterone in newborns and infants. Clin Endocrinol (Oxf) 31:201–207
15. **Greaves RF, Hunt RW, Chiriano AS, Zacharin MR** 2008 Luteinizing hormone and follicle-stimulating hormone levels in extreme prematurity: development of reference intervals. Pediatrics 121:e574–e580
16. **Tapanainen J, Koivisto M, Vihko R, Huhtaniemi I** 1981 Enhanced activity of the pituitary-gonadal axis in premature human infants. J Clin Endocrinol Metab 52:235–238
17. **Shinkawa O, Furuhashi N, Fukaya T, Suzuki M, Kono H, Tachibana Y** 1983 Changes of serum gonadotropin levels and sex differences in premature and mature infant during neonatal life. J Clin Endocrinol Metab 56:1327–1331
18. **Pihkala J, Hakala T, Voutilainen P, Raivio K** 1989 Characteristic of recent fetal growth curves in Finland. Duodecim 105:1540–1546
19. **Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, Skakkebaek NE, Toppari J** 2004 Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363:1264–1269
20. **Smith DP, Rickman C, Jerkins GR** 1995 Ultrasound evaluation of normal penile (corporeal) length in children. J Urol 154(2 Pt 2): 822–824
21. **Demir A, Alfthan H, Stenman UH, Voutilainen R** 1994 A clinically useful method for detecting gonadotropins in children: assessment of luteinizing hormone and follicle-stimulating hormone from urine as an alternative to serum by ultrasensitive time-resolved immunofluorometric assays. Pediatr Res 36:221–226
22. **Turpeinen U, Linko S, Itkonen O, Hämäläinen E** 2008 Determination of testosterone in serum by liquid chromatography-tandem mass spectrometry. Scand J Clin Lab Invest 68:50–57
23. **Mitrunen K, Pettersson K, Piironen T, Björk T, Lilja H, Lövgren T** 1995 Dual-label one-step immunoassay for simultaneous measurement of free and total prostate-specific antigen concentrations and ratios in serum. Clin Chem 41(8 Pt 1):1115–1120
24. **Bergadá I, Milani C, Bedecarrás P, Andreone L, Ropelato MG, Gottlieb S, Bergadá C, Campo S, Rey RA** 2006 Time course of the serum gonadotropin surge, inhibins, and anti-mullerian hormone in normal newborn males during the first month of life. J Clin Endocrinol Metab 91:4092–4098
25. **Forest MG, Sizonenko PC, Cathiard AM, Bertrand J** 1974 Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. J Clin Invest 53: 819–828
26. **Tomlinson C, Macintyre H, Dorrian CA, Ahmed SF, Wallace AM** 2004 Testosterone measurements in early infancy. Arch Dis Child Fetal Neonatal Ed 89:F558–F559
27. **Chemes HE, Chemes HE** 2001 Infancy is not a quiescent period of testicular development. Int J Androl 24:2–7
28. **Bidlingmaier F, Dörr HG, Eisenmenger W, Kuhnle U, Knorr D** 1986 Contribution of the adrenal gland to the production of androstenedione and testosterone during the first two years of life. J Clin Endocrinol Metab 62:331–335
29. **Heckmann M, Hartmann MF, Kampschulte B, Gack H, Bödeker RH, Gortner L, Wudy SA** 2006 Persistent high activity of the fetal adrenal cortex in preterm infants: is there a clinical significance? J Pediatr Endocrinol Metab 19:1303–1312
30. **Demir A, Dunkel L, Stenman UH, Voutilainen R** 1995 Age-related course of urinary gonadotropins in children. J Clin Endocrinol Metab 80:1457–1460
31. **Damgaard IN, Jensen TK, Petersen JH, Skakkebaek NE, Toppari J, Main KM, Nordic Cryptorchidism Study Group** 2008 Risk factors for congenital cryptorchidism in a prospective birth cohort study. PLoS One 3:e3051
32. **Kuijper EA, van Kooten J, Verbeke JI, van Rooijen M, Lambalk CB** 2008 Ultrasonographically measured testicular volumes in 0- to 6-year-old boys. Hum Reprod 23:792–796
33. **Main KM, Toppari J, Suomi AM, Kaleva M, Chellakooty M, Schmidt IM, Virtanen HE, Boisen KA, Kai CM, Damgaard IN, Skakkebaek NE** 2006 Larger testes and higher inhibin B levels in finnish than in danish newborn boys. J Clin Endocrinol Metab 91: 2732–2737
34. **Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, Damgaard IN, Kai CM, Chellakooty M, Skakkebaek NE, Toppari J, Main KM** 2006 Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. Eur J Endocrinol 154:125–129
35. **Antoniou A, Papanastasiou P, Stephanidis A, Diamandis E, Androulakakis PA** 2004 Assessment of serum prostate specific antigen in childhood. BJU Int 93:838–840
36. **Randell EW, Diamandis EP, Ellis G** 1996 Serum prostate-specific antigen measured in children from birth to age 18 years. Clin Chem 42:420–423
37. **Sato I, Yoshikawa A, Fugimoto M, Shimizu K, Ishiwari A, Mukai T, Iwamoto T** 2007 Urinary prostate-specific antigen is a noninvasive indicator of sexual development in male children. J Androl 28:150–154; discussion 155–157
38. **Goldfarb DA, Stein BS, Shamszadeh M, Petersen RO** 1986 Age-related changes in tissue levels of prostatic acid phosphatase and prostate specific antigen. J Urol 136:1266–1269
39. **Yeaney NK, Murdoch EM, Lees CC** 2009 The extremely premature neonate: anticipating and managing care. BMJ 338:b2325
40. **Hovi P, Andersson S, Eriksson JG, Järvenpää AL, Strang-Karlsson S, Mäkitie O, Kajantie E** 2007 Glucose regulation in young adults with very low birth weight. N Engl J Med 356:2053–2063
41. **Swamy GK, Ostbye T, Skjaerven R** 2008 Association of preterm birth with long-term survival, reproduction, and next-generation preterm birth. JAMA 299:1429–1436
42. **Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC** 2009 Influence of preterm birth and birth size on gonadal function in young men. J Clin Endocrinol Metab 94: 4243–4250
43. **Gluckman PD, Hanson MA, Cooper C, Thornburg KL** 2008 Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359:61–73
44. **Hines M** 2008 Early androgen influences on human neural and behavioural development. Early Hum Dev 84:805–807