GnRH agonist stimulation of the pituitary–gonadal axis in children: age and sex differences in circulating inhibin-B and activin-A

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BACKGROUND: Inhibin-B decreases and activin increases FSH secretion in adults. We investigated whether an FSH–inhibin/activin feedback loop exists before or during puberty. METHODS: FSH secretion was stimulated with 10 μg/kg leuprolide acetate (GnRH agonist) in 18 girls, ages 1.0–13.2 years, and 11 boys, ages 8.9–15.2 years, with variations in pubertal development, and in five normal 9- to 10-year-old girls. Blood, obtained at 0, 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 h after GnRH agonist, was analysed for LH, FSH, activin-A, inhibin-A, inhibin-B, follistatin 288 and estradiol/testosterone. RESULTS: FSH increased within 30 min of GnRH agonist administration with a peak greater in girls than boys (P < 0.0006). Baseline inhibin-B was greater in boys than girls (P < 0.01), while baseline activin-A concentrations were greater in girls. GnRH agonist-stimulated FSH increased inhibin-B in girls by 8 h and in boys by 20 h (P < 0.05), but did not affect activin-A. Inhibin-B increases were seen only in girls older than 5 years. CONCLUSIONS: An inhibin-B–FSH feedback loop exists prior to the onset of puberty in girls older than 5 years. Sex differences in activin-A and inhibin-B concentrations may be responsible for sex differences in serum FSH concentrations.

Key words: FSH/gonadal peptides/inhibin/puberty

Introduction

Boys and girls exhibit differences in the timing of pubertal maturation even though in both sexes puberty is coincident with a marked increase in the episodic release of LH (Marshall and Kelch, 1986) under the control of GnRH. Girls begin puberty on average 18 months before boys do, and have as much as a 5-fold increase in the incidence of precocious puberty as compared with boys (Grumbach and Styne, 1998). Both boys and girls have remarkable activity of the hypothalamic–pituitary–gonadal axis in early infancy, but, within 3 months after birth, activity wanes and remains quiescent until the time of puberty (Winter, 1982; Lee, 1985; Bergada et al., 1999). Throughout infancy and childhood, however, FSH is more readily released in girls than in boys (Grumbach et al., 1974; Rosenfield et al., 1989; Cuttler et al., 1993). Since FSH plays a key role in gonadal development in girls (Aittomäki et al., 1996; Themmen and Huhtaniemi, 2000; Barnes et al., 2002), differences in the regulation of FSH or its activity at the level of the gonad might contribute to sexual dimorphism in the timing of puberty.

FSH secretion is under the complex regulation of GnRH, which controls episodic release and is also released constitutively under the positive stimulation of the activating family of FSH-regulatory peptides (for reviews see Farnworth, 1995; Padmanabhan and Sharma, 2001). The activins are peptides in the transforming growth factor-β (TGF-β) family that are produced in gonadal, pituitary and extragonadal tissues (Ying, 1988). Although there are several known activin forms, serum assays have only been developed for activin-A and activin-AB. Only activin-A has been detected in human serum at concentrations above assay sensitivity (Knight et al., 1996; Evans et al., 1997). Human activin-A concentrations may vary in the menstrual cycle and perhaps with ageing, but have not been reported to fluctuate with pubertal maturation (Muttukrishna et al., 1996; Foster et al., 2000; Luisi et al., 2001). Inhibins, also members of the TGF-β family, are produced principally in the gonads and act to depress FSH secretion (Ying, 1988; Bilezikjian et al., 1994; Kishi et al., 1999). Two forms of inhibin, inhibin-A and -B, are produced in the ovary, while the testis produces only inhibin-B.
FSH regulatory peptides after GnRH agonist in children

Serum inhibin-A concentrations, produced from dominant follicles (Roberts et al., 1993), are at pubertal concentrations in girls during the 3 months after birth and then decline to low concentrations until late puberty (Bergada et al., 1999; Foster et al., 2000; Sehested et al., 2000; Raivio and Dunkel, 2002). Serum inhibin-B concentrations are produced from Sertoli cells in boys and small antral follicles in girls (Roberts et al., 1993). Serum inhibin-B concentrations are at pubertal levels for up to 3 months after birth in boys and girls (Bergada et al., 1999). Inhibin-B concentrations then decline to low levels in girls until the time of puberty (Bergada et al., 1999; Foster et al., 2000; Sehested et al., 2000). Inhibin-B concentrations are much greater in boys than in girls throughout childhood and exhibit a further increase in boys at the time of puberty (Andersson et al., 1997).

FSH is also regulated by follistatin, a monomeric peptide that binds activin and prevents activin binding to its receptor, thereby inhibiting FSH secretion (Robertson, 1992; de Winter et al., 1996; Phillips and de Kretser, 1998). Processing of mRNA results in at least two forms of follistatin, a 315 kDa amino acid (aa) peptide and a 288 kDa aa (Robertson, 1992; Phillips and de Kretser, 1998). Follistatins are made in the gonads, pituitary and extra-gonadal tissues (Robertson, 1992; Phillips and de Kretser, 1998). Serum concentrations of total follistatin decline in late puberty in girls (Foster et al., 1999; Phillips and de Kretser, 1998). Serum inhibin-A concentrations, produced from dominant follicles (Roberts et al., 1993), are at pubertal concentrations for up to 3 months after birth in boys and girls (Bergada et al., 1999). Inhibin-B concentrations then decline to low levels in girls until the time of puberty (Bergada et al., 1999; Foster et al., 2000; Sehested et al., 2000). Inhibin-B concentrations are much greater in boys than in girls throughout childhood and exhibit a further increase in boys at the time of puberty (Andersson et al., 1997).

The temporal changes in the serum concentrations of the FSH-regulatory peptides seem to be associated with changes in serum FSH concentration. Raivio et al. (2000) demonstrated that early pubertal boys exhibit an inverse relationship between baseline inhibin-B concentrations and peak FSH levels after GnRH stimulation, but the role of the other FSH-regulatory proteins in children has not been investigated. In puberty, FSH responses to GnRH may be regulated, not only by inhibins, but also by changes in other FSH-regulatory proteins. Gonadal maturation may be required before gonadal feedback of FSH-regulatory peptides can occur in response to FSH stimulation. As FSH secretion is controlled by the combined effects of the activins, inhibins, follistatins and sex steroids as well as GnRH, understanding the changes in gonadal production of FSH-regulatory peptides in the basal state and under GnRH-induced FSH stimulation should provide insights into the role of the gonad in FSH regulation during development. We hypothesized that the prepubertal and pubertal ovary and the pubertal testis would respond to FSH stimulation with changes in FSH-regulatory protein concentrations confirming the presence of a prepubertal feedback loop between FSH and FSH-regulatory peptides in boys and girls. To test this hypothesis, we increased FSH secretion in children by administering the GnRH agonist analogue, leuprolide acetate, and followed the changes in gonadotrophins, FSH-regulatory peptides and sex steroids over 24h.

Materials and methods

Subjects
We studied 29 children who had variations in growth and/or pubertal development and five normal 9- to 10-year-old girls. The clinical characteristics of the 18 girls and 11 boys with variations in development are shown in Table I. The five normal girls were 9 or 10 years of age and had bone ages of 9–10 years, consistent with their chronological ages. None of the girls had had onset of pubic hair growth or menses. Three girls had stage II breast development by the method of Tanner (1978) at the time of study. Two had onset of breast development within 6 months after the study. All were in good health.

Table I. Clinical characteristics of girls with variations of pubertal development who received GnRH agonist

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Chronological age (years)</th>
<th>Bone agea (years)</th>
<th>Breastb</th>
<th>Pubic hairb</th>
<th>Vaginal bleeding</th>
<th>Final diagnosisc</th>
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<td>SS</td>
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</table>

a Determined by the method of Greulich and Pyle (1959).
b Determined by the method of Tanner (1978).
c IPP = idiopathic precocious puberty; ND = not done; none = no diagnosis made; PP = precocious pubarche; PT = precocious thelarche; SS = short stature.
For the purpose of this study, precocious thelarche was defined as onset of breast development prior to 7 years of age in the absence of pubic hair, the absence of bone age advancement beyond 2 SD above the mean, and a growth velocity within 2 SD of the mean for age. Precocious pubarche was defined as the onset of pubic hair prior to 5 years of age and at least 2 years before the onset of breast development. Those children with precocious pubarche also had absence of bone age advancement beyond 2 SD above the mean, and a growth velocity within 2 SD of the mean for age. Precocious pubarche was one of these six boys had delayed adolescence without short stature. One of these six boys had early pubic hair growth without significant progression of breast or pubic hair growth or growth bone age acceleration. Three girls were referred for investigation of short stature.

Of the 11 boys (Table II), six were referred for short stature. Four of these six boys had delayed adolescence, one of these six boys had isolated growth hormone deficiency and, in one, no diagnosis was made. Two boys had delayed adolescence without short stature. Two boys had precocious puberty with advancement of bone age. One of these boys had late onset congenital adrenal hyperplasia and developed central puberty after suppression of his adrenal steroids. The final boy had early pubic hair growth without significant elevation of adrenal androgens. One boy had a prior orchidopexy for undescended testes. His phallus and gonad length were at the 10th percentile. Since his gonadotrophins, inhibin-B and activin-A concentrations were comparable with those of the other boys studied, his data were included in the analysis.

**Protocol**

The protocols were approved by the Institutional Review Board of the University of Michigan or the University of Chicago. Consent was obtained from a parent, and assent was obtained from the children who were older than 6 years of age. All studies were conducted in the General Clinical Research Centers (GCRCs) at the University of Michigan or the University of Chicago. An intravenous cannula was placed in a forearm vein and kept open with a dilute solution of heparin. At 09.00 h, 10 μg/kg leuprolide acetate (Lupron®, TAP Pharmaceuticals, Deerfield, IL) was administered subcutaneously. In the five normal girls studied, two received a dose of 1 μg/kg of leuprolide acetate and three received 10 μg/kg. The dose did not change the magnitude of the FSH response, so all data for the normal girls were pooled. These five girls had LH and FSH determined as per the 29 subjects with variations in pubertal maturation, but inhibin-A, inhibin-B and activin-A were determined at 0 and 4 h and in a serum pool of the 16–24 h samples. Estradiol concentrations were determined at 0, 16 and 24 h. In the 29 subjects with variations in pubertal development, blood samples were obtained at baseline, 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 h for determination of LH, FSH, activin-A, and inhibin-A and -B. Follistatin 288 and estradiol or testosterone were determined at 0, 4 and 24 h. Three girls and seven boys also had determinations of total follistatin in the 0 and 20 h samples. Sample volumes were insufficient to allow assays in the other subjects. Each child had a bone age examination performed to assess biological maturity. Bone age was determined by the method of Greulich and Pyle (1959).

**Assays**

Serum LH, FSH and testosterone were determined using Delphia® immunofluorometric assay kits (Wallac, Gaithersburg, MD) with assay sensitivities of 0.05 IU/l for LH and FSH and 0.1 nmol/l for testosterone. The intra- and inter-assay coefficients of variation (CVs) were 3.1 and 4.8% for LH, 3.9 and 4.8% for FSH, and 5.1 and 9.5% for testosterone. Estradiol, determined by radioimmunoassay kits (Diagnostic Products Corporation, Los Angeles, CA), had an assay sensitivity of 18 pmol/l, and intra- and inter-assay CVs of 5 and 9%, respectively.

**Table II. Clinical characteristics of boys with variations of pubertal development who received GnRH agonist**

<table>
<thead>
<tr>
<th>Subject number</th>
<th>Chronological age (years)</th>
<th>Bone age&lt;sup&gt;a&lt;/sup&gt; (years)</th>
<th>Pubic hair&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Testicular volume (ml)</th>
<th>Diagnosis&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>4</td>
<td>DA</td>
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</table>

<sup>a</sup>Determined by the method of Greulich and Pyle (1959).

<sup>b</sup>Determined by the method of Tanner (1978).

<sup>c</sup>UT = undescended testes s/p orchidopexy; ND = not done; CAH = congenital adrenal hyperplasia; PP = precocious pubarche; IGHD = isolated growth hormone deficiency; DA = delayed adolescence; SS = short stature.
Activin-A, inhibin-A and inhibin-B were determined using two-site enzyme-linked immunosorbent assays (ELISAs; Serotec, Raleigh, NC). The assay sensitivities were 0.2 ng/ml for activin-A, 8 pg/ml for inhibin-A and 15 pg/ml for inhibin-B. The intra- and inter-assay CVs were 6 and 16% for activin-A, 6 and 13% for inhibin-A, and 10 and 16% for inhibin-B.

Follistatin 288 was determined using a two-site ELISA, as described previously (Evans et al., 1998), with an assay sensitivity of 9.75 pg/ml. The intra- and inter-assay CVs were 12 and 16%, respectively. This assay cross-reacts by 10% with follistatin 315. Total follistatin concentrations were determined using a heterologous radioimmunoassay described previously (O’Connor et al., 1999), which employs dissociating reagents to remove the interference of bound activin. The rabbit polyclonal antiserum was raised against 35 kDa bovine follistatin, and human recombinant (hr)-follistatin 288 was used as both tracer and standard. Cross-reactivity is 100% for hr-follistatin 288 and 33% for hr-follistatin 315. The assay sensitivity was 1.7 ng/ml, and the intra- and inter-assay CVs were 6.7 and 4.8%, respectively.

Statistics
Analysis of sex differences was determined in age-matched boys and girls such that only data from those girls with bone ages of 7.9 years or more were compared with the data of the boys. The choice of 7.9 years as a cut-off was based on studies regarding the normal age of onset of pubertal development in girls (Wu et al., 2002). As many as 10% of normal girls have breast development by age 8. Comparisons between girls were made by grouping girls by bone age. Three groups were defined: bone ages 0.9–3 years (n = 5), bone ages 5.5–7.3 years (n = 6) and bone ages ≥7.9 years (n = 7). All hormone values were transformed logarithmically prior to analysis. Multiple comparisons were made by repeated measures of analysis of variance followed by a Tukey–Kramer test for post hoc significance. Significance was defined as P < 0.05. Both the significance within the repeated measures and the post hoc comparisons between values within an analysis are presented. Comparisons of peak, baseline, and percentage change values were performed by one-way analysis of variance followed by Fisher’s PLSD. A P-value of <0.05 was considered significant.

Results
GnRH agonist responses for girls with variations in development (Figure 1)
Administration of leuprolide acetate produced a significant increase in both serum LH and FSH concentrations (P < 0.0001). LH and FSH concentrations increased within 7.9 years as a cut-off was based on studies regarding the normal age of onset of pubertal development in girls (Wu et al., 2002). As many as 10% of normal girls have breast development by age 8. Comparisons between girls were made by grouping girls by bone age. Three groups were defined: bone ages 0.9–3 years (n = 5), bone ages 5.5–7.3 years (n = 6) and bone ages ≥7.9 years (n = 7). All hormone values were transformed logarithmically prior to analysis. Multiple comparisons were made by repeated measures of analysis of variance followed by a Tukey–Kramer test for post hoc significance. Significance was defined as P < 0.05. Both the significance within the repeated measures and the post hoc comparisons between values within an analysis are presented. Comparisons of peak, baseline, and percentage change values were performed by one-way analysis of variance followed by Fisher’s PLSD. A P-value of <0.05 was considered significant.

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Results
GnRH agonist responses for girls with variations in development (Figure 1)
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Figure 2. GnRH agonist response in girls with variations in pubertal development by bone age group. Leuprolide acetate (10 μg/kg) was administered at 09.00 h and blood obtained at the indicated times. Follistatin 288 and estradiol were measured only at 0, 4 and 24 h. Each point represents the mean ± SE. *Significant ($P < 0.05$) change from $t = 0$; †significant ($P = 0.008$) elevation with respect to the oldest girls; ‡significantly ($P = 0.04$) lower with respect to the oldest girls.
30 min, reaching maximal concentrations by 4 h. Inhibin-B concentrations increased significantly ($P < 0.0001$). The increase from baseline was significant 8 h after administration of GnRH agonist. There were no significant changes in activin-A or follistatin 288 concentrations throughout the 24 h time course of the study. Inhibin-A was at or below assay sensitivity for all children at all time points (data not shown). Total follistatin concentration decreased in the three girls in whom it was measured, from 3.78 to 3.57 ng/ml, from 5.01 to 3.88 ng/ml and from 8.76 to 4.01 ng/ml. These changes were not significant. Estradiol concentrations increased significantly ($P = 0.0017$), with the increase detected at 24 h after GnRH agonist administration.

**GnRH agonist responses in girls by bone age (Figure 2)**
The girls’ data were subdivided by bone age, a measure of biological maturity, for analysis of age-related changes. The peak FSH response to GnRH agonist was significantly greater in the youngest girls with respect to the oldest group of girls. An inverse correlation of $r = 0.59$ was present between the log of the peak FSH and bone age ($P = 0.01$). Inhibin-B concentrations did not increase significantly in the youngest girls, despite a vigorous FSH response. In the intermediate and oldest girls, inhibin-B concentrations increased significantly ($P < 0.0001$). There was a significant change from baseline by 8 h in the intermediate group and at 12 h in the oldest girls. There were no significant changes in activin-A or follistatin 288 concentrations within or between groups. Estradiol concentrations increased significantly only in the girls with bone ages of $\geq 7.9$ years ($P = 0.02$).

**GnRH agonist responses in normal girls (Figure 3)**
LH and FSH concentrations increased significantly in response to GnRH agonist ($P < 0.0001$). The increase was seen after 30 min and was maximal at 4 h, as in the subjects with variations in pubertal development. In contrast to our subjects with variations in development, inhibin-A concentrations increased significantly ($P = 0.02$) in the 16–24 h sample after GnRH agonist administration. Inhibin-B also increased significantly in the 16–24 h sample ($P = 0.0002$). Estradiol concentrations were significantly increased, compared with time 0, by 16 h. Activin-A concentrations did not change with GnRH agonist stimulation.

![Figure 3. GnRH agonist response in normal girls. Leuprolide acetate (1 or 10 μg/kg) was administered at 09.00 h and blood obtained at the indicated times. Inhibin-A, inhibin-B and activin-A concentrations were measured at 0 and 4 h and in a pool of samples at 16, 20 and 24 h. Estradiol concentrations were measured at 0 and 24 h. *Significant ($P < 0.05$) change from $t = 0$.](https://academic.oup.com/humrep/article-abstract/19/12/2748/2356308)
Hormone and peptide response to GnRH agonist in boys (Figure 4)

LH and FSH concentrations increased significantly \((P < 0.0001)\). The increase was first observed at 30 min and reached maximal concentrations at 2 and 4 h, respectively. Inhibin-B concentrations increased significantly \((P = 0.0003)\), and the increase above baseline occurred at 20 h. There were no significant changes in activin-A or follistatin 288 concentrations. Although mean total follistatin concentrations declined in six of seven boys in whom it was determined, the change of \(5.96 \pm 1.9 \text{ ng/ml} \text{ (mean } \pm \text{ SD)}\) at time 0 to \(5.28 \pm 1.7 \text{ ng/ml} \text{ (mean } \pm \text{ SD)}\) at time 20 h was not significant. Serum testosterone concentrations increased significantly \((P = 0.02)\), with the first significant change from baseline at 24 h with respect to baseline concentration.

Sex-dependent differences in hormone and peptide concentrations in response to GnRH agonist (Figure 5)

Peptide and hormone concentrations were compared in biologically age-matched boys and girls (those with bone ages of \(\geq 7.9\) years). Basal serum LH concentrations were similar between the sexes as were peak response and percentage change after GnRH agonist. Serum FSH concentrations were similar at baseline and with respect to percentage change, but maximal FSH concentrations in response to GnRH agonist were significantly \((P = 0.006)\) greater in girls than in boys. Boys, however, had significantly \((P = 0.01)\) greater baseline concentrations of inhibin-B than did age-matched girls. Peak concentrations of inhibin-B were not significantly different, but girls had a greater percentage change between basal and maximal values. Basal activin-A concentrations were significantly \((P = 0.04)\) greater in girls than boys. Follistatin 288 and total follistatin concentrations were not significantly different between boys and girls.

Discussion

The reproductive axis is remarkably active in the fetus. When separated from the placenta and negative feedback of sex steroids in the newborn period, gonadotrophin secretion, presumably under the influence of increased release of GnRH, occurs episodically in a manner similar to that seen in adults (Winter, 1982). Inhibin-A and -B concentrations are similar to those seen in pubertal boys and girls, suggesting that the gonads can produce FSH-regulatory peptides in a manner similar to that seen in adults (Bergadá et al., 1999). By 6 months of age, gonadotrophin and sex steroid concentrations decline to low levels (Winter, 1982) and inhibin-B concentrations decline to near assay detection levels in girls but concentrations are greater in boys (Bergadá et al., 1999). The decline in gonadotrophins is thought to be secondary to
a decrease in GnRH secretion (Winter, 1982). The resultant decrease in gonadotrophin secretion has thus been thought to account for the subsequent decline in sex steroids and inhibin-B concentrations. In infant girls, FSH is readily releasable in response to GnRH, achieving concentrations characteristic of post-menopausal women (Rosenfield et al., 1989). It remains unclear, however, whether the decline in inhibin-B in young girls is secondary to a decrease in FSH stimulation resulting from a decline in GnRH secretion or whether there is ovarian resistance to FSH stimulation of inhibin-B in the ovary in early childhood. A feedback loop between FSH and inhibin-B is suggested by the work of Raivio et al. (2000) who demonstrated that early pubertal boys exhibit an inverse relationship between baseline inhibin-B concentrations and peak FSH levels after GnRH stimulation.

To understand better the relationship between gonadal function and FSH secretion, we determined changes in several FSH-regulatory peptide concentrations after stimulating

Figure 5. Sex-dependent differences in hormone and peptide concentration at baseline, peak and percentage change in response to GnRH agonist. Baseline and peak levels were compared in boys (n = 11) and those girls who had bone age of ≥7.9 years (n = 7) by ANOVA with Fisher’s PLSD. The percentage change was calculated by taking [(peak–basal)/basal] × 100. P-values are provided where differences are significant.
FSH secretion with the GnRH agonist, leuprolide acetate, using a protocol similar to that described by others (Rosenfield et al., 1989; Garibaldi et al., 1993) in children with variations in pubertal development. Within 4 h of leuprolide acetate injection, there is a robust release of FSH which is greater in younger than older girls and is inversely correlated with bone age. Within 16–20 h of the maximal FSH concentration, inhibin-B concentrations increase in girls, but this is only seen consistently after 4 years of age. This suggests that the infantile ovary is not sufficiently developed to respond with inhibin-B production after FSH stimulation. In the ovary, inhibin-B is produced in antral follicles and the number of antral follicles increases with advancing age in girls. Thus, it is likely that insufficient antral follicles have formed in infants to allow for inhibin-B feedback on pituitary FSH secretion. The fact that GnRH agonist stimulation of FSH is greatest in young girls and declines with advancing age is best explained by an increasing ability of the ovary to produce inhibin-B as it matures throughout childhood. Since FSH stimulates inhibin-B concentrations in girls 5 years of age and older, an inhibin-B→FSH feedback loop can exist in girls before the onset of puberty. The majority of our studies were conducted in girls with variant forms of puberty. Many of the girls had precocious thelarche, a condition that is considered a variant of normal (Van Winter et al., 1990; Lee, 2003). Such girls usually have normal reproductive axes in adulthood, so it is likely that the hypothalamic–pituitary–gonadal axis functions in a manner similar to that seen in ‘normal’ children. We were able to perform a limited study in five completely normal early pubertal girls and were gratified to see a similar, significant, increase in inhibin-B concentrations. In contrast to the girls with variations of pubertal development, inhibin-A concentrations also increased in the normal girls. In our study, approximately half of the boys had constitutional delay of puberty. This is also considered a variant form of puberty and is associated with normal pubertal progression and reproduction despite delay in the timing of puberty (Lee, 2003). Altogether, our data indicate that the inhibin→FSH feedback axis is established before adulthood in both boys, as has been noted previously (Raivio et al., 2000), and girls.

The sex differences seen in the concentrations of FSH-regulatory peptides are intriguing. Although pubertal-aged boys respond to FSH stimulation with an increase in serum inhibin-B concentrations, the magnitude of the increase is significantly lower than what is seen in girls. Ambient inhibin-B concentrations, however, are much greater in boys than in girls, suggesting that boys have restrained FSH concentrations before and during early puberty, as compared with girls, because of a greater threshold of inhibin negative feedback. Girls begin puberty earlier than do boys, and girls have a 5-fold greater rate of idiopathic precocious puberty than do boys (Grumbach and Styne, 1998). Thus it is conceivable that the earlier timing of puberty in girls is related to the lesser inhibin restraint of constitutive FSH secretion leading to earlier gonadal stimulation of sex steroid secretion and subsequent sex steroid maturation of the hypothalamic–pituitary axis. Early sex steroid exposure of the hypothalamic–pituitary axis results in central sexual precocity in both boys and girls (Foster et al., 1984; Pescovitz et al., 1984).

FSH stimulation of the gonads in boys and girls is not associated with a change in activin-A concentration. This may be due to the fact that the prepubertal and pubertal gonadal production of activin-A is insensitive to FSH stimulation, or that gonadal-derived activin-A changes are masked by the extra-gonadal production of activin-A. Our previous observations, however, indicate that, at least in girls, follistatin concentrations decrease with the progression of puberty (Foster et al., 2000), suggesting that more free activin-A may be available to stimulate pituitary activin-A receptors. The similarity of follistatin 288 concentrations between boys and girls also allows for the possibility that girls could have higher free activin-A concentrations than boys, permitting greater stimulation of pituitary FSH and further accounting for sex differences between boys and girls.

Total follistatin concentrations were measured in 10 subjects at two time points. Although the results did not achieve significance, the fact that nine of 10 children had a decline in total follistatin concentrations suggests that acute stimulation by GnRH agonist may decrease production of total follistatin. In rats, fast frequency GnRH pulses are associated with an increase in follistatin mRNA (Kirk et al., 1994), suggesting that increased GnRH exposure could increase follistatin secretion. Whether the change in mRNA expression translates into changes in protein is not known. In our previous cross-sectional study of FSH-regulatory peptides in girls and women (Foster et al., 2000), total follistatin declined significantly between girls in early puberty and women in the follicular phase of the menstrual cycle. Girls with early puberty have fast frequency GnRH secretion only for 3–6 h at night and women in the follicular phase of the menstrual cycle have fast frequency GnRH secretion during the day with some slowing at night. Additional studies will be required to determine whether changes in serum concentration of total follistatin can be seen in boys across puberty and whether these changes have a physiological significance in either gender.

Direct comparisons of the concentrations of inhibin-B in boys and girls have not been performed in previous studies, but the available data support our observation that inhibin-B concentrations are much greater in boys than in girls. Manasco et al. (1997) demonstrated that total inhibin concentrations in boys exceeded those of girls throughout puberty. Companion papers by Crofton et al. (2002a,b) also suggest that girls have lower concentrations of inhibin-B than do boys, even though statistical comparisons were not made in these studies. The greater concentrations of inhibin-B in boys are likely to account for the lower baseline and blunted stimulated FSH concentrations we have observed in boys compared with girls.

It is of interest that activin-A concentrations were significantly greater in girls than boys in our study. This differs from previous studies which found no sex differences in activin-A in males and females during puberty (Luise et al., 2001) or in adulthood (Loria et al., 1998). The reason for this discrepancy in findings is unclear, but could relate to
the timing of serum sampling. Our comparisons were made at a uniform time of day, since we have noted previously that there may be diurnal variation in serum activin A concentrations (Foster et al., 1999). This variation may have masked the ability to detect sexual dimorphism in prior studies. The developing adrenal gland may also release activin-A into the circulation (Spencer et al., 1992) and might contribute to sexual dimorphism in activin-A concentrations. The fact that sexual dimorphism in activin-A concentrations exists in boys and girls is consistent with the possibility that circulating activin-A could stimulate the pituitary to produce the observed sexual dimorphism in FSH concentrations between boys and girls.

In summary, our results indicate that GnRH agonist-stimulated FSH concentrations are greatest in infant girls and decline steadily with age. GnRH agonist-stimulated FSH can increase inhibin B concentrations in mid childhood and in early puberty, but not in infant girls. The fact that stimulated inhibin-B concentrations are low in infancy but readily measurable in mid childhood, varying in the opposite direction to stimulated FSH concentrations, suggests that inhibin B production from the ovary in developing girls may serve a feedback regulatory role in FSH secretion. Further, the demonstration of sexual dimorphism of activin-A and inhibin-B concentrations in boys and girls also supports the role of these FSH-regulatory peptides in the prepubertal control of FSH secretion.

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