Glucagon Stimulation Test for Childhood Growth Hormone Deficiency: Timing of the Peak is Important

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Objectives In the glucagon stimulation test (GST) growth hormone (GH) secretion is considered sufficient when at least 1 value is >10 ng/mL. Because GH typically peaks at 90 or 120 minutes, we evaluated whether peak occurrence at other times ("atypical") signifies abnormal GH secretion.

Study design A retrospective review of 222 GSTs was conducted to determine these outcomes: 1) frequency of GH deficiency per confirmatory clonidine or arginine stimulation test in typical versus atypical GSTs, and 2) growth velocity standard deviation score (GVSDS) in patients with typical versus atypical GSTs.

Results Of 222 tests, 57 GST results (25.7%) were atypical, and 54 GST results (24.3%) were deficient. Atypical deficient tests had a higher chance than typical deficient tests of predicting GH deficiency per confirmatory test (15/21, 71.4% versus 14/33, 42.4%; $P < .05$). Patients with deficient atypical GST results and sufficient confirmatory tests (n = 6) had a lower GVSDS than patients with deficient typical GST results (n = 18; –1.58 [−3.2-1.76], versus 0.23 [–1.54-3.95], $P = .03$). Overall, 75% of atypical deficient GST results were followed by atypical timing in a clonidine test. Three of the 222 tests (1.3%) peaked at 180 minutes.

Conclusions The GST can be terminated at 150 minutes without sacrificing sensitivity. A GH peak at a time other than 90 or 120 minutes may be a new important indicator of GH deficiency. *(J Pediatr 2009;154:415-9)*

The glucagon stimulation test (GST) is a commonly used and reliable dynamic test for the evaluation of growth hormone (GH) deficiency.[1-3] In this test, blood samples are taken before intramuscular or subcutaneous (but not intravenous) administration of glucagon, and then every half hour for 3 hours.[4-6] The only known significant result is the maximum (peak) value of the 7 samples. In earlier reports of this test, the values for most children peaked at 90 and 120 minutes.[7,8] We hypothesized that timing of the peak value at times other than 90 and 120 minutes might be an important, hitherto overlooked indication of abnormal GH secretion. To investigate this hypothesis, we retrospectively examined all glucagon stimulation tests performed at one clinic between September 1998 and May 2005 and compared patients with peak values occurring at different times.

Another aim of this study was to examine whether the GST could be performed with fewer samples without compromising its diagnostic value. This was based on earlier suggestions for reducing the number of samples in the GST for adults[9] and for shortening the gonadotropin-releasing hormone agonist test[10] and the clonidine tests[11] in children.

METHODS

In the evaluation of GH deficiency at the Pediatric Specialists' Clinic, Clalit Health Services, Jeusalem, GST is performed as the first dynamic test. This is because glucagon is easy to administer, costs little, and concomitantly tests the pituitary-adrenal axis. Criteria for evaluation of GH status vary, but generally include height less than the third percentile or height standard deviation score (SDS) significantly lower than the target range, an abnormal growth rate, a delayed bone age, and exclusion of other causes of growth retardation such as anemia, celiac disease, hypothyroidism, renal or liver disease, and chronic inflammation. When the maximum GH level on this test is <10 ng/mL, it is considered indicative of GH deficiency (termed "deficient"). In such a case, a second confirmatory test, with either clonidine (most commonly) or arginine as the GH stimu-

GH	Growth hormone	IGF-1	Insulin-like growth factor 1
GST	Glucagon stimulation test	SDS	Standard deviation score
GVSDS	Growth velocity standard deviation score		

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Table I. Average plus or minus SD, range, and median of timed growth hormone samples in 222 glucagon stimulation test

	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes	150 minutes	180 minutes
Serum GH, (ng/mL)							
Average ± SD	3.6 ± 5.2	4.0 ± 4.5	3.1 ± 5.1	8.34 ± 8.1	12.4 ± 8.7	7.2 ± 7.4	3.9 ± 3.9
Range	0.05-34.40	0.05-25.00	0.1-40.0	0.01-40.00	0.1-40.0	0.1-40.0	0.1-23.5
Median	1.7	2.7	1.2	6.1	10.1	4.8	2.6
Serum cortisol, (μg/dl)							
Average ± SD	16.5 ± 8.5	ND	ND	ND	25.1 ± 10.6	29.5 ± 11.0	29.6 ± 12.2
Range	4.3-49.0				5.3-49.7	4.6-49.7	5.1-49.7
Median	14.22				25.2	29.3	30.1

ND, not done.

lant, is performed. When peak levels on both tests are <10 ng/mL, GH deficiency is diagnosed.

Results of all GSTs performed in our clinic between 1998 and 2005 were reviewed. GH measurements from patients who had finished their growth were excluded. In total, there were 228 GSTs. Four patients were lost to follow-up after the GST. Two patients were >18 years at the time of the test and were excluded. Therefore, data from 222 children were analyzed. The average age at testing was 9.7 ± 3.9 years (range, 0.2-17.2 years).

The test procedure was as follows. Stimulation of GH secretion was performed with intramuscular injection of 0.1 mg/kg glucagon to a maximum of 1 mg. Blood samples for GH, glucose, and cortisol levels were taken at 0, 30, 60, 90, 120, 150 and 180 minutes.[3] Prepubertal girls >8 years were primed with 2 mg beta estradiol (or equivalent estrogen) orally each day for 2 days until the day before the test, and prepubertal boys >9 years were given a single intramuscular injection of 100 mg testosterone enanthate 7 to 10 days before the test. Serum GH concentration was tested with the immulite 2000 analyzer 2-site chemiluminescent test (DPC, Germany). Insulin-like growth factor 1 (IGF-1) levels were measured before July 2003 with the DSL-5600 kit (Diagnostic Systems Laboratories, Webster, Texas; www.DSLabs.com). For tests performed with this method, SDS for age could be calculated. After July 2003, our laboratory used the immulite 2000 chemiluminescent kit to measure IGF-1 levels (DPC, Germany). Unfortunately, the new kit's results are not Gaussian and therefore cannot be transformed correctly to SDS.

RESULTS

Of the 222 glucagon tests, the highest average GH level was at 120 minutes (Table I). Ninety and 120 minutes were the most frequent peak times (in 74.3% of the tests; Table II). A total of 168 of 222 tests (75.6% of the tests; Table II) were sufficient (ie, with GH levels >10 ng/mL in at least 1 sample).

We separated test results into 2 groups according to the timing of the peak: 1) "typical," tests in which the highest GH level (peak) occurred at 90 or 120 minutes (the most frequent peak times), and 2) "atypical," tests in which the peak occurred at any time other than 90 or 120 minutes.

Frequency of Deficiency in the Glucagon Test

The peak in tests indicating GH sufficiency ("sufficient tests") tended to occur more often at typical times. Of the 168 sufficient tests, 132 (78.5%) occurred at typical times, compared with 33 of 54 (61.1%) deficient tests ($\chi^2 = 6.0$; $P < .025$).

Frequency of Deficiency in the Confirmatory Test

Patients who had deficient atypical GST results had nearly double the chance of having deficient confirmatory (arginine or clonidine) test results when compared with patients with deficient but typical GST results (15/21 [71.4%] versus 14/33 [42.4%]; $\chi^2 = 3.9$; $P < .05$). That is, atypical GST results are more frequently true positive indicators of GH deficiency.

Atypical Timing of Peak at Confirmatory Test

For 42 children, the confirmatory test was clonidine stimulation. We evaluated whether patients with atypical timing in the GST peaked at atypical times in the clonidine test. We did this analysis because the GH peaks in the clonidine test occur fairly consistently at 60 or 90 minutes, so tests with peaks at these times can be considered typical.[11] Of 16 atypical GST results, 12 (75%) confirmatory clonidine test results were also atypical. Conversely, of 26 typical GST results, the confirmatory clonidine test results were atypical in only 8 (30.8%; $P = .01$).

Growth and IGF-1 of Patients Who Are GH Sufficient with Typical or Atypical GST Results

We compared the growth velocity SDS (GVSDS) of 24 of 25 patients whose peak in the GST was <10 ng/mL, but was >10 ng/mL in a second diagnostic test (with either arginine or clonidine as the stimulant). These children were considered GH sufficient because their peak was higher than the threshold of 10 ng/mL in one test. One child underwent testing shortly after the first clinical examination, and GVSDS was not available for this patient. The GST results in 18 of the patients were typical. Average GVSDS was significantly lower for the 6 children whose GST results were atypical (Table III). We were able to calculate average IGF-1