Transient Postnatal Gonadal Activation and Growth Velocity in Infancy

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ABSTRACT

BACKGROUND AND OBJECTIVE: Transient activation of the hypothalamic-pituitary-gonadal axis with a sex steroid surge is observed in boys and girls during the first months of life. However, the role of sex steroids in the regulation of growth has not been substantiated in infancy. We tested the hypothesis that testosterone (T) surge, known to be higher in infant boys than in girls during the transient postnatal gonadal activation regulates linear growth in infants.

METHODS: To characterize in detail the linear growth velocity (GV) differences between genders in the normal population in early infancy, we evaluated growth of 18,570 healthy infants (51.0% boys) with 162,003 height measurements from birth to 12 months of age. GV was monitored and compared with serially measured urinary T and estradiol levels and serum insulin-like growth factor 1 levels in 84 healthy infants (45% boys) during the first 6 months of life.

RESULTS: GV was significantly faster from birth to 6 months of age in boys than in girls (P ≤ .01). The greatest GV difference, 4.1 cm per year, was observed at 1 month of age, simultaneously with the peak of postnatal gonadal activation. In the mixed model analysis, GV showed a significant positive association with T in both genders (parameter estimate up to 0.62, 95% confidence interval 0.44–0.81).

CONCLUSIONS: These results provide a new insight into the regulation of growth in infants and elucidate a novel biological role of the transient postnatal gonadal activation in growth regulation.

WHAT’S KNOWN ON THIS SUBJECT: A transient sex steroid surge occurs postnatally in both infant boys and girls, but whether this “minipuberty” has effects on linear growth, analogously to the puberty, remains unclear.

WHAT THIS STUDY ADDS: There is a discernible growth rate difference in infancy, with boys growing faster than girls. A positive association between testosterone level during the first months of life and growth rate was observed, independently of the growth hormone/insulin-like growth factor 1 axis.
Linear growth is mediated by the proliferative activity of chondrocytes in the growth plates and regulated by many hormones, with growth hormone (GH) and insulin-like growth factor 1 (IGF-1) being the most important regulators. Elevated sex steroid levels stimulate growth both by enhancing GH and IGF-1 synthesis and by direct effects in the growth plate. The first, transient, postnatal activation of the hypothalamic-pituitary-gonadal (HPG) axis occurs shortly after birth during the first months of life. In infant boys and girls, the HPG axis activation in infancy is involved in the regulation of linear growth. Our aim was, first, to characterize normative linear growth during the first year of life in detail in a large population-based cohort of healthy infants to be able to observe possible subtle and transient gender-specific phases of infantile growth; and second, to verify our hypothesis and evaluate the role of sex steroids in infant growth, a prospective observational study with serial sex steroid measurements was conducted.

METHODS

Subjects and Measurements

For detailed characterization of the normative growth in infancy, growth data of 18,570 healthy full-term infants (cohort 1; 51% boys) were analyzed from birth to 12 months. These infants were a subcohort of a larger child population from the city of Espoo, previously used in the generation of the new Finnish growth references. In brief, these infants were born from full-term singleton pregnancies, did not have intrauterine growth failure or exposure to maternal smoking, and did not have disorders or take medications that could potentially affect growth. Growth data were obtained from the electronic patient records of free-of-charge well-baby clinic visits that monitor the growth and well-being from infancy to childhood in Finland. During the first year of life, children have 10 scheduled visits: at 2 weeks of age and at 1, 2, 3, 4, 5, 6, 8, 10, and 12 months of age.

To assess the potential role of HPG axis activation in modulating growth in infancy, a cohort of healthy term and near-term newborn infants (cohort 2; n = 84; 45% boys, mean gestational age [SD] 38.0 [2.7] weeks; mean birth weight 2914 [819] g) was recruited at the Kuopio University Hospital, Finland, between August 2006 and March 2008. The infants were measured at 1 week of age (D7) and thereafter monthly until 6 months of age (M1–M6). In both cohorts, length was measured at birth and in the primary care or study visits to the nearest 0.1 cm by using standardized techniques and calibrated equipment described in the nationwide guidelines by the Finnish National Institute for Health and Welfare. Birth size in SD units was determined according to a contemporary Finnish birth size reference.

The Ethics Committee of the local Health Care District approved the study. The study was conducted according to the principles of the Declaration of Helsinki, and written informed consent was obtained from the parents of the subjects participating in the prospective study. Permission for the use of anonymized growth data of Cohort 1 was obtained from the city of Espoo Municipality Institutional Review Board.

Urine and Blood Sampling and Quantitative Hormonal Assays

In the 84 infants participating in the prospective part of the study, a spot urinary sample was collected with a plastic urine collection bag or with clean catch for sex steroid analyses at every visit from D7 to M6. At the D7 and M3 visits, a venous blood sample was drawn for the measurement of serum IGF-1 concentration.

Testosterone (T) and estradiol (E2) levels were measured in the urinary samples. To adjust the results to urine concentration, measured urinary T and E2 levels were corrected for urinary creatinine. An enzymatic method was used to analyze creatinine concentration before the urine was deep-frozen at −72 °C and stored for the later analyses of T and E2, which were measured by high performance liquid chromatography tandem mass spectrometry as described earlier. All results from T and E2 analyses under the detection limit of the assay were set to zero to avoid these results to falsely affect the statistical analyses.

Serum IGF-1 concentration was measured using an enzyme-linked immunosorbent assay method (Mediagnost IGF-1 Elisa cat E20, Mediagnost GmbH, Reutlingen, Germany). The within-run CV% was
Growth Modeling and Statistical Analyses

Linear growth in infancy occurs in an episodic manner, and GV shows major changes during the first months of life. In cohort 1 with 18,570 infants, the periodicity of infantile growth was smoothed based on the large number of subjects. Linear GV (cm/year) was calculated between height measurements of the consecutive scheduled visits by dividing the change in length by the time interval between the measurements. In cohort 1, a total of 162,003 height measurements were made from birth to the 12-month visit, but because of a lack of height data at certain points, it was possible to determine only 123,655 GVs. Because repeated observations from the same individuals were included, a linear mixed model was used to evaluate differences in mean GVs between genders at different time points. In the model, patient code identified multiple measurements between scheduled visits at different time points. GV was the dependent variable; the time point of the measurement, infant gender, and interaction between sex and measurement point were included as fixed factors.

To account for the periodicity of growth in the prospectively collected cohort 2 with 84 infants, the linear growth was modeled individually by fitting a second-degree polynomial function to the observed length data, where age was plotted on the x-axis and length on the y-axis. The fitting procedure was done by using the R software (R Foundation for Statistical Computing, Vienna, Austria). The derivative of the growth model function was used to determine the individual GVs from 1 to 6 months of age. Differences in GVs, T, E2, and IGF-1 levels between genders were analyzed using the linear mixed model with birth weight and length SDs as the fixed effects and twinness (ie, family code) as a random effect. The t test was used for comparison of correlation coefficients between boys and girls, and 2-sided P values are presented. To evaluate association between GV and hormonal factors independently from confounding factors, 2 linear mixed models were used with different combination of fixed effects. Model 1 included GV as the dependent variable and T and E2 levels (M1–M6) and serum IGF-1 level (M3) as explanatory variables. Model 2 was supplemented with birth weight and length SDs and gestational age at birth as explanatory variables. Urinary T and E2 levels were not normally distributed, and therefore, to achieve normality of the residuals in the linear mixed model, a decadic logarithmic transformation was used. To avoid zero values from dropping out, a constant value of 10 was added to the creatinine corrected T and E2 values before the logarithmic transformation. T, E2, IGF-1, and gestational age values were standardized in each time point to produce parameter estimates from the linear mixed model that are equivalent to partial correlation coefficients. Twinness was included in both models as a random factor to account for interdependencies between twins.

All statistical analyses were performed with IBM SPSS Statistics, Version 19 (IBM Corp, Armonk, NY).

RESULTS

Gender Differences in Linear Growth

GV of 9468 boys and 9102 girls from birth to 12 months is presented in Fig 1A. GV decreases after birth in both genders. In early infancy, until age 6 months, GV in girls was significantly slower than in boys (P < .01 for 2 weeks of age, P < .001 for M1–M5, P = .01 at M6). The GV difference between genders reached its peak at 1 month of age, when GV in boys was 4.1 cm per year faster than in girls (Fig 1B).

Growth velocities during the first months of life in 84 infants with hormonal measurements (cohort 2) were in line with the normative growth pattern in cohort 1. Also in this small cohort, there was a statistically significant difference in GV between gender, with GV being greater in boys at ages 1, 2, and 3 months (M1: P = .010, M2: P = .009, and M3: P = .018; data not shown).
During the first 5 months of life, a significant positive correlation was observed between T level and concurrent GV (Table 1). The strongest correlation was seen at M2 (Spearman ρ = 0.63, P < .001). The slope of the fit line was similar in both genders at the overlapping area of T levels, indicating similar relationships between T exposure and GV (difference between boys and girls statistically nonsignificant, P = .18; Fig 2). At M2, variation in T level attributed to 40% of variance of GV (48% in girls and 24% in boys; Table 1). From M2 to M5, the correlation between T and GV did not differ significantly between genders (P from 0.13 to 0.82; Table 1). At M1, the correlation between T and GV was significantly stronger in girls (P = .03), whereas at M6, the correlation was stronger in boys (P = .008).

To evaluate the possible independent association of T and concurrent GV, 2 linear mixed models were constructed (Table 2). In model 1, including T, E2, and IGF-1 levels, a significant positive association between T and GV was observed from M1 to M5 (parameter estimates 0.34–0.62, P < .05). The relationship between T and GV remained significant after including IGF-1 level in model 1 at M3 (parameter estimate 0.51, P < .001). Significant negative correlations were observed between both T level or GV and gestational age at birth and birth size (birth weight or length SDs). Therefore, model 2 was complemented with gestational age at birth, birth weight and length SDs, in addition to sex steroids and IGF-1. In model 2, the association between T and GV from M1 to M3 remained significant (parameter estimates 0.21–0.36, P < .05; Table 2). According to both models, the strongest association between T and GV was observed at M2. In addition to T level, IGF1 was positively associated with GV at M3 (in model 1, parameter estimate 0.30, P < .001; in Model 2, 0.28, P < .001). E2 level was not associated in GV at any time point.

**DISCUSSION**

We conducted a longitudinal cohort study to clarify 2 questions. First, we characterized in detail the magnitude and timing of sexual dimorphism in human growth during the first
months of life. Second, we looked for evidence for the transient postnatal HPG activation ("minipuberty") being involved in the regulation of linear growth during infancy.

Our study confirmed earlier observations that boys grow faster than girls in infancy during the first 6 months of life. Maximal difference in GV between genders was seen during the first 2 months of life. The difference in GV between genders is of the same magnitude as in puberty, that is, GV in infant boys is up to 4 cm per year faster than in girls. According to Finnish growth standards, the gender difference in GV during the first 6 months of life results in a 1.8-cm difference in median length between genders at 6 months of age (girls 67.6 cm, boys 69.4 cm), and this difference remains almost constant until age 12 months (girls 75.7 cm; boys 77.4 cm).13

Our longitudinal study, using serial samples, confirmed the earlier observations of a T peak during the first months of life. E2 surge was seen only in girls. We have previously shown that the postnatal sex steroid surge has biological effects on hormone-sensitive target tissues,5,16 including the prostate,24 which is consistent with the observed higher prostate-specific antigen rise from the prostate and growth in penile length.5

In contrast to elevated postnatal T levels in both genders, E2 surge was seen only in girls. We have previously shown that the postnatal sex steroid surge has biological effects on hormone-sensitive target tissues,5,16 including the prostate,24 which is consistent with the observed higher prostate-specific antigen rise from the prostate and growth in penile length.5

### Table 2: Association of Hormone Levels With GV in 84 Infant Boys and Girls During the Transient Postnatal Gonadal Activation From 1 to 6 Months of Age in 2 Linear Mixed Models

<table>
<thead>
<tr>
<th>Time</th>
<th>Model 1 factors</th>
<th>Model 2 factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>1 Month</td>
<td>T</td>
<td>0.51 (0.33 to 0.69)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>0.13 (–0.05 to 0.32)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>—</td>
</tr>
<tr>
<td>2 Months</td>
<td>T</td>
<td>0.82 (0.44 to 0.81)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>0.06 (–0.10 to 0.25)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>0.30 (0.14 to 0.46)</td>
</tr>
<tr>
<td>3 Months</td>
<td>T</td>
<td>0.51 (0.51 to 0.71)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>–0.16 (0.19 to 0.28)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>—</td>
</tr>
<tr>
<td>4 Months</td>
<td>T</td>
<td>0.40 (0.20 to 0.61)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>–0.14 (0.18 to 0.29)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>0.30 (0.14 to 0.46)</td>
</tr>
<tr>
<td>5 Months</td>
<td>T</td>
<td>0.34 (0.10 to 0.56)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>–0.14 (0.18 to 0.29)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>—</td>
</tr>
<tr>
<td>6 Months</td>
<td>T</td>
<td>0.08 (–0.14 to 0.32)</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>–0.24 (–0.24 to 0.20)</td>
</tr>
<tr>
<td></td>
<td>IGFB-1</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are expressed as parameter estimates and 95% confidence intervals (CIs). GA, gestational age. — indicates data not available.

a SDS, SD score, according to Finnish growth reference for birth size.11

Birth length SDS values are calculated as parameter estimates and 95% confidence intervals (CIs). GA, gestational age. — indicates data not available.

Birth weight SDS values are calculated as parameter estimates and 95% confidence intervals (CIs). GA, gestational age. — indicates data not available.

Birth weight SDS values are calculated as parameter estimates and 95% confidence intervals (CIs). GA, gestational age. — indicates data not available.
associated with androgen-mediated cutaneous manifestations, such as sebaceous gland hypertrophy and acne in both infant girls and boys.\(^1,8\) In girls, the subtle E2 surge in infancy is associated with the growth of E2 sensitive tissues, such as the uterus and mammary glands.\(^16\) Taken together, these data indicate that the transient sex steroid surge during minipuberty has biological effects on sensitive target tissues expressing androgen receptor (AR) and estrogen receptors. The current study shows evidence, for the first time, that the growth plates of long bones are also sensitive and influenced by the postnatal sex steroid surge.

The timing of the maximal GV difference between genders at 1 to 2 months of age observed in the present data matches perfectly with our observations of the timing of maximum postnatal HPG axis activation.\(^5\) This finding indirectly supports our hypothesis that differences in sex steroid levels contribute to differences in GV between infant boys and girls. Direct support for the role of sex steroids on infantile growth was provided by the positive associations between postnatal T levels with GV during the first months of life. In general, the strongest correlation was seen at 2 months of age, when variation of measured T level explained as much as 40% of the variance of GV. Even though boys had higher T levels than girls during the minipuberty, the relationship between T levels and GV seems to be aligned in the partially overlapping areas of T levels in boys and girls. This probably indicates that both sexes may respond similarly to T exposure during infancy. This is also supported by the observation that from M2 to M5, the strength of the correlation between T and GV did not differ significantly between boys and girls. However, we observed a stronger correlation in girls at M1 and in boys at M6. These temporal differences between genders might be due to differences in timing of the androgen surge during the first months of life or to type 1 error in our relatively small study cohort.

After birth, the majority of length gain is achieved in the metaphyses of long bones through proliferation, differentiation, hypertrophy, and subsequent calcification of the growth plate chondrocytes.\(^19,20\) During infancy, GH is the primary regulator of circulating IGF-1, and GH and IGF-1 are the 2 key hormones regulating linear growth. Sex steroids influence growth through the GH-IGF-1 system but probably have direct effects on the growth plate as well via AR and especially estrogen receptors.\(^21\) In the growth plate of both girls and boys, C19 androgens such as T are aromatized into estrogens, which are probably more important than androgens of the regulation of growth. Thus, the measurable T level is probably not a good surrogate of estrogen exposure in peripheral tissues such as the growth plate. In addition to the T level indicating availability of aromatizable androgens, gonadal estrogen production in girls (with much lower circulating levels than T) and the local aromatase activity in the growth plate, among other factors, contribute to the observed differences in GV.

Data from various experiments have provided some additional insight on the role of sex steroids in linear growth in infancy. Some,\(^22,23\) but not all,\(^24\) studies have shown increased birth size and a lack of typical sexual dimorphism in birth weight in patients with congenital adrenal hyperplasia exposed to androgen excess already in utero. Infants with a delayed diagnosis of this disorder and continuous androgen excess after birth grow faster than healthy children during the first year of life,\(^22\) supporting a role for adrenal steroids in linear growth. Boys with congenital hypogonadotropic hypogonadism who completely lack the postnatal T surge show stunted linear growth during minipuberty.\(^25\) A temporary increase in GV has been reported in infants and prepubertal boys exposed to repeated doses of intramuscular T for treatment of micropenis.\(^26\) These findings further support the view that androgens have a potential to enhance growth, either directly or through aromatization, also during infancy.

The complete androgen insensitivity syndrome (CAIS) constitutes a model to study the role of estrogens and androgens in the regulation of linear growth. Individuals with CAIS have 46,XY genotype, and, despite normal or high T levels, absent androgen action due to abnormal AR. However, a growth spurt during puberty can be normal in CAIS.\(^27\) This suggests that pubertal growth is primarily mediated by estrogens, which can be originated from local aromatization of androgens. Our data show no connection with E2 and GV in infancy, but it is probable that the concentration of E2 in urine does not reflect the concentration in the growth plate. It has been suggested that patients with CAIS do not have a postnatal sex steroid surge, and therefore this condition does not serve as a model to study the role of sex steroids postnatally.\(^28\) CAIS also constitutes a model to assess the nonhormonal factors that contribute to linear growth. The nonhormonal factors include growth promoting genes of the Y chromosome that may influence longitudinal growth independently of sex steroids.\(^29\) The 46,XY CAIS women have mean adult heights intermediate between average females and males.\(^27\) The shorter adult height compared with healthy men may indicate the impaired androgen action due to mutations in the AR gene, whereas the taller height compared with healthy women implies androgen-independent effects of
genes in the Y chromosome. Thus, both hormonal and nonhormonal factors seem to contribute to these sex-specific patterns of linear growth.

The limitation of the study was that we did not have population-based urinary hormone data from the large population in which the sexually dimorphic growth was described. Instead, we tested the hypothesis in the cohort of 84 infants recruited for the prospective longitudinal study on the mechanisms, characteristics, and roles of minipuberty, the postnatal HPG axis activation. However, the sexually dimorphic growth pattern during the first months of life was visible also in the prospective cohort.

**CONCLUSIONS**

Results from this study clarify differences in GV between genders in infancy, when boys grow faster than girls until age 6 months. The maximum difference in GV was observed at 1 month of age, simultaneously with the maximum postnatal T surge of the transient gonadal activation. Furthermore, a positive association between GV and postnatal T surge was demonstrated. The positive association between T and GV in infancy is evident even after adjustments for multiple confounding factors. Thus, the study suggests a new role for the postnatal gonadal activation in the regulation of growth.

**ACKNOWLEDGMENTS**

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**ABBREVIATIONS**

AR: androgen receptor  
CAIS: complete androgen insensitivity syndrome  
E2: estradiol  
GH: growth hormone  
GV: growth velocity  
HPG: hypothalamic-pituitary-gonadal  
IGF-1: insulin-like growth factor 1  
T: testosterone

**REFERENCES**


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