

## Hormonal changes throughout puberty in boys: an observational study on the treatment outcome of congenital hypogonadotropic hypogonadism

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*To the Editor:* It is difficult to capture the exact time of initiation of puberty in normal children, such as when breasts in girls and testicles in boys begin to grow, especially in boys. As a result, research on the detailed patterns of physical development during puberty and the cut-off values and changes of hormones at the initiation of puberty and developmental maturation is rare. It is generally believed that hormonal changes during puberty are a direct result of the stimulating effect of luteinizing hormone (LH) on initiation of puberty;<sup>[1]</sup> thus, LH is widely accepted as an indicator to evaluate initiation of puberty. However, researchers have not yet reached an agreement on the cut-off value of LH, especially in boys.

First, it is easy to determine testicle volume and penis length in boys. Second, increased testosterone (T) level is usually concordant with changes in secondary sexual characteristics in boys. Gonadotropin-releasing hormone (GnRH), via pulsed secretion from the hypothalamic neurons, binds to the transmembrane receptors on the anterior pituitary membrane to promote follicle-stimulating hormone (FSH) and LH secretion directly.<sup>[2]</sup> Some recent studies have shown that besides LH and FSH, anti-Mullerian hormone (AMH) and inhibin B (INH-B) are also involved in the initiation of puberty.<sup>[3]</sup> Hence, a series of hormonal changes are responsible for initiating puberty; however, systematic research on these changes is lacking. Studies have shown that pituitary pump therapy is effective and safe for the treatment of congenital hypogonadotropic hypogonadism (CHH).<sup>[4]</sup>

Male patients with chronological age and bone age  $\geq 12$  years who received GnRH pulse treatment (pump therapy; the details were shown in reference 5) for Kallmann

syndrome (KS)/CHH at Beijing Children's Hospital between January 2008 and March 2017 were included in this study to prospectively investigate the puberty onset and hormones changes. The patients were examined at baseline and followed up at  $1 \pm 1$  day,  $4 \pm 2$  days, and  $12 \pm 2$  days after treatment. Increased T with testicle volume  $> 3$  mL was used as the indicator for initiation of puberty. The receiver operating characteristic (ROC) curve which was used to evaluate the time points of initiation – development – maturation, was used to determine the cut-off hormones value at the initiation of puberty in the study. At the end of 12 weeks' study, we encouraged the patients to continue the same therapy and followed up on their development. The study was approved by the Ethics Committee of Beijing Children's Hospital (No. 2016-Y-005-D), which acts in compliance with ethical standards defined by the *Declaration of Helsinki*. Written informed consent was obtained by all participants and their parents or legal guardians.

A total of 24 patients with hypogonadotropic hypogonadism (HH; included 17 cases of KS) were eligible and included in this study. The average age at the first visit was  $14.01 \pm 1.40$  years. Moreover, 15 patients (62.5%) had a small penis, eight (33.3%) had a small penis and cryptorchidism, and one (4.2%) had a small penis and hypospadias. All 24 patients completed 4 weeks and 18 patients completed 12 weeks study.

During treatment, FSH and INH-B began to rise at week 1, with no changes in T, AMH levels, or testicle volume. LH started increasing with a significant increase in T level at the end of week 4, but no changes in testicle volume or AMH. At week 12, LH and INH-B continued to rise, but FSH stopped increasing at a high level, while T could be

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**Table 1: Hormone levels at different time points of LHRH treatment.**

Hormones	Time after LHRH treatment							
	W0		W1		W4		W12	
	Basal	Stimu	Basal	Stimu	Basal	Stimu	Basal	Stimu
LH, IU/L	0.16 ± 0.10	2.85 ± 2.42	1.30 ± 0.86	2.85 ± 2.49	2.96 ± 1.98	8.06 ± 4.22	4.22 ± 3.59	10.40 ± 5.29
FSH, IU/L	0.65 ± 0.58	3.61 ± 2.47	5.94 ± 2.86	6.95 ± 2.96	8.56 ± 7.53	11.43 ± 8.66	6.98 ± 6.19	9.86 ± 7.12
LH/FSH	0.37 ± 0.27	0.72 ± 0.36	0.26 ± 0.13	0.36 ± 0.17	0.43 ± 0.31	1.00 ± 0.91	0.65 ± 0.51	1.64 ± 1.26
T, ng/dL	<20.00	<20.00	21.38 ± 5.09	20.00 (20.00, 20.00)	20.00 (20.00, 50.10)	20.00 (20.00, 73.35)	72.20 (20.00, 157.75)	118.00 (51.60, 218.00)
INH-B, pg/mL	28.15 ± 17.03	22.23 ± 3.99	47.12 ± 31.12	49.95 ± 6.37	82.48 ± 45.61	79.22 ± 9.50	131.72 ± 75.56	138.98 ± 22.28
AMH, ng/mL	13.93 ± 9.96	13.23 ± 9.53	21.21 ± 3.58	21.19 ± 3.63	22.77 ± 0.47	21.45 ± 3.10	15.25 ± 4.61	13.87 ± 3.83

Data are presented as mean ± standard deviation or median ( $P_{25}$ ,  $P_{75}$ ). AMH: Anti-Mullerian hormone; FSH: Follicle-stimulating hormone; INH-B: Inhibin B; LH: Luteinizing hormone; LHRH: Luteinizing hormone-releasing hormone; KS: Kallmann syndrome; T: Testosterone; W: Week.

detected, the testicle volume was >3 mL, AMH began to decrease [Table 1; Supplementary Figure 1, <http://links.lww.com/CM9/A663>].

LH peaked at 30 minutes and FSH at 60 minutes after LH-releasing hormone (LHRH) stimulation. We take values at these two points, respectively, to analyze ROC. LH did not rise until week 4, but FSH increased significantly at week 1 and stabilized by week 4. The stimulation test showed that the LH/FSH ratio decreased at week 1, began to increase at week 4, and stabilized at week 12. T began to rise at week 4. These results indicated that puberty was initiated by week 4, and developmental maturation was achieved by week 12. No significant change was observed in the basal and stimulated T level on day 0 (before pump therapy). However, when the T could be detected, it peaked at 90 minutes, but no significant change was observed in AMH or INH-B [Table 1; Supplementary Figure 1, <http://links.lww.com/CM9/A663>]. After 12 weeks of pump therapy, patients reported testicle and penis growth, especially testicle volume, which increased more significantly and earlier than penis size. Moreover, beard or pubic hair appeared in 38.9% (7/18), and three patients reported initial seminal emission after 39 weeks.

At week 4, basal LH continued to rise and T levels rose in 12 patients with a significant positive correlation with LH levels ( $r = 0.736$ ,  $P < 0.01$ ). Therefore, week 4 was used as the puberty ignition time point for the ROC curve. Week 4 was used as the positive group and week 0 as the negative group to plot the ROC curve to determine the cut-off values of T, LH, LH/FSH, and INH-B before and after the stimulation.

The cut-off basal LH was 1.32 IU/L. The elevated T level was correlated with basal LH > 1.32 IU/L in all patients and it accounted for 65% (13/20) of the patients, indicating that 1.32 IU/L was the cut-off value. In the same method, we determined the cut-off value of basal LH/FSH was 0.34. Among patients with basal LH > 1.32 IU/L and T undetected, 62.5% (5/8) had LH/FSH > 0.34. The cut-off value of stimulated LH was 4.45 IU/L. The corresponding ROC curve showed that the cut-off value of LH/FSH after stimulation was 0.54. Similarly, the cut-off value of INH-B was 54.5 pg/mL at the initiation

of puberty [Table 1 and Supplementary Digital Content, Table 1, <http://links.lww.com/CM9/A663>].

At week 12 of treatment, FSH stabilized, INH-B continued to rise, and T levels increased, indicating that week 12 was the cut-off point for developmental maturation. The ROC curve analyses of T, LH, LH/FSH, and INH-B at week 12 are shown in Supplementary Digital Content, Table 1, <http://links.lww.com/CM9/A663>.

At week 1, INH-B rose and was followed by LH and FSH [Supplementary Figure 1, <http://links.lww.com/CM9/A663>]. LH/FSH decreased first then climbed up until T increase at last means puberty ignition. Therefore, the decrease in stimulated LH/FSH (0.37 to 0.26) and the increase in INH-B preceded puberty. The continued LH increase followed by an increase in LH/FSH was the most significant marker of initiation of puberty. Basal LH of 1.32 IU/L and 3.54 IU/L are the cut-off values of puberty starting and maturation. Before real initiation of puberty, a decrease in LH/FSH was followed by a continued LH increase and stabilized FSH, suggesting that Sertoli cells mature before Leydig cells, and sperm was produced after both Sertoli and Leydig cells reached maturation, thereafter seminal emission was the final milestone of developmental maturation.

Basal LH of 1.32 IU/L and LH/FSH of 0.34 have a high sensitivity and can enable the timely detection of early puberty. Grinspon *et al*<sup>[5]</sup> recommended basal LH of 0.1 IU/L and LH/FSH of 0.29 in CHH patients, which were in line with ours in this study (0.16 ± 0.10 IU/L, 0.37 ± 0.27). In Grinspon's study,<sup>[5]</sup> there were no specific time points for children with pump treatment, whereas we performed LHRH stimulation tests in patients receiving pump therapy to determine the cut-off values of hormone levels at specific follow-up time points. Thus, this prospective study was able to determine more relevant LH ranges to determine the initiation and maturation of the hypothalamic-pituitary-gonadal (HPG) axis.

INH-B is the primary active inhibin in males.<sup>[6]</sup> To date, a few studies have been conducted to investigate the effects of INH-B on puberty and development in males. This study showed that INH-B and FSH concordantly rose firstly and

were more sensitive than AMH. The cut-off value of INH-B was 54.5 pg/mL for initiation of puberty. At the end of week 12 of pump therapy, INH-B continued to rise, then T level increased, and FSH stabilized. INH-B produced by Sertoli cells was able to establish feedback, indicating developmental maturation. At this point, the cut-off value of INH-B was 79.6 pg/mL, which was close to the normal reference value for adults (>80 pg/mL).<sup>[7]</sup> Thus, INH-B from 54.5 to 79.6 pg/mL indicated a consistent developmental stage of puberty. INH-B could present the first signal of puberty and maturation of Sertoli cells.

Hormone basal levels are highly specific indicators for clinical manifestations but are less sensitive than stimulation tests for early detection of puberty. The cut-off value of stimulated LH was 4.45 IU/L and LH/FSH was 0.54. They are almost the same as the findings by Grinspon *et al*<sup>[5]</sup> (LH 1.4 IU/L and LH/FSH 0.63 after stimulation in CHH patients). Furthermore, this study was more rigorous and reliable. Thus, we recommend stimulated LH > 4.45 IU/L and LH/FSH > 0.54 as the indicators of initiation of puberty.

GnRH pump therapy is safe and effective, but it is important to determine whether pump therapy is indicated for patients with pituitary disorders. Zheng *et al*<sup>[6]</sup> retrospectively analyzed the effects of GnRH pump therapy in patients with congenital combined pituitary hormone deficiency (CCPHD) and found that LH < 2 IU/L after 1 month of treatment was associated with no increase in T levels, suggesting that pump therapy was contraindicated if LH < 2 IU/L, as it indicated a poor pituitary response. In this study, all of the patients reached puberty after pump therapy, indicating that all of the patients had normal pituitary responses and were indicated for pump therapy. So, we analyzed hormone levels in these patients. The median of stimulated LH is 2.41 IU/L. The 95% confidence interval is (1.81–3.17) IU/L. At the same time, we found that the excitation level of LH was 2.85 IU/L 1 week after treatment, and the basal LH was 2.96 IU/L at 4 weeks after treatment. These three data were almost at the same level. Combined with the adult study (LH < 2 IU/L), it was considered that the level of LH was about 2 IU/L, showed normal pituitary responses. Concurrently, we also analyzed LH/FSH. The results showed that the median LH/FSH was 0.65 and 95% confidence interval was 0.57–0.87 after stimulation.

In short, this is a prospective study with a novel design and reliable results with clinical value. The pre-puberty normal boys' basal LH level was  $0.16 \pm 0.10$  IU/L. FSH was  $0.65 \pm 0.58$  IU/L. During puberty, FSH is increased firstly, followed by INH-B and then LH and T. Finally, AMH

decreased. And the decrease in stimulated LH/FSH and the increase in INH-B preceded puberty. The cut-off LH values at the initiation of puberty and developmental maturation in boys, which were similar to the reference values in girls. Once the HPG axis was activated, there are no significant differences in hormones after stimulation. To further improve the reliability of diagnosis and scientific rigor, future research may validate the results with additional patients and the time points identified in this study.

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### Conflicts of interest

None.

### References

- Howard SR, Dunkel L. The genetic basis of delayed puberty. *Neuroendocrinology* 2018;106:283–291. doi: 10.1159/000481569.
- Thompson IR, Kaiser UB. GnRH pulse frequency-dependent differential regulation of LH and FSH gene expression. *Mol Cell Endocrinol* 2014;385:28–35. doi: 10.1016/j.mce.2013.09.012.
- Chada M, Prusa R, Bronský J, Kotaska K, Sídlová K, Pechová M, *et al*. Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol Res* 2003;52:45–51.
- Gong C, Liu Y, Qin M, Wu D, Wang X. Pulsatile GnRH is superior to hCG in therapeutic efficacy in adolescent boys with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2015;100:2793–2799. doi: 10.1210/jc.2015-1343.
- Grinspon RP, Ropelato MG, Gottlieb S, Keselman A, Martínez A, Ballerini MG, *et al*. Basal follicle-stimulating hormone and peak gonadotropin levels after gonadotropin-releasing hormone infusion show high diagnostic accuracy in boys with suspicion of hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2010;95:2811–2818. doi: 10.1210/jc.2009-2732.
- Zheng J, Mao J, Xu H, Wang X, Huang B, Liu Z, *et al*. Pulsatile GnRH therapy may restore hypothalamus-pituitary-testis axis function in patients with congenital combined pituitary hormone deficiency: A prospective, self-controlled trial. *J Clin Endocrinol Metab* 2017;102:2291–2300. doi: 10.1210/jc.2016-3990.
- Radicioni AF, Anzuini A, De Marco E, Nofroni I, Castracane VD, Lenzi A. Changes in serum inhibin B during normal male puberty. *Eur J Endocrinol* 2005;152:403–409. doi: 10.1530/eje.1.01855.

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