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Longitudinal Reproductive Hormone Profiles in Infants: Peak of Inhibin B Levels in Infant Boys Exceeds Levels in Adult Men*

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ABSTRACT

The gonads are usually considered quiescent organs in infancy and childhood. However, during the first few postnatal months of life, levels of gonadotropins and sex hormones are elevated in humans. Recent epidemiological evidence suggests that environmental factors operating perinatally may influence male reproductive health in adulthood. The early postnatal activity of the Sertoli cell, a testicular cell type that is supposed to play a major role in sperm production in adulthood is largely unknown. Recently, the peptide hormone inhibin B was shown to be a marker of Sertoli cell function in the adult male. In the adult woman, inhibin B is secreted by the granulosa cells. Longitudinal serum levels of inhibin B were measured in healthy boys (n = 15) and girls (n = 15), in cord blood, and every third month during the first 2 yr of life. In addition, serum levels of FSH, LH, and testosterone (boys) were measured in the same group of children. In boys, inhibin B, FSH, LH, and testosterone levels were all elevated at 3 months of age. However, the peak of inhibin B was unexpectedly high, into the supraadult range (mean ± SE, 378 ± 23 pg/mL) and persisted much longer than the elevation of FSH, LH, and testosterone. Thus, although levels of FSH, LH, and testosterone decreased into the range observed later in childhood by the age of 6–9 months, serum inhibin B levels remained elevated up to at least the age of 15 months. In girls, the hormonal pattern was generally more complex, with a high interindividual variation in levels of inhibin B, FSH, and LH within each age. In conclusion, the sustained elevation of inhibin B to supraadult levels in infant boys indicates that the neonatal period may be a developmental window important for Sertoli cell proliferation and maturation. Thus, the gonads may be potentially vulnerable to exogenous endocrine interference, *e.g.* from environmental factors during this period of life. Measurement of serum levels of inhibin B in infants may give clinical clues about developmental deficiencies in the gonads that otherwise only become apparent around puberty or later in life. (*J Clin Endocrinol Metab* **83:** 675–681, 1998)

IT IS WELL established that the hypothalamic-pituitary-gonadal hormonal axis is transiently activated during the first months of human postnatal life in a gender-specific pattern, exhibiting increased serum levels of gonadotropins and gonadal steroids (1–3). Inhibin is a gonadal peptide hormone that plays an important role in feedback regulation of the pituitary-gonadal hormone axis in puberty and adulthood (4). In the male, inhibin is believed to be produced chiefly by the Sertoli cells of the testes. However, human plasma contains several immunoreactive inhibin forms, including some that are believed to be biologically inactive (5). In its biologically active form, inhibin is a dimer consisting of an α-subunit linked to either a β_A-subunit (inhibin A) or a β_B-subunit (inhibin B). Immunoassays for inhibin have until recently suffered from cross-reaction with inactive monomeric precursor forms present in plasma and from lack of discrimination between inhibin A and inhibin B (6). Using a nonspecific inhibin assay, it has been suggested that inhibin levels are increased in humans during the first year of life (7). By the use of newly developed immunoassays that are specific for either the bioactive inhibin A or the inhibin B form (8), it has recently been demonstrated that it is inhibin B that is the principal inhibin form in men and in women during the follicular phase, whereas inhibin A is mainly present in the luteal phase of the female menstrual cycle and is absent in men (8, 9). In adult men, serum levels of inhibin B seem to be a promising marker of Sertoli cell function (10, 11). The aim of the present study was to evaluate secretion of the biologically active form, inhibin B, during the first 2 yr of life in healthy boys and girls. In addition, serum levels of FSH, LH, and testosterone (boys) were studied longitudinally in the same group of children.

Materials and Methods

The children who were followed from birth to 2 yr were participants in a diabetes prediction and prevention study (12). The complete (98%) birth cohort was tested at birth for risk of insulin-dependent diabetes mellitus. Two different human leukocyte antigen alleles in 1 locus were determined. The presence of 1 of the 2 alleles predicts a 3% risk, and the presence of both predicts a 7% risk. After screening and further information, 78% of the children at increased risk of developing diabetes attended the follow-up. The 15 girls and 15 boys included in the present

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study had a 3% risk of becoming diabetic. However, generally the children were healthy, and they all followed a normal growth pattern. None of them produced islet cell antibodies before 2 yr of age. One was operated upon for a ventricular septal defect. Two have had pyelonephritis, and in 1 of them bilateral vesico-ureteral reflux was detected. Two have had pneumonia. Several of the children have had several episodes of otitis. Sampling was never performed during acute sickness. None of the children have shown evidence of gonadal or other endocrine diseases.

The first blood sample was taken from umbilical vessels at birth. Thereafter, single blood samples were taken randomly between 0900–1500 h at 3-months intervals up to 2 yr of age. After centrifugation, sera were frozen and stored at −20 to −70 C.

Serum inhibin B was measured in duplicate in a double antibody enzyme-immunometric assay using a monoclonal antibody raised against the inhibin β_B-subunit in combination with a labeled antibody raised against the inhibin α-subunit, as previously described (8). This assay was recently used in our laboratory to measure levels of serum inhibin B in pubertal and adolescent boys (13). The detection limit was 18 pg/mL, and the intra- and interassay coefficients of variation were 15% and 18%, respectively.

Serum FSH and LH were measured by time-resolved immunofluorometric assay (Delfia, Wallac, Finland) with detection limits of 0.06 and 0.05 U/L, respectively. Intra- and interassay coefficients of variation were below 8% in both FSH and LH assays. The assay for LH measurements had a small (<1.5%) cross-reactivity with hCG. Considering the high levels of placenta-derived hCG in maternal and cord blood, this assay was thus unsuitable for LH measurements in cord blood. Testosterone was measured by RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA) with a detection limit of 0.23 nmol/L and intra- and interassay coefficients of variation below 10%. The testosterone assay had a low cross-reaction to other steroids, including estradiol, estrone, and androstenedione. This cross-reaction was at the level of 0.01–2%, which normally would not significantly affect the results. However, the high levels of placenta-derived steroid that are present in maternal and cord blood render the assay unsuitable for measurements in cord blood and during the first few weeks of life (14). (Due to the limited amount of sera, FSH, LH, and testosterone levels were the results of a single measurement.)

Results

Boys

The individual levels of inhibin B, FSH, LH, and testosterone in boys are plotted longitudinally in Fig. 1, a–d, and the mean values of inhibin B, FSH, LH, and testosterone at each age are plotted in Fig. 2. Table 1 shows the median and range for the four hormones within each age group; for comparison, the reference ranges in prepubertal children and adult men are also presented. In term cord blood, inhibin B

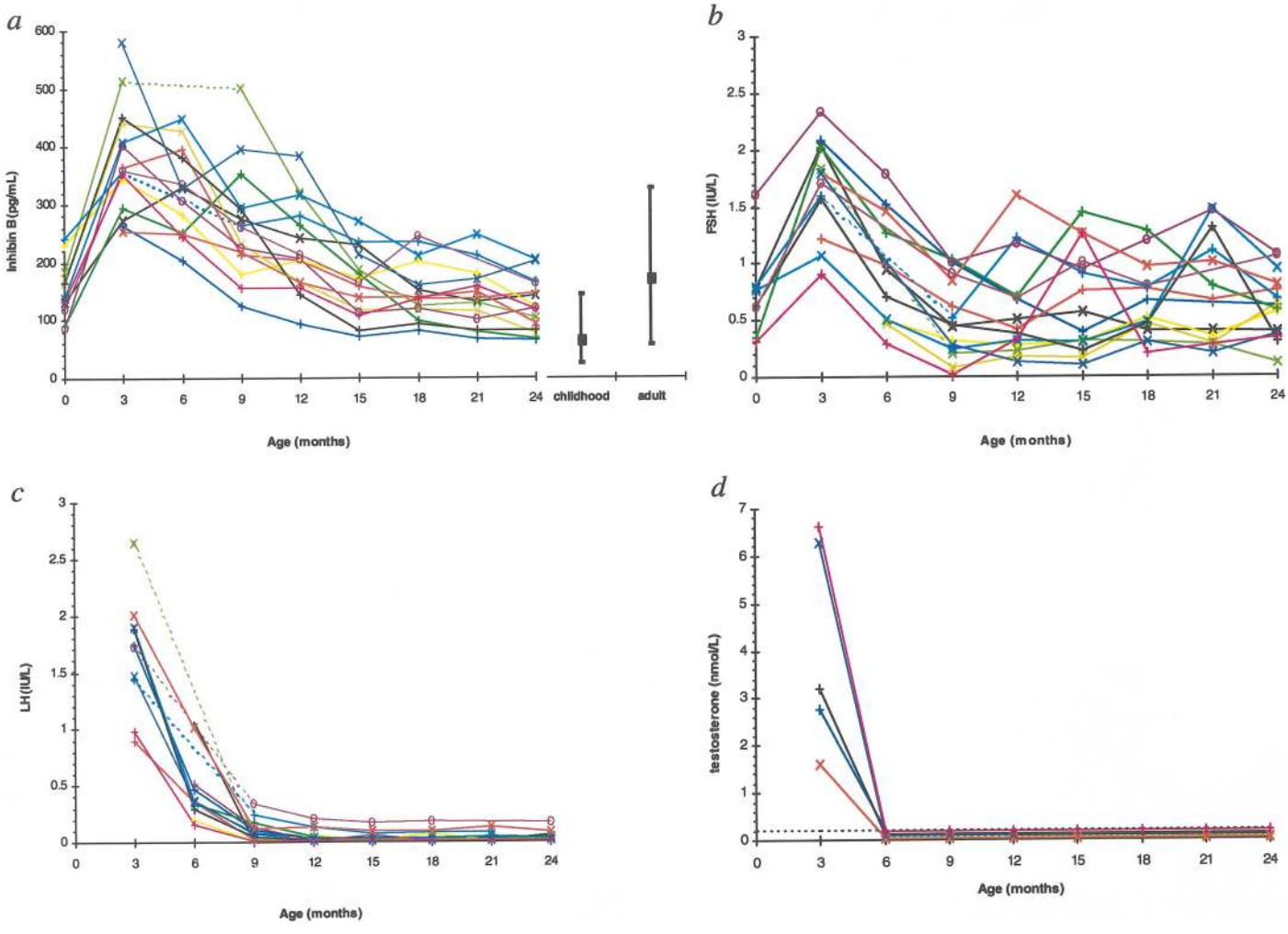

FIG. 1. Individual longitudinal levels of inhibin B (a), FSH (b), LH (c), and testosterone (d) in 0- to 24-month-old boys. For each boy, the levels of all four hormones are indicated by the same color and marker. In a, the normal ranges (median, 2.5 and 97.5 percentiles) for 5- to 10-yr-old boys (childhood) and adult men are indicated on the *left*. The *dotted line* in d represents the detection limit in the assay for testosterone.

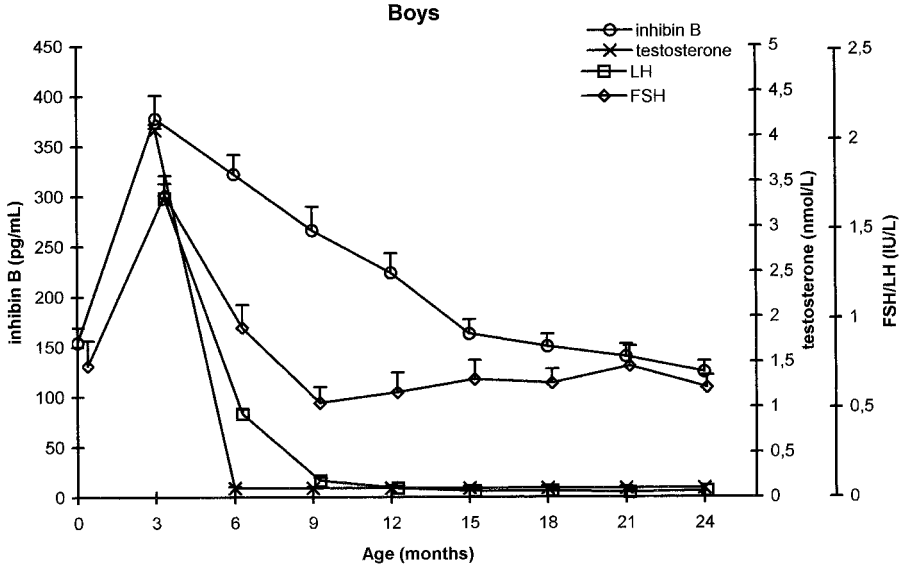

FIG. 2. The group mean values of inhibin B, FSH, and LH levels in boys plotted against age. The SE is indicated by the *error bars*.

levels (mean ± SE, 154 ± 15 pg/mL) were slightly above or in the high range of levels observed in 5- to 10-yr-old boys, whereas FSH levels (0.73 ± 0.14 IU/L) were comparable to those observed later in childhood. LH and testosterone levels were not determined in cord blood due to cross-reactivity with placenta-derived hormones in the assays for LH and testosterone. Serum levels of inhibin B, FSH, LH, and testosterone were all elevated at 3 months of age. Serum inhibin B levels (mean ± SE, 378 ± 23 pg/mL) were elevated to well above adult levels, whereas FSH and LH levels (1.78 ± 0.14 and 1.71 ± 0.50 IU/L, respectively) were elevated into the low adult range, and testosterone levels (4.41 ± 0.68 nmol/L) were below adult levels. Serum levels of FSH, LH, and testosterone decreased in the following 3–6 months. Serum levels of testosterone were undetectable from 6 months of age, whereas serum levels of FSH and LH (0.67 ± 0.17 and 0.1 ± 0.03 IU/L, respectively) were decreased to the range observed later in childhood at 9 months of age. In 12 of 15 boys, the individual maximum level of serum inhibin B was observed at 3 months of age, with declining levels in the following months. In the remaining three boys, the individual maximum level of serum inhibin B was observed at 6 months of age. Although serum inhibin B levels decreased from 3 (or 6) months of age, they remained elevated even after the levels of the other three hormones had decreased to within the range observed later in childhood. In the majority of the boys (n = 10), there was a change in the slope of the declining values around 15–18 months of age, with a slower subsequent fall in inhibin B levels, whereas the decrease was even more in the remaining boys. At 24 months of age, serum levels of inhibin B (124 ± 11 pg/mL) were either above (n = 2) or in the high range (n = 13) for 5- to 10-yr-old boys. No significant correlation between individual levels of inhibin B and FSH was observed at any of the ages studied.

Girls

The individual levels of inhibin B, FSH, and LH in girls are plotted longitudinally in Fig. 3, a–c, and the mean values for inhibin B, FSH, LH, and testosterone at each age are plotted in Fig. 4. The hormonal patterns were generally more complex in girls than in boys. In girls, levels of inhibin B in cord blood were undetectable in all measured samples (n = 11), and FSH levels were either undetectable (n = 3) or in the range observed later in prepubertal childhood (n = 6; mean ± SE, 0.17 ± 0.09 IU/L). At 3 months of age, three girls had inhibin B levels that were elevated (157 ± 34 pg/mL), but to a lesser degree than in boys, four girls had low but detectable inhibin levels (38 ± 5 pg/mL), and four had undetectable levels (three samples missing at this age). At 6 and 9 months of age, there was still high interindividual variation in the levels of inhibin B in girls, although inhibin B was detectable in most samples. From 12 months of age onward, levels of inhibin B were either undetectable or low (37 ± 2 pg/mL). At 3 months of age, serum FSH levels increased to a mean of 2.67 ± 1.91 IU/L, corresponding to the high range of FSH levels observed later in childhood in girls. FSH levels remained in this range in all subsequent samples, with some individual variation (see Fig. 3b). Serum LH levels were generally undetectable or very low in all samples from 3–24 months of age, corresponding to levels observed later in childhood. However, at 3 months of age, one girl diverged from the general FSH/LH pattern. This girl had a serum FSH level of 24.0 IU/L and a LH level of 1.0 IU/L at this age. Inhibin B and estradiol (not shown) levels in the same sample were undetectable. In subsequent samples this girl did not diverge from the general hormonal pattern observed in infant girls. There was no significant correlation between individual levels of inhibin B and FSH in girls at any age, although a nonsignificant negative trend was apparent.

Discussion

Using a newly developed immunoassay specific for the bioactive inhibin B form, we observed surprisingly high levels of inhibin B in boys at 3–6 months of age. The elevated levels of inhibin B in these boys persisted for a much longer period than the neonatal increases in FSH, LH, and testosterone. In neonatal girls, elevated levels of inhibin B were also observed in some individuals. To our knowledge, this lon-

TABLE 1. Median and range for inhibin B, FSH, LH, and testosterone during the first 2 yrs of life in reference to 5- to 10-yr-old prepubertal children and adult males

Age (months)	Boys				Girls		
	Inhibin B (pg/mL)	FSH (IU/L)	LH (IU/L)	Testosterone (nmol/L)	Inhibin B (pg/mL)	FSH (IU/L)	LH (IU/L)
0 (cord blood)	140 (87–243)	0.70 (0.32–1.61)			<18	0.06 (<.06–.69)	
3	361 (254–513)	1.79 (0.90–2.93)	1.74 (0.90–2.64)	4.02 (1.83–6.54)	32 (<18–226)	2.57 (0.48–24.0)	0.08 (<0.05–1.00)
6	330 (204–427)	0.96 (0.29–1.78)	0.36 (0.16–1.07)	<0.23	80 (<18–208)	3.05 (1.68–8.71)	<0.05 (<0.05–0.29)
9	262 (126–501)	0.45 (<0.06–1.03)	0.07 (<0.05–0.34)	<0.23	50 (<18–152)	3.55 (1.53–9.52)	<0.05 (<0.05–0.16)
12	206 (94–383)	0.41 (0.13–1.60)	<0.05 (<0.05–0.21)	<0.23	24 (<18–67)	3.97 (0.68–11.80)	<0.05 (<0.05–0.26)
15	166 (71–272)	0.56 (0.10–1.44)	<0.05 (<0.05–0.17)	<0.23	24 (<18–52)	3.58 (0.30–5.21)	<0.05 (<0.05)
18	137 (96–245)	0.52 (0.19–1.28)	<0.05 (<0.05–0.18)	<0.23	26 (<18–52)	3.31 (1.24–7.73)	<0.05 (<0.05–0.05)
21	136 (80–248)	0.66 (0.20–1.47)	<0.05 (<0.05–0.13)	<0.23	27 (<18–52)	3.72 (1.62–5.24)	<0.05 (<0.05–0.17)
24	121 (71–204)	0.61 (0.11–1.07)	<0.05 (<0.05–0.17)	<0.23	26 (<18–66)	2.75 (1.13–5.61)	<0.05 (<0.05)
5- to 10-yr-old	64 (<18–258)	0.57 (<0.06–1.84)	0.05 (<0.05–0.42)	<0.23 (<0.23–0.58)	<18 (<18–120)	1.22 (0.15–5.61)	<0.05 (<0.05–0.98)
Adult	165 (31–443)	4.03 (0.73–18)	3.31 (0.77–8.02)	17.03 (1.25–35.7)			

Reference ranges in prepubertal children (5–10 yr old) and adult men are based on 114 boys and 142 girls in the age range of 5–10 yr and 358 males in the age range of 25–50 yr. Reference samples were analyzed in the same laboratory and by the same method as the 0- to 2-yr-old children's samples. Eighty-seven of these 114 boys were included in a previous study of serum inhibin B levels (13).

gitudinal study is the first investigation of inhibin B in infants. We show that explicit differences in the levels not only of gonadotropins but also of inhibin B exist between infant boys and girls.

Boys

In studies using less specific assays for inhibin that comeasure inhibin forms that are not biologically active, it has previously been demonstrated that inhibin levels are elevated in parallel with FSH and LH during the first months of life in both humans (7) and primates (15). The significance of this early postnatal elevation in immunoreactive inhibin levels is emphasized by our results, which show that this elevation in human male infants most likely represents a genuine elevation in inhibin bioactivity conferred by the inhibin B form. In contrast, it was recently shown that serum levels of inhibin B in 1- to 2-month-old male rhesus monkeys are at the same level as in juvenile monkeys, using the same specific inhibin B assay as that used in our study (16, 17). This result indicates that inhibin B levels in male rhesus monkeys, in contrast to those in humans, do not exhibit an early postnatal peak followed by a decrease to a lower level later in childhood. Nevertheless, FSH, LH, testosterone, and nonspecific inhibin levels all follow this pattern during this neonatal period in both humans and rhesus monkeys. As inhibin B seems to be a marker of Sertoli cell function, this difference in neonatal levels of inhibin B may reflect differences in Sertoli cell maturation between the two species. In humans, the total number of Sertoli cells increases 5- to 6-fold during the first year of life (18, 19), whereas in primates the mitotic activity of Sertoli cells seems to be limited during this period (20). A second period of postnatal Sertoli cell proliferation occurs around puberty in both humans (18) and primates (20), when the pituitary-gonadal axis is (re)activated, suggesting that activation of this axis may be important for Sertoli cell proliferation (21). As the number of Sertoli cells is believed to be a determinant of spermatogenic potential, adverse effects on Sertoli cell proliferation may be expected to result in impaired sperm output in adulthood. In adult men with hypogonadotropic hypogonadism, basal inhibin B levels have been shown to correlate to prior endogenous gonadotropin stimulation, but not to prior postpubertal exogenous gonadotropin stimulation (22). Furthermore, baseline inhibin levels in these patients seemed to predict the spermatogenic response to gonadotropin treatment (23). It has been suggested that there may be a developmental window during which gonadotropin stimulation of the testis is critical to Sertoli cell function later in life (22). Lack of gonadotropin stimulation in men with congenital or early onset of hypogonadotropic hypogonadism may limit the number or development of Sertoli cells during these developmental periods and thereby limit inhibin B secretion. The neonatal period of endocrine activity of the hypothalamic-pituitary-gonadal axis is possibly such an important developmental window, although fetal, childhood, or early pubertal gonadotropin secretion may also play a role. Thus, treatment of prepubertal hypogonadotropic hypogonadal boys with recombinant human FSH stimulated the production of inhibin B, which presumably reflected an increased Sertoli cell func-

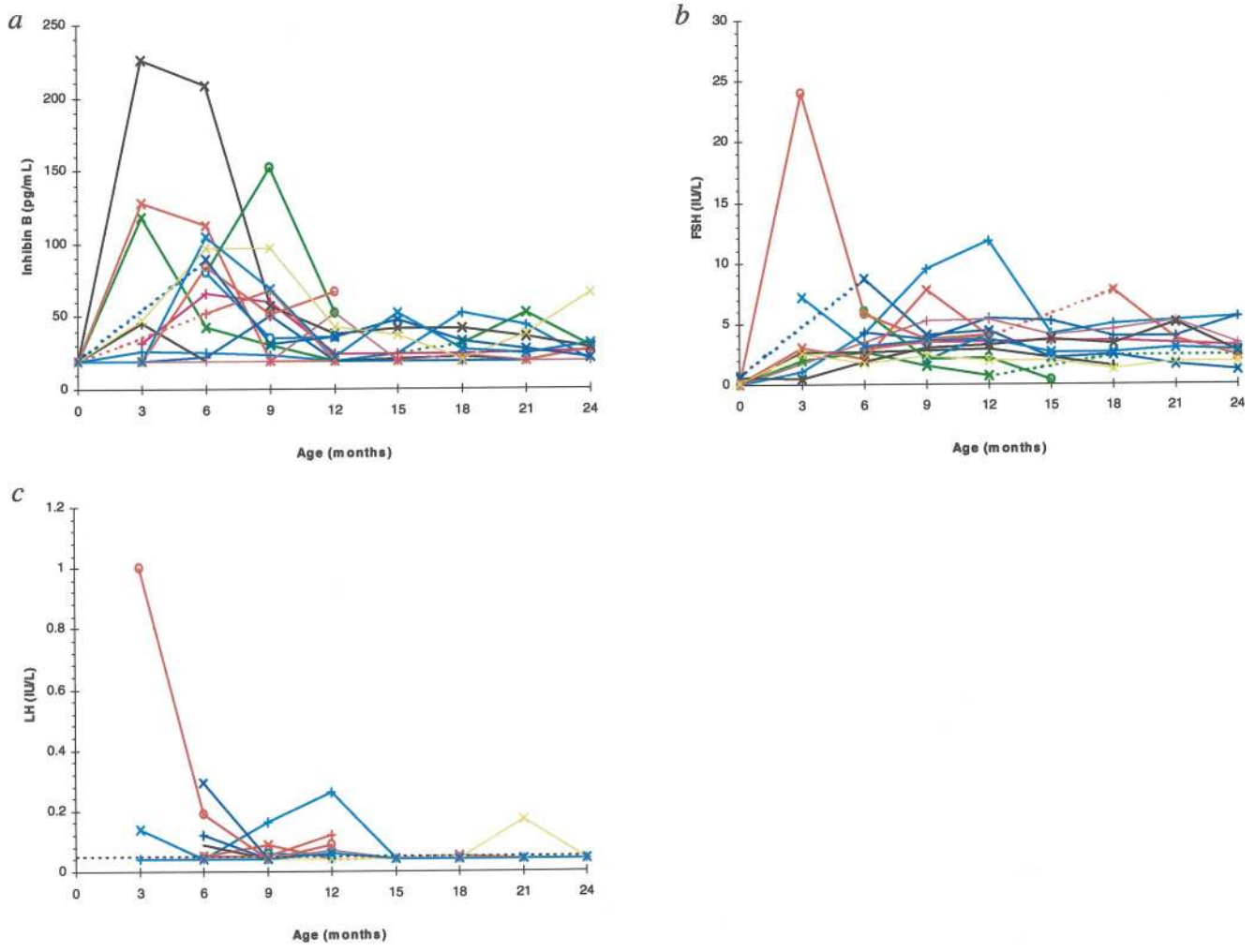

FIG. 3. Individual longitudinal levels of inhibin B (a), FSH (b), and LH (c) in 0- to 24-month-old girls. For each girl, the levels of all four hormones are indicated by the same color and marker. The *dotted line* in c represents the detection limit in the assay for LH.

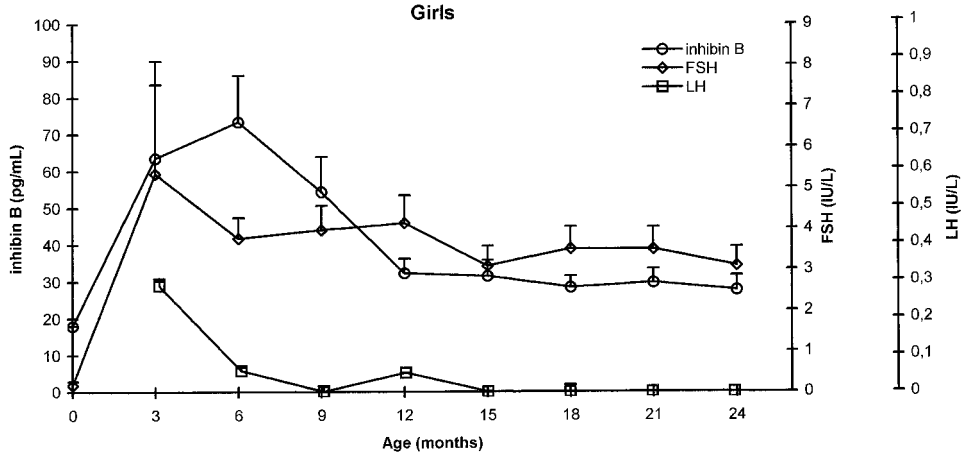

FIG. 4. The group mean values of inhibin B, FSH, and LH levels in girls plotted against age. The SE is indicated by the *error bars*.

tion, and induced growth of the testes (21). Similarly, induced precocious puberty in rhesus monkeys was associated with marked proliferation of Sertoli cells (20). Interestingly, reversible blocking of the pituitary-gonadal axis during the first 4 months of life in monkeys resulted in lower sperm counts in treated animals than in control animals, indicating that the normal activation of the pituitary-gonadal axis during this period also in primates may be important for subsequent spermatogenesis (24).

Sertoli cells are, however, not the only testicular cell type

that increases in number during and shortly after the early postnatal activation of the hypothalamic-pituitary-gonadal axis. A quantitative study of cell numbers in testes obtained at autopsy showed that the total number of germ cells in boys increased until 100 days of age, with a successive decrease in germ cell number in boys older than 100 days (25). Likewise, a pronounced rise in the number of fetal Leydig cells has been shown to take place during the third month after birth, followed by a rapid decrease (26). Furthermore, Leydig cells at this age are larger than those found in the preceding or following weeks. This transient Leydig cell development is presumably stimulated by the elevated LH levels observed at this age and is most likely responsible for the transient elevated testosterone levels. This elevation in testosterone levels has been suggested to be involved both in testicular changes, such as the increase in germ cell number (25), and in sexual differentiation of the central nervous system (27).

No correlation was observed between inhibin B and FSH in boys. Experimental studies in monkeys suggest that mechanisms that regulate the hormonal interactions between the pituitary and the gonad are established in the newborn primate. GnRH antagonist treatment, which blocks FSH and LH release, results in severely suppressed testosterone (28) and inhibin B (16) levels in infant male rhesus monkeys, indicating that testosterone and inhibin B production and secretion at this age, as in the adult male, are stimulated by gonadotropins. However, in our study, inhibin B levels, although slowly decreasing, remained above or in the high range of inhibin B levels observed later in childhood even a year after gonadotropins had reached low childhood levels. This indicates that inhibin B production, once activated by gonadotropin stimulation, can continue autonomously or under the influence of other unknown factors for a period. The negative gonadal feedback mechanisms seem also to be operating in the neonate, as gonadectomy of newborn male and female rhesus monkeys results in dramatically elevated gonadotropin levels (29, 30). Both gonadal steroids and inhibin B are likely gonadal feedback regulators of gonadotropin secretion. This closed feedback loop of the pituitary-gonadal axis is presumably also established in the human newborn. For example, we have observed highly elevated FSH levels in a 3-month-old boy with gonadal dysfunction, reflected in undetectable serum inhibin B levels (unpublished observation). The failure of these pituitary-gonadal interactions to be reflected in a significant correlation between serum inhibin B and FSH levels may be explained by the dramatic hormonal changes occurring during a relatively short period. Even within a given age group, *e.g.* 3–6 months, the activity of the pituitary-gonadal axis may be increasing in some individuals and may be on the decline in others. Thus, individual hormone levels at a given age may reflect different stages of hormonal interaction. Alternatively, additional regulatory factors that interact with the pituitary-gonadal axis may be operating in the newborn. Thus, unknown nongonadal factors seem to be responsible for the subsequent hiatus in gonadotropin secretion later in childhood (31).

Girls

Little is known about the physiological significance of the early postnatal activation of the hypothalamic-pituitary-gonadal hormone axis in girls, and to our knowledge, no studies of GnRH antagonist treatment during the neonatal period in female primates have been presented.

In the normal immature ovary, ovulation does not occur, but different stages of follicular maturation are frequently observed during childhood (32). There seems to be a definite increase in follicle maturation with age. However, the most rapid increase takes place during the first 4 months of postnatal life, concurrent with activation of the pituitary-gonadal axis (32). In a study of prepubertal ovaries obtained at autopsy, the incidence of polycystic ovaries in girls was shown to peak around the age of 4 months, presumably as a result of the increased gonadotropin stimulation (32). In contrast, an ultrasonic study indicated that the incidence of polycystic ovaries did not change during the first 2 yr of life, although the incidence of cystic ovaries with macrocyst was much higher in the first, than in the second, year of life (33). Furthermore, inhibin activity and estradiol levels in follicular fluid tend to be elevated at 0–2 months of age and decrease thereafter (34). In our study, one girl had dramatically elevated FSH and LH levels at the age of 3 months. In the same sample, inhibin B and estradiol were undetectable, a finding that might indicate gonadal dysfunction in this girl. However, in subsequent samples from this girl, FSH and LH were at levels similar to those in the other girls, as was inhibin B. Furthermore, no other clinical data suggested that this girl was endocrinologically abnormal. An alternative explanation for the apparently deviant hormone pattern of this girl at the age of 3 months could be that girls at this age have brief episodes of gonadotropin peaks. Thus, this apparently deviant hormone pattern might be perfectly normal, although it was not observed in the other girls due to the sampling frequency. Gonadotropin levels in the same range as that in this girl have previously been measured in serum from girls under the age of 1 yr (7). Along the same lines, it may be speculated that the large interindividual variation in inhibin B levels at the age of 3–9 months may reflect large intraindividual variation, perhaps in a pseudocyclic pattern. However, a sampling frequency of 3 months clearly only offers a crude indication of the hormonal changes that take place during the first year of life, and more detailed studies of hormone levels in infant girls are needed to further elucidate these issues.

In conclusion, the first 1–2 yr of life, in particular the first few months, are characterized by high gonadal endocrine activity, including a supraadult secretion of inhibin B in boys. This early hormonal activation seems to be important for sexual development and may be potentially vulnerable to endocrine interference, *e.g.* from suspected impact of environmental factors (35).

Our results may also be important from a clinical point of view. Identification and delimitation of the developmental periods receptive to gonadotropin stimulation should lead to improved endocrine management of patients with congenital or early onset of gonadal failure. Identification of inadequate activation of the hypothalamic-pituitary-gonadal axis

during the neonatal period may provide clues about developmental deficiencies that otherwise only become apparent around puberty or later in life. Furthermore, in some cases (*e.g.* in gonadotropin deficiency) identification of inadequate infant activation of the hypothalamic-pituitary-gonadal axis might enable the initiation of treatment at the most responsive periods in development.

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