

Serum Bioactive Luteinizing and Follicle-Stimulating Hormone Concentrations in Girls Increase during Puberty¹

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ABSTRACT. FSH plays an essential role in folliculogenesis and ovarian growth. However, cross-sectional studies have not shown an increase in bioactive FSH (B-FSH) during puberty. To eliminate intersubject variability, we used a longitudinal design and tested the hypothesis that B-FSH increases during puberty. Thirty normal, healthy girls were enrolled in a longitudinal study from pubertal stages I to IV. The subjects were evaluated at 6-mo intervals; each visit consisted of pubertal staging, bone age determination by x-ray, measurements of serum immunoreactive FSH (I-FSH) and B-FSH ($n = 14$) or immunoreactive LH (I-LH) and bioactive LH (B-LH) ($n = 18$), and adrenal and ovarian steroids. All girls had clinical and hormonal characteristics of puberty. Both I-FSH and B-FSH levels were relatively elevated before puberty, whereas serum I-LH and B-LH were low. From pubertal stages I to III, there was a modest yet significant rise in serum I-FSH ($p < 0.001$) and serum B-FSH ($p < 0.01$). Serum I-LH and B-LH concentrations showed the expected increases with puberty ($p < 0.001$), with serum B-LH concentrations exhibiting a greater rise than I-LH ($p < 0.001$). Our results demonstrate that serum B-FSH and I-FSH increase during puberty. Relatively elevated B-FSH concentrations from early to midpuberty may be an important factor for ovarian growth while circulating LH and estrogen are still low. As puberty progresses, the continued and selective increase in LH induces a rise in estradiol and ultimately leads to ovulation. (*Pediatr Res* 34: 829-833, 1993)

Abbreviations

I-LH, immunoreactive luteinizing hormone
B-LH, bioactive luteinizing hormone
I-FSH, immunoreactive follicle-stimulating hormone
B-FSH, bioactive follicle-stimulating hormone
GnRH, gonadotropin-releasing hormone
DHEA, dehydroepiandrosterone

DHEAS, dehydroepiandrosterone sulfate
 $\Delta 4A$, delta-4-androstenedione
 E_2 , estradiol

The prepubertal or inhibitory stage of sexual maturation is characterized by low serum gonadotropin levels (1). In contrast, the pubertal phase of sexual maturation is distinguished by a sleep-entrained increase in LH concentration (2). Animal studies have shown a strong correlation between GnRH secretory patterns and peripheral LH concentrations (3, 4). Derived from these animal models, the current neuroendocrine concepts of human puberty are based on the assumption that LH pulse frequency reflects GnRH secretory patterns (5). Such a strong correlation has not been demonstrated for FSH as measured by RIA (6). In light of the essential role of FSH in folliculogenesis and ovarian growth (7-9), this apparent lack of FSH increase during the puberty-related gonadal changes was unexpected.

The emerging concept of microheterogeneity of gonadotropins highlights the importance of qualitative changes of circulating hormones as an additional modulator of target site responses. *In vitro* bioassays for measurement of bioactive changes in gonadotropins (10, 11) have shown, as a general rule, that measurements obtained by *in vitro* bioassays were higher than those obtained by RIA. Several studies have conclusively demonstrated that B-LH concentrations during puberty follow changes in I-LH concentrations but with a greater magnitude (12-14). In contrast, very little information is available regarding B-FSH secretory profiles during puberty. We have reported normative cross-sectional data on mean B-FSH levels through puberty (6, 15). Although B-LH is an established sensitive index of sexual maturation, B-FSH does not appear to be affected by the changes of the neuroendocrine milieu characteristic of puberty according to those cross-sectional studies.

Several important considerations have led us to the present study. First, the sample size in our cross-sectional study was small and therefore included significant intersubject variability. Second, Hassing *et al.* (16) have shown a sleep-entrained night time increase in B-FSH in early pubertal boys at a time when a nocturnal augmentation in LH secretion occurs (17). Third, an increase in urinary B-FSH excretion during puberty has been reported in three girls by Fauser *et al.* (18). In this study, we reduced intersubject variability with a longitudinal design and tested the hypothesis that qualitative changes in FSH, reflected by B-FSH, occur during pubertal maturation in girls.

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MATERIALS AND METHODS

Subjects. Thirty normal, healthy girls for whom sufficient serum was available were enrolled from a larger longitudinal study conducted from 1971 through 1977 (19). In view of the longitudinal nature of the study, separate aliquots were stored at -20°C until *in vitro* bioassay methodologies became available. They were followed prospectively at 6-mo intervals from a prepubertal stage until completion of sexual maturation. At each visit, they underwent a thorough physical examination including pubertal staging using a modification of Tanner's criteria (20), growth velocity based on the previous visit or the last 6 mo, and a wrist x-ray study documenting skeletal maturation using the standards of Greulich and Pyle (21). Blood samples were obtained for the determinations of serum concentrations of prolactin, DHEA, DHEAS, $\Delta 4\text{A}$, and E_2 by RIA. The mean \pm SEM ages for pubertal stages I, II, III, IV, and V, were 9.1 ± 0.2 , 10.7 ± 0.1 , 11.5 ± 0.1 , 12.0 ± 0.2 , and 13.4 ± 0.2 y, respectively. Because sufficient serum was not available for characterizing changes in both LH and FSH, subjects were randomly allocated to either an FSH or an LH group. Blood samples for both groups of girls were drawn in the morning between 0800 and 1100 h. In the first group ($n = 18$), the serum samples were assayed for I-LH by RIA and for B-LH by the rat interstitial cell testosterone production assay. In the second group ($n = 14$), the serum samples were assayed for I-FSH by RIA and B-FSH by the rat Sertoli cell aromatase induction assay. Two girls from whom sufficient serum was available had their blood samples analyzed for both LH and FSH and were therefore included in both groups.

RIA. Sera were kept frozen at -20°C until assayed by RIA using previously described methods for prolactin (22), DHEA, DHEAS, and $\Delta 4\text{A}$ (19, 23, 24); briefly, DHEA, and $\Delta 4\text{A}$ were separated from the plasma using a celite chromatography system, and eluates were measured by RIA using a specific antibody. E_2 was purified and separated from estrone by celite chromatography (ethyl acetate-isooctane) (25) and assayed according to the method of Abraham (25). I-LH was measured using two standards (2nd IRP-hMG and LER 960). This latter precaution was taken to ensure that the microheterogeneity associated with a given LH standard would not affect the outcome of the measurements. The assay sensitivities were 1.0 IU/L and 0.1 ng/mL, respectively, for the 2nd IRP-hMG and LER 960 standards. The standard and antisera used for I-FSH were National Institute of Diabetes and Digestive and Kidney Diseases hFSH-3 and 510 (26), respectively. All samples for I-FSH were measured in one assay, and the assay sensitivity was 0.2 ng/mL. Intraassay coefficients of variation for LH and FSH RIA were below 6% and 8%, respectively.

In vitro bioassays. Separate aliquots of available sera that were stored frozen at -20°C were assayed for bioactive gonadotropin concentrations, once the methodology became available. B-LH was measured by the production of testosterone by rat interstitial cells as previously validated by Dufau *et al.* (11). The sensitivity of the assay was 0.5 IU/L with 2nd IRP-hMG standard and 0.2 ng/mL for LER 960. The intraassay and interassay coefficients of variation were less than 7% and 10%, respectively, for the two standards.

B-FSH was measured by a rat Sertoli cell aromatase assay (10). The sensitivity of the assay was 1.0 ng/mL of hFSH-3 and intraassay and interassay coefficients of variation were $13.2 \pm 2.7\%$ (mean of five assays) and $16.1 \pm 1.5\%$ (based on four quality controls and five assays), respectively.

Statistical analyses. The value of assay sensitivity was assigned to data with less than assay sensitivity. For each subject, the mean of all values within each pubertal stage was calculated. To adjust for heterogeneity of variance, I-LH, B-LH, I-FSH, and B-FSH values were logarithmically transformed. An *F* test of the equivalence of the stages was tested by fitting a general linear model to the data and adjusting for subject to subject differences; this model uses all information available from each individual

even when the individual does not have data for all stages. In addition, for the six subjects who had data in stages I through IV, a repeated-measures analysis of variance was performed to compare stages. The trend of these results was similar to that obtained by the use of the general linear model and is not presented.

RESULTS

Clinical and hormonal characteristics. Table 1 summarizes the clinical characteristics (height, weight, bone age, and growth velocity) and serum concentrations of adrenal steroids (DHEA, DHEAS, and $\Delta 4\text{A}$), prolactin, and E_2 in all subjects. From pubertal stages I through V, there were significant progressive increases in bone age, height, mean growth velocity, and serum concentrations of prolactin and adrenal and ovarian steroids ($p < 0.001$). The mean age of menarche was $13 \text{ y} \pm 2 \text{ mo}$.

Mean immunoreactive and bioactive gonadotropins. Progressive changes in mean serum gonadotropin and E_2 concentrations during puberty for both the LH and FSH groups are displayed in Table 2 and Figure 1. Serum B-LH concentrations as measured by both 2nd IRP and LER 960 standards showed progressive increases with pubertal stages ($p < 0.0001$) (Table 2). Similarly, I-LH concentrations as measured by both standards also exhibited progressive increases ($p < 0.0001$). The changes in B-LH concentrations were larger than those in I-LH concentrations. The difference between B-LH and I-LH concentrations became more pronounced as puberty progressed (Fig. 1, panel A). The B-LH/I-LH ratio showed a significant increase with the progression of puberty ($p < 0.0001$). Serum E_2 showed the expected gradual pubertal increase throughout sexual maturation (Fig. 1, panel A).

The changes in serum B-FSH and I-FSH concentrations and E_2 concentrations for the 14 girls in the FSH-only group are summarized in Table 2 and Figure 1B. Mean serum I-FSH concentrations showed a significant increase ($p < 0.0001$) from pubertal stages I to III; thereafter, the serum concentrations plateaued. Similarly, serum B-FSH levels rose from pubertal stages I to III ($p < 0.01$); thereafter, it seems from the small number of subjects, that serum B-FSH concentrations plateaued. The B-FSH/I-FSH ratio averaged 1.4 ± 0.2 , 1.1 ± 0.1 , 1.0 ± 0.1 , 0.8 ± 0.1 , and 1.2 ± 0.2 for the five pubertal stages and decreased from pubertal stages I through IV ($p < 0.01$). As seen in the LH group, serum E_2 concentrations exhibited the expected steady increase characteristic of puberty.

DISCUSSION

The results demonstrate that despite the relatively elevated levels of serum I-FSH and B-FSH in prepubertal girls, serum B-FSH and I-FSH concentrations increase during early pubertal stages. Differential regulation of B-FSH and B-LH becomes evident as puberty progresses: B-LH and E_2 secretion increase markedly, whereas B-FSH levels stabilize and reach a plateau. These results contrast with our earlier results from a cross-sectional study of boys and girls in which serum B-FSH concentration remained constant throughout puberty (15). The longitudinal component of the present study reduced intersubject variability, thus unmasking modest but significant increases in serum B-FSH concentration. It is possible that a longitudinal experimental design incorporating overnight frequent sampling may show more pronounced changes in the pattern of B-FSH secretion. The single daily sampling performed in this study did not allow for the assessment of gonadotropin pulsatility, nor was it possible to determine whether sleep-entrained changes occurred. A sleep-entrained nighttime rise of B-FSH was previously demonstrated in early pubertal boys (16), and girls with precocious puberty have increased excretion of urinary B-FSH (18).

As expected, the B-LH signal increased more than I-LH, and as a consequence the B-LH/I-LH ratio increased with progression

Table 1. Clinical characteristics and hormone concentrations expressed as mean \pm SEM from pubertal stages P I through P V, after F test analysis throughout pubertal stages*

	Pubertal stage					p value
	I	II	III	IV	V	
Age (y)	9.1 \pm 0.2	10.7 \pm 0.2	11.5 \pm 0.1	12.0 \pm 0.2	13.4 \pm 0.2	<0.0001
Height (cm)	136.9 \pm 1.4	145.3 \pm 1.1	152.2 \pm 0.9	157.0 \pm 1.2	160.2 \pm 1.4	<0.0001
Weight (kg)	30.8 \pm 1.5	35.7 \pm 1.4	40.8 \pm 1.6	43.6 \pm 1.3	50.3 \pm 2.3	<0.0001
Bone age (y)	9.1 \pm 0.2	10.8 \pm 0.1	12.0 \pm 0.1	12.8 \pm 0.1	14.2 \pm 0.2	<0.0001
GV (cm/6 mo)	2.6 \pm 0.1	3.1 \pm 0.1	3.5 \pm 0.8	3.6 \pm 0.2	2.5 \pm 0.2	<0.0001
E ₂ (pmol/L)	59.8 \pm 7.4	116.2 \pm 13.3	117.2 \pm 14.8	264.2 \pm 20.3	301.9 \pm 23.7	<0.01
Prolactin (μ g/L)	5.6 \pm 0.4	6.2 \pm 0.5	7.0 \pm 0.6	7.7 \pm 1.0	9.0 \pm 0.6	<0.01
n	24	30	24	15	21	
DHEA (nmol/L)	3.5 \pm 0.3	8.0 \pm 1.0	11.2 \pm 1.7	10.5 \pm 1.7	12.2 \pm 1.7	<0.0001
Δ 4A (nmol/L)	0.7 \pm 0.0	1.4 \pm 0.3	2.3 \pm 0.3	3.4 \pm 0.7	3.1 \pm 0.3	<0.0001
n	15	17	13	6	9	
DHEAS (μ mol/L)	0.5 \pm 0.1	1.0 \pm 0.2	0.9 \pm 0.1	1.5 \pm 0.3	1.4 \pm 0.2	<0.0001
n	17	19	15	8	10	

* GV, growth velocity. Mean age of menarche was 13 \pm 2 mo.

Table 2. Serum LH and FSH concentrations expressed as mean \pm SEM from pubertal stages I through V after analysis by F test throughout pubertal stages

	Pubertal stage					p value
	I	II	III	IV	V	
I-LH* (mIU/mL)	1.1 \pm 0.1	1.7 \pm 0.2	2.5 \pm 0.3	4.9 \pm 0.6	7.7 \pm 1.0	<0.001
I-LH† (ng/mL)	0.8 \pm 0.2	1.1 \pm 0.3	2.4 \pm 0.5	8.5 \pm 1.7	12.1 \pm 1.6	<0.001
B-LH (ng/mL)	1.3 \pm 0.5	2.0 \pm 0.4	6.6 \pm 1.3	17.5 \pm 2.1	29.1 \pm 4.0	<0.001
n	12	18	13	10	14	
I-FSH (ng/mL)	2.0 \pm 0.2	2.5 \pm 0.2	3.7 \pm 0.4	3.3 \pm 0.2	3.6 \pm 0.4	<0.001
B-FSH (ng/mL)	2.6 \pm 0.4	2.8 \pm 0.4	3.7 \pm 0.8	2.8 \pm 0.7	3.9 \pm 0.6	<0.01
n	13	14	12	6	9	

* LH standard = 2nd IRP hMG.

† LH standard = LER 960.

of puberty (12–14, 27). In contrast, the B-FSH signal showed only a modest rise through pubertal stage III, but as a result the B-FSH/I-FSH ratio decreased through pubertal stage IV. The secretory patterns of B-FSH and its isoform distribution during puberty need further investigation. The divergence of the gonadotropin bioactive/immunoreactive ratios (increased LH versus decreased FSH) provides additional support for the differential regulation of LH and FSH.

The differences in magnitude of B-FSH and I-FSH increases could result from several possible sources. First, there could be a change in the quality of the FSH secreted by the pituitary gland. Several observations support the hypothesis that GnRH and/or estrogens modulate FSH bioactivity or create a shift of FSH isoform distribution toward the relatively more basic forms. For example, Wide *et al.* (28) showed qualitative changes in the FSH secreted in response to GnRH stimulation in girls with Turner's syndrome; the newly secreted isoforms had a less negative median charge. In the intact female lamb, experimentally induced puberty induces an increase in circulating B-FSH, which also correlates with a shift in circulating isoform distribution toward a relatively more basic pI (29). Relatively more basic isoforms have greater *in vitro* bioactivity (30). Finally, estrogen treatment of postmenopausal women was associated with a shift in the excretion of FSH toward a relatively more basic and biopotential isoform mix (6). It will be important to determine in future studies whether the changes in B-FSH observed in this study reflect shifts in FSH isoform distribution throughout puberty in girls.

A second mechanism by which B-FSH secretion would be selectively altered could be mediated by growth factors. Indeed, gonadal growth factors such as IGF-I, transforming growth factor, and epidermal growth factor may have a modulatory role in

the mediation of FSH action (7). At the gonadal level, nonspecific effects of serum growth factors may contribute to the observed discrepancies between I-FSH as measured by RIA and B-FSH as measured by the Sertoli cell aromatase assay (one of several biological end measures of FSH action in the Sertoli cells). Adashi *et al.* (31) found IGF-I to be a powerful potentiator of the actions of FSH in rat granulosa cells. It has been well documented that IGF-I increases dramatically during puberty (32), a time also characterized by peak growth velocities. Although IGF-I levels were not measured in this particular study, our subjects did show the progressive clinical and hormonal changes characteristic of sexual maturation, notably the significant increase in growth velocity. Therefore, puberty-associated changes in circulating levels of IGF-I could theoretically interfere with our FSH bioassay measurements. However, in the *in vitro* B-FSH system, because insulin is added to the medium, further addition of IGF-I to the Sertoli cell cultures does not alter the levels of FSH-induced aromatase (33).

Because we are measuring E₂ as an end point of FSH action, circulating levels of E₂ could contribute to the final B-FSH measurement (34). However, in the pubertal samples used in the present study, circulating levels of E₂ ranged from 20 to 100 pg/mL. Because we are using 10 μ L of serum in the bioassay, the absolute amount of E₂ added ranges from 0.2 to 1.0 pg and has no effect on the final measurement of serum B-FSH concentrations. Furthermore, in the luteal phase of the menstrual cycle, E₂ levels are high, yet B-FSH levels are low (30). Considering the longitudinal nature of the study, prolonged storage may have deleterious effects on the bioactive gonadotropin isoforms. However, quality control serum pools from both male and female subjects measured in the FSH bioassay over the last 4-y period (1989–1992) showed no evidence of deterioration of B-FSH with long-term storage.

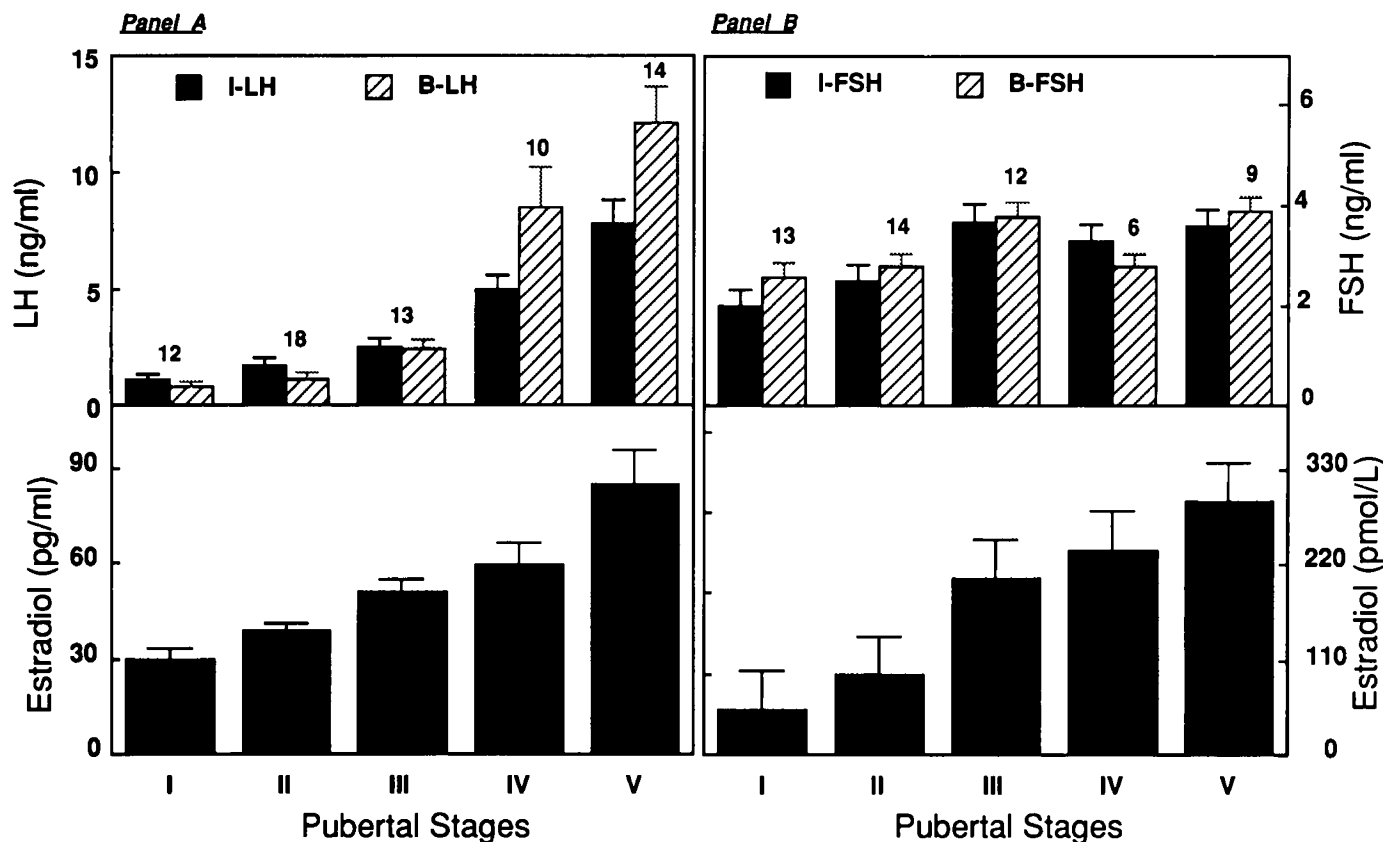


Fig. 1. Panel A, Mean B-LH, I-LH using the LER 960 standard, and E_2 concentrations relative to pubertal stages I through V. Panel B, Mean B-FSH, I-FSH, and E_2 concentrations relative to pubertal stages I through V. The numbers above the bars in the top graphs represent the number of subjects at each pubertal stage.

It appears that FSH has a trophic role in the ovary in the absence of pubertal LH and E_2 secretion. Ovarian size increases steadily and limited ovarian follicular growth occurs during the prepubertal years when the reproductive axis is in a relatively quiescent state (35). Unsustained sexual precocity such as breast budding is not uncommon in girls (36). Furthermore, it appears that recombinant FSH alone can induce growth of preovulatory follicles in the absence of optimal E_2 concentrations. After such treatment, Shoot *et al.* (37) observed that intrafollicular $\Delta 4A$ was low in a woman with hypogonadotropic hypogonadism. These findings would indicate that follicular growth and steroidogenic activity are differentially regulated in granulosa cells.

All subjects in our study showed the expected clinical and hormonal changes of puberty: increase in growth velocity and advancement of skeletal maturation, as well as increased production of gonadal and adrenal steroids. The patterns of serum B-FSH secretion in our subjects are in keeping with earlier results from studies in normal women and patients with gonadal dysgenesis in whom rising peripheral concentrations of either endogenous or exogenous E_2 led to a nonparallel rise in both I-FSH and B-FSH (30). Some of our physically immature subjects had E_2 values compatible with an early pubertal range, and it is possible that these pubertal stage I subjects on the verge of puberty blunted the magnitude of B-FSH increase in comparison with latter maturational stages.

In conclusion, the results of this study highlight the complexity of the regulation of FSH secretion and its role in the maturing ovary. These regulatory mechanisms are still poorly understood but undoubtedly play an indispensable role in the maintenance and development of ovarian follicles.

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