EVALUATION OF THE BUSERELIN STIMULATION TEST IN DIAGNOSING GONADOTROPIN DEFICIENCY IN MALES WITH DELAYED PUBERTY

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Objective **To assess the efficacy of the gonadotropin-releasing hormone (GnRH) agonist buserelin in a stimulated gonadotropin test for the investigation of delayed puberty in males.**

Study design **Prepubertal males (n = 31; age range, 10.3 to 17.2 years) were studied; buserelin (100 μg) was administered subcutaneously, with blood sampling at 0 and 4 hours for serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH). At follow-up (mean, 4.2 years), 8/31 (26%) failed to progress into puberty, constituting hypogonadotropic hypogonadism (HH), but 23/31 (74%) had testicular enlargement (≥8 mL) consistent with a normal hypothalamic-pituitary-gonadal (HPG) axis.**

Results **Stimulated serum LH response to buserelin was lower in males with HH (mean ± standard error under the mean for HH, 1.4 ± 0.5 U/L, compared with a normal HPG axis of 17.4 ± 2.0 U/L; $P < .0001$). Stimulated serum FSH response was nondiscriminatory (HH, 7.7 ± 2.2 U/L; normal HPG axis, 11.5 ± 1.6 U/L; $P = .27$). All males with HH had a stimulated serum LH level <5 U/L, whereas only 1/23 with a normal HPG axis had a stimulated serum LH below this level. Using this value as the criterion for diagnosing HH, the buserelin stimulation test yielded a sensitivity of 100%, specificity of 96%, and positive predictive value of 89%.**

Conclusions **The buserelin stimulation test is a highly specific and sensitive GnRH agonist test for the investigation of males with delayed puberty.** ***(J Pediatr 2006;148:89-94)***

Distinguishing males with hypogonadotropic hypogonadism (HH) when evaluating delayed puberty is difficult. Although males cannot be defined as having delayed puberty until age 14 years, clinicians are frequently faced with males presenting in the early teenage years with anxiety due to their relative short stature and lack of pubertal and physical development in comparison to their peers. A simple and reliable means is needed to distinguish males with HH from those with a normal hypothalamic-pituitary-gonadal (HPG) axis including constitutional delay of growth and development (CDGD). Males with HH have an abnormality of the pituitary or hypothalamus, which may be associated with other pituitary or central nervous system abnormalities. Individuals with HH require life-long hormone treatment for development and maintenance of secondary sexual characteristics, specialized fertility treatment, and further pituitary evaluation.

There is a small, but significant percentage (5%) of males who have delayed onset of puberty (beyond age 14 years).[1-5] Most of these males have CDGD, a normal variant characterized by delayed bone age, shorter stature than genetic potential, normal growth velocity, and usually a family history of delayed onset of puberty.[1,6,7] Although puberty is delayed, progression and virilization eventually proceed without intervention. A minority of males (<1%) do not progress into puberty by age 18, however, and half of these have HH.[2-5]

BMI	Body mass index	HH	Hypogonadotropic hypogonadism
CDGD	Constitution delay of growth and development	HPG	Hypothalamic-pituitary-gonadal
FSH	Follicle-stimulating hormone	LH	Luteinizing hormone
GH	Growth hormone	SDS	Standard deviation score
GnRH	Gonadotropin-releasing hormone		

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Traditionally, the gonadotropin-releasing hormone (GnRH) stimulation test has been used to differentiate males with delayed puberty. But the predictive value of this test is low, with a negligible stimulated serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) response seen in 1/3 of males with CGGD.[2,5,8,9] Other investigations, such as overnight frequent LH sampling,[10] urinary LH evaluation,[11] and repetitive intravenous GnRH testing,[12-14] either have similar efficacy or are not practical to perform in the clinical setting.

Over the last decade, the development and use of GnRH agonists in the investigation of delayed puberty has shown promise, with good diagnostic efficacy demonstrated in differentiating HH from CDGD.[2,15-18] More potent and with a longer half-life than GnRH,[19-21] these agonists more effectively stimulate pituitary gonadotrophs and promote a more sustained secretion of LH and FSH. Buserelin ([D-Ser (Bu1)6] GnRH nonapeptide ethylamide) is a potent GnRH agonist used to treat gynecologic disorders, hormone-dependent tumors, precocious puberty, and infertility.[19]

The purpose of this study was to evaluate the efficacy of the buserelin stimulation test in identifying males with HH in the peripubertal years. We hypothesized that males with a final diagnosis of HH will be biochemically distinct from those with a normal HPG axis (including CDGD), and, as such, a diagnostic criterion (ie, cutoff value) can be established. In addition, we aimed to establish whether there are any baseline clinical differences in males with HH that can be used as clinical criteria.

METHODS

Clinical data were reviewed on all males referred from pediatric endocrinology clinics at Starship Childrens' Hospital, Auckland, New Zealand, for a buserelin stimulation test between 1997 and 2002. The cohort comprised both a group of males at high risk for HPG axis abnormalities (due to comorbidities such as pituitary hormone deficiency) and a group of males with relative short stature. All of the subjects had prepubertal sexual development (defined as testicular volumes $\leq$ 3 mL) and were age > 10 years at baseline. Apart from those subjects receiving growth hormone supplementation, the growth velocity of the peripubertal males before investigation was between the 25th and 75th percentiles (standard deviation score [SDS], -0.7 to $+0.7$), consistent with a diagnosis of CDGD. Baseline clinical data included age, height (measured with a Harpenden stadiometer), weight, testicular size (assessed by an orchidometer), birth weight, information on comorbidities, and bone age assessment.[22]

The buserelin stimulation test consisted of a baseline blood sample for serum LH and FSH measurement, followed by subcutaneous administration of 100 μg of buserelin and a further blood sample for stimulated serum LH and FSH measurement taken 4 hours later. This protocol was established based on the work of Ghai et al,[2] who demonstrated that peak stimulated serum LH and FSH levels occurred 4 hours after administration of the GnRH analogue, nafarelin. Zamboni et al[15] also used the 4-hour stimulated serum LH and FSH levels to investigate delayed puberty with triptorelin. Although no pharmacokinetic data regarding buserelin stimulation of LH secretion are available, buserelin has peak plasma levels similar to those of triptorelin and nafarelin,[19,23,24] and, given this, the 4-hour level was adopted.

After the buserelin stimulation test, and at a minimum age of 12.5 years, all males were offered low-dose testosterone treatment. Low-dose testosterone (intramuscular testosterone enanthate 50 to 100 mg monthly or oral testosterone undeconoate 40 to 80 mg daily, for 3 to 6 months) promotes virilization and growth in normal peripubertal boys without compromising final height.[25,26] More important, it precipitates maturation and activation of the HPG axis, promoting normal pubertal progression.[27,28] It does not suppress central HPG axis activation, and in individuals with an intact HPG axis, testicular growth indicative of FSH and LH stimulation occurs within 12 to 24 months of commencement of testosterone therapy.[27,28] Pubertal development, particularly testicular size (by orchidometer), was assessed at 3 monthly intervals in this cohort of males. When the testes enlarged to 6 to 8 mL in volume, the testosterone was discontinued.

Clinical progression was available for a minimum of 2 years after the buserelin stimulation test. Males with sustained and progressive enlargement of testes to a volume of $\geq$ 8 mL were considered to have normal puberty and were classified as having a normal HPG axis. In contrast, those males who failed to experience sustained and progressive testicular enlargement to a volume of 8 mL were defined as HH.

Serum FSH and LH concentrations were determined by a fully automated immunometric chemiluminescent assay (Immulite 2000; Diagnostic Products, Los Angeles, Calif). The calibration range and analytical sensitivity for FSH was 0.1 to 170 IU/L, and that for LH was 0.05 to 200 IU/L. The mean intraassay and interassay coefficients of variation were 2.7% and 3.5% for FSH and 3.2% and 3.6% for LH.

Body mass index (BMI) was calculated (weight [kg]/height [m^2]). BMI and height were converted to SDSs for purposes of comparison.[29]

Analysis was performed using GraphPad Prism software (GraphPad, San Diego, Calif). Clinical characteristics of the 2 groups were expressed as means with ranges (except for noncontinuous data, which are expressed as median and range). Hormonal data were documented as a mean ± standard error under the mean. Clinical characteristics and the hormonal data of the 2 groups were compared using the Mann-Whitney *U* test. Statistical significance was accepted at $P < .05$.

The study design was approved by the Northern X Regional Ethics Committee, Auckland, New Zealand.

RESULTS

Thirty-one prepubertal males, age 10.3 to 17.2 years, underwent a buserelin stimulation test between 1997 and 2002. The average length of follow-up after buserelin stimulation testing was 4.2 years (range, 2 to 8 years). Eight of the subjects failed to progress into puberty (testicular size < 8 mL),