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Age-Related Change of Pulsatile Gonadotropin Secretion in Turner Syndrome

AKIO HOSODA, KENJI FUJIEDA, NOBUO MATSUURA, AKIHIRO OKUNO, AND KENJI YURI

Department of Pediatrics, Hokkaido University, School of Medicine, Sapporo, Japan

ABSTRACT. In an attempt to understand the dynamic change of the gonadotropin-releasing hormone-pituitary axis during the transitional stage from prepuberty to puberty, we investigated gonadotropin secretory patterns using a highly sensitive assay system and frequent blood sampling technique in children with Turner syndrome aged 5–17 y. Blood samples were collected every 20 min for 24 h in 16 cases, or every 30 min for 9 h (daytime 5 h, nighttime 4 h) in nine cases. Serum LH and FSH were measured by time-resolved fluoroimmunoassay. A 24-h profile of LH and FSH was analyzed by a computerized pulse detection program (PC-PULSAR). As early as 5 to 6 y of age, mean daytime LH concentration was significantly higher than nighttime concentration and pulsatile LH secretion existed throughout the day and night. At about 9 to 11 y of age, corresponding to the early stage of puberty, a dramatic increase in LH concentration and amplitude was observed, and both concentration and pulse amplitude were much higher during the night than during the day. However, these day-night differences became less clear at ages corresponding to late pubertal stages. Pulse frequency of LH secretion remained almost constant throughout the day and night at all ages investigated. As for FSH concentration, a trend similar to that of LH was observed, although day-night differences and age-related changes were less remarkable. Furthermore, pulsatile FSH secretion was detected in only a small number of the cases. These findings suggest that in Turner syndrome the hypothalamic gonadotropin-releasing hormone oscillator is functioning actively with constant frequency before the onset of puberty. Although we know that the day-night rhythm of gonadotropin secretion exists long before the onset of puberty, what controls the day-night rhythm of gonadotropin in the younger girls and the factors responsible for the dramatic increase of gonadotropin around the age of early puberty remain to be determined. (*Pediatr Res* 29: 196–200, 1991)

Abbreviations

Gn-RH, gonadotropin-releasing hormone

The mechanism for the onset of puberty is not yet fully understood, although maturation of the hypothalamic-pituitary-gonadal axis plays a major role in initiating the pubertal process. To appreciate the complex mechanism controlling the onset of puberty, it is of great importance to analyze functional changes of the hypothalamus throughout maturational stages of puberty. It is a well-recognized concept that sleep-enhanced, periodic, pulsatile discharge of LH is initiated in early puberty (1, 2); however, there is no general agreement as to the secretory pattern of gonadotropins in the prepubertal stage before appearance of any appreciable development of secondary sexual characteristics.

In Turner syndrome, where ovarian function is genetically impaired, intensified gonadotropin secretion at the age that corresponds to puberty is also initiated in a similar but more pronounced manner than in normal puberty (3–5). These findings suggest that Turner syndrome represents a typical model of nature to investigate maturation of the Gn-RH-pituitary component of the control system that governs gonadal function under reduced or absent influence of gonadal steroid hormone.

Recently, a very sensitive gonadotropin assay system based on time-resolved fluoroimmunoassay was developed for practical use (6). This assay system is about 100 times more sensitive than previous assay systems. This makes it possible to investigate more definitively the pattern of gonadotropin secretion at low concentration. Accordingly, we used this assay system to investigate age-related changes in the gonadotropin secretory pattern of girls with Turner syndrome. Our special interest was whether or not day-night rhythm and pulsatile gonadotropin secretion exist in prepubertal children.

MATERIALS AND METHODS

Subjects. Twenty-three girls with Turner syndrome, aged from 5 y 6 mo to 17 y 11 mo, were recruited for our study. Diagnosis was confirmed by chromosome analysis of peripheral mononuclear cells. Their karyotypes and clinical characteristics are shown in Table 1. Cases 2 and 8 were studied twice at different ages. None of them showed any spontaneous development of secondary sexual characteristics at the time of our study. Replacement therapy of sex steroid hormones, performed in cases 22 and 23 for the purpose of developing secondary sexual characteristics after they were 15 y old, was discontinued at least several months before the study. Informed consent was obtained from both patients and parents.

Protocol. The experimental protocol was approved by the Human Subjects Investigation Committee of Hokkaido University Hospital. Blood samples were obtained from 16 cases through an indwelling venous catheter every 20 min for 24 h using Cormed's pump. From the other nine cases, blood samples were drawn every 30 min manually for 9 h during daytime hours from 1000 to 1500 h and during nighttime hours from 2100 to 0100 h. The patients' activities were not restricted. Blood samples were collected into the tubes containing EDTA, and the plasma was separated and frozen until assay. Plasma LH and FSH measurements were performed with a time-resolved fluoroimmunoassay kit (Delfia LH and FSH, Pharmacia, Uppsala, Sweden). Calibrated standards were used for LH against WHO-lst IRP68/40 and FSH against WHO-2nd IRP78/549.

Pulse analysis. Pulse analysis was performed using a computerized program, PC-PULSAR, developed by Merriam and Wachter (7). Detection of pulse peaks of secretion as well as

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Correspondence and reprint requests: Akio Hosoda, M.D., Department of Pediatrics, School of Medicine, Hokkaido University, North-15 West-7 Kitaku Sapporo, Japan 060.
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Table 1. *Subjects studied*

Case no.	Age	Bone age	Sampling condition*	Karyotype
1	5 y 6 mo		A	45X/46XX
2-a	6 y 10 mo	6 y 6 mo	A	45X/46XX
2-b	8 y 6 mo	8 y 0 mo	B	
3	8 y 7 mo	6 y 10 mo	B	46Xi(Xq)
4	8 y 8 mo	8 y 0 mo	A	45X
5	9 y 5 mo	7 y 6 mo	A	46Xi(Xq)
6	9 y 8 mo	9 y 0 mo	B	46Xi(Xq)
7	10 y 8 mo	10 y 6 mo	B	46Xi(Xq)
8-a	11 y 0 mo		A	45X
9	11 y 0 mo	11 y 0 mo	B	46Xi(Xq)
10	11 y 8 mo	11 y 6 mo	B	46X/45X+mar
11	11 y 8 mo	11 y 0 mo	B	45X
12	11 y 11 mo	11 y 0 mo	B	45X
13	11 y 11 mo	10 y 0 mo	B	45X
14	12 y 8 mo	12 y 6 mo	B	45X/46X,r(X)
15	12 y 19 mo	11 y 0 mo	B	45X
16	13 y 2 mo	13 y 6 mo	B	46XXp-
17	13 y 7 mo	11 y 0 mo	B	44Xt(13q14q)/45Xi(Xq)t(13q14q)
8-b	13 y 7 mo		B	
18	13 y 8 mo		A	45X
19	14 y 7 mo	11 y 0 mo	A	45X/46XYq-
20	14 y 7 mo		A	45X
21	14 y 8 mo	13 y 0 mo	B	45X/46X+mar
22	16 y 6 mo	15 y 0 mo	B	45X/46X,r(X)
23	17 y 11 mo	13 y 10 mo	A	45X/46Xi(Xq)

* A, 9 h every 30 min; B, 24 h every 20 min.

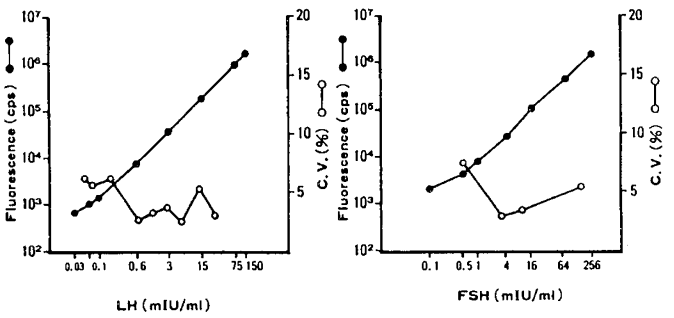

Fig. 1. Standard curves and assay coefficients of variation of time-resolved fluoroimmunoassay for LH and FSH.

calculation of mean pulse amplitude was performed only for the cases with 20-min sampling schedules for 24 h. Default values of parameters for calculation in PC-PULSAR were not modified other than those for least detectable concentration. Assay SD approximated by a linear function of concentration constructed from the values of SD over the measured ranges of concentration to be analyzed were used as a scale factor for the detection of peaks in the program.

Figure 1 shows the standard curves and assay coefficients of variation at various concentrations of LH and FSH. Intraassay coefficient of variation was 2–7% for LH and 3–8% for FSH over the wide range of concentrations. Assay sensitivity, determined as twice the SD at zero concentration, was 0.03 IU/L for LH and 0.1 IU/L for FSH.

Mean concentration during daytime (0800 to 2000 h) and nighttime (2000 to 0800 h) was calculated in every case.

Statistical analysis. Wilcoxon's rank-sum test was used to verify the difference between day and night concentrations.

RESULTS

Representative examples of actual gonadotropin secretory patterns are shown in Figure 2*A–D*. Individual results of the day and night concentrations and pulse analysis of gonadotropins are summarized in Tables 2 and 3. All the girls with Turner

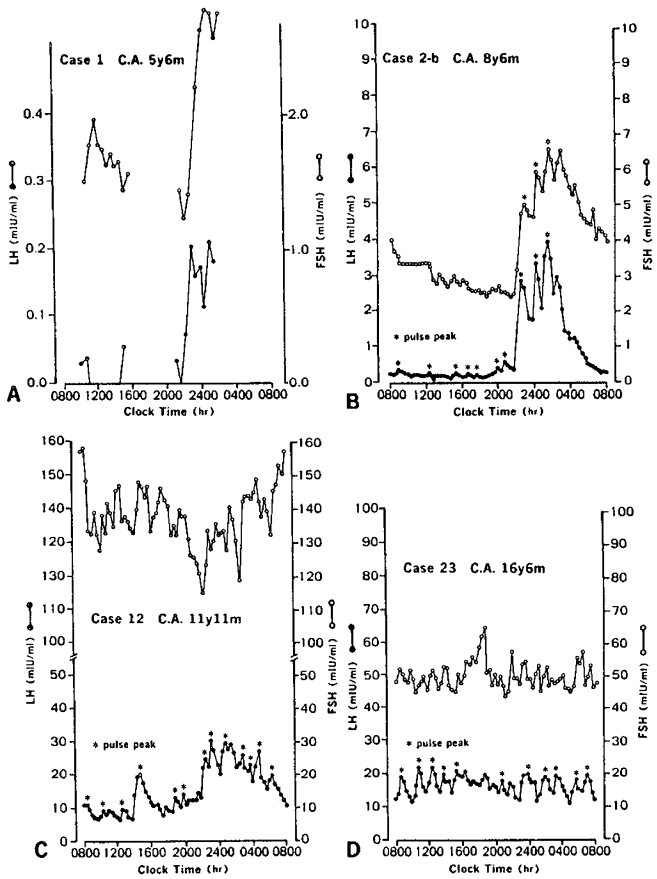

Fig. 2. Representative examples of gonadotropin secretory pattern. Vertical scales of concentration are not uniform. At the age of early prepuberty, day-night rhythm of gonadotropins, especially LH, is already seen, although their absolute value is low (*A*, *B*). After the age of early puberty, gonadotropin concentrations become much higher (*C*); however, day-night rhythm becomes vague by the age of late puberty (*D*). Pulse peaks of LH are seen during both day and night and through all ages.

Table 2. *Mean concentration, number of pulse peaks, and mean pulse amplitude of LH*

Case no.	Mean concentration (mIU/mL)		No. of peaks		Mean pulse amplitude (mIU/mL)	
	Day (mean ± SD)	Night (mean ± SD)	Day	Night	Day (mean ± SD)	Night (mean ± SD)
1	0.03 ± 0.07	0.14 ± 0.07*				
2-a	0.06 ± 0.01	0.29 ± 0.09*				
2-b	0.19 ± 0.05	1.56 ± 1.15*	6	4	0.07 ± 0.05	1.99 ± 1.30*
3	0.51 ± 0.09	1.24 ± 0.75*	3	5	0.15 ± 0.08	1.09 ± 1.16*
4	0.03 ± 0.01	0.05 ± 0.03*				
5	0.08 ± 0.01	1.02 ± 0.60*				
6	16.49 ± 2.77	26.47 ± 6.75*	6	9	6.45 ± 0.99	14.10 ± 3.21*
7	0.24 ± 0.07	0.51 ± 0.18*	6	5	0.09 ± 0.08	0.41 ± 0.08*
8-a	0.02 ± 0.03	0.03 ± 0.04†				
9	9.31 ± 1.81	15.39 ± 5.08*	5	7	4.87 ± 0.75	8.26 ± 4.74†
10	20.79 ± 3.67	27.07 ± 9.46*	8	7	8.48 ± 2.37	17.45 ± 11.56‡
11	16.79 ± 2.94	20.74 ± 5.31*	6	5	6.20 ± 2.59	11.27 ± 3.00‡
12	9.97 ± 3.28	19.92 ± 5.56*	6	7	4.50 ± 3.74	9.55 ± 3.41‡
13	21.27 ± 6.13	31.35 ± 9.08*	6	5	15.03 ± 5.41	19.55 ± 6.47†
14	25.15 ± 4.97	27.72 ± 7.01†	5	5	12.27 ± 3.14	13.60 ± 5.94†
15	22.33 ± 4.55	27.68 ± 4.24*	7	7	5.12 ± 1.04	8.11 ± 1.52*
16	10.34 ± 1.67	14.42 ± 4.24*	9	6	3.67 ± 1.04	7.30 ± 5.71†
17	1.36 ± 0.40	5.24 ± 3.78*	4	4	0.80 ± 0.42	8.22 ± 5.28‡
8-b	18.06 ± 3.40	25.63 ± 7.43*	7	6	7.49 ± 2.35	14.70 ± 8.11‡
18	17.18 ± 4.33	17.22 ± 4.55†				
19	24.62 ± 3.96	24.15 ± 4.11†				
20	20.14 ± 2.17	22.11 ± 3.90†				
21	6.74 ± 0.88	8.12 ± 2.39*	5	6	1.61 ± 0.63	4.47 ± 2.35*
22	16.83 ± 2.58	15.87 ± 2.37†	5	7	6.19 ± 1.64	5.09 ± 1.57†
23	14.64 ± 1.55	14.67 ± 20.4†				

* $p < 0.01$ (day < night).
† NS.
‡ $p < 0.05$ (day < night).

Table 3. *Mean concentration, number of pulse peaks, and mean pulse amplitude of FSH*

Case no.	Mean concentration (mIU/mL)		No. of peaks		Mean pulse amplitude (mIU/mL)	
	Day (mean ± SD)	Night (mean ± SD)	Day	Night	Day (mean ± SD)	Night (mean ± SD)
1	1.68 ± 0.15	2.20 ± 0.65*				
2-a	2.78 ± 0.20	3.86 ± 0.34†				
2-b	2.99 ± 0.35	4.67 ± 1.23†	0	3		2.26 ± 0.42
3	8.55 ± 0.81	9.17 ± 1.13‡	2	2	1.95 ± 1.35	2.43 ± 0.04‡
4	1.65 ± 0.40	1.54 ± 0.09‡				
5	9.03 ± 0.61	10.28 ± 2.10‡				
6	69.93 ± 4.54	76.40 ± 4.96†	0	0		
7	12.13 ± 1.57	14.13 ± 1.46†	1	1	6.38	3.70§
8-a	0.48 ± 0.08	0.37 ± 0.04‖				
9	92.98 ± 9.21	92.54 ± 10.10‡	1	0	42.26	
10	68.49 ± 4.09	66.21 ± 8.61‡	0	0		
11	111.92 ± 7.28	113.01 ± 14.99‡	0	0		
12	138.68 ± 6.93	134.34 ± 9.50§	0	0		
13	113.10 ± 5.52	121.79 ± 14.62†	1	0	30.99	
14	57.47 ± 3.84	66.67 ± 6.25†	0	1		11.26
15	130.06 ± 8.73	131.07 ± 10.87‡	0	0		
16	75.19 ± 5.80	75.78 ± 20.26‡	0	0		
17	24.34 ± 2.19	29.00 ± 5.51†	0	4		9.88 ± 1.98
8-b	88.39 ± 6.75	93.82 ± 7.54†	0	0		
18	74.54 ± 2.59	72.58 ± 2.93‖				
19	93.29 ± 3.57	88.89 ± 4.01§				
20	87.43 ± 5.94	85.57 ± 3.07‡				
21	104.67 ± 23.19	100.09 ± 10.54‡	0	0		
22	49.74 ± 4.42	49.56 ± 5.78‡	1	1	15.17	26.08†
23	55.75 ± 4.13	52.31 ± 2.74§				

* $p < 0.05$ (day < night).
† $p < 0.01$ (day < night).
‡ NS.
§ $p < 0.01$ (day > night).
‖ $p < 0.05$ (day > night).

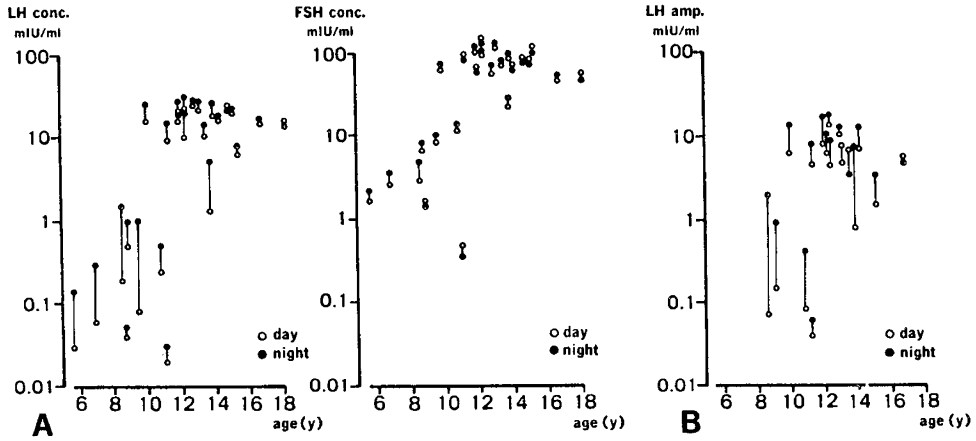

Fig. 3. *A*, individual day and night mean concentrations of LH and FSH. Vertical axis drawn in logarithmic scale. Concentration of LH increases dramatically around the ages of 9 to 11 y. *B*, individual day and night mean amplitudes of LH pulse. The amplitude increases coinciding with the concentration.

syndrome from prepuberty to puberty showed episodic LH secretion. Also, distinct day-night rhythm of LH was seen in most cases of prepubertal age.

Nighttime concentration of LH was significantly higher than daytime concentration in younger aged cases whose levels of LH concentration were relatively low. Both day and night, mean concentration increased dramatically around the ages of 9 to 11 y. The day-night concentration difference became less clear in older age cases (Fig. 3*A*). Pulse peaks of LH were detected throughout the day and night in every case investigated. Furthermore, the profile of LH in cases 1 and 2 suggested existence of pulsatility, although their 30-min sampling interval was too long to analyze by PC-PULSAR (Fig. 2*A*). Mean pulse amplitude of LH correlated with corresponding LH concentration, and amplitude also increased around the ages of 9 to 11 y, following the same trend as LH concentration (Fig. 3*B*). Pulse frequency of LH was10–15 peaks/24 h (mean 11.8 ± 2.2/24 h), and this frequency was independent of age or the concentration of LH. Distribution of pulse peaks during every 4 h stayed almost constant throughout the day (from 1.7 ± 0.8 during h 12–16 to 2.1 ± 1.0 during h 16–20). Case 17 showed relatively low concentration and amplitude of LH for her chronologic age, although her bone age was somewhat younger.

FSH showed a trend similar to that of LH (Fig. 3*A*). However, the increase of FSH concentration around the age of early puberty was less dramatic. Day-night difference was also less prominent. FSH concentration was relatively high compared to LH concentration in the younger group of cases. Only small numbers of FSH pulse peaks, almost all of which coincided with LH pulse peaks, were detected in several cases.

DISCUSSION

By using frequent blood sampling, a highly sensitive gonadotropin assay, and a computerized pulse detection algorithm, we demonstrated that significant day-night rhythm of LH concentration exists at the prepubertal stage as early as 5 y of age in girls with Turner syndrome. Furthermore, we demonstrated that, at the age of 8 y or more, LH secretion was pulsatile in nature. Pulse frequency did not change throughout the transition phase from prepuberty to puberty. In addition, marked increment of LH concentration around the age of early puberty coincided with a marked increase of pulse amplitude. At the stage of late puberty, day-night difference of LH concentration became less prominent.

Similar findings in principle were reported by Ross *et al.* (8). However, there are minor differences between their findings and ours. We observed more marked day-night rhythmicity in prepubertal stages. Furthermore, no apparent difference of pulse frequency of LH was observed during the stages of pubertal development. Ross claimed that the combined pulse frequency from both LH and FSH did not change, although LH frequency increased and FSH frequency decreased with advancing age. These differences might be due to several factors such as difference in assay systems or methods of pulse analysis. It is most probable that the conventional RIA methods used by Ross *et al.* (8) were not sensitive enough to evaluate lower LH concentrations. This might lead to underestimation of day-night difference in LH concentration and LH pulse frequency in the prepubertal stage.

Assuming that the pattern of LH release from the pituitary gland closely reflects the pattern of Gn-RH release from the hypothalamus (9), our results suggest that the hypothalamic oscillator mechanism is functioning far before the onset of puberty, at least in Turner syndrome.

Whether day-night rhythm and pulsatility of gonadotropin secretion exists in normal prepubertal children is still not settled. However, the more sensitive the assay system used, the more day-night rhythm and pulsatility found. For instance, Jackachi *et al.* (10) found day-night rhythm and pulsatile secretion of LH in half of the prepubertal children with bone age from 3 to 9.5 y by using a conventional RIA system but improving its sensitivity several-fold. Furthermore, Wennik *et al.* (11), using an immunoradiometric assay with a sensitivity of 0.1 IU/L of 1st IRP68/40, detected nocturnal pulsatility of LH in three normal prepubertal boys, whereas LH levels during the daytime remained below assay sensitivity. It is probable that pulsatility might have been detected in the daytime if the assay were sensitive enough.

From a viewpoint of hormonal environment, Turner syndrome has reduced feedback effect of gonadal steroids compared to that in normal children. It might be interesting to know how sex steroid hormones modulate episodic gonadotropin secretory pattern. Indeed, Mauras *et al.* (12) found that a low dose of estrogen administered to prepubertal girls with Turner syndrome reduced gonadotropin concentration and pulse amplitude, but did not modulate pulse frequency. If additional studies confirm that day-night rhythm and pulsatile secretion of gonadotropins exist in both normal prepubertal children and prepubertal girls with Turner syndrome and a similar pronounced increase in secretion is seen at the onset of puberty, changing level of threshold to the gonadal steroid hormones in the hypothalamus and pituitary gland might not play a major role in the onset of puberty.

The hypothalamic-pituitary component of the control system

that governs gonadal function attains a high degree of organization already during the fetal period. Also, it was recently reported that chemical stimulation of the hypothalamus by N-methyl-D-asparic acid can easily result in precocious puberty through activation of the Gn-RH-pituitary system in rat and rhesus monkey (13, 14). In light of these findings, our results may indicate that the difference between prepubertal and pubertal states is not qualitative but rather quantitative in nature.

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