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Blood Spot Follicle-Stimulating Hormone during Early Postnatal Life in Normal Girls and Turner's Syndrome*

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ABSTRACT

Although FSH has previously been found to be elevated during infancy in agonadal subjects, it is not known whether perinatal FSH levels are also increased. Neonatal blood spot FSH levels were studied retrospectively in nine full term girls born with Turner's syndrome and compared with presumably normal full term girls born the same week. FSH was measured using a highly specific immunoradiometric assay adapted to blood spots collected at the time of systematic neonatal screening. On day 5–6 after birth, FSH was undetectable (<1 IU/L) or low (1–4.4 IU/L) in normal girls. Among the nine patients with Turner's syndrome, five had FSH levels below 3 IU/L, and four showed slightly elevated levels, ranging from 4.3–10.9 IU/L. These differences in FSH secretion were not related to differences in karyotype. Among five patients studied longitudinally during the first 6 weeks of life, three showed increases in FSH levels to 14.9–15.9 IU/L during the second week of life. However, this increase was comparable to that seen in some normal girls sampled on a second occasion during the first weeks after birth. One patient with Turner's syndrome still had low FSH (2.5 IU/L) on day 23, but showed some increase to 8.5 IU/L on day 30. We conclude that 1) in Turner patients, perinatal changes in FSH secretion are similar to those in normal girls, although there is already a lack of feedback control by gonadal hormones on the hypothalamo-pituitary axis; and 2) the FSH assay cannot be used for neonatal screening of Turner's syndrome. (*J Clin Endocrinol Metab* **78:** 978–981, 1994)

IN TURNER'S syndrome, the serum concentrations of LH and FSH are elevated from infancy up to 4 yr of age (1). Although elevated FSH levels have been observed by Conte *et al.* (1) as early as 3 days of age, the early postnatal changes in FSH have not been studied in a significant number of patients with Turner's syndrome. In addition, comparison with data obtained in normal girls of the same age is critical, because normal girls are known to show an early postnatal increase in serum FSH (2–4). In this study, we have developed a blood spot assay for FSH that can measure FSH in samples obtained at the time of systematic neonatal screening. The aim was to determine whether on day 5–6 after birth, FSH secretion was greater in Turner's syndrome than in normal girls; such a finding could provide the basis for a systematic neonatal screening for Turner's syndrome.

Subjects and Methods

Patients

In nine full term patients with Turner's syndrome (45,X karyotype, n = 5; mosaic 45,X/46,XX, n = 4), we studied retrospectively the blood spots collected on day 5–6 of life, at the time of systematic screening for phenylketonuria and hypothyroidism. The time between blood spot collection and assay varied from 1–29 months. For each patient, the blood spots obtained in 8–10 presumably normal girls born the same week were used as controls. In 5 patients with Turner's syndrome, we studied additional blood spots collected between 1–5 weeks after birth. Parental consent was obtained for these patients.

We studied retrospectively the blood spots collected at the time of systematic screening (day 5–6 of life) in 46 premature female newborns (mean gestational age ± SD, 32.8 ± 2.7) and 155 full term female newborns (including the time-matched controls). In 18 premature girls and 13 full term girls, FSH could be measured on a second occasion before the age of 6 weeks. This was made possible using the blood spots obtained after recall to check on the blood level of phenylalanine or TSH in the framework of the systematic neonatal screening.

Methods

The reagents of an immunoradiometric assay for serum FSH (Medgenix Diagnostics, Fleurus, Belgium) were adapted for working with 8-mm diameter dried blood spots. The assay consisted of a single overnight incubation at room temperature. The blood spots were constantly agitated in tubes coated with a monoclonal anti-FSH antibody and containing a second monoclonal anti-FSH antibody labeled with ^{125}I diluted in 0.3 mL phosphate buffer (0.05 mol/L; pH 7.5). Using either blood spots or serum samples collected at the same time in 81 subjects or patients, a close linear relationship was found between the data obtained using the blood spot assay and those obtained with the standard serum assay of FSH ($y = 1.05x - 1.19$; $r = 0.984$; $P < 0.001$); the concentrations ranged from 0.5–40 IU/L serum. The data from the blood spot assay were expressed with reference to the standard preparation (MRC 68/39) as international units per L serum, assuming a hematocrit of 50%. The limit of detection of the blood spot assay was 1 IU/L, and the interassay coefficient of variation was 15.1%. The cross-reactivity of α-subunit in the assay was less than 0.1%. There was no cross-reactivity with hCG (hCGβ) or LH. The validity of the measurement of FSH on blood spots obtained up to 29 months before the assay was documented by finding a similar distribution of the data obtained in the 8–10 control girls born in the same week as in the patients with Turner's syndrome regardless of the storage time.

Statistics

Results are expressed as the mean ± SD. Differences between different groups of patients were determined by analysis of variance, followed by *post-hoc* Fisher protected least significant difference tests.

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Results

Data obtained retrospectively using time-matched blood spots from normal girls sampled 1–29 months before this study were not different from those obtained from freshly collected blood spots (Fig. 1). In the normal girls studied on day 5–6 after birth, blood spot FSH concentrations decreased with gestational age (Fig. 1). In severely preterm girls (gestational age, 26–32 weeks), the mean ± SD FSH concentration was 7.9 ± 5.5 IU/L (Fig. 2). This value was greater than that observed in the mild preterm girls (gestational age, 33–36 weeks; 3.8 ± 4.1 IU/L). After 36 weeks gestational age, 77% of the girls had undetectable FSH, whereas low values (1–4.4 IU/L) were observed in the remaining subjects (Fig. 1). Among the nine patients with Turner's syndrome studied on day 5–6 after birth, five had blood spot FSH concentrations below 3 IU/L, and four showed slightly elevated levels, ranging from 4.3–10.9 IU/L (Fig. 1). These variations in FSH concentration were not related to the differences in karyotype. The differences were significant among severe preterm, mild preterm, and full term girls ($P < 0.0001$) as well between full term girls and patients with Turner's syndrome ($P < 0.001$; Fig. 2).

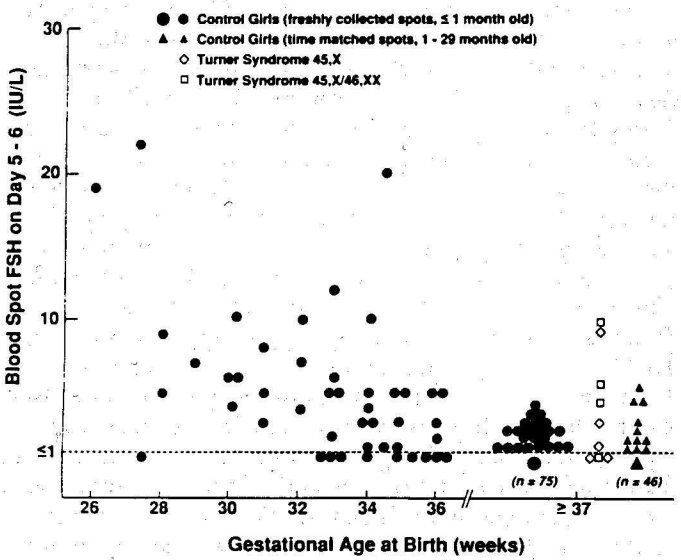

FIG. 1. Individual blood spot FSH concentrations on days 5–6 of life as a function of the gestational age at birth in 46 preterm and 155 full term phenotypically normal girls and in 9 full term patients with Turner's syndrome.

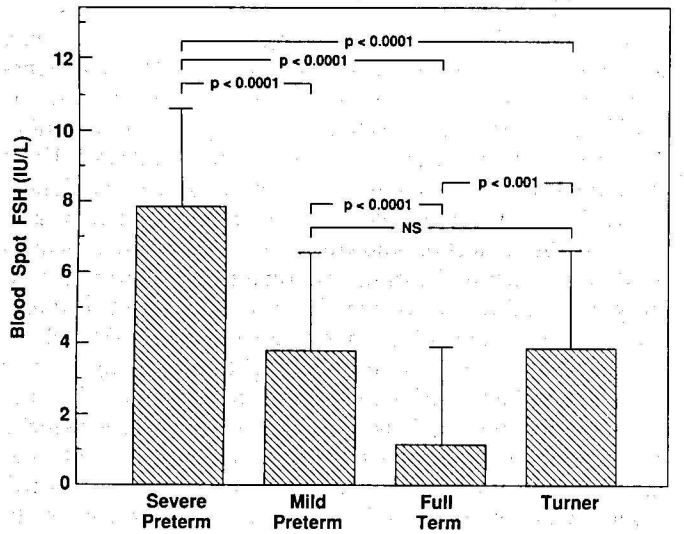

FIG. 2. Blood spot FSH (mean ± SD) in severe preterm (26–32 weeks gestation), mild preterm (33–36 weeks gestation), full term control girls, and full term patients with Turner's syndrome.

Among the 18 normal premature girls studied longitudinally, the vast majority (14 of 18) showed increases in blood spot FSH levels above 15 IU/L in the second sample obtained between days 12–41 after birth (Fig. 3A). Among the 13 normal full term girls studied longitudinally, 9 showed blood spot concentrations of FSH persistently below 3 IU/L during the first month of postnatal life (Fig. 3B). In the 4 remaining girls, FSH increased to a level of 11–22 IU/L during the first month of postnatal life (Fig. 3B). Among the 5 patients with Turner's syndrome studied longitudinally before the age of 6 weeks, one had elevated FSH (10.9 IU/L) on day 5–6, which rose to 20.4 IU/L on day 33. In 3 patients, FSH was low on day 5–6, and it increased to 14.9–15.9 IU/L during the second week of life. This increase was similar to that seen in some normal full term girls. The remaining Turner patient who had a classical 45,X karyotype still showed a low FSH level (2.5 IU/L) on day 23, and an increase to 8.5 IU/L was seen on day 30 (Fig. 3B). In 5 patients, FSH could be measured later during the first year of life. Two showed a clear increase during the first year of life (from 8.5 IU/L at 1 month to 14.9 and 26.3 IU/L at 5 and 12 months, and from 14.1 IU/L at 1 month to 37.0 and 45.3 at 7 and 13 months, respectively). However, the increase in FSH was modest in 2 other patients. One showed similar levels of 14.5 and 14.9 mIU/L at 2 weeks and 6 months. In another, FSH levels were 1.9 and 11.4 IU/L at 6 and 12 months, respectively. The last patient showed a decrease in FSH, from 14.9 IU/L at the age of 10 days to 6 IU/L at 9 months.

Discussion

In this study, we measured FSH in female newborns, using blood spots obtained for the mass systematic neonatal screen-

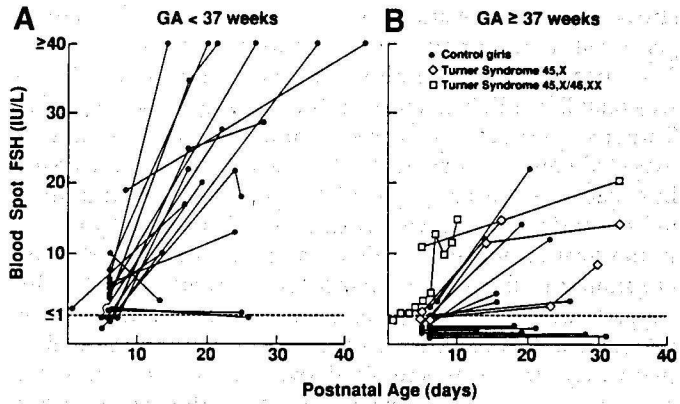

FIG. 3. Longitudinal variations in blood spot FSH concentration measured on two or three occasions during the first weeks of life in 18 premature girls (A) and 18 full term girls (B), either phenotypically normal (n = 13) or with Turner's syndrome (n = 5). GA, Gestational age.

ing of phenyketonuria and hypothyroidism. Although the sensitivity of the blood spot assay was less than that in serum, this was not a limiting factor because we were attempting to detect increased FSH levels. The specificity, particularly the absence of cross-reactivity of hCGβ and the α-subunit, was an important consideration, because these hormones may be elevated at that early stage after birth.

It has been reported previously that during fetal life, FSH is present in the pituitary gland as early as 70 days, whereas serum FSH is detectable as early as 84 days gestation. Then, concentrations rise until midgestation to approach adult castrate values (5). In cord blood, FSH has been found to be elevated at the beginning of the third trimester of pregnancy, whereas undetectable levels are seen at full term (2, 4–8). A similar pattern of secretion of bioactive FSH has been observed with gestational age, although FSH bioactivity was more elevated than immunoreactive FSH (9). In a cross-sectional study, Winter *et al.* (2) observed high FSH levels postnatally in full term girls, with marked individual variations. During the first 10 weeks after birth, serum FSH has been reported to reach levels 10–20 times higher in premature girls than full term girls (3, 4). Our data confirm that serum FSH levels are usually greater in premature girls than in full term girls, who show very low values. In addition, FSH levels show a postnatal rise, which is more marked in premature girls than in full term neonates. Animal studies also indicate that the serum FSH concentrations decline during late pregnancy and increase during early postnatal life, as shown in the female ovine fetus (6) and in one female infant chimpanzee studied longitudinally (2).

In this paper, we show that blood spot FSH concentrations are normal on day 5–6 after birth in most girls with Turner's syndrome. Although slightly elevated FSH levels are found in some patients, their increase is comparable to that seen in some normal full term girls during early postnatal life. Most patients with Turner's syndrome develop primary gonadal deficiency after the first trimester of gestation, with an increase in connective tissue of the gonads (10). In postmortem studies of neonates with Turner's syndrome, one of two patients was found to have a normal ovary (11), whereas streak ovaries or ovaries with a considerable reduction in germ cell number have been described by Carr *et al.* (12). This suggested that the important period for germ cell loss was fetal life (12). Therefore, in the majority of patients with Turner's syndrome, increased serum FSH levels might be expected perinatally on account of the absence of or decrease in negative feedback by gonadal hormones, as seen in most infants and teenagers with Turner's syndrome (1). However, in the ovariectomized female ovine fetus, serum FSH levels are similar to those in the intact female fetus (13, 14). This suggests that the negative feedback of the ovaries on FSH secretion does not operate during fetal life. At the end of pregnancy, the ovariectomized ovine fetus even shows a decrease in serum FSH levels, suggesting the existence of a central maturational process independent of the gonads or the possible role of extraovarian factors, such as the placental hormones. Our data in patients with Turner's syndrome are consistent with those experimental observations, indicating that the ovaries do not supply significant negative feedback on the hypothalamo-pituitary axis, either prenatally or neonatally. In contrast, postnatal agonadism results in increased serum FSH levels, as shown in the rhesus monkeys ovariectomized 1 week after birth. In these animals, serum FSH increases obviously within the next 2 weeks (15). In patients with Turner's syndrome, serum FSH increases during infancy or early childhood (1, 16), although the time of this increase has not been clearly defined. It is noteworthy that in the study of Conte *et al.* (1), no control FSH values were obtained before the age of 1 yr. Taking into consideration the reference data published by others (2–4), it is possible that the FSH values observed in some young infants with Turner's syndrome are in the normal range. Interestingly, the data that we have obtained during the first year of life in five patients indicate that the increase in serum FSH levels can still be modest at 6–12 months of age. Therefore, we assume that a clear-cut pathological rise in serum FSH is likely to occur during late infancy or possibly early childhood in some girls with Turner's syndrome. Massarano *et al.* (17) reported that the proportion of nonstreak ovaries at pelvic ultrasound decreases between 0–2 and 4–6 yr. Based on a reduction in gonadal function between 2 and 6 yr, accounting for a decrease in negative feedback control of FSH secretion, a concomitant increase in FSH levels would be expected. In fact, the cross-sectional data of Conte *et al.* (1) showed high FSH by 2 yr of age and subsequent reduction between 2 and 6 yr. Thus, there are two possibly independent processes, central changes in sensitivity to gonadal feedback and peripheral reduction of gonadal cell content, so that the link between the two events is difficult to establish. In this respect, early longitudinal studies in patients with Turner's syndrome and normal subjects could be helpful. After definition of the age of the clear-cut pathological rise in serum FSH, a blood spot assay of FSH could provide a useful method to screen for Turner's syndrome at that age. Unfortunately, the blood spots obtained routinely on day 5–6 after birth cannot be used in this respect.

The utility of systematic screening for Turner's syndrome is a debatable issue. As reported recently, 50% of patients with Turner's syndrome are diagnosed after 10 yr of chronological age (18). Thus, it is likely that a systematic screening could result in earlier diagnosis of Turner's syndrome. Early awareness of the affection may allow early compassionate counseling of the patients and their families. It may also permit earlier detection and treatment of associated medical conditions, such as hearing abnormalities or learning disability. It is still unknown whether the early onset of therapeutic management of Turner's patients with GH will be correlated to an increase in final height and whether it will result in a reduction of the psychosocial problems related to short stature. So far, it is known that the height velocity during GH therapy is inversely correlated with chronological age at the onset of therapy (19). It is possible that the early onset of GH therapy would account for an increase in height at the time of adolescence. Then, treatment with sex steroids could be started at a normal chronological age for the induction of puberty, with possible auxological and psychological

benefits. Before considering a possible systematic screening for Turner's syndrome, the questions raised about the impact of age at the onset of GH therapy should be answered. In addition, the possible age-related differences in acceptability of GH therapy should be evaluated.

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