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Insulin-Like Factor 3 Levels in Cord Blood and Serum from Children: Effects of Age, Postnatal Hypothalamic-Pituitary-Gonadal Axis Activation, and Cryptorchidism

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Context: The Leydig cell hormone insulin-like factor 3 (INSL3) is important for testicular descent. Currently INSL3 levels in cord blood, in serum throughout childhood, and in relation to congenital cryptorchidism are unknown.

Objective: The objective of the study was to characterize INSL3 levels in cord blood during the postnatal activation of the hypothalamic-pituitary-gonadal axis and in later childhood in normal boys and girls and cryptorchid boys.

Design and Participants: Serum from 267 3-month-old boys of a prospective study with standardized cryptorchidism classification was analyzed for INSL3 (of these, 99 also had cord blood samples). Testicular position was known in 151 controls and 54 transiently cryptorchid and 62 persistently cryptorchid subjects. Eight infant girls, 26 boys (4.1–10.1 yr), and 13 girls (3.7–8.7 yr) were also included.

Outcome Measure: INSL3, age, testicular position, LH, and testosterone were measured.

Results: INSL3 levels were significantly higher ($P < 0.001$) in cord blood and 3-month-old boys as compared with older prepubertal boys. At 3 months of age, INSL3 correlated significantly with LH in healthy boys. Cord blood INSL3 was significantly reduced in persistently cryptorchid boys ($P = 0.001$), and 3-month-old persistently cryptorchid boys had a significantly increased LH to INSL3 ratio ($P = 0.014$). INSL3 was unmeasurable in girls at all ages.

Conclusions: In boys, early postnatal INSL3 is markedly higher as compared with later childhood, presumably because it is stimulated by the transient postnatal LH peak. INSL3 was unmeasurable in girls at all ages. Reduced cord blood INSL3 and an increased LH to INSL3 ratio at 3 months of age in persistently cryptorchid boys suggest impaired Leydig cell function in cryptorchid boys already in the perinatal period. **(*J Clin Endocrinol Metab* 92: 4020–4027, 2007)**

THE HORMONE insulin-like factor 3 (INSL3) is derived almost exclusively from Leydig cells (1) and is released into the circulation, resulting in considerable serum concentrations in adult men (2–4). The hormone is produced in pre- and postnatal Leydig cells (1). It is, at least postnatally, related to the state of Leydig cell differentiation (4, 5), which again, is LH dependent. This is reflected by increasing INSL3 serum concentrations during puberty (6, 7), suppression of INSL3 in response to gonadotropin suppression, and subsequent increase in INSL3 after human chorionic gonadotropin (hCG) stimulation (8) and undetectable INSL3 serum levels in men with hypogonadotropic hypogonadism (2). Thus, INSL3 is, at least from puberty onward, dependent on functional integrity of the hypothalamic-pituitary-gonadal (HPG) axis.

Postnatally expressed INSL3 has been suggested to protect germ cells from undergoing apoptosis (9, 10). However, expression of the gene encoding the INSL3 receptor, *LGR8*, in various human tissues (10–12) combined with high circulating INSL3 levels in adult men (2, 12) supports the hypothesis that INSL3 may also exert other, as yet unknown, endocrine functions.

Prenatally expressed INSL3 is, according to animal studies, central in the process of testicular descent (13, 14). Mutation analyses performed in men with a history of maldescensus testis suggest a corresponding function for INSL3/LGR8 in human embryogenesis (15, 16) although mutations in human *INSL3/LGR8* are rare in human cryptorchidism (17, 18).

The process of testicular descent involves a transabdominal and an inguinoscrotal phase (19). The transabdominal part comprises anchoring of the testes close to the inguinal ring, a process mediated primarily by INSL3 via the outgrowth of gubernaculum (14, 20). The inguinoscrotal phase is primarily facilitated by androgens and comprises migration of the gubernaculum and enlargement of the processus vaginalis invagination of the gubernaculum and the processus vaginalis down into the developing scrotum (19). The question has been raised whether INSL3, along with androgens, also exerts a function in this final part of testicular descent (21). Transgenic female mice with INSL3 overex-

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Abbreviations: CV, Coefficient of variation; hCG, human chorionic gonadotropin; HPG, hypothalamic-pituitary-gonadal; INSL3, insulin-like factor 3.

pression appear not only with ovaries descending to the base of the abdominal cavity but furthermore show development of processus vaginalis (22) and inguinal hernia (23).

Failure of complete testicular descent, or cryptorchidism, is one of the most common congenital malformations in infant boys (24, 25) and a risk factor for reduced semen quality and testicular cancer in adult life (26, 27). We have, in a large prospective cohort study of Danish and Finnish boys, evaluated the reproductive organs of male infants in the two countries. Major country differences are evident, including a substantially higher incidence of cryptorchidism in Danish boys (25, 28–30). Functional integrity of the HPG axis, which is fundamental for testicular descent (31), have been assessed and related to cryptorchidism in the male infant cohort, at the time of the postnatal hormonal surge (32). INSL3 levels are, however, still uncharacterized in early life. We therefore analyzed INSL3 levels in cord blood and serum during the postnatal transient hormonal peak in a large subgroup of these boys (29, 32, 33) as well as in older prepubertal boys. For gender comparison, INSL3 serum levels were analyzed in cord blood from girls and serum from 3-month-old and older prepubertal girls. INSL3 levels were related to LH and other reproductive hormones as well as testicular position.

Subjects and Methods

Study populations

All newborns and 3-month-old boys and girls participated in a joint prospective cohort study performed 1997–2001 at the Turku University Hospital in Turku, Finland, and the University Hospital in Copenhagen, Denmark. The study aimed at investigating temporal and geographical trends in the prevalence of genital malformations (25, 28, 30). Recruitment and inclusion criteria of the cohort study were described in details earlier (25).

Data concerning parity, maternal smoking, and diabetes mellitus were obtained from prenatal questionnaires and hospital records. Birth weight was obtained from birth records, whereas at 3 months of age the boys were weighed by us on a digital baby scale (Baby Scale Model; Solotop Oy, Helsinki, Finland). The children were examined at birth and at 3 months of age, and nonfasting venous blood samples were collected at 3 months of age. For some, also cord blood was collected at birth. Blood samples were taken between 1200 and 1800 h in Finland and between 0800 and 1700 h in Denmark. The samples were centrifuged after clotting, and serum was stored at −20 C.

The present study included serum samples from Danish and Finnish cryptorchid boys of whom we had available serum aliquots that had not previously been thawed and refrozen. The Danish controls were selected randomly from the original cohort, however, with preference to control boys from whom also cord blood was available. Originally the Finnish cohort control group was formed of matched controls (one to two controls per case was selected using the following matching criteria: date of birth (±14 d), parity, gestational age (±7 d), maternal smoking, and diabetes) and random controls. However, for the present study, we combined all the controls into one group. Only full-term boys (gestational age 37–42 wk, as based on routine ultrasonography at gestational age 18–20 when available, and otherwise on the last menstrual period) were included in the present study. After exclusion of boys with testicular ascent before 3 months of age and boys with blood samples taken outside the determined 3-month time frame (2.5–3.5 months), a total of 267 boys (179 Finnish, 88 Danish) were included in the final analysis. Of these, 99 boys (61 Finnish, 38 Danish) also had cord blood samples taken. None of the included boys had genital malformations other than cryptorchidism, and none had an orchidopexy performed before 3 months of age.

To get an impression of INSL3 serum levels in relation to exact age around 3 months of age, we also included six Danish boys (all healthy and born full term) who had blood samples taken outside the determined 3-month time frame. (These six boys appear only in Fig. 2 and were not included in any statistical analyses.)

To establish gender differences, samples from eight girls (all Danish) taken at birth (cord blood) and 3 months of age were analyzed for INSL3. These girls were also included in the joint prospective cohort study, and thereby recruitment, inclusion criteria, and examination time points were as defined for the boys.

The study was conducted according to the Helsinki II Declaration and was approved by the local Finnish ethics committee, the local Danish ethics committee, and the Danish Data Protection Agency. Written informed consent was given by the parents.

Evaluation of testicular position

Testicular position was assessed at birth and at 3 months of age. The examination technique developed by Scorer was applied and completely standardized between researchers from both countries (for details, see Ref. 25). Testicular position was divided into five categories: normal (scrotal or retractile), high scrotal, suprascrotal, inguinal, and nonpalpable. Boys with at least one high scrotal testis were categorized as cryptorchid. In cases of bilateral cryptorchidism, classification was made according to the most pathological position. Of all 267 boys included, 151 boys (100 Finnish, 51 Danish) had fully descended testes both at birth and at 3 months of age (controls), 54 (28 Finnish, 26 Danish) were cryptorchid at birth but had spontaneous descent before 3 months of age (transiently cryptorchid), and 62 boys (51 Finnish, 11 Danish) were cryptorchid at birth and at 3 months of age (persistently cryptorchid). In the subgroup of boys who had blood samples drawn both at birth and at 3 months of age, there were 46 control boys (20 Finnish, 26 Danish), 32 transiently cryptorchid boys (21 Finnish, 11 Danish), and 21 persistently cryptorchid boys (20 Finnish, one Danish).

Prepubertal children

Twenty-six healthy Danish prepubertal boys (aged 4.1–10.1 yr) and 13 healthy Danish prepubertal girls (aged 3.7–8.7 yr) were included in a study on analysis of reproductive hormones in prepubertal children. All children had a general medical examination and a blood sample taken. All children were in Tanner stage 1 and all boys had fully descended testes. Blood samples were collected from a peripheral vein, centrifuged after clotting, and serum stored at −20 C. The study was approved by the local Danish ethics committee and written informed consent was given by the parents.

Hormone analyses

Serum samples were analyzed in duplicates and blinded for outcome by a few skilled technicians at one laboratory (Rigshospitalet, Copenhagen, Denmark). Each assay run contained serum samples from both countries to minimize interassay variation.

INSL3 was measured using a time-resolved fluorescence immunoassay with a detection limit of 0.05 ng/ml and intra- and interassay coefficients of variation (CV) of 8.0 and 11.3%, respectively. Development and evaluation of the assay has been described in detail previously (2). Testosterone was determined by a RIA (Coat-a-Count; Diagnostic Products, Los Angeles, CA). The detection limit was 0.23 nmol/liter and the intra- and interassay CVs were less than 10%. Inhibin B was analyzed by a double-antibody enzyme immunometric assay (Oxford Bio-Innovation, Oxford, UK) with a detection limit of 20 pg/ml and intra- and interassay CVs less than 15 and 18%, respectively. LH and FSH were analyzed using time-resolved immunofluorometric assays (Delfia; Wallac, Turku, Finland) with a detection limit of 0.05 and 0.06 IU/liter for LH and FSH, respectively. Both gonadotropin assays had intra- and interassay CVs less than 5%.

Statistics and calculations

Analyses were performed country-wise due to previously reported discrepancies in the reproductive organs of Danish and Finnish boys. Characteristics (Table 1) are given as mean ± SD and hormone levels (Table 2) are given as median (range). The categorical variables parity, maternal smoking, and maternal diabetes were tested in a Kruskal

TABLE 1. Characteristics of Finnish and Danish controls, transiently cryptorchid, and persistently cryptorchid boys

	Controls	Transiently cryptorchid	Persistently cryptorchid	*P*
Finland (n)	100	28	51	
Parity				
1	46 (46.0)	9 (32.1)	30 (60)	
2	33 (33.0)	12 (42.9)	13 (26)	
3	16 (16)	4 (14.3)	5 (10)	
More than 4	5 (5.0)	3 (10.7)	2 (4)	0.62
Maternal diabetes	4 (4.0)	4 (14.3)	9 (17.6)	<0.01[a], 0.04[b]
Smoking during pregnancy				
Yes	17 (17.0)	5 (17.9)	10 (20.0)	
No	83 (83.0)	23 (82.1)	40 (80.0)	0.67
Gestational age (d)	280.6 ± 7.5	272.8 ± 9.4	281.6 ± 7.3	<0.001[b,c]
Birth weight (kg)	3.7 ± 0.4	3.4 ± 0.6	3.6 ± 0.5	0.04[b]
Weight at 3 months (kg)	6.6 ± 0.6	6.6 ± 0.8	6.4 ± 0.8	0.28
Age at blood sampling (months)	3.0 ± 0.1	3.0 ± 0.2	3.0 ± 0.2	0.77
Denmark (n)	51	26	11	
Parity				
1	37 (72.5)	14 (53.8)	6 (54.5)	
2	9 (17.6)	9 (34.6)	4 (36.4)	
3	4 (7.8)	3 (11.5)	1 (9.1)	
More than 4	1 (2.0)			0.22
Maternal diabetes				
Smoking during pregnancy				
Yes	21 (41.2)	11 (42.3)	3 (27.3)	
No	30 (58.8)	15 (57.7)	8 (72.7)	0.61
Gestational age (d)	282.8 ± 7.1	279.7 ± 6.7	284.6 ± 4.2	0.08
Birth weight (kg)	3.7 ± 0.5	3.7 ± 0.5	4.0 ± 0.5	0.20
Weight at 3 months (kg)	6.7 ± 0.7	6.6 ± 1.0	6.6 ± 0.8	0.94
Age at blood sampling (months)	3.0 ± 0.3	3.0 ± 0.3	2.8 ± 0.2	0.20

Data represent number (percent) or mean ± SD. Parity, maternal diabetes, and smoking during pregnancy were tested in a Kruskal-Wallis test. If *P* values below 0.05 were obtained, Mann-Whitney *U* test was performed. Gestational age, birth weight, weight at 3 months of age, and age at blood sampling were tested by one-way ANOVA and Scheffe *post hoc* test.

[a] *P* values between controls and persistently cryptorchid boys.

[b] *P* values between controls and transiently cryptorchid boys.

[c] *P* values between transiently and persistently cryptorchid boys.

TABLE 2. Serum hormone levels related to Leydig cell function in Finnish and Danish controls, transiently cryptorchid, and persistently cryptorchid boys at birth (cord blood) and 3 months of age

	Controls	Transiently cryptorchid	Persistently cryptorchid	*P*
Cord blood				
Finnish boys				
n	20	21	20	
INSL3 (ng/ml)	0.13 (<0.05 to 0.34)	0.09 (<0.05 to 0.23)	0.08 (<0.05 to 0.31)	0.033[a], 0.001[b]
Danish boys				
n	26	11	1	
INSL3 (ng/ml)	0.14 (0.06–0.39)	0.17 (0.10–0.21)	<0.05	0.179
3 months of age				
Finnish boys				
n	100	28	51	
INSL3 (ng/ml)	0.13 (<0.05 to 0.60)	0.13 (<0.05 to 0.37)	0.11 (<0.05 to 0.30)	0.341
LH (IU/liter)	1.67 (0.48–5.52)	1.78 (0.89–6.73)	1.97 (0.84–6.14)	0.099
Testosterone (nmol/liter)	3.38 (0.64–9.91)	4.15 (1.24–7.39)	3.37 (0.35–12.70)	0.104
LH to INSL3 ratio	12.38 (1.92–77.60)	14.15 (5.41–51.77)	19.00 (3.63–61.20)	0.002[b]
LH to testosterone ratio	0.49 (0.09–2.84)	0.44 (0.18–2.35)	0.65 (0.20–5.31)	0.036[b]
				0.018[c]
Danish boys				
n	51	26	11	
INSL3 (ng/ml)	0.15 (<0.05 to 0.34)	0.17 (<0.05 to 0.36)	0.12 (<0.05 to 0.25)	0.247
LH (IU/liter)	1.63 (0.41–4.87)	1.84 (0.51–4.33)	2.28 (0.48–3.40)	0.195
Testosterone (nmol/liter)	3.14 (0.34–8.91)	3.47 (0.92–6.84)	2.72 (0.01–7.21)	0.726
LH to INSL3 ratio	10.00 (4.28–54.11)	11.26 (2.86–43.30)	13.50 (9.60–68.00)	0.044[b]
LH to testosterone ratio	0.51 (0.19–1.94)	0.54 (0.10–2.23)	0.72 (0.29–2.19)	0.326

Data are shown as median (range). Serum hormone levels in controls and transiently cryptorchid and persistently cryptorchid boys were compared in a Kruskal-Wallis test. If $P < 0.05$ was obtained, a Mann-Whitney *U* test was performed to pair-wise test for differences between the individual groups.

[a] *P* values between controls and transiently cryptorchid boys.

[b] *P* values between controls and persistently cryptorchid boys.

[c] *P* values between transiently and persistently cryptorchid boys.

Wallis test, and if significance was obtained, a Mann-Whitney *U* test was performed to pair-wise test for differences. The continuous variables gestational age, birth weight, weight at 3 months of age, and exact age at blood sampling in the three groups were tested by one-way ANOVA and Scheffé *post hoc* tests. The influence of confounders was tested in a univariate general linear model. Because serum INSL3 was not normally distributed in the individual groups, nonparametric tests were used to evaluate hormone data: Wilcoxon signed ranks test was used to compare INSL3 in the same boys at birth and 3 months of age; Kruskal Wallis and Mann-Whitney *U* tests were used to compare hormone serum levels and their ratios in controls and transiently and persistently cryptorchid boys and to compare country-specific hormone levels. Spearman rank correlation coefficients were calculated to evaluate hormone correlations. In all statistical analyses, INSL3 values below the detection limit of the assay are given the value of the detection limit (0.05 ng/ml). Two-sided $P < 0.05$ was considered significant. The Statistical Package for Social Sciences (version 15.0; SPSS Inc., Holte, Denmark) was used for calculations and statistical analyses.

Results

Characteristics

Characteristics of controls, transiently, and persistently cryptorchid boys are given in Table 1. In either Finnish or Danish boys, the groups did not differ with respect to parity, maternal smoking, weight at 3 months of age, and exact age at blood sampling. This was also true for Danish boys with respect to gestational age and birth weight. Although all boys were full term, Finnish transiently cryptorchid boys had a significantly lower gestational age, compared with Finnish control boys and Finnish persistently cryptorchid boys ($P < 0.001$ for both) and a lower birth weight, compared with Finnish persistently cryptorchid boys ($P = 0.04$). Neither gestational age nor birth weight was found to influence serum INSL3 levels in any of the groups. In Denmark, none of the mothers of included boys had diabetes during pregnancy. In Finland, mothers of transiently and persistently cryptorchid boys had diabetes during pregnancy more often, compared with control boys ($P = 0.04$ and $P < 0.01$, respectively). There were no significant differences in INSL3 serum levels at birth or at 3 months of age in boys born from diabetic mothers and boys born from nondiabetic mothers in any of the groups. Factors listed in Table 1 were not included in further comparisons of controls or transiently and persistently cryptorchid boys.

Hormone serum levels, ratios, and correlations

Serum INSL3 levels are depicted in Figs. 1 and 2, and hormone levels and their ratios are described in Table 2.

In the group of Finnish boys, cord blood INSL3 levels were higher in control boys than boys with cryptorchidism at the time of blood sampling (transiently cryptorchid: $P = 0.033$, persistently cryptorchid: $P = 0.001$). In control boys, there was no individual change in INSL3 levels between birth and

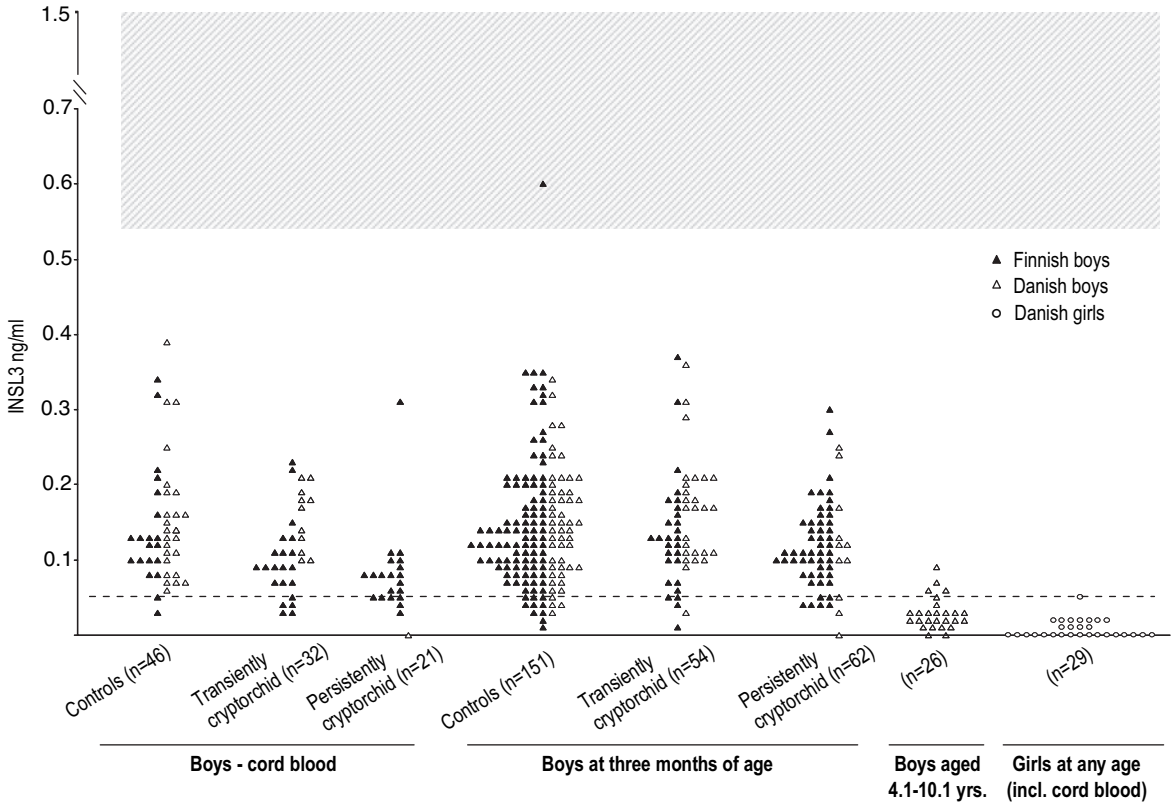

FIG. 1. INSL3 in boys and girls in cord blood and serum at 3 months of age and in older nonpubertal children. INSL3 serum levels in Finnish (▲) and Danish (△) boys at birth (cord blood), at 3 months of age, and later in childhood. At birth and 3 months of age, boys were divided into three groups based on the position of their testes: scrotal (controls), cryptorchid at birth but with spontaneous testicular descent before 3 months of age (transiently cryptorchid), or cryptorchid at birth and at 3 months of age (persistently cryptorchid). All boys in the age group 4.1–10.1 yr had fully descended testes. All samples from girls (○) are pooled. These include cord blood samples, samples from 3-month-old girls and older nonpubertal girls (aged 3.7–8.7 yr). The *gray toned area* indicates the normal range for INSL3 in adult men (note the broken y-axis). The *dotted line* indicates the detection limit of the assay.

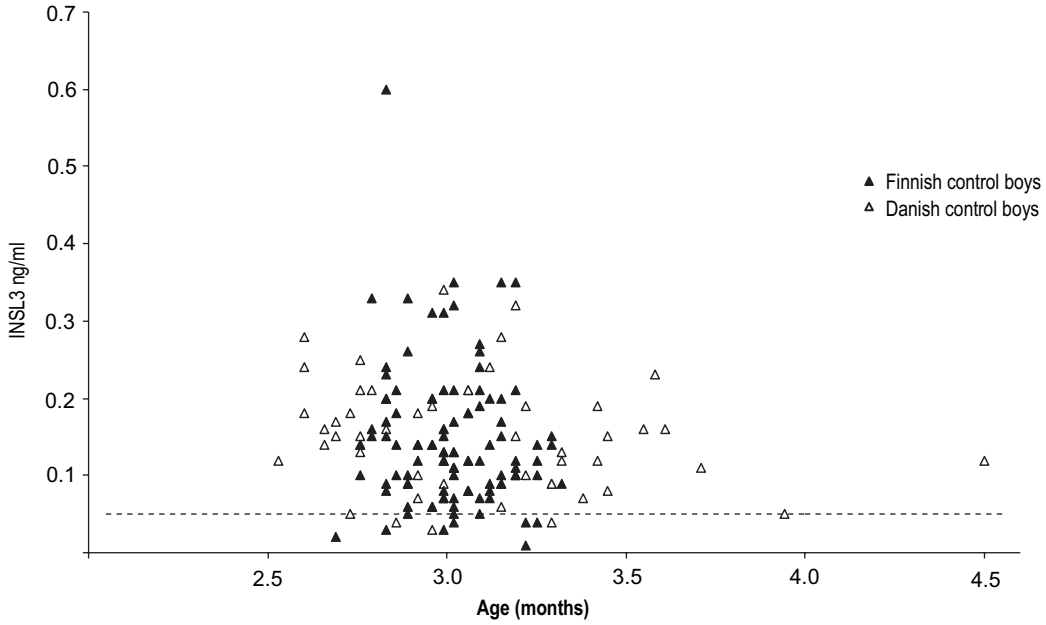

FIG. 2. INSL3 serum levels in healthy boys aged 2.5–4.5 months. INSL3 serum levels in Finnish (▲) and Danish (△) control boys in relation to exact age. To illustrate the tendency of INSL3 serum levels to peak around 3 months of age, we also included in this figure six boys who had blood samples taken after the defined 3-month time frame. The *dotted line* indicates the detection limit of the assay.

3 months of age. Individual INSL3 levels in cryptorchid boys increased significantly between birth and 3 months of age, for both those with transient cryptorchidism ($P = 0.016$) and those with persistent cryptorchidism ($P = 0.015$). No significant difference was observed in INSL3, testosterone, or LH levels between controls and transiently and persistently cryptorchid boys at 3 months of age, although there was a tendency toward lower INSL3 and higher LH levels in persistently cryptorchid boys, reflected by a significantly increased LH to INSL3 ratio ($P = 0.002$) and a significantly increased LH to testosterone ratio ($P = 0.036$), compared with control boys.

In Danish cord blood samples, there were no significant differences in INSL3 with respect to testicular position, and no individual changes were observed in INSL3 levels between birth and 3 months of age in control or transiently cryptorchid boys. Because serum from only one persistently cryptorchid boy was analyzed at both time points, no statistics were carried out in this group. At 3 months of age, none of the hormone levels differed with respect to testicular position; however, the LH to INSL3 ratio was significantly increased in persistently cryptorchid boys *vs.* control boys at 3 months of age ($P = 0.044$).

Danish transiently cryptorchid boys had significantly higher INSL3 cord blood levels, compared with Finnish transiently cryptorchid boys ($P = 0.002$). In the same boys, 38% of the Finnish (eight of 21) as opposed to only 18% of the Danish (two of 11) had severe (suprascrotal or worse) as opposed to mild (high scrotal) cryptorchidism. Otherwise, there were no significant country differences for INSL3, testosterone, LH, or their ratios in any of the groups.

In neither Finnish nor Danish boys was there any relation between INSL3 levels and the severity (severe or mild) or laterality (uni- or bilateral) of cryptorchidism (data not shown).

Correlation analyses were performed for INSL3 and testosterone, inhibin B, LH, and FSH at 3 months of age. In both Finnish and Danish control boys, there was a significant positive correlation between INSL3 and, respectively, LH ($r = 0.19$, $P = 0.06$ for Finnish boys and $r = 0.32$, $P = 0.03$ for Danish boys), testosterone ($r = 0.30$, $P = 0.003$ for Finnish boys and $r = 0.45$, $P = 0.001$ for Danish boys), and inhibin B ($r = 0.20$, $P = 0.05$ for Finnish boys and $r = 0.32$, $P = 0.03$ for Danish boys). No significant correlation was found between INSL3 and any other reproductive hormone in transiently or persistently cryptorchid boys.

All infant girls had INSL3 levels at or below the detection limit of the assay, both at birth and at 3 months of age. Likewise, prepubertal girls all had nondetectable INSL3, whereas the levels in prepubertal boys were between less than 0.05 and 0.09 ng/ml. Serum INSL3 in prepubertal boys was significantly lower than serum INSL3 at birth ($P < 0.001$) and in 3-month-old control boys ($P < 0.001$) (all Danish).

Discussion

Our study, which included a large group of normal children and cryptorchid boys, revealed significantly higher levels of INSL3 in cord blood and serum from 3-month-old boys, compared with older nonpubertal boys. In contrast, the levels of INSL3 were undetectable in girls at all ages studied. Interestingly, cord blood INSL3 was significantly reduced in boys with persistent cryptorchidism, compared with healthy boys. Moreover, at 3 months of age, the persistently cryptorchid boys had a significantly increased LH to INSL3 ratio, compared with healthy boys. As we and others have reported, serum INSL3 levels increase during normal puberty (6, 7) and remain high in adult men (range 0.55–1.73 ng/ml) (2). By combining previously reported levels of INSL3 in adolescence and adulthood with our novel data on INSL3

levels in cord blood during infancy and in childhood, it is now possible to establish a first general impression of the age-dependent dynamics of serum INSL3 from birth to adulthood.

Our data suggest that postnatal INSL3 production is dependent on LH stimulation. At 3 months of age when the transient postnatal surge in gonadotropins peaks (34, 35), serum INSL3 correlated positively with both LH and testosterone serum levels in the healthy boys. Moreover, INSL3 levels tended to be lower in boys who had blood samples drawn at age 3.5–4.5 months, when the levels of gonadotropins decline (35). This finding may suggest that the drop toward the INSL3 levels found in later childhood follows the reported decline in LH levels.

It has previously been shown that INSL3 might be used as a marker of Leydig cell function in adults (5, 8). Our new data suggest that INSL3 may also serve as a marker of presence of functioning testicular tissue in infants, particularly during the transient HPG axis activation in early infancy. Thereby the hormone may also be of clinical value in the evaluation of patients with disorders of gonadal development.

The Sertoli cell hormone inhibin B has been reported to exceed adult levels during the postnatal hormonal surge. In comparison, levels of INSL3 and testosterone only reach levels close to or in the lower part of the adult normal range (2, 35), perhaps suggesting that Leydig cells may be activated to a lower extent than Sertoli cells in response to gonadotropins. The positive correlation found between INSL3 and not only testosterone and LH but also inhibin B in healthy boys may be indicative of an overall hormonal balance between the different testicular compartments, which is present irrespective of different degrees of stimulation of Sertoli and Leydig cells.

Interestingly, INSL3 levels in cord blood from healthy boys were very similar to the levels found at 3 months of age. INSL3 in cord blood is likely stimulated by a combination of maternal hCG and fetal LH. High INSL3 levels in perinatal and early postnatal life raises the question of the physiological importance of INSL3 at this developmental stage. The transabdominal part of testicular descent, for which INSL3 is a prerequisite (14), takes place already before midgestation in humans. Therefore, it appears unlikely that INSL3 levels in cord blood are functionally related to these events. The inguinoscrotal phase, on the other hand, is normally completed in the last part of intrauterine life and predominantly androgen dependent (19). The high INSL3 levels detected in cord blood could support the hypothesis that INSL3, along with androgens, also exerts a role in this second phase of testicular descent (21–23). Perinatally secreted INSL3 may also be coupled to the suggested function of INSL3 in germ cell survival (9). Indeed the population of germ cells fluctuates considerably in the early postnatal period (36).

A large portion of the boys included in our study were born with cryptorchidism. Notably, significantly reduced INSL3 levels were found in cord blood from boys with persistent cryptorchidism. Moreover, the LH to INSL3 ratio was significantly increased at 3 months of age in persistently cryptorchid boys, suggesting some degree of Leydig cell dysfunction. The significant positive correlations found in healthy boys between INSL3 and LH, testosterone, and inhibin B, respectively, were not observed in boys with cryptorchidism, indicating an overall disturbed hormonal balance in cryptorchid boys.

In the group of boys with cryptorchidism at birth and spontaneous testicular descent before 3 months of age, cord blood INSL3 levels were higher in Danish boys, compared with Finnish boys. This may be because the group of Danish boys with transient cryptorchidism was rather small or because a larger part of the Finnish boys selected for analysis had severe cryptorchidism, compared with the Danish boys. The difference could also be merely coincidental. Within the Finnish groups of boys with transient or persistent cryptorchidism, INSL3 levels increased significantly between birth and 3 months age, suggesting that the postnatal LH surge was capable of stimulating INSL3 production toward normal levels. The INSL3 increase was, however, more pronounced in boys with spontaneous descent between birth and 3 months of age, compared with those with persistent cryptorchidism. We speculate that some of the transiently cryptorchid boys were born with undescended testes due to suboptimal LH/hCG stimulation in late fetal life. The postnatal LH surge combined with well-functioning testes in these boys would result in spontaneous completion of testicular descent during the first months of life.

The physiological role of INSL3, except for its role in transabdominal testicular descent, is poorly understood. We speculate that it may also, along with testosterone, be needed for the inguinoscrotal phase and thereby the normal perinatal positioning of the testes in scrotum. Our findings of reduced INSL3 levels in cord blood and increased LH to INSL3 ratio at 3 months of age in persistently cryptorchid boys seem to be in line with this hypothesis. In addition we have (unpublished) data suggesting that boys in whom the testes ascend after birth may have INSL3 levels in the lower normal range at 3 months of age. However, the hormone may also have other roles in neonatal life, including a stimulatory role on the germ cells (9). Thus, there could be a physiological link between the previously reported reduction in germ cell number in neonates with undescended testes (37) and reduced cord blood INSL3 levels in cryptorchid boys. Further studies are clearly needed to elucidate these aspects.

There were no differences between healthy and cryptorchid boys with respect to the majority of the tested confounding factors. The increased rate of diabetes in mothers of cryptorchid boys in this cohort has been discussed previously (38). Importantly, there were no differences in serum INSL3 in sons of diabetic and nondiabetic mothers in any of the three groups, indicating that maternal diabetes during pregnancy does not affect serum INSL3 in the sons. Likewise, the significantly lower gestational age (although all full term) and birth weight in Finnish transiently cryptorchid boys did not influence the INSL3 levels measured.

In addition to Leydig cells, the ovarian theca cells have been reported to produce small amounts of INSL3 (39). Female infants have, like boys, a transient postnatal activation of the HPG axis (40). INSL3 serum levels were at or below the detection limit in all girls studied, including in cord blood, at 3 months of age, and in older nonpubertal girls. This

suggests that, provided any INSL3 is produced from the postnatally active ovaries, this production is very modest and did not result in clearly detectable serum levels.

In conclusion, we report significantly higher INSL3 levels in cord blood and serum from 3-month-old boys, compared with levels in older nonpubertal boys. On the other hand, no INSL3 was detected in girls at all ages studied. Our data suggest that in boys, LH stimulates INSL3 during the postnatal surge in gonadotropins. By combining the current available knowledge of serum INSL3 in normal pubertal and adult life with our novel data on INSL3 in early infancy and childhood, we now have an overview of the dynamics in serum INSL3 from birth to adulthood. Our study also revealed reduced cord blood INSL3 levels and an increased LH to INSL3 ratio at 3 months of age in persistently cryptorchid boys, compared with healthy boys, suggesting that cryptorchidism may be associated with a mild degree of Leydig cell dysfunction already during the perinatal period.

Note Added in Proof

Supporting the theory that INSL3 is involved in the continued postnatal positioning of the testes in scrotum is a recent paper (41) reporting strong expression of LGR8, the receptor for INSL3, in adult mouse cremaster.

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