Chapter 2

Urine gonadotropin and testosterone levels in male very-low-birth-weight infants

Miranda de Jong, Joost Rotteveel, Annemieke C. Heijboer, Anneke Cranendonk, Jos W.R. Twisk, Mirjam M. van Weissenbruch

ABSTRACT

Background/Aims
The postnatal activation of the hypothalamic-pituitary-gonadal axis is more exaggerated in preterm than in full-term born infants and may be important for reproductive function. Our objective was to investigate this activation of the hypothalamic-pituitary-gonadal axis in male very-low-birth-weight infants.

Methods
Twenty-one very-low-birth-weight boys (gestational age 26.0-30.0 weeks), participating in the NIRTURE trial, were included. Gonadotropin and testosterone levels were measured in serial urine samples collected at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months.

Results
Longitudinal analysis shows that after birth LH and FSH levels peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks) and decrease until 38 weeks postnatal age (corrected age of 6 months). Testosterone levels decrease with increasing age and this decrease is faster in infants receiving early insulin therapy.

Conclusions
Serial urine sampling for measurement of gonadotropin and testosterone levels provides accurate information about the postnatal activation of the hypothalamic-pituitary-gonadal axis in very-low-birth-weight boys. FSH and LH levels peak at 1 to 4 weeks of age. Insulin treatment causes faster decrease of testosterone levels.
**INTRODUCTION**

The postnatal activation of the hypothalamic-pituitary-gonadal axis that is observed during the first months of life, is considered as an important phase in the maturation of this axis. In boys, this activation has been shown to be associated to the development of the testes as well as the penis and the scrotum (1-5) and as a consequence the postnatal activation may be of importance for reproductive function in adult men.

The postnatal peak has been demonstrated in term as well as in preterm born boys (3, 6-17). In comparison with full-term boys, preterm born boys have higher levels of gonadotropins and testosterone during the first postnatal months (3, 10, 14). Data about the postnatal activity of the hypothalamic-pituitary-gonadal axis in preterm boys born at a gestational age less than 30 weeks are limited.

The pattern of hormone secretion in individual infants during this postnatal activation of the hypothalamic-pituitary-gonadal axis can accurately be described by serial hormone sampling. Most of the aforementioned studies in boys are cross-sectional and report serum levels of hormones. The use of urine samples makes it possible to collect serial measurements without the burden of frequent blood sampling and measurement of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in urine samples was previously shown to be reliable (18, 19).

The aim of the present study was to describe the postnatal activation of the hypothalamic-pituitary-testicular axis in male very-low-birth-weight (VLBW) infants by serial measurement of gonadotropin and testosterone levels in urine samples from birth to 9 months of age.

**METHODS**

**Study population**

The subjects were part of the Neonatal Insulin Replacement Therapy in Europe (NIRTURE) trial. This was an international multicenter randomized controlled trial investigating the role of early insulin therapy in VLBW infants (20). After informed parental consent was obtained, VLBW infants younger than 24 hours of age were randomized to receive continuous intravenous infusion of insulin for the first 7 days of life or standard neonatal care with insulin treatment only in case of hyperglycemia. Exclusion criteria included maternal diabetes and major congenital anomalies.

The 21 male infants participating in the NIRTURE trial in our neonatal intensive care unit were included in the present study. They had a mean gestational age of 28.2 weeks (range 26.0-30.0 weeks) and a mean birth weight of 1102 g (range 670-1460 g). Nine infants were assigned to the early-insulin group and 12 infants received standard neonatal care.
care. After discharge, all patients were followed in the outpatient clinic. Approval from the local ethics committee was obtained.

**Urine samples**

Urine samples were collected in a pediatric urine collection pouch at 1 and 4 weeks postnatal age, at 32 weeks postmenstrual age, at expected date of delivery and at the corrected age of 3 and 6 months. Samples were stored at -20 °C until analysis.

**FSH, LH and testosterone measurement**

FSH and LH concentrations were measured by immunometric assays (Architect, Abbott Laboratories Diagnostics Division, Abbott Park, Illinois, USA), as validated and described earlier (19). For FSH lower limit of quantitation is 0.1 IU/l, intra-assay coefficients of variation are 5% at a level of 0.3 IU/l, 3% at 1.8 IU/l, 5.5 IU/l, 25 IU/l and 75 IU/l and inter-assay coefficients of variation are 6% at 5 IU/l and 5% at 18 IU/l. For LH lower limit of quantitation is 0.1 IU/l, intra-assay coefficient of variation is < 4% at 0.2 IU/l, 1.5 IU/l, 5 IU/l, 40 IU/l and 75 IU/l and inter-assay coefficients of variation are 7% at 4 IU/l and 6% at 23 IU/l.

After adding a sodiumacetate buffer (pH 4.8) and deuterated internal standard (testosterone-2,2,4,6,6-D5; D5T; obtained from CDN Isotopes, Pointe-Claire, Quebec, Canada), samples were hydrolyzed with helix pomatia juice (Pall Biosepra, Cergy-Saint-Christophe, France). The supernatant was injected to a Symbiosis online Solid Phase Extraction (SPE) system (Spark Holland, Emmen, The Netherlands). Online SPE with C18 cartridges (Spark Holland) was performed for further purification of the samples. Separation was achieved on a C18 analytical column (Kinetex, 2.1x75 mm, 2.6 µm particle size; Phenomenex, Utrecht, The Netherlands) by gradient elution, using 0.1% formic acid in water and 0.1% formic acid in acetonitrile. A Quattro Premier XE tandem mass spectrometer (Waters Corporation, Milford, Massachusetts, USA) with electrospray in positive mode was used for detection. Operating conditions were as follows: capillary voltage, 1.0 kV; cone voltage, 40 V; source temperature, 130 °C. Argon was used as collision gas. For each component (analyte and internal standard), two transitions were monitored: m/z 289>97 and m/z 289>109 for testosterone; m/z 294>100 and m/z 294>113 for D5T. The first transitions in each set were used for quantification, the second transitions for confirmation. Data acquisition and processing were done with Masslynx 4.1 software (Waters Corporation). Total analysis time was 9 minutes. Linearity was tested by dilution between 3 and 43 nmol/l (R2 was 0.9992). Lower limit of quantitation was 1 nmol/l, intra-assay coefficient of variation for the whole range was < 7%. All analyses were performed in one run.

Gonadotropin and testosterone levels were corrected for creatinine levels. Creatinine concentrations were measured by the Jaffé method (Modular, Roche Diagnostics, Mannheim, Germany). Inter-assay coefficients of variation are 2.2% at 5.9 mmol/l and 1.7% at 12.5 mmol/l.
**Statistical analysis**

Statistical analyses were performed using the Statistical Package of Social Sciences software for Microsoft Windows version 17 (SPSS Inc., Chicago, Illinois, USA). At first FSH, LH and testosterone levels of infants treated with insulin were compared with hormone levels of infants receiving standard care. Because the distribution of hormone levels was skewed, a log transformation was performed before analysis. FSH, LH and testosterone levels were analyzed for postmenstrual age as well as for postnatal age, taking into account that both maturation and birth itself are important for activation of the hypothalamic-pituitary-gonadal axis. Longitudinal analyses were performed with generalized estimating equations, a method that takes into account that the repeated observations in one child are correlated. For gonadotropin levels below the limit of quantitation, a value of 0.01 U/l was used to discriminate these results from the values exactly on the limit of quantitation. Levels below the limit of quantitation were not corrected for creatinine. P values < 0.05 were considered as significant.

**RESULTS**

From our study population of 21 male VLBW infants, a total of 108 urine samples were collected for determination of LH, FSH and testosterone levels. At 1 week postnatal age, at expected date of delivery and at the corrected age of 3 months, urine samples were collected from all infants. At 4 weeks postnatal age, 32 weeks postmenstrual age and 6 months corrected age, samples were obtained from 19, 12 and 14 infants, respectively. In one sample collected at 6 months corrected age, only gonadotropin levels could be measured, because the volume was too small for all measurements.

**Levels of LH, FSH and testosterone for postmenstrual age**

The 108 urine samples were divided into six age groups, based on the postmenstrual age on the day of collection of the sample. The details of the age groups with mean postmenstrual age and number of samples as well as the median levels of FSH, LH and testosterone are shown in table 1. Because LH and FSH levels and patterns were not different between the early-insulin and standard care group, the groups were taken together for further analyses.

Figure 1 shows the median LH and FSH levels for increasing postmenstrual age. Longitudinal analysis showed that after birth LH levels significantly increased from 28 to 30 weeks postmenstrual age (corresponding to mean postnatal ages of 1 and 2 weeks) and significantly decreased from 32 until 66 weeks postmenstrual age (corrected age of 6 months) (corresponding to 4 until 38 weeks postnatal age); levels between 30 and 32 weeks postmenstrual age were not significantly different. FSH levels showed a
significant peak at 32 weeks postmenstrual age (4 weeks postnatal age) and thereafter significantly decreased until 66 weeks postmenstrual age (corrected age of 6 months or 38 weeks postnatal age).

Figure 2 shows the median testosterone levels with increasing postmenstrual age, for both the early-insulin and standard care group. In both groups longitudinal analysis showed that the observed peak is not significant; from 41 to 66 weeks postmenstrual age (13 to 38 weeks postnatal age), testosterone levels decreased significantly. Comparison between the early-insulin group and standard care group showed no significant differences between testosterone levels at all six mean postmenstrual ages, but the decrease from the highest levels was significantly faster in the early-insulin group.

Table 1. FSH, LH and testosterone levels for samples collected from six age groups based on postmenstrual age

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Postmenstrual age (weeks)</th>
<th>FSH (IU/mmol creatinine)</th>
<th>LH (IU/mmol creatinine)</th>
<th>Testosterone (nmol/mmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I total</td>
<td>11</td>
<td>28.2 (27.0-29.3)</td>
<td>0.1 (0.01-1.3)</td>
<td>0.5 (0.01-3.6)</td>
<td>24.8 (7.1-102.0)</td>
</tr>
<tr>
<td>standard</td>
<td>7</td>
<td>28.3 (27.0-29.3)</td>
<td>0.01 (0.01-0.9)</td>
<td>0.3 (0.01-2.4)</td>
<td>22.4 (10.9-102.0)</td>
</tr>
<tr>
<td>insulin</td>
<td>4</td>
<td>28.0 (27.1-29.0)</td>
<td>0.5 (0.01-1.3)</td>
<td>1.0 (0.01-3.6)</td>
<td>32.1 (7.1-97.5)</td>
</tr>
<tr>
<td>II total</td>
<td>17</td>
<td>30.4 (29.6-31.3)</td>
<td>0.6 (0.01-2.6)</td>
<td>1.3 (0.01-11.2)</td>
<td>31.9 (1.7-160.0)</td>
</tr>
<tr>
<td>standard</td>
<td>9</td>
<td>30.5 (29.6-31.1)</td>
<td>0.7 (0.01-2.6)</td>
<td>1.3 (0.01-11.2)</td>
<td>31.1 (19.8-160.0)</td>
</tr>
<tr>
<td>insulin</td>
<td>8</td>
<td>30.4 (29.6-31.3)</td>
<td>0.5 (0.01-2.5)</td>
<td>1.5 (0.01-8.2)</td>
<td>40.6 (1.7-93.8)</td>
</tr>
<tr>
<td>III total</td>
<td>24</td>
<td>32.5 (31.4-34.0)</td>
<td>1.2 (0.2-5.6)</td>
<td>1.6 (0.09-11.2)</td>
<td>35.6 (2.4-87.3)</td>
</tr>
<tr>
<td>standard</td>
<td>12</td>
<td>32.4 (31.7-34.0)</td>
<td>1.4 (0.2-5.6)</td>
<td>1.8 (0.2-11.2)</td>
<td>37.1 (24.9-87.3)</td>
</tr>
<tr>
<td>insulin</td>
<td>12</td>
<td>32.5 (31.4-33.6)</td>
<td>0.9 (0.01-1.8)</td>
<td>1.3 (0.09-3.5)</td>
<td>30.3 (2.4-60.5)</td>
</tr>
<tr>
<td>IV total</td>
<td>21</td>
<td>41.3 (39.6-44.7)</td>
<td>0.5 (0.2-1.4)</td>
<td>0.7 (0.01-5.7)</td>
<td>29.1 (2.5-61.7)</td>
</tr>
<tr>
<td>standard</td>
<td>12</td>
<td>41.0 (39.6-44.4)</td>
<td>0.6 (0.2-1.3)</td>
<td>0.6 (0.09-5.7)</td>
<td>31.8 (11.9-61.7)</td>
</tr>
<tr>
<td>insulin</td>
<td>9</td>
<td>41.0 (39.7-43.0)</td>
<td>0.5 (0.2-1.4)</td>
<td>0.7 (0.01-2.0)</td>
<td>20.9 (2.5-54.2)</td>
</tr>
<tr>
<td>V total</td>
<td>21</td>
<td>53.7 (52.0-57.0)</td>
<td>0.2 (0.01-0.7)</td>
<td>0.09 (0.01-0.8)</td>
<td>11.1 (2.3-32.8)</td>
</tr>
<tr>
<td>standard</td>
<td>12</td>
<td>53.7 (52.0-57.0)</td>
<td>0.2 (0.01-0.7)</td>
<td>0.1 (0.01-0.8)</td>
<td>11.6 (4.7-32.8)</td>
</tr>
<tr>
<td>insulin</td>
<td>9</td>
<td>53.7 (52.0-57.0)</td>
<td>0.2 (0.01-0.5)</td>
<td>0.01 (0.01-0.4)</td>
<td>7.0 (2.3-26.2)</td>
</tr>
<tr>
<td>VI total</td>
<td>14</td>
<td>66.0 (65.0-67.0)</td>
<td>0.03 (0.01-0.4)</td>
<td>0.01 (0.01-0.04)</td>
<td>2.7 (0.4-8.8)</td>
</tr>
<tr>
<td>standard</td>
<td>7</td>
<td>66.1 (66.0-67.0)</td>
<td>0.01 (0.01-0.3)</td>
<td>0.01 (0.01-0.04)</td>
<td>3.1 (2.1-8.8)</td>
</tr>
<tr>
<td>insulin</td>
<td>7</td>
<td>65.9 (65.0-67.0)</td>
<td>0.2 (0.01-0.4)</td>
<td>0.01 (0.01-0.03)</td>
<td>2.4 (0.4-4.9)</td>
</tr>
</tbody>
</table>

Hormone levels are expressed as median and range, postmenstrual age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and testosterone levels did not differ significantly between the standard care and early-insulin group.
Figure 1. Median FSH and LH levels for samples collected from six age groups with increasing mean postmenstrual age.

Figure 2. Median testosterone levels for samples collected from six age groups with increasing mean postmenstrual age and subdivided into early-insulin group and standard care group.

Table 2. FSH, LH and testosterone levels for samples collected from five age groups based on postnatal age

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Postnatal age (weeks)</th>
<th>FSH (IU/mmol creatinine)</th>
<th>LH (IU/mmol creatinine)</th>
<th>Testosterone (nmol/mmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I total</td>
<td>28</td>
<td>1.5 (1.0-3.6)</td>
<td>0.4 (0.01-2.5)</td>
<td>1.0 (0.01-11.2)</td>
<td>40.5 (7.1-160.0)</td>
</tr>
<tr>
<td>standard</td>
<td>15</td>
<td>1.4 (1.0-3.4)</td>
<td>0.2 (0.01-2.2)</td>
<td>1.0 (0.01-11.2)</td>
<td>33.3 (10.9-160.0)</td>
</tr>
<tr>
<td>insulin</td>
<td>13</td>
<td>1.6 (1.0-3.6)</td>
<td>0.5 (0.01-2.5)</td>
<td>0.9 (0.01-8.2)</td>
<td>44.3 (7.1-97.5)</td>
</tr>
<tr>
<td>I total</td>
<td>24</td>
<td>4.3 (4.0-5.9)</td>
<td>1.2 (0.2-5.6)</td>
<td>1.7 (0.2-11.2)</td>
<td>27.5 (1.7-87.3)</td>
</tr>
<tr>
<td>standard</td>
<td>13</td>
<td>4.2 (4.0-5.7)</td>
<td>1.3 (0.2-5.6)</td>
<td>1.6 (0.2-11.2)</td>
<td>29.5 (19.8-87.3)</td>
</tr>
<tr>
<td>insulin</td>
<td>11</td>
<td>4.3 (4.0-5.9)</td>
<td>0.9 (0.4-2.2)</td>
<td>1.8 (0.7-3.5)</td>
<td>21.2 (1.7-51.4)</td>
</tr>
<tr>
<td>III total</td>
<td>21</td>
<td>13.1 (10.1-18.3)</td>
<td>0.5 (0.2-1.4)</td>
<td>0.7 (0.01-5.7)</td>
<td>29.1 (2.5-61.7)</td>
</tr>
<tr>
<td>standard</td>
<td>12</td>
<td>13.3 (10.6-18.3)</td>
<td>0.6 (0.2-1.3)</td>
<td>0.6 (0.09-5.7)</td>
<td>31.8 (11.9-61.7)</td>
</tr>
<tr>
<td>insulin</td>
<td>9</td>
<td>12.8 (10.1-15.0)</td>
<td>0.5 (0.2-1.4)</td>
<td>0.7 (0.01-2.0)</td>
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</tr>
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<td>IV total</td>
<td>21</td>
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<td>0.09 (0.01-0.8)</td>
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<td>insulin</td>
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<td>7.0 (2.3-26.2)</td>
</tr>
<tr>
<td>V total</td>
<td>14</td>
<td>37.9 (35.7-40.0)</td>
<td>0.03 (0.01-0.4)</td>
<td>0.01 (0.01-0.04)</td>
<td>2.7 (0.4-8.8)</td>
</tr>
<tr>
<td>standard</td>
<td>7</td>
<td>38.1 (36.6-40.0)</td>
<td>0.01 (0.01-0.3)</td>
<td>0.01 (0.01-0.04)</td>
<td>3.1 (2.1-8.8)</td>
</tr>
<tr>
<td>insulin</td>
<td>7</td>
<td>37.8 (35.7-39.9)</td>
<td>0.2 (0.01-0.4)</td>
<td>0.01 (0.01-0.03)</td>
<td>2.4 (0.4-4.9)</td>
</tr>
</tbody>
</table>

Hormone levels are expressed as median and range, postnatal age as mean and range. For each age group, results are given for all samples together, for samples of infants in the standard care group and for samples of infants in the early-insulin group. In all age groups, gonadotropin and testosterone levels did not differ significantly between the standard care and early-insulin group.
Levels of LH, FSH and testosterone for postnatal age

For this analysis, the 108 urine samples were divided into five age groups, based on the postnatal age at the time of collection of the sample. Table 2 shows the details of these age groups with mean postnatal age, number of samples and median levels of FSH, LH and testosterone. LH and FSH levels and patterns were not different between the early-insulin and standard care group and the groups were taken together for further analyses.

Figure 3 shows the median LH and FSH levels for increasing postnatal age. Longitudinal analysis showed that after birth LH levels significantly decreased from 4 until 38 weeks postnatal age (corresponding to 32 until 66 weeks postmenstrual age); levels between 1 and 4 weeks postnatal age (29 and 32 weeks postmenstrual age) were not significantly different. FSH levels showed a significant peak at 4 weeks postnatal age (32 weeks postmenstrual age) and significantly decreased until 38 weeks postnatal age (66 weeks postmenstrual age or corrected age of 6 months).

Figure 4 shows the median testosterone levels with increasing postnatal age, for both the early-insulin and standard care group. From 1 to 4 weeks postnatal age (29 to 32 weeks postmenstrual age), there was a significant decrease only in the early-insulin group. Although the median testosterone level at 1 week postnatal age was higher in the early-insulin group, the difference with the standard care group was not significant. In both groups testosterone levels significantly decreased from 13 until 38 weeks postnatal age (41 until 66 weeks postmenstrual age). There were no significant differences in testosterone levels between the early-insulin and standard care group at all five mean postnatal ages.

![Figure 3](image1.png)

**Figure 3.** Median FSH and LH levels for samples collected from five age groups with increasing mean postnatal age.

![Figure 4](image2.png)

**Figure 4.** Median testosterone levels for samples collected from five age groups with increasing mean postnatal age and subdivided into early-insulin group and standard care group.
DISCUSSION

The present study provides an accurate description of the postnatal activation of the hypothalamic-pituitary-testicular axis in VLBW boys, based on serial measurements of gonadotropins and testosterone. The use of urine samples made it possible to collect these serial measurements without the disadvantages of serial blood sampling. FSH peaked at a mean postmenstrual age of 32 weeks (mean postnatal age of 4 weeks) and peak levels of LH were measured at mean postmenstrual ages of 30 and 32 weeks and mean postnatal ages of 1 and 4 weeks. Testosterone levels decreased with increasing age.

The first studies in preterm born male infants, performed more than 30 years ago, showed higher serum testosterone levels, a more prolonged increase with peak testosterone levels at 3 to 4 months of age and a slower decrease than in term born males (10, 14). The postnatal pituitary-gonadal activation is probably caused by loss of inhibitory feedback as a result of the disappearance of placental estrogens after birth. With increasing age, gonadotropin levels decline due to maturation of the inhibitory feedback system mediated by gonadal steroids and inhibin B (6, 10, 16, 17). The prolonged and more marked testicular activity in preterm born male infants is probably caused by the more immature state of the hypothalamic-pituitary-gonadal axis in preterms, which could be less sensitive for negative feedback by sex steroids and needs more time for full maturation of the inhibitory feedback system (10, 14).

The postnatal activation of the hypothalamic-pituitary-testicular axis is associated with an increase in numbers of Sertoli cells, Leydig cells and germ cells and testicular volume increases during infancy (1, 2, 5, 21). These testicular changes suggest that infancy is an important period for testicular development (22). The activation of the hypothalamic-pituitary-testicular axis with testosterone secretion in the first postnatal months is also important for a normal development of the external genitalia, suggested by lack of penile growth and involution of the scrotum in infants with hypogonadism (4). The importance of the exaggerated activation of the pituitary-testicular axis in preterm boys for reproductive function is still unclear.

Kuiri-Hanninen et al. (3) recently also used serial urine samples to study the postnatal activation of the hypothalamic-pituitary-gonadal axis in preterm born boys. They not only confirmed higher testosterone levels in preterm born boys compared to term born boys, but also showed that preterm born boys have higher levels of gonadotropins, which was not found in earlier studies (14, 23). They also found that the increased postnatal hypothalamic-pituitary-gonadal axis activation in preterm boys is associated with faster testicular and penile growth compared to full-term boys (3).

The study population of Kuiri-Hanninen et al. (3) is more heterogeneous than our study population and consists of preterm boys born at 24.7 to 36.6 weeks gestational age; less
than half was born at less than 32 weeks gestational age. We studied the hypothalamic-pituitary-gonadal axis using serial urine samples in preterm boys all born between 26 and 30 weeks gestational age. Concerning the gonadotropin levels, postnatal age at the peak levels and height of these levels in our study are in accordance with the results of Kuiri-Hanninen et al. (3). We did not find a significant peak in testosterone levels. Our analysis of testosterone levels for postmenstrual age shows highest levels at 30 and 32 weeks postmenstrual age; this is where peak levels are expected because the high LH levels at this age will probably cause Leydig cell stimulation. The absence of a significant peak in this analysis could be caused by the small number of infants. Our analysis of testosterone levels for postnatal age shows that levels are highest in the youngest age group (mean postnatal age of 1.5 weeks) and decrease with increasing age. This is in contrast with the results of Kuiri-Hanninen et al. (3), which showed peak testosterone levels at 1 month, and with the results of earlier studies (10, 14), where peak values were found at 3 to 4 months of age. This difference can be caused by the different study population as we studied only VLBW infants less than 30 weeks gestational age, whereas the other studies included only (10, 14), or also (3) older premature infants. It is also possible that we did not find a peak because of the small number of infants or the lack of data between 4 and 13 weeks postnatal age.

Testosterone levels were not significantly different between the early-insulin and standard care group, which could also be caused by the small number of patients. However, the decrease of testosterone levels was significantly faster in the early-insulin group. This could possibly be the result of a greater amount of adipose tissue and associated with this higher leptin levels in the infants treated with insulin. High leptin levels reduce testosterone production, probably by both a direct effect on the Leydig cells and by diminishing gonadotropin-releasing hormone secretion (24, 25). Ertl et al. (26) demonstrated an inverse relationship between leptin and testosterone levels during the first 5 weeks of life in male preterm born infants. In addition, as aromatase is mainly located in adipose tissue, increased aromatization of testosterone into estradiol could cause inhibition of gonadotropin release by estradiol in infants in the early-insulin group (24). In our study, we did not find evidence for a role of the amount of adipose tissue: there were no significant differences in body weight and total body fat, calculated according to Dauncey et al. (27) from skinfold thickness measurements and body dimensions, between infants in the early-insulin and standard care group (data not shown). We also did not find a difference in gonadotropins between the early-insulin and standard care group, which could have indicated an effect of leptin or increased aromatization. The absence of these differences could be caused by the small number of infants. The effect of insulin on testosterone levels could also be mediated by its effect on sex hormone-binding globulin as the negative correlation between insulin and sex hormone-binding globulin is already present at birth (28).
Our study is limited by the small number of infants and the lack of data from term born male infants. The results have to be confirmed in a larger group of VLBW infants. To make a good comparison with the hypothalamic-pituitary-testicular activation in term born boys, a study should be performed using serial urine samples of term born male infants to measure gonadotropins and testosterone using the same assays.

In conclusion, by using urine samples for serial measurements of gonadotropins and testosterone, we were able to get an accurate impression of the postnatal activation of the hypothalamic-pituitary-testicular axis in VLBW boys. Levels of LH and FSH peak at a mean postnatal age of 1 to 4 weeks (mean postmenstrual age of 30 to 32 weeks). Testosterone levels do not show a significant peak and decrease with increasing age; this decrease is faster in infants receiving early insulin therapy compared to those receiving standard care.
REFERENCES


