

# PROVOCATIVE TESTING OF HUMAN GROWTH HORMONE RELEASE IN SHORT STATURED CHILDREN

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## Abstract

We have studied the combined use of two procedures, i.e, L-Dopa stimulation and exercise stimulation as provocative tests of hGH release in establishing the diagnosis of hGH deficiency in 30 short statured children. This combination was found to be a simple, reliable and practical regimen for routine use as an outdoor diagnostic procedure. Our initial experience with the "Cold pressor test", a new test of hGH stimulation is also reported (JPMA 37:256 ,1987).

## INTRODUCTION

When a child falls repeatedly below the third percentile of height standard in relation to its age, his growth may be considered to be abnormal. Children with growth failure and dwarfism without a clearly defined abnormality should be studied for hGH deficiency.

The growth hormone content of human serum may be measured by bioassay, radioreceptor assay, or radioimmunoassay (RIA). RIA was first applied to hGH by Greenwood and his colleagues<sup>1</sup>. Prior to the introduction of RIA techniques for measuring hGH levels in plasma, the definite diagnosis of pituitary dwarfism presented great difficulties. The use of RIA for the measurement of hGH levels in the serum has brought about major impact on physiological studies and clinical practice. In most normal subjects, the basal secretion of hGH is low and it is not possible to differentiate with confidence the normal from hypopituitarism by a single determination of plasma hGH. To evaluate the adequacy of hGH secretion, dynamic testing of hGH release in response to suitable provocative tests has now become established as an effective means to assess the hGH secretory capacity of anterior pituitary or the presence of an abnormality of hGH control mechanism.

A large number of stimulatory tests have been employed to aid in the assessment of possible hGH deficiency in the short statured (Table 1).

**TABLE – I**  
**Stimulation Tests of Growth Hormone Release.**

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1. Physiological stimuli
    - a. Exercise
    - b. Sleep
  2. Nutrient challenge
    - a. Arginine infusion
    - b. Insulin-induced hypoglycaemia
  3. Pharmacological stimuli
    - a. L-Dopa
    - b. Clonidine
    - c. Propranolol
    - d. Diazepam
    - e. Metoclopramide
  4. Hormonal stimuli
    - a. Androgen/Estrogen primed tests
    - b. Glucagon
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For our study, we selected the L-Dopa Stimulation and the exercise stimulation tests, from among the range of established stimulatory tests, on account of their ease of performance, simplicity, reliability and lack of necessity of continuous medical surveillance.

This study was aimed at evaluating, in routine outpatient clinic environment the role of the above tests in investigating hGH deficiency in short statured children. Furthermore, we report our initial experience with the “Cold pressor test”, a new test for hGH stimulation.

#### **MATERIALS AND METHODS**

Thirty short statured children ranging in age from 3.5 to 18 years, with a mean age of  $10.41 \pm 3.80$  years referred to the Nuclear Medical Centre, A.F.I.P, Rawalpindi, for investigations of growth

hormone deficiency were studied. Serum samples for the estimation of basal resting hGH levels were obtained from all patients in the morning after an overnight fast. Growth hormone stimulation tests were then performed in these patients. L—Dopa stimulation test was performed in 29 patients, exercise stimulation in 25 patients and 10 patients were subjected to the “Cold pressor” stimulation test. 26 children received a combination of two provocative tests and 4 children underwent all three provocative procedures.

## **STIMULATION TESTS**

### **1. Exercise stimulation test**

The patients were exercised maximally on a treadmill at a speed and gradient varying with their physical size and condition and blood samples obtained at the end of the exercise period.

### **2. L-Dopa stimulation test**

An oral dose of L-Dopa was administered according to the weight of the patient as follows:

Less than 20 Kg—125mg.

From 20 to 35 Kg — 250 mg.

Over 35 Kg—500mg.

Blood samples were taken 90 min. later.

### **3. Cold pressor test**

The patients were made to place one hand in crushed ice for a period of 4-5 min. and blood samples for hGH estimation were obtained 5 min. later.

## **IMMUNOASSAY FOR HUMAN GROWTH HORMONE**

Dac-Cel-hGH-MCA by Wellcome, an immunoradiometric assay kit utilising antibody to human growth hormone was used to quantify serum hGH.

The kit utilises the two-site immunoradiometric assay (sandwich) principle in which two different antibodies, combining with distinct antigenic determinants on the growth hormone molecule, are used<sup>2</sup>. The various stages of the assay take place sequentially. hGH in the sample is first incubated with an excess of labelled antibody to hGH and the labelled antibody-hGH complex is then reacted with a second (unlabelled) antibody to hGH. The labelled antibody-hGH-second antibody complex is then separated from free labelled antibody using an anti-rabbit serum covalently coupled to cellulose. After solid-phase separation of this “sandwich”, the distribution of radioactivity is determined. The amount of radioactivity increases proportionately with the concentration of hGH in the sample, which is determined by reference to a standard curve set up at the same time. The labelled antibody to hGH is of monoclonal origin conferring high specificity on the assay. The intra-assay precision (agreement between replicate measurements) was about 3.9%, and the interassay precision (repeatability of assayed values) was about 6.1%.

## **RESULTS**

Based on the results of the stimulation tests for hGH, the patients were divided into 3 groups. Group 1 included patients who showed normal response to a stimulation test in that their serum hGH level increased to 15 mIU/L or more as a result of the stimulus; Group 2 included patients who showed a response of 10 mIU/L or more but less than 15 mIU/L and these were classified as partially deficient; Group 3 included those patients who failed to achieve a level of 10 mIU/L in response to any of the stimulation tests.

**TABLE II**  
**Data for Group 1 Patients.**

S. No.	Sex	Age (Yr)	Height Percentile	Growth	Hormone	Levels (MIU/L)	
				Basal	After L-Dopa	Stimulation Exercise	C. Pressor
1.	F	12.5	<3P	3.0	20.0	11.0	—
2.	F	18	<3P	7.2	10.0	15.0	—
3.	M	3.5	<3P	0.76	46.0	2.6	—
4.	F	5	<3P	10.2	60.0	44.0	—
5.	F	10	<3P	2.2	21.0	18.0	—
6.	F	14	<3P	3.3	19.0	16.0	—
7.	F	6.4	<3P	10.0	17.0	15.0	—
8.	F	17	<3P	2.8	5.0	15.0	9.0
9.	F	9.0	10P	10.0	16.0	22.0	—
10.	M	12	3P	2.0	12.0	—	24.0
11.	M	8	<3P	9.0	24.0	—	20.0
12.	F	9	<3P	1.2	7.0	12.0	22.0

Table II lists the relevant clinical details, the basal hGH values, and the results of the stimulation tests for the patients in group 1,

**TABLE III**  
**Data for Group 2 Patients.**

S. No.	Sex	Age (Yr)	Height Percentile	Growth	Hormone	Levels (MIU/L)	
				Basal	After L-Dopa	Stimulation Exercise	C. Pressor
1.	M	8	—	3.2	7.0	10.5	—
2.	M	7.5	3P	2.0	10.0	11.0	—
3.	M	15	<3P	2.2	11.0	5.6	2.9
4.	M	14	<3P	3.0	14.0	8.8	—
5.	F	7	<3P	5.8	14.0	—	8.8

table III for the patients in group 2, and table IV for the patients in group 3.

**TABLE IV**  
**Data for Group 3 Patients.**

S. No.	Sex	Age (Yr)	Height Percentile	Growth	Hormone	Levels	(MIU/L)
				Basal	After L-Dopa	Stimulation Exercise	C. Pressor
1.	M	10	<3P	1.6	1.8	2.8	—
2.	M	4	10P	3.0	3.8	4.0	—
3.	M	12	10P	2.0	5.2	3.0	—
4.	F	13	<3P	3.0	5.2	5.0	—
5.	F	14	<3P	1.4	4.2	2.0	—
6.	M	10	<3P	1.6	8.0	6.4	—
7.	F	6	<3P	3.0	6.2	9.0	—
8.	F	9	<3P	0.60	—	0.65	0.90
9.	M	15	<3P	1.6	2.8	4.4	—
10.	F	5.5	<3P	2.0	2.2	5.0	7.0
11.	M	10	<3P	1.8	4.4	6.0	—
12.	F	12	<3P	3.0	3.8	—	4.4
13.	M	14	3P	2.0	5.0	—	2.4

#### GROUP 1 PATIENTS

40% (n12) of the patients tested showed a normal response to at least one provocative test. The mean basal hGH value in this group was  $5.14 \pm 3.6$  mIU/L. The mean hGH level in response to L-Dopa (n12) was  $213 \pm 15.4$  mIU/L, with a mean rise of 810% over the basal level; the mean hGH value in response to exercise stimulation (n10) was  $17.1 \pm 10.7$  mIU/L with a mean rise of 355% over the basal value; and the mean hGH value in response to cold pressor (n=4) was  $18.7 \pm 5.8$  mIU/L with a mean rise of 794% over the basal value.

#### GROUP 2 PATIENTS

16.7% (n5) of the patients showed partial hGH deficiency and the mean basal hGH level in this group was  $3.2 \pm 1.4$  mIU/L. After L-Dopa stimulation the mean hGH value increased to  $11.2 \pm 2.6$  mIU/L, showing a mean increase of 321% over the basal value. In response to exercise stimulation the mean hGH value rose to  $8.9 \pm 2$  mIU/L with a mean increase of 256% over the basal value, and in response to cold pressor the percentage increase was only 42% with a mean hGH value of 5.8 mIU/L.

#### GROUP 3 PATIENTS

43.3% (n=13) of the patients tested proved hGH deficient with a mean basal hGH level of  $2 \pm 0.7$  mIU/L. After L-Dopa stimulation, the mean hGH value rose to  $4.4 \pm 1.6$  mIU/L, showing a mean percentage rise of 118% over the basal value. After exercise stimulation, the hGH value rose to  $4.4 \pm 2.2$  mIU/L showing a mean percentage rise of 121% over the basal value and after cold pressor stimulation the mean hGH value was found to be  $3.7 \pm 2.33$  mIU/L with a mean percentage rise of 29% over the basal value.

75% of the patients in groups 1 and 2 as compared to 30% of the patients in group 3 had basal levels of serum hGH greater than 2.0 mIU/L.

17% (n=5) of the patients tested with L-Dopa failed to respond to this stimulus, but showed a normal response to other provocative tests. Similarly, 20% (n=5) of the exercise test results also showed a failure to respond, as did 33% of those subjected to cold pressor test.

## DISCUSSION

Impairment of hGH secretion either due to abnormal function or destruction of somatotrophic cells of the anterior pituitary, or due to lack of hypothalamic stimulation in childhood leads to the impaired growth called pituitary dwarfism. However, in only a minority of these patients there is evidence of pituitary destruction; 10% are familial with transmission as a recessive gene, most cases have no familial basis and are not the result of any disease process affecting the pituitary; in about one third of the cases isolated hGH deficiency is present; and in the remaining, other pituitary deficiencies exist<sup>3</sup>. Thus the estimation of plasma hGH appears to have a decisive role to play in establishing the diagnosis of pituitary dwarfism.

The characteristic secretory pattern of hGH is one of low basal levels with secretory episodes associated with sleep, exercise, stress, and high protein meals<sup>3</sup>. In our study the basal level of serum hGH was seen to be less than 2 mIU/L in 70% of the patients in group 3 as compared to 25% of the patients in groups 1 and 2. Thus although the basal levels of hGH tend to be lower in patients with hGH deficiency than in the normal patients, simple hGH levels alone are not sufficient to arbitrarily decide the issue and the diagnosis of hGH deficiency must be considered in the light of dynamic testing of hGH release and the clinical picture.

L-Dopa, a precursor of catecholamines, induces a significant rise in the peripheral hGH levels and is thought to act via its conversion to dopamine and the subsequent perturbation of the hypothalamic catecholamine<sup>4</sup>s. The manner in which exercise stimulates hGH release is not known, although it has been observed that for a reliable response the subject should be exercised to exhaustion.<sup>5</sup>

The criteria applied to the assessment of hGH stimulation tests are largely empirical. Most workers would accept the failure to achieve a peak level of 10.0 mIU/L as evidence of hGH deficiency. A further category of partial hGH deficiency has been accepted by consensus, although the cut.off for this group varies from 14 to 20 mIU/L in different centres. The normal response of normal subjects to hGH stimulation tests ranges from 50-90%, with most of the tests recording a positive response in approximately 75% of normal subjects if a peak response of 15 mIU/L is to be achieved. This means that one in four of the normal subjects will have partial hGH deficiency on the basis of one test. For this reason it is customary to perform a series of tests to reduce the probability of a false interpretation.<sup>6</sup> Our results of the three provocative tests show that the mean response to L-Dopa in the non-deficient and partially deficient patients is higher than that obtained with the other two procedures, and that the mean percentage rise over the basal value by L-Dopa is also greater in these patients. The results of the exercise stimulation in the partially hGH deficient patients were not satisfactory in that 50% of these patients failed to respond adequately to the stimulus (see figure).

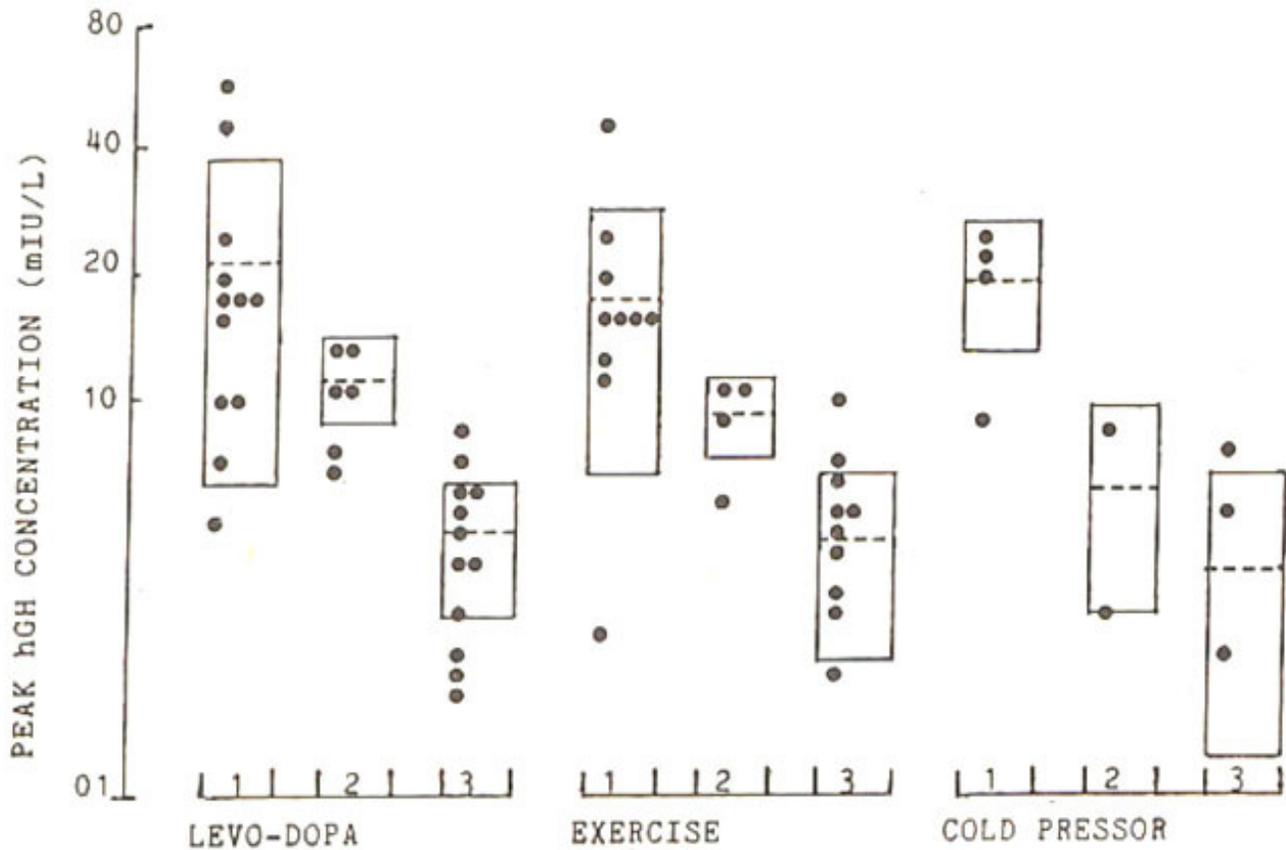


Figure. Results of exercise stimulation in partially hGH deficient patients.

The superiority of L-Dopa is also reported by other workers. In a comparative study of 5 provocative test procedures conducted in normal volunteers to ascertain the relative effectiveness of each stimulus, Eddy et al found that the most consistent stimulus for producing a positive hGH response was L-Dopa (95%), followed by insulin (90%), arginine (80%), vasopressin (60%), and glucagon (55%). They further reported that the side effects encountered with L-Dopa stimulation were considerably less frequent and less consequential than those with insulin induced hypoglycaemia<sup>7</sup>

None of the three stimulation tests in this study produced a positive response in 17-33% of the hGH non-deficient patients (see table II). However the patients in this group, who failed to respond to L-Dopa, responded adequately to exercise and vice versa, and those who did not respond to cold pressor responded well to exercise and/or L-Dopa. These results agree with the reports in the literature and confirm that failure to respond to one stimulus does not necessarily imply a lack of responsiveness to another, nor does it unequivocally establish the diagnosis of hypopituitarism.

Faraser and Fass et al have reported that of normal children tested with L-Dopa stimulation, 10% showed a response of less than 15 mIU/L<sup>8,9</sup>

Weldon et al in studying the response of short statured children to various provocative tests found a false negative response to L.Dopa in 13% and to insulin in 15% of those proved to be hGH non-deficient<sup>10</sup> Similarly Lacey et al and Buckler et al have reported hGH levels of less than 10 mIU/L after exercise in 20-30% of the normal children tested<sup>11,12</sup>

Our study has highlighted the effectiveness of the combined use of two simple tests of hGH

stimulation, i.e., L-Dopa and exercise stimulation, for evaluating hGH deficiency and established their suitability for routine use as an outdoor diagnostic procedure. Although insulin-induced hypoglycaemia has been regarded as the standard hGH stimulation test, the incidence and severity of side effects and the need for constant medical surveillance often curtail its usefulness. L-Dopa has proved to be a safe and reliable stimulus of hGH release, with few side effects, and the additional use of exercise testing has effectively reduced false interpretation.

The number of patients on whom cold pressor test was performed is as yet too small to merit definite conclusions but the initial results appear promising. It evoked a higher response than L-Dopa in 5 out of 9 patients who underwent both tests, and also a greater response than to exercise in 3 out of 4 patients subjected to both exercise and cold stimulation. Four patients in group 1 were subjected to cold stimulation and the diagnostic response of greater than 15 mIU/L was elicited by this test in two patients who had failed to respond adequately to L-Dopa and exercise stimulation. These results indicate that this simple, safe, and less time consuming test may be valuable in discriminating patients with hGH deficiency from the non-deficient patients.

## REFERENCES

1. Greenwood, F.C., Hunter, W.M., and Glover, J.S. The preparation of <sup>131</sup>I-labelled human growth hormone of high specific activity. *Biochem. J.*, 1963; 89: 114.
2. Peake, G.T., Morris, J. and Buckman, M.T. Growth Hormone. in *methods of hormone radioimmuno assay*. Edited by Jaffe, B.M., Behrman, H.R. New York, Academic Press, 1979, p. 223.
3. Williams, R.H. *Textbook of endocrinology*. Edited by Robert H. Williams 6th ed. Philadelphia, Saunders, 1981, p. 97.
4. Eddy, R.L., Jones, A.L., Chakmakjian, Z.H. and Silverthorne, M.G. Effect of levodopa (L-Dopa) on human hypophyseal trophic hormone release. *J. Clin. Endocrinol. Metab.*, 1971; 33:709.
5. Richard, A.D. *Endocrine disorders; a guide to diagnosis*. Edited by Richard, A.D. London, Butterworths, 1984, p. 639.
6. Richard, A.D. *Endocrine disorders; a guide to diagnosis*. Edited by Richard, A.D. London, Butterworths, 1984, p. 292.
7. Eddy, R.L., Gilliland, P.F., Ibarra, J. D. Jr., McMurry, J. F. Jr., and Thompson, J.Q. Human growth hormone release; comparison of provocative test procedures. *Am. J. Med.*, 1974; 56: 179.
8. Fraser, S.D. A review of growth hormone stimulation tests in children. *Paediatrics*, 1974; 53: 929.
9. Fass, B., Lippe, B.M. and Kaplan, S.A. Relative usefulness of three growth hormone stimulation screening tests. *Am. J. Dis. Child.*, 1979; 133:931.
10. Weldon, V.V., Gupta, S.K., Haymond, M.V., Pagliara, A.S., Jacobs, L.S. and Daughaday, W.P. The use of L-dopa in the diagnosis of hypopituitarism in children. *J. Clin. Endocrinol. Metab.*, 1973; 36:42.
11. Lacey, K.A., Hewison, A. and Parkin, J.M. Exercise as a screening test for growth hormone deficiency in children. *Arch. Dis. Child.*, 1973; 48:508.
12. Buckler, J.M.H. Plasma growth hormone response to exercise as a diagnostic aid. *Arch. Dis. Child.*, 1973; 48:565.