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Comparison between Spontaneous Gonadotropin Concentration Profiles and Gonadotropin Response to Low-Dose Gonadotropin-Releasing Hormone in Prepubertal and Early Pubertal Boys and Patients with Hypogonadotropic Hypogonadism: Assessment by Using Ultrasensitive, Time-Resolved Immunofluorometric Assay

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ABSTRACT. To assess whether nocturnal gonadotropin concentration profiles in children could be predicted by measurement of peak gonadotropin levels after gonadotropin-releasing hormone (GnRH) administration, we measured spontaneous gonadotropin levels every 20 min and the gonadotropin responses to low-dose GnRH using an ultrasensitive, time-resolved immunofluorometric assay in 61 boys with short stature and/or delayed puberty. Spontaneous nocturnal LH pulses were observed in 58 out of 61 patients. After GnRH administration in a dose of 25 ng/kg, all of the 61 patients had significant LH and FSH responses, and GnRH-stimulated peak LH and FSH levels were highly correlated with maximal spontaneous nocturnal LH and FSH levels, respectively ($r = 0.83$ for LH and $r = 0.91$ for FSH; $p < 0.00001$). Analysis of individual subjects revealed that GnRH-stimulated peak LH levels were almost identical to maximal nocturnal LH levels in the subjects whose GnRH-stimulated peak LH levels were between 5 and 10 IU/L, whereas GnRH-stimulated peak LH levels tended to be higher than maximal nocturnal levels in the subjects whose GnRH-stimulated peak LH levels were 5 IU/L or lower. To determine if there were any parameters in the gonadotropin response to GnRH that might be useful in distinguishing early pubertal boys from prepubertal boys, we evaluated the gonadotropin response to GnRH in 44 prepubertal and 10 early pubertal normal short boys. Although maximal nocturnal LH levels did not overlap between prepubertal and pubertal groups, GnRH-stimulated LH peak levels overlapped considerably between the two groups. Even the GnRH-stimulated peak LH to peak FSH ratio overlapped between the two groups. In conclusion, although the use of an ultrasensitive assay enabled us to detect gonadotropin response to GnRH stimulation in a dose of 25 ng/kg in all of the subjects, it was difficult to predict the precise changes in spontaneous circulating gonadotropin concentrations around the onset of puberty simply by measurement of gonadotropin peak levels after GnRH administration. (*Pediatr Res* 31: 535–539, 1992)

Abbreviations

GnRH, gonadotropin-releasing hormone

Previous studies have shown that gonadotropin secretion is pulsatile and shows marked circadian rhythm due to nocturnal augmentation of gonadotropin secretion well before the onset of puberty and that this nocturnal secretion is dramatically increased with puberty (1–7). Therefore, investigation of the precise pattern of changes in circulating gonadotropin concentrations in children around pubertal onset is essential for us to understand the neuroendocrinologic mechanism in the onset of puberty and may contribute to early diagnosis of potential abnormalities of the onset of puberty. However, studies of spontaneous circulating gonadotropin concentrations in children are often difficult because of a large amount of blood sampling. The GnRH test is less cumbersome and more cost-effective than nocturnal frequent sampling. The choice of GnRH doses has been widely discussed, and large doses of approximately 100 $\mu g/m^2$ were commonly used to obtain a maximal release of gonadotropin. However, the diagnostic reliability of such a standard GnRH bolus test is often questionable in children because the dosage of GnRH in the standard test produces supraphysiologic stimulation to pituitary gonadotrophs (8, 9). In the present study, we measured the gonadotropin response to a single physiologic dose of GnRH (10) by using an ultrasensitive, time-resolved immunofluorometric assay in 61 children investigated for potential problems with growth and/or development. Concurrently, we studied nocturnal spontaneous circulating gonadotropin concentrations for comparison with the pituitary responsiveness to the single physiologic dose of exogenous GnRH and assessed whether gonadotropin response to the single physiologic dose of GnRH provides an analogy of spontaneous gonadotropin concentration profiles.

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SUBJECTS AND METHODS

Subjects. Sixty-one Japanese boys attending the Kobe Children's Hospital for investigation of short stature and/or delayed puberty were studied. Clinical characteristics of these subjects are summarized in Table 1. Out of 61 subjects, 54 were short but found to be endocrinologically normal (normal short boys), four had idiopathic hypogonadotropic hypogonadism, and three had multiple pituitary hormone deficiency. Pubertal development was assessed according to Tanner's criteria (11). Among 54 normal short boys, 44 boys were prepubertal [G (genital development) = 1, P (pubic hair distribution) = 1] and the remaining 10 boys were early pubertal (G = 2–3, P = 2–3). All of the patients with idiopathic hypogonadotropic hypogonadism and all of the patients with multiple pituitary hormone deficiency had no evidence of pubertal onset. Bone age was evaluated according to the standards of Greulich and Pyle (12). Informed consent was obtained from all children and/or parents. The protocol was approved by the ethical committee of the Kobe Children's Hospital.

Protocol. Blood samples were drawn for LH and FSH measurement at 20-min intervals for 25 h starting at 1000 h from 44 out of 61 patients. After the 25-h period of blood sampling was completed, GnRH (LHRH; Tanabe, Tokyo, Japan) was administered i.v. in a dose of 25 ng/kg, and plasma LH and FSH were measured every 20 min for the next 2 h. From the remaining 17 patients, blood samples were drawn at 20-min intervals for 3 h in the daytime starting at 1000 h and for 8 h in the nighttime starting at 2200 h. At 1100 h the following morning, GnRH was administered i.v. in a dose of 25 ng/kg, and blood samples were drawn at 20-min intervals for 1 h before and for 2 h after GnRH injection.

Hormone assay. Plasma LH and FSH concentrations were measured in duplicate by a time-resolved immunofluorometric assay using a DELFIA LH kit and an FSH kit (Pharmacia, Turku, Finland). The assay standards were calibrated against the World Health Organization Second International Reference of Pituitary LH 80/552 for LH and the Second International Reference of Pituitary FSH/LH 78/549 for FSH. The intraassay coefficients of variation with LH concentrations of 0.09, 0.54, 1.1, 5.4, 10.9, and 20.8 IU/L were 7.8, 6.6, 2.6, 1.7, 1.7, and 2.9%, respectively. For FSH concentrations of 0.07, 0.38, 0.86, 4.2, 9.5, and 21.0 IU/L, they were 11.0, 8.0, 5.6, 3.6, 2.5, and 5.4%, respectively. The interassay coefficients of variation with LH concentrations of 3.4, 19.8, and 59.6 IU/L were 7.0, 6.0, and 5.3%, respectively. For FSH concentrations of 5.1, 11.7, and 27.1 IU/L, they were 5.5, 5.0, and 4.1%, respectively. The detection limit of the assay for LH was 0.02 IU/L, and for FSH it was 0.03 IU/L as defined by mean + 3 SD of 10 replicates of the zero standard provided by the manufacturer. Values below the detection limit were assigned the value of the detection limit. The recovery of purified LH standard varied from 98 to 110% when 22.5, 37.5, and 62.5 IU/L LH standards were added to samples. The recovery of purified FSH standard varied from 100 to 108% when 8.0, 32.0, and 80.0 IU/L FSH standards were added to samples. All samples of one patient were run in one assay.

Table 1. *Clinical characteristics of study subjects**

Study group	*n*	CA (y)†	BA (y)‡	Testicular size (mm)§
Normal short boys				
Prepubertal	44	9.0 ± 0.4 (3.2–12.5)	6.7 ± 0.4 (1.0–10.5)	20.1 ± 0.7
Early pubertal	10	13.1 ± 0.3 (11.9–14.8)	11.0 ± 0.3 (10.0–12.8)	29.2 ± 1.9
Idiopathic hypogonadotropic hypogonadism	4	15.7 ± 1.0 (13.6–18.5)	14.1 ± 0.7 (12.0–15.8)	17.5 ± 2.2
Multiple pituitary hormone deficiency	3	12.2 ± 0.7 (10.5–13.2)	8.2 ± 1.1 (5.5–9.5)	18.3 ± 0.7

* Values are the mean ± SEM (range).
† Chronological age.
‡ Bone age estimated by the method of Greulich and Pyle (12).
§ Greatest diameter.

Analysis of data. Pulses within the LH data series were evaluated using Cluster analysis (13). A 1 × 1 (test nadir 1, test peak 1) cluster size was used, and the *t* statistic was set at 2.5 for both upstroke and downstroke, so that the false-positive rate would be less than 2% on signal-free noise generated by assaying 100 replicates of a single serum pool. Because plasma FSH has a longer circulating half-life and does not show as distinct a pulsatile pattern as plasma LH, we did not evaluate pulses within the FSH data series using Cluster analysis. GnRH-induced gonadotropin responses were considered significant only if they reached a maximum within 40 min for LH and 60 min for FSH and if plasma gonadotropin levels obtained for 2 h after GnRH administration (six samples) were significantly higher than those obtained for 2 h at the same hour on the previous day. Nighttime values corresponded to the period 2200–0600 h, and the relationship between gonadotropin concentration profiles during the nocturnal 8-h period and the GnRH-stimulated gonadotropin peak level was examined by linear regression analysis. Because there was great variation in the number of subjects among the four groups (Table 1), statistical comparisons of gonadotropin pulse properties among the four groups were not performed. All data are shown as the mean ± SEM.

RESULTS

Nocturnal gonadotropin concentration profiles. Spontaneous nocturnal LH pulses were observed in 58 patients, including four patients with idiopathic hypogonadotropic hypogonadism. Peak height of LH in these 58 patients widely ranged from 0.13 to 11.44 IU/L. The remaining three patients, who had multiple pituitary hormone deficiency, showed no detectable LH pulsations, giving extremely low mean nocturnal concentrations of 0.02 to 0.06 IU/L. Except for the three patients with multiple pituitary hormone deficiency and the four patients with idiopathic hypogonadotropic hypogonadism, all of the patients showed nocturnal augmentation of LH concentrations. Detailed properties of the nocturnal gonadotropin concentration profiles are presented in Table 2. Although statistical comparisons of gonadotropin pulse properties among the four groups were not performed (see Subjects and Methods), nocturnal 8-h mean LH and FSH concentrations, mean LH amplitude, and mean LH nadir in early pubertal boys tended to be higher than those in all other groups. Conversely, there was little difference in nocturnal LH pulse frequency and pulse interval among the four groups.

Gonadotropin response to GnRH. After GnRH administration in a dose of 25 ng/kg, all of the 61 patients had a significant LH and FSH response within 40 min and 60 min, respectively ($p < 0.05$). As shown in Figure 1, peak gonadotropin level after GnRH administration was highly correlated with maximal nocturnal gonadotropin level ($r = 0.83$ for LH and $r = 0.91$ for FSH, $p < 0.00001$). Representative spontaneous concentration profiles of gonadotropin and gonadotropin response to low-dose GnRH from the study subjects are shown in Figure 2. In two early pubertal normal short boys (Fig. 2*c* and *d*), GnRH-stimulated peak LH levels were almost identical to the maximal spontaneous nocturnal LH levels. Conversely, in a prepubertal normal short boy (Fig. 2*a*) and a patient with hypogonadotropic hypogonadism (Fig. 2*b*), GnRH-stimulated peak LH levels were higher than maximal nocturnal LH levels. Figure 3 illustrates the comparison between maximal nocturnal gonadotropin level and peak gonadotropin level after GnRH administration in each of the 61 subjects. In the subjects whose peak LH levels after GnRH

Table 2. *Detailed properties of nocturnal gonadotropin concentration profiles from four study groups**

Study group	LH					FSH
	Mean concentration (IU/L)	Pulse amplitude (IU/L)	Nadir (IU/L)	Pulse frequency (/h)	Pulse interval (min)	Mean concentration (IU/L)
Normal short boys						
Prepubertal	1.02 ± 0.18 (0.07–4.42)	0.70 ± 0.10 (0.05–2.35)	0.87 ± 0.15 (0.02–3.41)	0.47 ± 0.03 (0.14–0.88)	122.5 ± 12.3 (40.0–280.0)	2.14 ± 0.20 (0.37–6.21)
Early pubertal	4.94 ± 0.38 (3.08–7.90)	3.53 ± 0.50 (1.71–6.20)	3.70 ± 0.41 (1.52–6.69)	0.57 ± 0.05 (0.33–0.83)	101.6 ± 9.6 (65.0–180.0)	5.25 ± 0.60 (2.56–9.32)
Idiopathic hypogonadotropic hypogonadism	0.14 ± 0.03 (0.10–0.24)	0.08 ± 0.01 (0.06–0.10)	0.09 ± 0.03 (0.02–0.17)	0.38 ± 0.04 (0.25–0.50)	138.3 ± 3.8 (130.0–150.0)	0.53 ± 0.10 (0.28–0.84)
Multiple pituitary hormone deficiency	0.04 ± 0.01 (0.03–0.06)					0.15 ± 0.03 (0.09–0.21)

* Values are the mean ± SEM (range). All LH pulse properties were evaluated using Cluster analysis (15). Patients with multiple pituitary hormone deficiency had no detectable LH pulsations. Because there was great variation in the number of subjects among the four groups, statistical comparisons of gonadotropin pulse properties among the four groups were not performed.

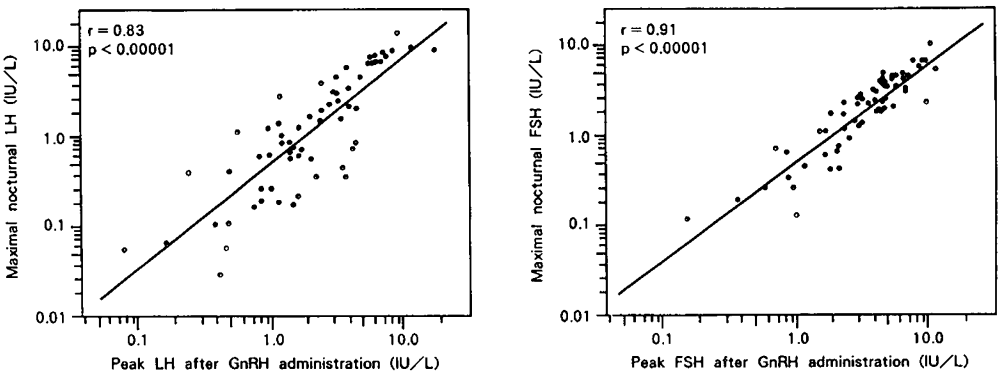

Fig. 1. The relationship between maximal nocturnal gonadotropin levels and peak gonadotropin levels after GnRH administration in a dose of 25 ng/kg. GnRH-stimulated peak LH and FSH levels were highly correlated with maximal spontaneous nocturnal LH and FSH levels, respectively ($r = 0.83$ for LH and $r = 0.91$ for FSH, $p < 0.00001$).

administration were approximately 5–10 IU/L, GnRH-stimulated LH peak levels were almost identical to maximal nocturnal LH levels. There was no significant difference between them: the GnRH-stimulated LH peak level was 7.08 ± 0.36 IU/L and the nocturnal maximal LH level was 7.37 ± 0.76 IU/L ($p = 0.56$, by paired t test). On the other hand, in the subjects whose peak LH levels after GnRH were 5 IU/L or lower, the GnRH-stimulated LH peak levels tended to be higher than maximal nocturnal levels and maximal nocturnal values spread so widely that nocturnal secretion appeared quite different in some cases, even with almost equal GnRH-stimulated peak LH levels.

Inasmuch as the GnRH-stimulated peak LH to peak FSH ratio has been used as a diagnostic test of the pubertal onset of the GnRH neuron (14, 15), we assessed whether the low-dose GnRH test could predict nocturnal mean LH to mean FSH ratio. In Figure 4, the nocturnal mean LH to mean FSH ratio was plotted on the vertical axis, and the GnRH-stimulated peak LH to peak FSH ratio was plotted on the horizontal axis. When this figure was divided into four parts at 1.0 on each axis, the upper left quarter contained no points. This means that nocturnal concentration profiles of gonadotropin were always FSH predominant in subjects who showed an FSH-predominant response after GnRH administration. However, no particular tendency was observed in nocturnal concentration profiles in subjects who showed an LH-predominant response after GnRH administration.

Changes in circulating gonadotropin concentrations around puberty. In the investigation of 54 normal short boys, all of them showed nocturnal pulsatile secretion of LH. The maximal spontaneous nocturnal LH levels in prepubertal and early pubertal normal short boys were within the ranges of 0.15–6.24 and 6.25–13.8 IU/L, respectively, and there was no overlapping between the two groups. Peak LH levels after GnRH administration in prepubertal group and early pubertal group were within the ranges of 0.23–6.61 and 6.07–18.1 IU/L, respectively. Three of the former group and two of the latter group overlapped (Fig. 5). With regard to GnRH-stimulated peak LH to peak FSH ratio, 10 out of 44 prepubertal boys showed an LH-predominant response, and one out of 10 early pubertal boys showed an FSH-predominant response. Therefore, it was impossible to distinguish prepubertal boys from early pubertal boys without overlapping by the low-dose GnRH test.

DISCUSSION

In the present study, we examined whether spontaneous circulating gonadotropin concentrations could be predicted by gonadotropin response to a single physiologic dose of GnRH. With the ultrasensitive assay, we could analyze spontaneous gonadotropin concentration profiles in detail. As a result, spontaneous nocturnal LH pulses were observed in all of the prepubertal normal short boys. Furthermore, even in the four patients with idiopathic hypogonadotropic hypogonadism, significant LH pulses were detected by Cluster analysis, and nocturnal mean concentrations in these patients were within the range of those in prepubertal normal short boys. Interestingly, these four patients did not show nocturnal augmentation of LH concentrations, whereas prepubertal boys did show significant nocturnal augmentation of LH concentrations. These findings are in agreement with a previous report (7) and may contribute to early diagnosis of idiopathic hypogonadotropic hypogonadism.

In contrast to nocturnal mean LH level and mean LH amplitude, there was little difference in nocturnal LH pulse frequency among the four groups in this study. This finding is in close

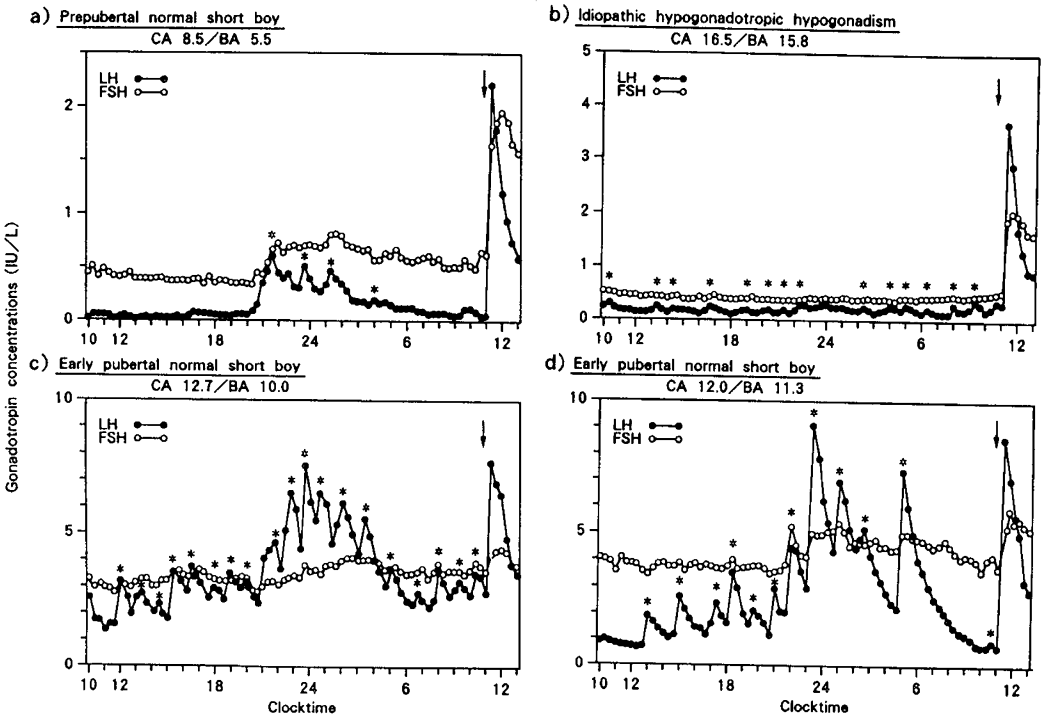

Fig. 2. Representative concentration profiles of gonadotropin in a prepubertal boy (*a*), a patient with idiopathic hypogonadotropic hypogonadism (*b*), and two early pubertal boys (*c* and *d*). Blood samples were drawn for LH and FSH measurement at 20-min intervals for 25 h starting at 1000 h. After the 25-h period of blood sampling was completed, GnRH was administered i.v. in a dose of 25 ng/kg, and plasma LH and FSH were measured every 20 min for the next 2 h. *Arrows* indicate GnRH administration. *Asterisks* indicate significant LH pulses. Note different scales on the vertical axes.

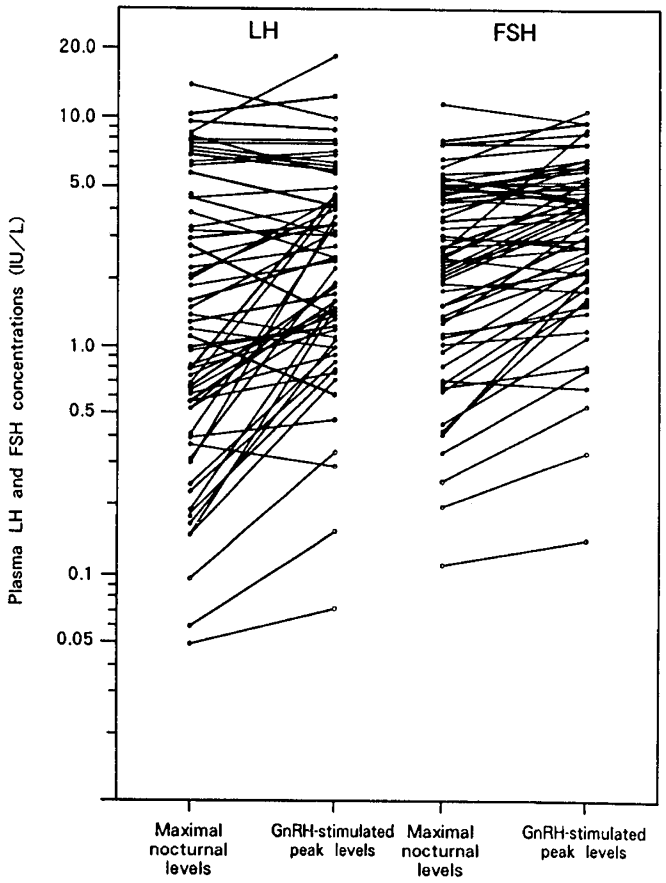

Fig. 3. Comparison between maximal nocturnal gonadotropin level and peak gonadotropin level after administration of 25 ng/kg GnRH in each of the 61 boys.

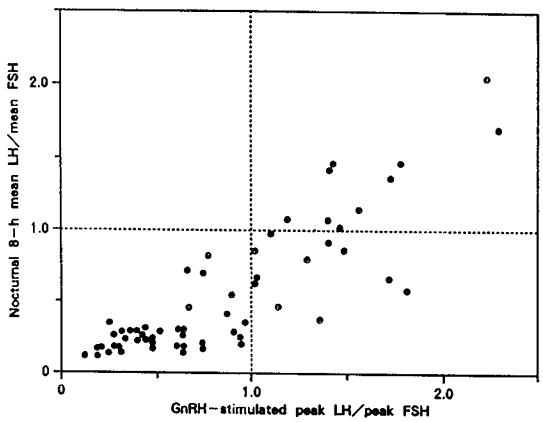

Fig. 4. Comparison between nocturnal mean LH to FSH ratio (*vertical axis*) and GnRH-stimulated peak LH to FSH ratio (*horizontal axis*).

agreement with the previous report that LH peak amplitude increased significantly with increasing pubertal stage, whereas LH peak frequency did not change (4, 15). This implies that an alteration in circulating LH levels during physiologic and pathologic puberty is through a change in LH pulse amplitude rather than pulse frequency. The LH pulse interval in our 44 prepubertal boys was significantly negatively correlated with bone age ($r = -0.46$, $p < 0.001$); however, the LH pulse interval in prepubertal boys who had bone ages of older than 5 y was not significantly correlated with bone age (data not shown). These findings suggest that even an ultrasensitive assay could not detect very small LH pulses in very young boys who had bone ages of younger than 5 y and that the pulse interval may not change during prepubertal period.

With the ultrasensitive assay, we could detect significant gonadotropin response to GnRH in a dose as low as 25 ng/kg in all of the subjects. As a result, highly positive correlation was observed between maximal nocturnal gonadotropin levels and

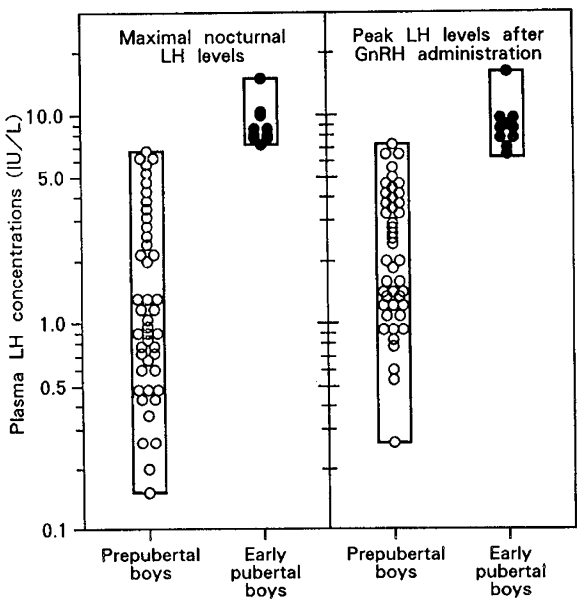

Fig. 5. Comparison of maximal nocturnal LH levels (*left panel*) and of GnRH-stimulated peak LH levels (*right panel*) between prepubertal boys and early pubertal boys.

GnRH-stimulated peak gonadotropin levels. Particularly in the subjects whose GnRH-stimulated peak LH levels were approximately 5–10 IU/L, GnRH-stimulated peak LH levels were almost identical to maximal nocturnal LH levels. This suggested that in such cases the administration of GnRH in a dose of 25 ng/kg gave peripheral plasma concentrations close to the physiologic portal plasma concentration of GnRH (10). Conversely, in the subjects whose GnRH-stimulated peak LH levels were 5 IU/L or lower, maximal nocturnal LH levels were often lower than GnRH-stimulated peak levels. In the case of low nocturnal LH secretion, the low level of spontaneous GnRH secretion was able to maintain the synthesis of gonadotropin in the pituitary gland; however, such a low level of GnRH secretion failed to release enough gonadotropin. Therefore, a relatively large, releasable gonadotropin pool may have been formed. Because this gonadotropin pool was released by exogenous GnRH, GnRH-stimulated peak LH levels may become higher than nocturnal maximal LH levels (10, 16). In other words, in the subjects whose GnRH-stimulated peak LH levels were 5 IU/L or lower, peripheral plasma concentration of GnRH obtained by 25 ng/kg GnRH administration may be higher than the physiologic pituitary portal plasma concentration of GnRH. Furthermore, the overlapping in GnRH-stimulated peak LH levels observed between prepubertal boys and early pubertal boys may have occurred because the GnRH-stimulated peak LH level in prepubertal boys became higher than the maximal LH levels of nocturnal circulating concentrations. In some cases, however, the maximal nocturnal LH level was higher than the GnRH-stimulated peak LH level. This suggested that in such cases the gonadotropin pool had not been formed in gonadotrophs because of an impairment in the pituitary gonadotrophs.

It is known that the gonadotropin response to exogenous GnRH changes from an FSH-predominant pattern to an LH-predominant pattern with the onset of puberty (14, 15). In addition, it is reported that spontaneous nocturnal concentration profiles of gonadotropin change from an FSH-predominant pattern before puberty to an LH-predominant pattern after the onset of puberty (17). The present study disclosed that nocturnal spontaneous concentration profiles of gonadotropin were always FSH-predominant in subjects who showed an FSH-predominant response after GnRH administration. However, we failed to find any particular tendency in nocturnal gonadotropin concentration profiles in subjects who showed an LH-predominant response after GnRH administration. These findings suggested that an LH-predominant pattern appears in the gonadotropin response to exogenous GnRH at an earlier age than it does in nocturnal spontaneous concentration profiles of gonadotropin. In the investigation of 54 normal short boys, 10 out of 44 prepubertal boys showed an LH-predominant response to GnRH administration. These 10 had a significantly ($p < 0.05$) older mean bone age than the remaining 34 prepubertal boys. Only 1 out of 10 early pubertal boys showed an FSH-predominant response. These findings obtained from 54 normal short boys may apply more generally to normal healthy boys, because the short boys studied here were endocrinologically normal without any evidence of systemic disease, malnutrition, or psychosocial disturbances. Therefore, we consider it difficult to detect the onset of puberty by GnRH-stimulated peak LH to peak FSH ratio, at least in boys.

In conclusion, although a significant gonadotropin response to GnRH administration in a dose of 25 ng/kg was detected by using ultrasensitive assay in all subjects, it was difficult to predict changes in spontaneous gonadotropin concentration profiles around puberty by measurement of peak gonadotropin level after GnRH administration alone. Further detailed study may be necessary on dose, route, and time of GnRH administration to establish more accurate hormonal criteria for detecting pubertal onset.

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