What is a normal stimulated growth hormone concentration?

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ABSTRACT

In a retrospective analysis, we have compared the response of serum GH concentration to insulin-induced hypoglycaemia in 148 short prepubertal children (114 males, 34 females) aged between 3-9 and 11-9 years with the growth rate of the individual to determine 'cut-off' values for the diagnosis of GH insufficiency.

Sixty-three children grew with a height velocity standard deviation score (SDS) greater than −0·8 (group 1), which represents the growth velocity of children progressing along or closely parallel to the third height centile. Eighty-five children had a height velocity SDS of less than −0·8 (group 2). Median peak serum GH concentration responses to insulin-induced hypoglycaemia were 19·9 mU/l (range 1·5–54·4) in group 1 and 9·9 mU/l (range 0·7–46·2) in group 2 (Mann–Whitney; P < 0·001).

Using growth rate as the determinant of normality, the efficiency, sensitivity and specificity of the insulin-induced hypoglycaemia test were calculated using different serum GH concentration cut-off values to diagnose GH insufficiency. In our (Hybritech) assay, a cut-off value of 13·5 mU/l provided optimal performance in terms of efficiency (66%), sensitivity (64%) and specificity (70%).

The response of serum GH concentration to insulin-induced hypoglycaemia in short children growing at different growth rates was continuous. Each laboratory measuring serum GH concentrations needs to construct its own 'normal' cut-off value.

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INTRODUCTION

In the assessment of a child with short stature, the monitoring of growth rate over a period of time defines normality. However, there may sometimes be a need to investigate a child with provocative tests of growth hormone (GH) secretion without prior acquisition of auxological data. The insulin-induced hypoglycaemia provocation of GH secretion (ITT) remains the most widely used test for the assessment of hypothalamo–pituitary function. There are a number of limitations to the test for diagnosing GH insufficiency and some authors have even suggested that physiological assessment of GH secretion (12–24 h profiles) may be a more relevant method of identifying children who might benefit from treatment with GH (Spiliotis, August, Hung et al. 1984; Bercu, Shulman, Root & Spiliotis, 1986; Zadik, Chalew, Gilula & Kowarski, 1990). We do not consider this to be realistic.

One definition of GH insufficiency has been a peak serum GH concentration response to pharmacological testing of less than 15 mU/l in a short child who is growing slowly and in whom no other cause for growth failure has been identified (Milner, Russell-Fraser, Brook et al. 1979). Since the growth rate will increase in all children treated with GH, there is a need to re-evaluate the use of such 'cut-off' values (Van Vliet, Styne, Kaplan & Grumbach, 1983; Gerner, Genel, Gianfredi et al. 1984; Hindmarsh & Brook, 1987; Albertsson-Wikland & Hall, 1987; Ackland, Jones, Buckler et al. 1990). Further, the wide range of methods now available for measuring serum GH concentrations (radioimmunoassay, immunoradiometric assay (IRMA) and enzyme-linked systems) does not suggest that a universal cut-off value will be clinically appropriate (Reiter, Morris, MacGillivray & Weber, 1988; Celniker, Chen, Wert & Sherman, 1989). We report the relationship between stimulated GH responses to insulin-induced hypoglycaemia and growth in 148 children using an IRMA with a view to establishing the most appropriate cut-off value for the diagnosis of GH insufficiency in our laboratory.


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MATERIALS AND METHODS

Subjects
A retrospective analysis of 148 short prepubertal children (114 boys, 34 girls) aged 3-9 to 11-9 years was performed. The children had presented to the growth disorder clinic at The Middlesex Hospital between June 1984 and June 1990 and Turner Syndrome, skeletal dysplasias and other causes of short stature were excluded. Short stature was defined as a height less than 2-5 standard deviation scores (SDS) below the mean. A diagnosis of panhypopituitarism was subsequently made in two of the children.

Methods
Heights were measured using a Harpden standiometer and height velocities calculated over 1 year were expressed as an SDS to take into account differences between ages and sexes (Tanner, Whitehouse & Takaishi, 1966). The coefficient of variation of the measurement of height was 0-05% at 122-5 cm and 0-09% at 90 cm. Insulin-induced hypoglycaemia stimulation of GH secretion was used to assess GH secretion and subsequently compared with the growth velocity.

After an overnight fast, an intravenous cannula was inserted and insulin (Novo; 0-15 U/kg) administered intravenously. Samples for blood glucose and serum GH concentrations were taken at 0, 30, 60, 90 and 120 min. Adequate hypoglycaemia (less than or equal to 2 mmol/l) was achieved in all cases.

Assay
Serum GH concentrations were measured using the Hybritech Tandem-R IRMA. Tandem-R is a solid phase sandwich IRMA (Hybritech (Europe), Liege, Belgium) using two mouse monoclonal IgGs recognizing distinct antigenic sites on the human GH molecule. One antibody is coated on to a plastic bead (solid phase) and the other labelled with 125I in a protein matrix (horse serum) containing a blue dye and 0-1% sodium azide. The standards were calibrated against the pituitary-derived reference preparation HS2243E (NIH) and were made up in protein matrix containing 0-1% sodium azide. These were recalibrated in our laboratory against the pituitary-derived International Reference Preparation (IRP) 66/217 and International Standard (IS) 80/505 (NIBSC, South Mimms, Herts, U.K.) to give results in mU/l. The sensitivity of the assay was 0-5 mU/l. The within-assay coefficients of variation were 10-6, 4-9, 5-2, 4-9 and 5-0% at serum concentrations of 1-4, 3-4, 14-4, 26-4 and 99-4 mU/l. The between-assay coefficients of variation were 10-5, 7-2 and 5-4% at serum concentrations of 6-0, 13-2 and 33-3 mU/l. The long-term performance of the assay assessed by the percentage recovery of the 1st International Reference Preparation 66/217 was 100, 99-9, 98-6, 98-5 and 99-4 in the years 1985 through 1989.

Blood glucose concentrations were measured using the glucose oxidase method (Clenden Glucose Auto Stet GA-1120, Kyoto, Japan).

Statistics
The performance of the test was assessed by calculating the efficiency, sensitivity and specificity (Sox, 1986). Efficiency was the percentage of all results which were true positive or negative, a negative result being defined when an arbitrary concentration was exceeded in a child who proved to have a normal growth velocity. A positive result was defined by a peak GH value lower than the arbitrary cut-off value in a child with a suboptimal height velocity. The sensitivity of the test was calculated as the number of patients growing poorly with a positive test, divided by the total number of children growing poorly. The specificity was calculated as the number of children growing normally with a negative test, divided by the total number of children growing normally. Group data were compared using the Mann–Whitney test.

RESULTS
Sixty-three children were found to have a height velocity SDS greater than -0-8 (normal) and 85 had a height velocity SDS less than -0-8, this latter group including the two children with panhypopituitarism. The peak serum GH concentration responses to ITT are shown in Fig. 1. The median peak serum GH concentration response was 19-9 mU/l (range 1-5–54-4) in the group growing normally and 9-9 mU/l (range 0-7–46-2) in the slowly growing group (P<0.001). There was considerable overlap between the groups and the distribution of values was continuous when a frequency plot of peak serum GH concentration was made.

Table 1 shows the effect of choosing different peak GH values as cut-offs using the ITT for diagnosing GH insufficiency as compared with the gold standard of growth rate. Using the Hybritech IRMA, the optimal cut-off value was 13-5 mU/l, which generated an efficiency of 66%, a sensitivity of 64% and a specificity of 70%. Increasing the cut-off value led to an improvement in sensitivity but a reduction in specificity, whereas the converse was the case if a cut-off value lower than 13-5 mU/l was chosen.

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DISCUSSION

These data demonstrate that the peak serum GH concentration response to ITT is continuous in a large group of short children with varying growth rates. No clearly demarcated subpopulations existed, unlike the population distributions of other measurements such as blood pressure and intelligence quotient. These observations are similar to those obtained in the analysis of GH secretion and GH secretory dynamics assessed by direct and indirect means (Hindmarsh, Smith, Brook & Matthews, 1987; Albertsson-Wikland & Rosberg, 1988; Rose, Ross, Uriarte et al. 1988; Blum, Ranke, Kietzmann et al. 1990).

The wide overlap between values obtained in the two groups of short children suggested that establishing a cut-off value for the diagnosis of GH insufficiency to be applied to an individual would be difficult. In the assessment of any test, attention should be paid to the efficiency, sensitivity and specificity of that investigation as compared with an acceptable gold standard. Although ITT is generally accepted as the gold standard for hormonal evaluation, it is clear from this study that discrepancies between the test result and growth rate, which is what matters, can be marked.

A number of reasons have been proposed to explain the discordance between the results obtained from GH testing as compared with the long-term observation of growth of the child. Kaplan, Abrams, Bell et al. (1968) were among the first to recognize that a high serum GH concentration at time 0 during the test led to considerable difficulty in its interpretation. Secondly, the test was designed to evaluate the secretory pathway and, in particular, the readily releasable pool of GH; if there has recently been a pulse of GH and the pool is low, the response will be attenuated (Devesa, Lima, Lois et al. 1989; Suri, Hindmarsh, Brain et al. 1990). Thirdly, children's growth varies with the seasons (Marshall, 1975) and there is evidence to suggest cycles of periodicity greater than 1 year (Butler, McKie & Ratcliffe, 1990).

As GH secretion is related to growth rate, it is possible that some of the discordance could be explained by the performance of the ITT during a period of relatively good growth when GH secretion was improved whereas the annualized growth rate might indicate a different picture of overall growth failure. There remains, therefore, the difficulty of equating 1-year growth data with a 1-day stimulation test. This criticism is applicable to any test proposed for the investigation of GH insufficiency.

Finally, the reproducibility of the test result needs to be considered. A number of studies have demonstrated that the reproducibility of physiological and pharmacological methods of assessing GH secretion is poor in terms of the individual's position within an expected range (Hattori, Shimatsu, Kato et al. 1989; Hoche, Valido & Matussek, 1988; Fornito, Caldgero, Mongioi et al. 1990).

Our data suggest an additional problem: the variety of GH assays available employ differing reaction matrices and are almost certain to use antisera with differing epitope specificity. Using the Hybritech IRMA we achieved optimal test performance with a serum GH concentration cut-off value of 13-5 mU/l, but care must be exercised in interpreting results from different laboratories. Many of the commercially available kits are targetted towards the traditional cut-off value of 15 mU/l which may be inappropriate.

The product licence for the prescription of biosynthetic human GH in the United Kingdom requires the demonstration of GH insufficiency. Our observations highlight the problems associated with using absolute cut-off values for a condition where considerable overlap exists. If cut-off values are to be used, a careful analysis of the performance of the test chosen to

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<th>GH conc. (mU/l)</th>
<th>Efficiency (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>10</td>
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FIGURE 1. Box plot of peak serum GH concentrations achieved following insulin-induced hypoglycaemia in 63 short normal and 85 short slowly growing children. Lines demarcating the shaded areas represent the 25th, 50th and 75th centiles of the two groups, with the shaded areas representing quartiles.
assess GH insufficiency is required and every physician and associated laboratory must define its own appropriate value.

Finally, we would like to emphasize that the ITT, like other methods of investigation of GH reserve, is a potential cause of morbidity and mortality in childhood, particularly if performed by inexperienced persons. We therefore recommend that children requiring investigation of growth failure should be referred to specialist centres.

REFERENCES


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