Clinical Applications of Growth Hormone Assays

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This brief review of the use of growth hormone assays in clinical practice starts off by making several important assumptions—

1. That determinations of plasma or serum growth hormone (GH) are available freely to those who require them.
2. That everyone now uses a radioimmunoassay (RIA) for growth hormone measurement.
3. That there are no longer any methodological problems with this RIA.
4. That everyone uses and quotes numerical results measured in terms of the first international reference preparation (IRP) for GH distributed by the MRC (either in ng/ml or μU/ml; for practical purposes 1 ng = 2 μU).
5. That we do not have to understand exactly what growth hormone does before we measure it in plasma and use these results with confidence in planning and monitoring treatment of patients with a variety of diseases.

Before describing the interpretation of results, it is important to understand something about growth hormone.

Growth hormone is a polypeptide of molecular weight 21,500 containing 190 amino acids. It is stored in bulk in the acidophil cells of the anterior pituitary where it is synthesised. In terms of synthesis and storage, GH resembles insulin and differs from parathyroid hormone. This means that extracts of the gland are a clinically useful source of GH and, as a result of the widespread co-operation of pathologists throughout the nation, there exists an impressive stockpile of GH for clinical use. This is kept under lock and key by the MRC and, regrettably, is not available for general use.

Approximately 10 mg of growth hormone are normally stored within the pituitary gland and, although the secretion rate of the hormone from the gland can fluctuate 1,000 fold in a few minutes, roughly 1 mg per day is secreted in a normal subject. This is sufficient to maintain an average blood concentration of 3 ng/ml throughout the twenty-four hour period in a healthy 70 kg subject.

Acute stimuli of growth hormone secretion can only lead to the release of a very small fraction of the total amount of hormone stored in the gland. This
is analogous to the synthesis, storage and secretion of insulin but differs markedly from some other hormones, noticeably the adrenal steroids, where very little is stored and most is synthesised 'on demand'.

The rate of growth hormone secretion by the pituitary varies remarkably during the twenty-four hour cycle. Unlike ACTH (and subsequently cortisol) there is no true diurnal rhythm. There is, however, a nyctohemeral (night-day) pattern, with characteristic nocturnal peaks of secretion leading to plasma GH concentrations as high as 100 ng/ml, which usually occur during periods of deep (stage III or IV) sleep. This results in a higher mean plasma GH concentration during the night than the day, giving scientific credence to mother’s advice when children that we wished to grow up to be tall. It remains to be proven that this is also the basis of the proverb: ‘Early to bed and early to rise, makes a man healthy, wealthy and wise’. The major nyctohemeral peaks in GH secretion occur early during sleep and are less commonly seen in the few hours before waking (Fig. 1).
There are many other known factors that may stimulate GH secretion in everyday life, some being listed in Table 1(a). There are fewer known factors

Table 1a. Factors associated with increased GH secretion

| Factor | Remarks |
|--------|---------|
| Acute hypoglycaemia* (Insulin, tolbutamide, post glucose) | |
| Stress (metabolic, physical and psychological) | |
| Exercise* | |
| Fasting/starvation | |
| Sleep* | |
| Fall in plasma-free fatty acids | |
| α stimulation (and β blockade) | |
| L-Dopa* | |
| Amino acid infusions* | |
| Protein meal* (including Bovril?) | |
| Glucagon* | |
| Vasopressin | |
| Fever/pyrogen* | |
| Anaesthesia/operations | |
| Oestrogens (and androgens at puberty?) | |
| 2-deoxy-D-glucose | |
| * Indicates responses that are sufficiently reproducible to be clinically useful. | |

Table 1b. Factors associated with suppression of GH secretion

| Factor | Remarks |
|--------|---------|
| Hyperglycaemia* (and carbohydrate feeding—particularly glucose) | |
| Rest | |
| Increase in plasma FFA* | |
| Obesity | |
| Hypothyroidism | |
| Corticosteroid excess (endogenous or exogenous) | |
| β stimulation (and α blockade) | |
| Somatostatin* | |
| Centrally active drugs—chlorpromazine reserpine | |
| * Indicates responses that are sufficiently reproducible to be clinically useful. | |

that suppress GH secretion (Table 1(b)). (For an excellent current review, please see Stuart Mason, 1972.) Although it was thought until very recently that GH secretion was mainly under the control of a hypothalamic releasing hormone (GHRH), recent events have shown that there exists a hypothalamic peptide that inhibits the release of GH (SRIF; somatotrophin release inhibitory factor—somatostatin). Somatostatin, a simple polypeptide with 14 amino acids, has been isolated, characterised, synthesised and shown to be biologically highly active, in what must be one of the greatest contemporary ‘scoops’ in endocrine research (Brazeau et al., 1973). Whatever proves to be the eventual roles of GHRH and SRIF, it is already clear that the hypo-
thalamus and the central nervous system play a key role in regulating GH secretion. The hypothalamus contains a high concentration of bioamines and it has been postulated that they also are involved in the regulation of GH secretion. It is possible to demonstrate in many ways that sympathetic α and β receptors modify the GH response to various stimuli. Reciprocally to their role in modifying insulin secretion from the β cell, α stimulation (and conversely β blockade) leads to augmentation of GH release, while β stimulation (and α blockade) reduce GH release.

The effect of stress is of considerable practical significance in evaluating GH secretory patterns, and the avoidance of repeated venepunctures (by the use of indwelling plastic needles) is to be preferred. Although hypoglycaemia is normally a very potent stimulus to GH secretion, chronic hypoglycaemia, as seen with islet cell tumours of the pancreas, is usually associated with very low plasma GH concentrations. The administration of a non-metabolisable glucose analogue (2-deoxy-D-glucose) leads to GH release by interfering with glucose metabolism in the gluco-receptors of the hypothalamus. The effects of the sex hormones on GH secretion are not understood; oestrogens tend to enhance GH secretion and are thought to account for the more widely fluctuating patterns of GH secretion seen in the female; androgens undoubtedly influence GH secretion in the male, and delayed puberty may mimic partial GH deficiency.

**Clinical Applications**

**Acromegaly**

Since there are wide variations in the concentration of GH in fasting normal subjects, random GH measurements are of little value in establishing the diagnosis of acromegaly. The diagnosis can nearly always be made with a single 100 g oral glucose tolerance test, but samples must be taken frequently for at least three hours after the administration of glucose. Normal subjects, whatever their fasting GH concentrations, show suppression of GH release, with plasma concentrations falling to less than 1 ng/ml, usually between 90 and 120 minutes after glucose. In fact, in my experience of over fifty 100 g oral glucose tolerance tests in normal subjects, plasma GH has always fallen to less than 0.2 ng/ml in at least one blood sample. It is with this knowledge that it is possible to be confident about the diagnosis of acromegaly. In patient G.R., (Figs 2a, b, c), plasma GH concentrations varied between 7.8 and 2.3 ng/ml in two glucose tolerance tests performed with an interval of a year between them (on no occasion did serum GH fall below 2.3 ng/ml); despite a pituitary fossa reported as being normal, tomography demonstrated a small but unequivocal erosion in the posterior part of the floor (Fig. 2b).

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Fig. 2. (a) Patient G.R., aged forty-five. Lateral view of pituitary fossa, initially reported as normal; (b) Lateral tomogram of pituitary fossa, showing localised tumour extending posteriorly which can be seen in Fig. 2(a).
Occasionally one sees a patient with clinical features of acromegaly in whom repeated measurements of plasma GH fail to demonstrate detectable GH. The patient whose results are shown in Figs 3a, b was diagnosed clinically as having acromegaly in 1967 at which time he had a diabetic glucose tolerance test, and an enlarged pituitary fossa. He presented to us with a proven subarachnoid haemorrhage in December 1967. Measurement of GH during two subsequent glucose tolerance tests and three insulin tolerance tests failed to demonstrate plasma GH concentrations greater than 0-2 ng/ml on any occasion. The soft tissue changes have regressed and he has become hypogonadal. Cortisol production remains unimpaired. We consider that this man most likely developed spontaneous pituitary apoplexy resulting in partial hypopituitarism. There is controversy concerning the term ‘burnt out acromegaly’—it includes patients who have suffered pituitary apoplexy and also some with very slowly progressive disease and low plasma GH concentrations, such as G.R. in Fig. 2. Most commonly, plasma GH concentrations are high in the fasting state and show little or no suppression following the administration of glucose (Fig. 4). Occasionally, the fasting concentration may be low and show a paradoxical rise after glucose administration (Fig. 5). It can be seen that the two peaks of GH secretion in this patient coincide with
the rise in plasma insulin concentration, a feature often seen after insulin administration to diabetics (Sönksen et al., 1972) suggesting that on some occasions, insulin may, in the absence of hypoglycaemia, stimulate GH release.

Since plasma GH concentration may vary considerably from day to day in any given patient, serial glucose tolerance tests are probably the best way of assessing progress of the disease (Sönksen et al., 1967). When, as in patient B.R., shown in Fig. 4, a rise in mean plasma GH concentration is accompanied by a significant deterioration in glucose tolerance, this can usually be
Fig. 4. In patient B.R., two glucose tolerance tests over interval of 15 months demonstrate the development of chemical diabetes with progressive increase in insulin and GH concentrations. Plasma GH concentration did not fall significantly following glucose administration—the usual pattern in acromegaly. Within two months of trans-sphenoidal hypophysectomy, blood sugar and plasma insulin had returned to normal and there was a satisfactory fall in plasma GH, although there was still incomplete suppression of GH secretion. Note reactive hypoglycaemia which became more marked after hypophysectomy.

Fig. 5. Although fasting plasma GH was only modestly raised in a cromegalic patient S.H., there were two 'paradoxical' peaks of GH which coincided with similar peaks in blood sugar and plasma insulin, suggesting that insulin may have directly stimulated GH secretion.
taken as an indication of the progression of the disease, and expansion of the pituitary fossa can almost invariably be demonstrated. On this occasion, trans-sphenoidal hypophysectomy resulted in a dramatic reduction of GH values to near normal, with marked improvement in glucose tolerance. The improvement in glucose tolerance is related to the success of the operation and the fall in plasma GH. Hypophysectomy is not always as successful as this. The results shown in Fig. 6 are from a 37-year-old acromegalic patient who had a trans-sphenoidal hypophysectomy in 1967; plasma GH concentration remained elevated post-operatively and the disease has progressed relentlessly. Cobalt 60 telotherapy (4,000 R) failed to reduce GH secretion over a period of eight months. This patient may not be representative of the usual response to external pituitary irradiation that has been shown to reduce GH secretion substantially in many patients (Roth et al., 1970; Hunter et al., 1971).

It will probably be another ten years before we can be confident that alterations in GH secretion in acromegaly following treatment is a reliable assessment of the efficiency of the therapeutic manoeuvre. In the meanwhile,

![Fig. 6. Patient M.T. had an incomplete hypophysectomy in 1967. Plasma GH concentrations showed minor fluctuations over an 18-month period of observation. In each GTT there was incomplete suppression of plasma GH. Conventional external pituitary irradiation was without demonstrable effect on GH secretion. Post-glucose insulin concentrations varied considerably between tests and exaggerate relatively small differences in blood glucose concentrations.](image)
it seems that a reasonable objective is to lower the plasma GH to zero. In practice, this very rarely happens; occasionally, plasma GH concentrations actually rise post-operatively (Sönksen et al., 1967), more commonly they fall, but rarely to zero. It is difficult to understand what happened to patient D.R., shown in Fig. 7. Trans-sphenoidal hypophysectomy resulted in apparent normalisation of his GH secretion both in response to oral glucose and intravenous insulin (Fig. 8). It is interesting that although GH and cortisol responses to intravenous insulin were completely normal 5 months post-operatively and he remains euthyroid, he has become hypogonadal, plasma testosterone falling to 1.9 ng/ml (NR for a male, 3 to 8 ng/ml). That plasma GH response to treatment is a useful index of the success or failure of treatment is shown by the improvement in glucose tolerance that almost invariably accompanies a substantial reduction in plasma GH (Figs 4 and 7 and Sönksen et al., 1967). However, the improvement in glucose tolerance and insulin
secretion may be delayed as long as a year after effective treatment (Sönksen, 1972 and Sönksen et al., 1972).

**Hypopituitarism**

Although the clinical value of GH assays must be greatest in the management of patients with acromegaly, determination of plasma GH response to provocative stimuli has proved to be of great help in the evaluation of hypopituitarism. In recent years, with the increasingly widespread availability of sophisticated biochemical tests of pituitary function, hypopituitarism has become one of the most intriguing aspects of endocrinology. Isolated hormone defects are now being recognised, as are a spectrum of linked hormone deficiencies. We are also able to differentiate partial hypopituitarism from normal and from total pituitary deficiency. In the evaluation of pituitary function, plasma GH measurements following provocative stimuli have two
major uses: (1) to detect the patients deficient in GH who may benefit from GH treatment (Tanner et al., 1971); this, of course, is only relevant to those in whom the epiphyses have not yet fused; (2) as one direct index of pituitary function in an overall assessment of pituitary performance.

The radioimmunoassay of GH remains the easiest and most sensitive assay of all the anterior pituitary hormones. Unlike assays for thyrotrophin (TSH), luteinising hormone (LH), follicle stimulating hormone (FSH), adrenocorticotrophin (ACTH), where there are considerable methodological problems in distinguishing normal basal levels from zero, the high sensitivity and specificity of the GH assay allows the assessment of absolute GH deficiency without any technical ambiguity.

Undoubtedly, the most reliable provocative test of GH secretion now available is the response to insulin-induced hypoglycaemia.

The insulin tolerance test (ITT) has proved to be reliable, reproducible, and safe. Provided blood glucose falls to 20 mg/100 ml or lower one can be confident that there has been an adequate stimulus to GH release. We routinely monitor blood glucose with a reflectance meter and give an additional dose of insulin if adequate hypoglycaemia is not achieved (Fig. 8). We feel that this is more reliable than the appreciation of symptoms by the patient. The magnitude of the rise in plasma GH is dependent on the extent of the induced hypoglycaemia (Sönksen et al., 1972, 1973). A total failure of GH secretion is easy to detect but the range of normal response is much less easy to define. We have found that a rise in plasma GH to 10 ng/ml or greater in response to a blood glucose fall to 20 ng/100 ml or less, is a good working definition of a normal response. There is clinical ambiguity in responses that do not meet this definition of normal (Joss and Zuppinger, 1972). On some occasions, a repeat test will produce an unequivocally normal response; this is always worth doing. If the result of the repeat test remains ambiguous, it may be helpful to use alternative stimuli to GH secretion, such as the administration of arginine (Merimee et al., 1967), glucagon (Av Ruskin et al., 1971) or a sleep study (Takahashi et al., 1968). In our limited experience, the last of these alternatives is, although rather tedious, the most helpful.

In a number of clinical conditions the plasma GH responses may be in the ambiguous range and the failure of GH secretion relative rather than absolute. These include:

1. Hypothyroidism
2. Obesity
3. Cushing’s syndrome
4. Steroid treatment
5. Chronic hypoglycaemia
6. Partial hypopituitarism—(LH/FSH and GH)
7. Delayed puberty
The last of these is probably the most difficult to be sure about. In a few patients with delayed puberty and short stature, GH secretion is in the ambiguous range but returns to normal after the spontaneous occurrence of puberty. It is, therefore, extremely difficult to differentiate prospectively between partial pituitary failure with incomplete gonadotrophin and GH deficiency and simple delayed puberty, a diagnosis that can really only be made with confidence retrospectively. In fact, they may both be the same condition, with the primary abnormality in the hypothalamus.

**Short Stature**

Assessment of GH secretion is of primary importance in the investigation of patients with short stature. The fact that the MRC growth study group still demands a period of a year's anthropomorphic measurements before starting GH treatment in GH deficient subjects suggests that they are still not confident about a diagnosis of short stature due to growth hormone deficiency that is based only on biochemical testing. In the opinion of others, the demonstration of absolute GH deficiency on repeated testing is sufficient evidence to justify immediate treatment with GH, and any delay that occurs after the diagnosis has been established could well be interpreted as negligence by the physician concerned. It is also argued that, in cases of partial GH deficiency associated with short stature, growth hormone treatment should be given without delay since the efficacy of treatment is often limited by the time when the epiphyses fuse and any delay in starting treatment will probably limit the eventual height reached. It is of fundamental importance that other causes of impaired GH responses—particularly hypothyroidism—are excluded as the cause of short stature because they will respond well to treatment of the underlying disease and GH secretion returns to normal (Tunbridge et al., 1973).

There is often real difficulty in deciding whether or not partial GH deficiency exists in children with short stature and delayed puberty. One such problem is illustrated in Fig. 9. In patient R.M., height corrected for bone age rather than chronological age fell on the 50th percentile. At the age of 17, two ITTs and a prolonged GTT produced peak GH concentrations of only 3 ng/ml and the diagnosis of partial GH and gonadotrophin deficiency seemed likely. He then spontaneously developed his pubertal growth spurt, and repeat testing one year later when plasma testosterone had risen spontaneously from 0.8 to 1.9 ng/ml, produced a peak GH concentration after insulin of 8.6 ng/ml and during sleep of 13 ng/ml. If current progress is maintained he will be of normal stature.

Patient F.B., whose results are shown in Fig. 1, has in the past had many
operations for hypospadias. When seen at the age of 15\(\frac{1}{2}\) years, he was complaining of short stature and delayed puberty. Peak GH after good insulin-induced hypoglycaemia was only 3-4 ng/ml. One year later, he had begun to develop puberty spontaneously and his peak GH response to insulin had increased to 20 ng/ml, while peak values during a sleep study exceeded 50 ng/ml on two occasions. It would seem reasonable to conclude that in both these patients, a functional abnormality within the hypothalamus was responsible for the delay in puberty and the partial failure of GH secretion. Similar studies in short patients with treated juvenile onset Cushing’s syndrome and the adrenogenital syndrome have shown normal GH secretion and suggest that end organ resistance to the action of GH in the former and premature closure of the epiphyses in the latter were responsible for their short stature.

There are other situations in which short stature is associated with normal or even elevated plasma GH concentrations. These include the African pygmies (Rimoin et al., 1967), Laron dwarfs (Laron et al., 1966), patients with cachexia or malnutrition from whatever cause, and patients with chronic debilitating diseases such as renal and hepatic failure. It should be mentioned that in these conditions high plasma GH concentrations may not be suppressed normally with oral glucose administration. This is particularly true.
in anorexia nervosa where extremely high concentrations of GH are commonly seen. Short stature in children with home and parental problems may be associated with apparent GH hyposecretion imitating GH deficiency—these children may grow normally and regain normal GH secretion when removed from their adverse environment (Powell et al., 1967). It seems likely that this is a self-limiting functional abnormality of the hypothalamus as suggested in patients R.M. and F.B., and that removal from the adverse environment may have coincided with spontaneous resolution of the problem.

**OTHER ENDOCRINE DISEASES**

**Thyroid Disease**
GH secretion appears to be normal in hyperthyroidism but is impaired in myxoedema. It returns to normal when the hypothyroidism is corrected.

**Cushing’s syndrome**
GH secretion is commonly impaired in this condition but whether this is due to the over-production of cortisol or to the commonly associated obesity is not clear. Patients treated by adrenalectomy, who subsequently become pigmented, often show impaired GH secretion; it is not clear whether or not this is caused directly by the presence of a pituitary tumour. Suppression of GH secretion is also commonly seen in patients with iatrogenic Cushing’s syndrome.

**Diabetes Mellitus**
High and fluctuating GH concentrations are seen in poorly controlled diabetics. GH secretion returns to near normal with intensive treatment. Brisk elevations in plasma GH are seen during the treatment of uncontrolled diabetics with insulin and probably represent a direct response to insulin administration rather than a response to a fall in blood glucose (Sönksen et al., 1972; Alberti and Hockaday, 1973).

**Hypogonadism**
Secretion of GH appears to be normal in both adult males and females with hypogonadism although sex hormones appear to influence GH secretion at the time of puberty.

**Galactorrhoea**
GH secretion is usually normal in these patients, unless the condition is associated with acromegaly.
Pregnancy
Although special precautions are necessary to measure plasma GH during pregnancy (because human placental lactogen (HPL) is present in plasma in very high concentrations in pregnancy and interferes with the GH assay), basal secretion appears to be normal although a blunted response to hypoglycaemia has been reported.

Drugs and GH secretion
Centrally acting drugs influence growth hormone secretion, probably by acting on the hypothalamus. Thus, phenothiazine derivatives cause hypothalamic depression. Chlorpromazine has been shown to lower GH concentrations (Sherman et al., 1971), as well as alter temperature regulation and suppress other aspects of hypothalamic endocrine function.
Reserpine, which depletes the hypothalamus of catecholamines, inhibits GH secretion in response to hypoglycaemia (Müller et al., 1967) while the administration of L-Dopa by mouth or intravenously leads to a brisk rise in GH secretion (Boyd et al., 1970). α adrenergic stimulation, such as with infusions of adrenaline and a β-blocker, causes marked GH release (Massara and Camanni, 1972). Likewise, the administration of β-blockers greatly enhances the GH response to insulin induced hypoglycaemia.

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References
Alberti, K. G. and Hockaday, T. D. (1973) Diabetologia, 9, 13.
Av Ruskin, T. W., Crigler, J. F., Sonksen, P. H. and Soeldner, J. S. (1971) Excerpta Medica (Amsterdam) International Congress Series, 238, 395.
Boyd, A. E., Lebovita, H. E. and Pfeiffer, J. B. (1970) New England Journal of Medicine, 283, 1425.
Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. and Guillemin, R. (1973) Science, 179, 77.
Hunter, W. M., McGurk, F. M., McLelland, J., Hibbert, D. J., Strong, J. A., Gillingham, F. J. and Harris, P. (1971) Excerpta Medica (Amsterdam) International Congress Series, 236, 168.
Sir Henry Lunn achieved lasting fame as travel agent extraordinary. Founder of the Hellenic Travellers’ Club and many others, he was known as the King of Clubs. His early career gave promise of his ability. At school he was not content with the usual barter of pets, and started his own mouse colony, selling the mice through Exchange and Mart. In his eighteenth year he lost £3 on the three-card trick and decided to recoup by selling apparatus for the new-fangled game of lawn tennis. He also invented and sold a scoring dial to fix on to the racket. He started this business in 1877, the year of the first Wimbledon championship, and sales were immediately brisk. He sold out a flourishing business to his father for £1,000 and used the money to go to Dublin where he qualified as a doctor, intent on devoting his life to being a medical missionary. His first posting to India was a disaster to his health and his relationships with other missionaries. Returning to England he never again practised as a doctor but launched a one-man ecumenical movement. His organisation of parties going to Switzerland for religious meetings was so good that he was persuaded to form travel clubs. His first venture was taking a party of 440 people to Rome for Easter at a cost of 20 guineas a head. Mr Cook said that it could not be done for under 25 guineas but Lunn made a profit and went on from there.