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The Diagnosis of Growth Hormone Deficiency in Children and Adults

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I. Introduction

GH REPLACEMENT therapy has been offered to GH-deficient children for more than 30 yr, but it only became a licensed indication for GH-deficient adults in the United States, a number of European countries, and New Zealand in 1996. Thus, in contrast to the longstanding pediatric literature and interest in the biochemical diagnosis of GH deficiency (GHD), the concerns of the endocrinologist treating adults have been addressed only recently.

The type of underlying pathophysiology differs in childhood-onset compared with adult-onset GHD. In childhood, the commonest etiology is isolated idiopathic GHD (1, 2), a blanket term, which includes some children with distinctive pathophysiology that may be demonstrated radiologically, and others in whom the pathological insult is unknown and the explanation for the GHD ill-understood. In contrast, adult-onset GHD is most frequently due to a pituitary adenoma and/or treatment with surgery or radiotherapy (3, 4); isolated idiopathic GHD acquired in adult life has never been reported. In childhood the differential diagnosis is dominated by alternative causes of poor growth (5–9), whereas difficulties in the diagnosis of adult-onset GHD (10–12) exist in the obese and in the elderly. The purpose of this review is to evaluate the usefulness of a variety of clinical and biochemical approaches to the diagnosis of GHD in children and adults. In studies that contain GH measurements in milliunits/liter, the conversion into nanograms/ml has assumed an equivalence of 2 U/mg unless stated otherwise by the authors.

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II. GHD in Children

GH secretion is a continuum (13) between normality and abnormality (Fig. 1); therefore, with rare exception, the diagnosis of GH deficiency must be made on arbitrary grounds. The more severe the GHD, the less arbitrary the diagnosis, whereas the "lesser degrees of GHD" (GH insufficiency) merge into normality. Until 1985, replacement therapy consisted of pituitary-extract GH obtained from human cadavers; the supply was limited and thus the diagnostic strategy was directed initially at detecting severe GHD. Subsequently, recombinant DNA-derived GH became available, the supply of which is unlimited. Unfortunately, compared with other endocrine therapies, GH replacement is expensive. Thus, at the present time in certain countries, children with all forms of GH insufficiency from severe to mild are considered for GH replacement, whereas in other countries, because of economic restraints, only those with severe GHD have a chance of receiving GH replacement. These observations explain some of the variation in diagnostic criteria applied to the diagnosis of GHD in different parts of the world.

A. Clinical aspects

Children with GHD usually present with short stature and a low growth velocity for age and pubertal stage. Alternative causes of poor growth need to be considered and excluded. Age at presentation can vary from the first few months of life to adolescence. The variability and age at presentation are highly influenced by the time of onset and the degree of GHD (14). Thus the impairment in growth velocity is correlated with the severity of GHD. Individuals with complete absence of GH secretion associated with a GH gene deletion present before the age of 3 yr with a height SD score (SDS) below −3 and a growth velocity below the third centile for age. Other children with GH insufficiency present at an older age with less growth retardation and a growth velocity below the 25th

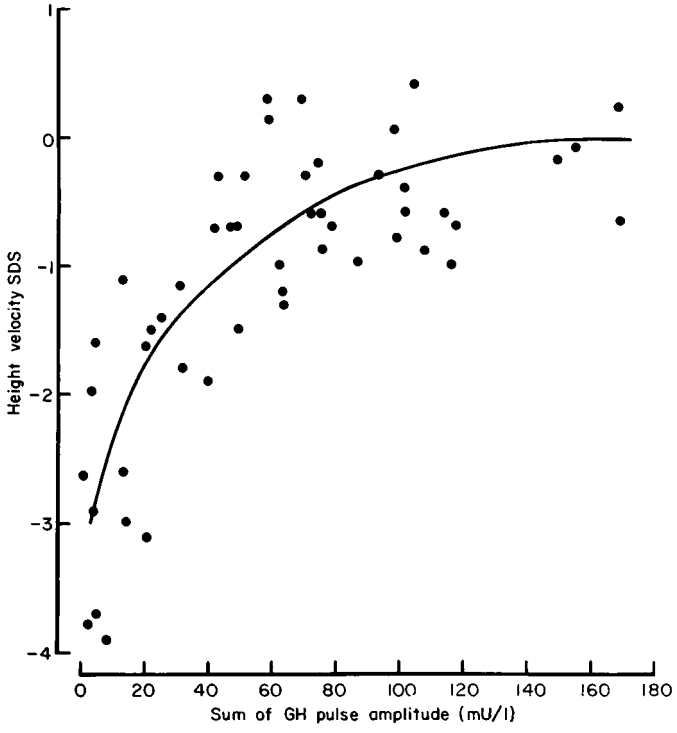

FIG. 1. Relationship between height velocity SDS for chronological age and sum of GH pulse amplitudes. [Reproduced with permission from P. C. Hindmarsh *et al.*: *Clin Endocrinol (Oxf)* 27:581–591, 1987 (13).]

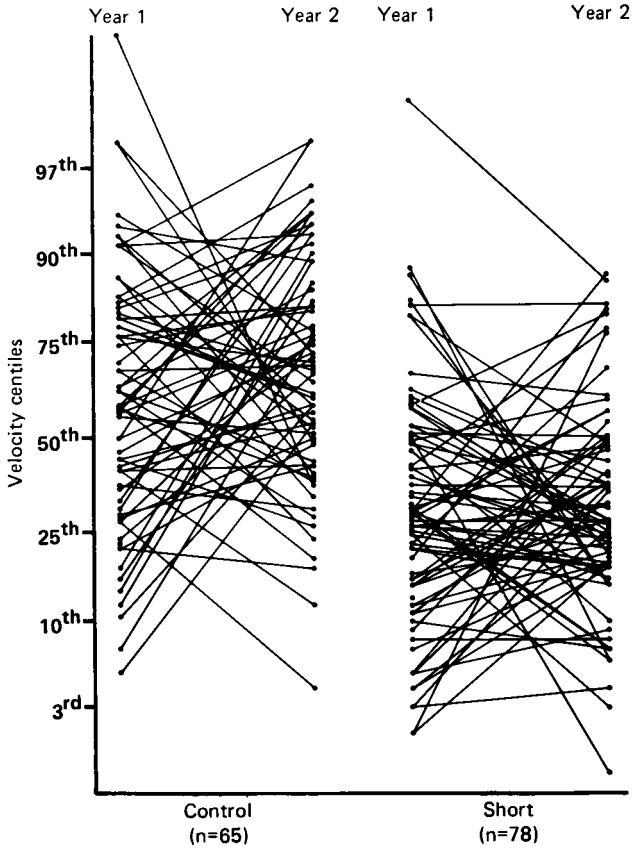

FIG. 2. Correlations between consecutive 12-month height velocity values in the group of short normal children (≤3rd centile for height) and their controls (10th to 90th centile for height). [Reproduced from L. D. Voss *et al.*: *Arch Dis Child* 66:833–837, 1991 (16) with permission from the BMJ Publishing Group.]

centile for their age. The common clinical dilemma is how to distinguish between GH insufficiency and variants of normality whereas the philosophical dilemma is whether or not making the distinction is worthwhile. One variant of normality is idiopathic short stature, a working term to describe a group of short children with no definitely recognizable underlying disease, and another is constitutional delay in growth and puberty. In such patients the prediction of adult height may be inaccurate, vary with the methods used, and lack precision (15). The acquisition of accurate growth data is crucial; however, even growth velocity measurements over a full year cannot always distinguish between the child with GH insufficiency and the short normal child. Voss *et al.* (16) have shown with serial height velocity calculations in a cohort of 78 short normal children that there is no significant correlation in individual velocities from year to year, suggesting that velocity is unable to predict future growth (Fig. 2). Thus, although the proportion of this cohort of short children lying beneath the 25th centile for velocity remained constant from year to year, the identity of the individuals comprising that proportion changed, a phenomenon that could be largely explained by the random error associated with height velocity.

Apart from growth there may be other markers in the history and clinical examination that point to a diagnosis of idiopathic GHD or organic GHD. There is a well recognized association between idiopathic GHD and a history of perinatal trauma such as breech delivery (17). Genetic forms of GHD may mean that the diagnosis is already established in other members of the family. The presence of hypoglycemia in the neonatal period, septo-optic dysplasia (18), and midline facial defects such as cleft palate (19) and solitary central incisor (20) will suggest the possibility of hypopituitarism. A history of previous cranial irradiation (21), histiocytosis X (22, 23), or the presence of symptoms of a mass lesion in the hypothalamic-pituitary region, such as headaches and visual deterioration, will support the probable diagnosis of GHD in a poorly growing child. In children with organic GHD of recent onset, the growth velocity will be subnormal, but insufficient time will have elapsed for short stature to be present. Under these circumstances the growth velocity is more informative than the current height centile position.

Typically the GH-deficient child has increased subcutaneous fat especially around the trunk. The face is immature with a prominent forehead and depressed midfacial development; this is related to the lack of GH effect on endochondral growth at the base of the skull, occiput, and the sphenoid bone. Dentition is delayed. In males the phallus may be small, and the average age of pubertal onset is delayed in both boys and girls.

B. Radiology

Bone age is delayed, and the degree of delay is related to the severity and duration of GHD. Magnetic resonance imaging of the hypothalamic-pituitary region may reveal a

mass lesion such as a craniopharyngioma or thickening of the pituitary stalk due to an infiltrative condition such as histiocytosis X. The anterior pituitary may appear aplastic or hypoplastic, and the posterior pituitary may be ectopically sited. The more severe the radiological abnormality, the more likely that additional pituitary hormone deficiencies, other than GHD, are present (24–26). Septo-optic dysplasia is hallmarked by varying degrees of hypoplasia of the optic nerves, chiasm, and infundibular region of the hypothalamus. The septum pellucidum may be absent.

C. GH provocative tests

GH secretion is pulsatile and serum concentrations are low during many hours of the day. Thus provocative tests of GH release, rather than a single basal GH estimation, were introduced to determine GH status. The commonly used immunoassays for determination of GH levels are the RIAs and immunometric assays. The first GH estimations were by RIAs using polyclonal antibodies. Due to the low specificity of these assays and the several molecular forms of GH, higher GH levels were obtained than with the later immunometric assays employing two highly specific monoclonal antibodies. The variability between assays increases the need for empiric GH cut-off levels, and the definitions of normality and abnormality should be established in each laboratory. In a study in which the GH level was estimated in the same serum samples in four different laboratories utilizing six different immunoassays, there was a high degree of agreement on the distribution of normal and abnormal values despite great variation in the actual cut-off level between laboratories (27). Thus pediatric endocrinologists need to know exactly what is being measured in their own GH assay and to define their own cut-off values. In practice, however, this is not happening: in a recent audit of practice (28), 251 of 413 US pediatric endocrinologists were surveyed about their current GH treatment practice. More than 80% used GH stimulation tests to decide whether to start GH therapy in a child with a growth disorder; however, 37% did not even know which type of GH assay their laboratory used and 82% used GH in short poorly growing children regardless of stimulation test results (28).

The first established pharmacological stimulus introduced for assessment of GH status was insulin hypoglycemia [insulin tolerance test (ITT)]. Advantages of this test include the facts that 1) the ACTH-adrenal axis can be assessed at the same time, 2) it constitutes a powerful stimulus to GH release, meaning that the range between normal and severely GHD is large, and 3) moderate hypoglycemia is sufficient to elicit maximal GH responses (29). The main disadvantages include the lack of normative data in children, a characteristic it shares with many other pharmacological tests, and the unpleasant nature of the test, which in inexperienced hands is frankly dangerous (30). In addition, growing awareness that any normal child might "fail" any single GH provocative test led to the evolving strategy of submitting a child with a growth disorder to two GH provocative tests (31, 32). It should be remembered that the main reason for this strategic development in a child who had "failed" the first GH stimulation test was to exclude normal children ("passed" the second test) from being exposed to a trial of GH therapy rather than adding confirmatory weight to the diagnosis of severe GHD ("failed" the second test).

Thus, a whole series of other pharmacological stimuli were introduced into the GH diagnostic investigational arena including L-dopa (33), arginine (34), glucagon (35), propranolol (33), and clonidine (36). A variety of combinations of these tests has been used (37) and, in some centers, two provocative stimuli are administered sequentially or in combination (33, 38–40). The latter approach may be time-saving and more economical, but there is no evidence to suggest that the results are more meaningful if the tests are performed in combination rather than individually.

Sex steroid priming with estrogen (41) or androgen (42) administered for a few days before carrying out a GH provocative test is an additional maneuver designed to distinguish between genuine GHD and constitutional delay in growth and puberty (CDGP) (43, 44). At puberty in normal children, there is a marked amplitude-modulated increase in GH secretion (45–47) directly due to the marked rise in sex steroid concentrations. Teenage children with CDGP often exhibit very low GH secretion while still prepubertal or in early puberty but show a normal increase in GH secretion with progress through puberty. Their GH responses to unprimed provocative stimuli mirror the physiological changes in spontaneous GH secretion. Thus, in a child of peripubertal age with a growth disorder, CDGP is a real possibility in the differential diagnosis. In such a child, priming with sex steroids before carrying out GH provocative tests will delineate severe GHD (minimal GH response) and a significant proportion of those with CDGP (pronounced GH response) providing sufficient data exist to define the degree of change in GH responsiveness to sex steroid priming (40). Nonetheless the gray zone at the interface between the diagnoses of GH insufficiency and CDGP will not be unraveled.

There is a paucity of normative data about the GH response to the entire range of provocative stimuli (32). Therefore the threshold level used to define a normal GH response was defined arbitrarily. Initially it was set at 5 ng/ml, based on the results of studies such as those performed by Kaplan *et al*. (48, 49) in which 80 of 91 (88%) children who were not GH-deficient showed a peak GH response to an ITT $\geq$ 5 ng/ml whereas all 53 GH-deficient children had a peak GH response below 5 ng/ml to the same stimulus. The GH response set-point was gradually moved to 7 ng/ml (32) as GH testing became more common, and finally reached 10 ng/ml with the increased availability of biosynthetic GH. Not only is normality defined arbitrarily, but the same threshold GH level is used to define normality irrespective of the pharmacological stimulus applied. In fairness, more normative data have become available recently (50–52), but they have not resolved the question of threshold definition (Fig. 3).

D. Physiological assessment of GH secretion

The artificial nature of pharmacological tests and the possibility that they might not always reflect GH secretion under normal physiological circumstances provided the impetus to

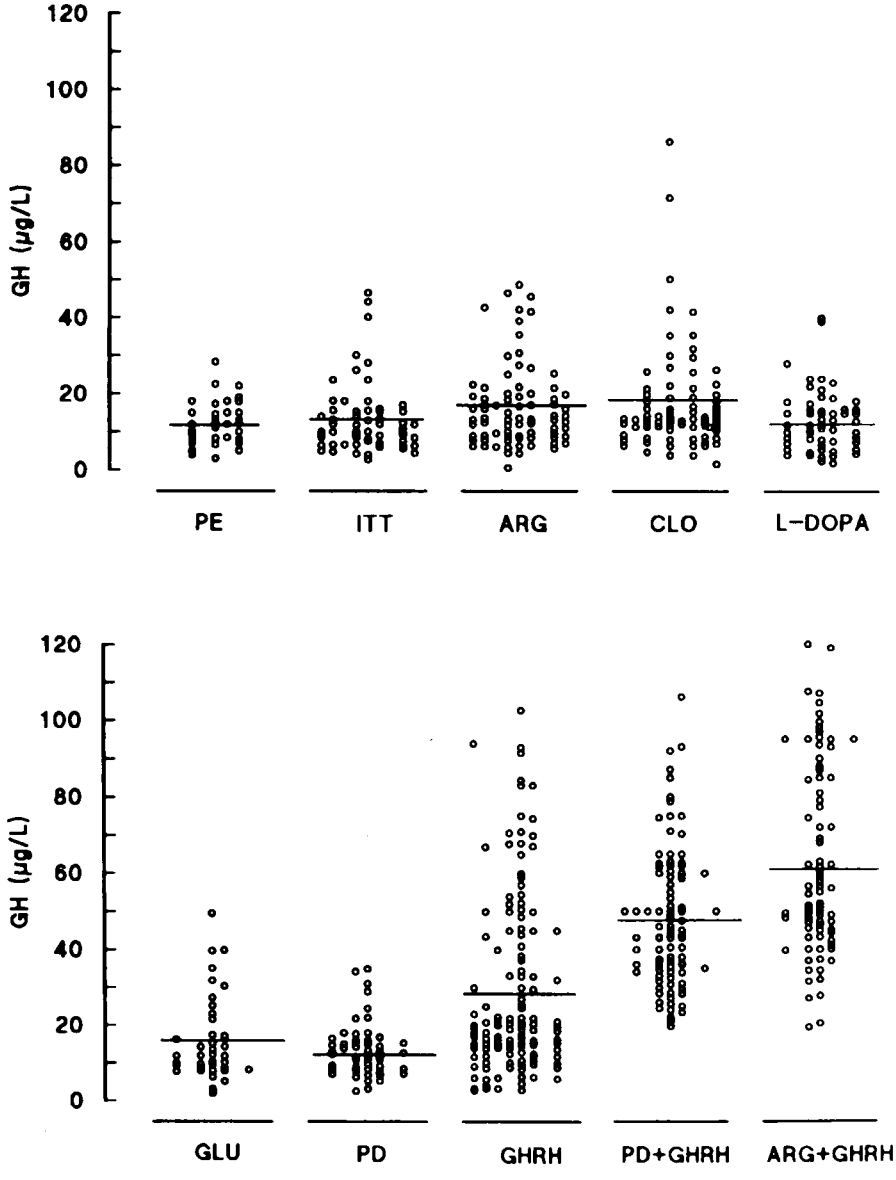

FIG. 3. Individual peak GH responses to physical exercise, ITT, arginine, clonidine, L-dopa, glucagon, pyridostigmine, GHRH, pyridostigmine and GHRH, and arginine and GHRH in normal children of either short or normal height. Serum GH levels were measured in duplicate at each time point by immunoradiometric assay. The sensitivity of the assay was 0.1 μg/liter, whereas the inter- and intraassay coefficients of variation were between 4.9 and 6.5% and 1.5 and 2.9%, respectively. Samples from different individuals for a single provocative stimulus or from the same individual over multiple provocative stimuli were not run in the same GH assay. [Reproduced from E. Ghigo *et al.*: *J Clin Endocrinol Metab* 81: 3323–3327, 1996 (52). © The Endocrine Society.]

examine the potential of the exercise test, 24-h GH profiling, and urinary GH estimation in the diagnosis of GHD.

The exercise test is safe, simple to perform as an outpatient procedure, and inexpensive (53–56). The results are highly influenced by the standardization of the exercise test and the degree of work performed by the subject. Unfortunately, an absent GH response to exercise may occur in up to a third of normal prepubertal children (56). However, in a recent very large survey (52) of the reliability of 10 provocative tests to assess GH secretory status carried out in 472 normally growing children, the mean peak GH response to exercise was not significantly different from that seen in response to an ITT or to arginine, clonidine, L-dopa, and glucagon tests. The exercise test, however, is no longer used very often in clinical practice (37).

Many of the criticisms and limitations of provocative GH testing also characterize measurements of spontaneous GH secretion. Such tests typically require blood sampling every 20 min for a minimum of 12–24 h and will require hospitalization. Assays of serum samples (n = 36–72) for GH concentration are expensive. Although continuous serum sampling through a constant blood withdrawal system can be employed, such testing also requires an indwelling catheter and does not permit analysis of GH pulsatility (57, 58).

There have been several reports in children of a poor correlation between spontaneous GH secretion and GH concentrations after provocative tests (59–61). In some of these studies a group of poorly growing children were described as exhibiting GH neurosecretory dysfunction (GHNSD) (62, 63). These children are characterized by normal GH responses to provocation tests but reduced mean 24-h serum GH concentrations; there may be several aetiologies for the latter combination of GH results including cranial irradiation (64). The exact prevalence of GH neurosecretory dysfunction among children with abnormalities of growth is unknown, and the phenomenon is yet to be described in adult patients.

The pathophysiology remains unknown, and very little of scientific or practical value has been gained from the introduction of this expression (GHNSD) into the endocrine literature.

The controversy over whether or not a 24-h spontaneous GH profile is more useful for assessing GH status than provocative GH tests is unresolved. It is clear, however, that while 24-h GH profile group data are highly reproducible, the intraindividual variation is wide, although no greater than that seen with the GH response to a variety of provocative tests (65, 66). This means that the interpretation of a single GH profile is difficult and, in a child being investigated for growth failure, must be interpreted with caution. Although a combination of nighttime spontaneous GH profiles with other indices of GH status, such as insulin-like growth factor I (IGF-I) measurement, demarcated more effectively between normality and short or slowly growing children compared with the GH profile alone (67). From a pragmatic viewpoint, however, very few pediatric centers in the world have the staff, time, or financial resources to carry out 24-h GH profiles, and even fewer centers have normative data for comparative purposes. Therefore, with rare exception, 24-h GH profiles remain a research investigation.

Before 1970, attempts to quantitate GH output in the urine failed because of insensitive assays. Since 1985 the development of ultrasensitive enzyme-linked immunosorbent assay or immunoradiometric assays have enabled detection of urine GH levels as low as 0.4 to 4 pg/ml. This has led to renewed interest in the use of urinary GH estimation in the diagnosis of GHD (68–73). The test is inexpensive, easy to perform, and noninvasive. Limitations in the interpretation of results relate to large inter- and intraindividual variation (74) and the effect of renal function, which accounts for 52% of the variability in urinary GH measurement. Initial reports were encouraging, but it has since become clear that although urinary GH estimation may match other tests in its ability to confirm severe GHD, it contributes little to the distinction between GH insufficiency and idiopathic short stature (75, 76).

E. IGFs

The IGFs are related GH-dependent peptide factors believed to mediate many of the anabolic and mitogenic actions of GH (77). The serum level of the major GH-dependent peptide IGF-I is stable during the day, due mainly to the complexing of IGF peptides with a family of IGF-binding proteins (IGFBPs) (78). Thus the potential for assessing GH status with a single estimation of the circulating IGF-I level proved attractive and gave rise to the hope that eventually dynamic GH provocation tests may become unnecessary. Initially IGF assay problems were caused by interference from the presence of IGFBPs (78, 79). This problem has been overcome by using a variety of approaches including acid size exclusion chromatography before the IGF assay, blocking IGFBP-binding sites with an excess of the nonmeasured IGF peptide (excess IGF-II for an IGF-I assay) or IGF analogs that do not bind to IGFBPs as radioligands (80). Additional problems remain, however, including lack of specificity. A reduced IGF-I level may occur in a child with malnutrition (81), hypothyroidism (82), hepatic disease, or diabetes mellitus (83), as well as GHD. The IGF-I level is influenced markedly by age and pubertal development (84–86). Particularly low concentrations of IGF-I occur in normal children younger than 5 yr of age. Therefore, the use of IGF-I estimation to distinguish between normal and GH-deficient children is less successful in this age group.

Thus the use of age and puberty-corrected IGF-I values improves the diagnostic use of IGF-I estimation, but overlap with normality remains a problem. Using GH testing as the gold standard to distinguish between 155 children with GHD and 219 with normal short stature, an IGF-I estimation at the fifth percentile level of the normal range provided a sensitivity of 95%, specificity of 60%, and accuracy of 75%, while improved figures of 79%, 95%, and 89%, respectively, were associated with an IGF-I cut-off level of 0.1 centile (87). The IGF-I results discriminated better in those over 8 yr compared with those under 8 yr of age (87). Juul and Skakkebaek (88) also concluded that measurement of IGF-I was useful in the diagnosis of childhood GHD but, unlike the findings reported by Blum (87), more so in younger than older children. They reported an accuracy (predictive value) of 88.8% and 52.3% in children aged less than 10 yr and between 10 and 20 yr, respectively (88). In the latter studies (87, 88) the IGF-I values were corrected for age and sex but not pubertal status.

Despite the promise of these results, multiple studies exist that indicate the serum IGF-I concentration does not correlate perfectly with GH status, as determined by provocative GH testing (76, 89, 90). To improve the diagnostic value of IGF-I measurements, the additional measurement of IGF-II has been recommended (91). Despite the fact that IGF-II is less GH dependent than IGF-I, the normal range for IGF-II levels is relatively high in young children and shows little age-dependence. Thus, although the accuracy of serum IGF-II measurement alone in detecting GHD is moderate, the combined use of IGF-I and IGF-II improves their value as a diagnostic parameter. In a study of 68 GH-deficient children, 197 normal-statured children, and 44 normal short children, 18% of the GH-deficient children had serum IGF-I levels within the normal range, whereas 32% of normal short children exhibited low IGF-I concentrations. Low IGF-II levels were found in 52% of GH-deficient children but also in 35% of normal short children. Utilizing the results from both IGF assays, however, revealed that 4% of GH-deficient children had normal IGF-I and IGF-II levels but only 0.5% of normal children and 11% of normal short children had reduced concentrations of both IGF-I and IGF-II (91). In reality, however, very few pediatric endocrine centers use IGF-II assays in the assessment of children with short stature.

F. IGFBPs

Of the six known IGFBPs, IGFBP-3 is normally the major serum carrier of IGF peptides (92). IGFBP-3 circulates as part of a ternary complex consisting of IGFBP-3, an IGF peptide, and an acid-labile subunit (93, 94). Both acid-labile subunit and IGFBP-3 are GH dependent. Since IGFBP-3 determinations reflect combined IGF-I and IGF-II concentrations, age dependency of IGFBP-3 is less striking than for IGF-I. Sim-

ilarly the influence of nutritional status on IGFBP-3 levels is less than for IGF-I levels. The IGFBP-3 assays do not require separation of the IGFBPs from IGF peptides and are technically easier to perform.

Yet again, the possibility of a single measurement of a GH-dependent peptide with a long half-life as a measure of GH status proved attractive. The close correlation between molar concentrations of IGFBP-3 and the sum of molar concentrations of both IGF-I and IGF-II suggested that IGFBP-3 estimation could reflect GH status more closely than IGF-I estimation alone. Initial (95) and some subsequent (96) reports were highly positive. Blum *et al*. (95) reported 128 of 132 children with GHD had serum IGFBP-3 levels below the fifth percentile. In contrast, 124 of 130 'normal' children had IGFBP-3 concentrations above the fifth percentile. In a more recent evaluation of the usefulness of IGFBP-3 measurement in the diagnosis of GHD, Juul and Skakkebaek (88) reported sensitivity and specificity figures of 60% and 97.9% in children less than 10 yr of age and 56.5% and 78.7%, respectively, in those aged between 10 and 20 yr. These results could not be reproduced in other studies (76, 97–100). Furthermore, the IGFBP-3 estimation discriminated particularly poorly between GHD and normality in pubertal children and those with radiation-induced GHD (76, 101).

It has been proposed that the measurement of the IGFBP-2 concentration adds to the value of IGF-I and IGFBP-3 assays in the diagnosis of GHD (102). IGFBP-2 values are usually elevated in patients with GHD (102, 103). Despite the fact that in most patients there is agreement between the IGFBP-2/IGF-I ratio, IGFBP-3 measurements, and the results of GH testing, the IGFBP-2/IGF-I ratio was discordant from the GH response in 21% of 80 patients (57 GHD and 23 idiopathic short stature), and IGFBP-3 results were discordant in 18% (102).

The proponents of IGF-I and IGFBP-3 estimations as the best choice of initial investigations in a child with possible GHD point out that in individuals with genetic forms of GH insensitivity (GHI), all have a low IGFBP-3 and most have a low IGF-I concentration (104, 105). By definition, these individuals have elevated GH concentrations associated with a mutation or deletion of the gene for the GH receptor, which renders them insensitive to GH action. Furthermore, despite the universally low IGFBP-3 concentrations and the characteristically severe growth failure, serum IGFBP-3 concentrations still correlate significantly with the height SDS (104, 106).

Thus, low IGF-I and IGFBP-3 concentrations are reliable guides to the diagnosis of severe GHD, providing the investigator considers the alternative possibilities of malnutrition, hypothyroidism, liver disease, and GHI. Discrepancies between IGF-I and IGFBP-3 results compared with the GH responses to dynamic tests exist, however, in those children believed to have lesser degrees of GHD.

The inadequacies of dynamic provocative tests of GH release, outlined previously, may explain the discrepancies in the GH status defined by the GH and IGF axis results. Alternatively, it may be that IGF-I and IGFBP-3 estimations are less useful than the peak GH response to provocative testing in children with GH insufficiency.

G. Newer strategies for assessment of GH status

The effects of the commonly used provocative tests of GH release, such as arginine, clonidine, and the ITT, are mediated through hypothalamic mechanisms. The availability of GH releasing hormone (GHRH) provided a means of assessing the secretory capacity of the pituitary somatotrope directly. Although in normal subjects, the GHRH test provokes a greater release of GH than several conventional provocative stimuli, there is great variability in the GH response to GHRH probably related to variation in endogenous somatostatin tone (107, 108).

The use of GHRH in combination with substances that act via inhibition of endogenous somatostatin, such as pyridostigmine (a cholinesterase inhibitor) and arginine, has been explored (109, 110). The suppression of hypothalamic somatostatin release with the latter substances enables GHRH to provoke a much greater GH response, and the GH response to the combined test (GHRH and arginine) shows much reduced inter- and intraindividual variability (111). In a recent comparative study of the reliability of various GH provocative tests, Ghigo *et al*. (52) studied the GH response to exercise, an ITT, arginine, clonidine, L-dopa, glucagon, pyridostigmine, GHRH plus pyridostigmine, and GHRH plus arginine in 472 children with normal stature or normal short stature. In this normal cohort, the combination of GHRH plus either arginine or pyridostigmine not only provoked a significantly greater GH response than the remainder of the GH provocative stimuli but the minimum individual response in 175 tests was 19 ng/ml! Despite these impressive "stimulated" GH levels in normal children, GHRH alone or in combination with pyridostigmine does not meet the essential requirements for distinguishing between children with GHD and normal subjects. As the authors themselves indicate (52), a normal GH response to these tests cannot exclude GHD due to hypothalamic dysfunction. This is a crucial limitation in that in most children with isolated GHD, the site of abnormal pathophysiology is believed to be hypothalamic rather than pituitary. It is not clear whether the same reservation applies to the combined GHRH and arginine test.

In contrast to the combined approach of a bolus of GHRH plus suppression of endogenous somatostatin tone with pyridostigmine or arginine, there is evidence that somatostatin pretreatment enhances the subsequent GH response to an acute bolus of GHRH 5 h later in normal children (112). The exact mechanism is not clear but possibilities include somatostatin blocking the release of stored intracellular GH without preventing accumulation, chronic somatostatin exposure resulting in somatostatin receptor desensitization, or a central action of somatostatin within the hypothalamus. The results of this new diagnostic approach discriminated well between children with GHD and normal short children, but the numbers were small as only 10 children with normal short stature were studied (112). Six of these children had normal GH responses to standard pharmacological tests and four had borderline GH responses (112).

Maghnie *et al*. (113) recommended metabolic rather than neuropharmacological pretreatment to enhance the GH response to provocative stimuli. They showed that the stim-

ulated GH response is strongly calorie dependent and that 3 days of a hypocaloric diet can increase the number and amplitude of spontaneous GH pulses and the total GH responses to arginine and to an ITT (113). The exact implications for a child who shows a prediet poor GH response to pharmacological stimuli and a "normal" GH response after the diet are unclear. Therefore, a 3-day hypocaloric diet before GH testing cannot be recommended in the diagnostic assessment of GH status in a child with a growth disorder.

An alternative therapeutic strategy, independent of the GH status of the child, has been introduced in Australia (114). The latter approach has been advocated since 1988 when the guidelines were revised to allow eligibility for GH therapy on auxological criteria alone. Initial entry criteria were height less than the third centile and growth velocity less than 25th centile for bone age. GH testing was continued, however, in most children even though it was not mandatory for the prescription of GH. More than 3100 children have been treated since 1988 including 35% with GHD (peak GH response <10 ng/ml), 12.5% with Turner syndrome, and 52% with other non-GHD forms of short stature. In 1994 the guidelines were revised to restrict the use of GH therapy to subjects with height less than the first centile, and cessation of GH therapy was brought forward to bone age 13.5 yr for girls and 15 yr for boys. New patient accruals have decreased since 1992 from 100/yr to less than 50/yr. Expenditure for GH between 1990–1991 and 1994–1995 has been halved and is in the midrange internationally (114).

The Australian auxology-based approach together with a comprehensive national database has worked well in a single country with a small number of prescribers and a tight audit system. Nonetheless, the identification of children with GHD remains important not only because of awareness of the potential for associated pathology and other hormone deficiencies but also as a predictor of significantly lower GH dose requirements for maximal growth response in children with severe GHD (peak GH response <5 ng/ml) (114). It should be pointed out, however, that the conditions necessary for the Australian system to work successfully would exclude many countries in the world from adopting this strategic approach.

H. Practical approach to the diagnosis of GHD

The usual circumstance in which GH status needs to be assessed is the child with standing height SDS below -2 and a growth velocity below the 10–25th centile in whom other causes of poor growth have been excluded. The major diagnostic difficulty is to distinguish between idiopathic GHD and idiopathic short stature; the reasons include 1) lack of age- and puberty-related normative data to define the threshold for subnormal IGF-I and IGFBP-3 levels and GH responses to provocative tests, 2) application of a single fixed cut-off level for GHD independent of the GH provocative test in use, and 3) poor reproducibility of GH provocative tests or spontaneous GH profiles. The most important reason, however, is biological in that GHD and normal short stature do not represent cleanly demarcated entities, a view supported by the evidence of overlap of all auxological and biochemical parameters in children with these two diagnostic labels.

GH treatment may improve the short-term growth velocity in any individual short child. Thus, one cannot use the growth response to GH during the first year of GH therapy to confirm the diagnosis of the GH insufficiency. Furthermore, after 3 yr of GH therapy the growth response of children with peak GH responses between 5 and 10 ng/ml is not significantly different from that of children with peak GH responses in excess of 10 ng/ml (115).

The most common approach to the practical assessment of GH status will not utilize 24-h spontaneous GH profiles and will not rely on GHRH tests in combination with arginine or pyridostigmine, in view of the inability to detect GH insufficiency associated with hypothalamic dysfunction reliably with this test.

This leaves IGFs, IGFBPs, dynamic GH provocative tests, and urinary GH estimation. It is the degree of GHD that dictates the extent of the diagnostic confusion; the child with severe GHD is least likely to be confused with the normal short child. Thus, measurement of IGF-I and IGFBP-3 and dynamic tests of GH release all perform best in those children with severe GHD. Urinary GH estimation appears to be a useful diagnostic discriminator only in those children with severe GHD and is singularly unhelpful in those with a lesser degree of GHD.

In practice, most information is gained by the performance of a single IGF-I and IGFBP-3 estimation in combination with a single dynamic GH provocative test. The performance of a second GH provocative test will add little to the diagnosis of severe GHD. In a poorly growing child with normal IGF-I and IGFBP-3 levels but a subnormal peak GH response to a provocative test, a second GH provocative test will help to distinguish between GH insufficiency and normal short stature. If the result of the second GH provocative test is normal, it suggests that the diagnosis of GH insufficiency has been refuted. If both provocative tests produce subnormal GH responses, the diagnosis of GH insufficiency will have been supported. It might be argued that this approach is wasteful because there may be very little difference in the growth response to GH therapy between children with these two sets of biochemical parameters. In the latter child defined as being GH insufficient, however, further biochemical assessment of pituitary function and neuroradiology are required. Furthermore, in this situation we may need to consider biological endpoints other than growth. Compared with the child who shows a normal GH response to at least one provocative test, it is more likely, but unproven, that a child with moderately reduced GH responses to two provocative tests is more prone to failure of acquisition of peak bone mass if left untreated.

A combination of endocrine results, less commonly seen and not yet considered, is that of a low IGF-I level in the presence of normal or exaggerated GH responses to provocative stimuli. The latter biochemistry may be associated with a number of clinical states associated with acquired GH insensitivity such as a chronic systemic disease, *i.e.*, chronic liver disease (116), severe chronic infection (116), and drug therapy, *i.e.*, chemotherapy for a brain tumor (117), but under these circumstances the clinical picture is dominated by the primary disorder and differential diagnosis is not an issue. There are, however, a variety of genetic defects in the GH-IGF-I axis that may result in a short poorly growing child

with a low IGF-I level and normal or exaggerated GH responses to provocative stimuli.

The reported genetic defects include a mutation in the GH gene resulting in biological inactivation of GH leaving its immunological properties intact (118), mutations in the GH receptor gene (119, 120), and intrauterine growth retardation and postnatal growth failure associated with deletion of the IGF-I gene (121).

In its classic form, Laron's syndrome (GH receptor gene mutation) is inherited as an autosomal recessive trait and is characterized by severe postnatal growth failure and low IGF-I and IGFBP-3 levels despite normal or elevated GH secretion (119, 120). The initial cases were all associated with low serum concentrations of GH-binding protein (GHBP), indicating genetic abnormalities in the extracellular domain of the GH receptor. Subsequently, several hundred cases have been identified, some of whom have had normal serum concentrations of GHBP, indicating either defects in the portion of the extracellular domain of the GH receptor molecule that is needed for dimerization or intracellular defects that may affect the signal transduction pathway (122).

Further biochemical investigations to distinguish other genetic defects in the GH-IGF-I axis from that of GH receptor gene mutations are not always helpful in that the GH gene mutation associated with a biologically inactive GH molecule may, like the GH receptor gene mutation, be associated with a low GHBP level and a failure to show an IGF-I response to short-term administration of exogenous GH (118).

The one child thus far described with deletion of the IGF-I gene had normal GHBP and IGFBP-3 levels associated with severe intrauterine growth retardation, sensorineural deafness, and mental retardation (121).

Further investigation of the short slowly growing child with a definitely low IGF-I level and normal or exaggerated GH secretion is likely to consist of screening for either a molecular defect of the GH receptor gene or IGF-I gene, or isoelectric focusing of serum GH.

Increasingly, less classic molecular defects of the GH receptor (123) are being reported, leading to extreme difficulty in determining the contribution of such defects to the growth problem of the child under investigation.

Many areas of the growth/GH/IGF-I field require further research. If one adopts an arbitrary cut-off for the biochemical definition of GH insufficiency, what is the long-term growth response and final height of children below the cut-off, who receive a conventional dose of GH replacement, compared with those children just above the cut-off, who remain untreated? Are there differences in the social or educational performances of these children? Is one group "happier" than the other in the short term, long term, or very long term?

What physiological explanation lies behind the short-term and long-term variation in growth in normal children? Why do children with radiation-induced GHD rarely exhibit a low IGFBP-3 level (76, 101) and more frequently exhibit an impaired GH response to an ITT than to other GH provocative tests (124, 125)? How do children previously treated for a craniopharyngioma grow in the presence of low GH and IGF-I levels (126)? What is the mechanism for suppression of GH secretion in children with obesity (47, 127)? These and many other research questions remain unanswered and will require a combination of the biochemical investigations discussed in this review and new ideas.

In practical terms, in the meantime, we should accept that 100% discrimination between GHD and normality does not exist. The overlap between normality and abnormality is more likely to be reduced, however, if investigators acquire their own age- and puberty-defined normal data for their IGF-I and IGFBP-3 assays and their chosen dynamic GH tests rather than seeking more complicated variations on the theme of conventional testing or new multiple combinations of estimates of GH status.

III. GHD in Adults

Until the last 10 yr, the main reason for performing an ITT in adults with disease of the hypothalamic-pituitary axis was to assess ACTH secretion to determine the need for glucocorticoid replacement. At a time when no function was ascribed to GH in adult life, there was little interest in the GH data provided by the ITT, with the exception that an impaired GH response to an ITT did constitute a marker of hypothalamic-pituitary disease in the era before the development of sophisticated neuroradiology. More recently, with the demonstration that GH replacement therapy benefits adults with GHD and the availability of unlimited supplies of synthetically derived GH, endocrinologists have become interested in the diagnosis of GHD in adults. Increasingly, therefore, the diagnostic strategies applied to the poorly growing child have been explored in adults with known or suspected hypothalamic-pituitary disease.

A. Clinical aspects

GHD in adult life is associated with increased fat mass, particularly distributed in the truncal region (128, 129), reduced lean mass (128, 129), osteopenia (130–134), an adverse lipid profile (135–137), glucose intolerance (138), insulin resistance (139), impaired fibrinolysis (140), altered cardiac structure and function (141–144), reduced exercise capacity (145, 146), and reduced quality of life (147–149). Despite the impressive list of clinical features, there is no single symptom or sign that is pathognomonic of GHD in adult life; this is particularly true of patients with multiple pituitary hormone deficits in whom T_4, glucocorticoids, and sex steroids may be under- or overreplaced. There is increasing evidence, however, that the profile of adverse sequelae associated with GHD in adult life differs in adults with childhood-onset compared with adult-onset disease (150).

There is no single biological endpoint in an adult suspected of being GH-deficient that offers the same diagnostic usefulness as the growth rate of a child. There is, however, the natural history of evolution of hypopituitarism in patients with a mass lesion of the hypothalamic-pituitary region or in those who have undergone surgery and/or radiotherapy to this region. GH is usually the first of the anterior pituitary hormones to be affected by these various pathological insults, which means that in a patient with multiple pituitary hormone deficits the probability of GHD being present is extremely high (151, 152). Unlike the situation in

the pediatric age group in whom pituitary adenomas are rare, this observation is of enormous importance in adults in whom nonfunctioning pituitary adenomas and/or their treatment are the most common causes of GHD (4).

B. GH provocative tests

Over a number of years the ITT has been the gold standard. For the reasons mentioned earlier, this is partly because the ITT is the only GH provocative test in which vast experience has been gained by different investigators. With the synacthen test gaining gradual acceptance as a reliable substitute for the ITT in the assessment of the pituitary-adrenal axis in significant numbers of patients with pituitary disease, an alternative test of GH status will be required in such patients (153–155).

There have been few studies of the GH response to different pharmacological stimuli in normal adults (156–163). Cain *et al*. (156) assessed the GH status of 20 normal young subjects (14 males) aged 21 to 34 yr by challenging with glucagon, arginine, ITT, and tolbutamide-induced hypoglycemia. They observed a significant GH increment from baseline to peak after each agent and concluded that glucagon was at least as good, if not better than the ITT, at stimulating GH release. Their study, however, reported the mean change in serum GH, from baseline to peak, and mean peak GH response to each agent but did not compare one agent with another. Only 10 subjects had an ITT and although the mean serum GH peak was not significantly different from the response to glucagon, none of the subjects had what Cain *et al*. (156) considered to be a subnormal GH response to the ITT whereas the "failure rates" (peak GH response or rise in serum GH from baseline to peak of less than 5 ng/ml) after arginine and glucagon were three subjects and one subject, respectively. No information was provided about the sex of the 10 subjects who underwent the ITT; this may be relevant as Hoeck *et al*. (164) have recently demonstrated that the peak GH response to an ITT is lower in female subjects than in males. This is in contrast to the greater GH response to arginine that is seen in women compared with men (160, 165, 166); thus, there is a gender-related effect on the GH response to certain provocative stimuli that varies in direction depending on the stimulus.

Certain conclusions can be drawn from the few studies that have been carried out. The ITT does appear to be a more effective stimulus for GH release compared with glucagon, arginine, L-dopa, and clonidine. In response to an ITT, all 19 healthy individuals in the Lin and Tucci study (157) achieved a peak GH response of greater than 5 ng/ml and, in the 18 healthy young male students studied by Rahim *et al*. (161), all but one (10.7 ng/ml) responded with a peak GH concentration in excess of 15 ng/ml. Comparison of the numerical results of these studies, however, is hindered by the fact that the early studies used GH assays that are now obsolete.

Oral clonidine in doses of up to 150 $\mu g/m^2$ proved singularly unimpressive in stimulating GH release (160, 161). Rahim *et al*. (161) showed that clonidine (100–200 μg) elicited a GH response no greater than placebo. Intramuscular glucagon (1 mg) stimulated a greater GH response than intravenous arginine (20 g/m^2 over 30 min) with 16 of the 18 students achieving a peak GH response in excess of 7.7 ng/ml and the remaining two between 3.8 and 7.7 ng/ml to glucagon. Two of the 18 subjects failed to achieve a peak GH response in excess of 1.9 ng/ml in response to arginine (161).

All the limitations of the GH provocative tests discussed in the pediatric section of this review apply to the diagnosis of GHD in adults, but one major difference is in the degree of GHD required to be present before GH replacement is initiated. In many countries, children with all grades of GHD ranging from complete absence of GH secretion to GH insufficiency are offered a trial of GH replacement. Thus far, GH replacement therapy has only been recommended for adults with severe GHD. This reduces the scale of the diagnostic problem in defining GHD in the adult with pituitary disease. Nonetheless, controversy over the definition remains an issue. In six recent clinical trials of GH replacement, the diagnostic criteria for GHD varied between a peak GH response to a provocative test (usually ITT) of less than 0.5 and 5 ng/ml (128, 167–171). To bring some consistency to the field, a recent consensus statement recommended that severe GHD be defined as a peak GH response of less than 3 ng/ml during an ITT (172). The findings of Hoffman *et al*. (173) support the choice of this diagnostic definition; they studied a variety of methods of diagnosing GHD in 23 hypopituitary patients and 35 normal subjects carefully matched for age, sex, and body mass index (BMI). Compared with their normal counter parts, the patients with organic hypopituitarism had significantly lower peak GH responses to an ITT, lower integrated GH concentration (IGHC) from 20-min sampling over 24 h, and lower IGF-I and IGFBP-3 levels (Fig. 4). There was a complete segregation of the peak GH response to an ITT between the normal and GH-deficient subjects irrespective of age or adiposity. No normal subject had a GH response below 5 ng/ml, and no subject with organic hypopituitarism had a response greater than 3 ng/ml. In contrast to the ITT, there was a significant overlap of IGHC, IGF-I, and IGFBP-3 values between groups (173).

There are, however, problems with the recommendation of the consensus meeting. Even though an ITT carried out in an experienced endocrine unit is associated with a low risk of complications (174, 175), there are certain categories of patients in whom this test should not be performed. Patients with documented ischemic heart disease or epilepsy should not undergo an ITT and because of the potential risk of occult ischemic heart disease with increasing age, we do not perform an ITT in patients over the age of 65 yr. Furthermore, the lack of standardization of GH assays means that each endocrine unit must establish their diagnostic threshold equivalent of 3 ng/ml. This brings the argument back to the need in adults with pituitary disease to establish an equivalent clinical endpoint that might match the use of the growth rate in pediatrics. We believe that the natural history of the evolution of adult hypopituitarism meets this need, the key feature being that in all the common forms of organic hypothalamic-pituitary disease in adult life, GHD is the earliest feature of anterior pituitary hypofunction and in individuals with multiple pituitary hormone deficits, GHD is almost inevitable.

In adult patients with known pituitary disease, not only the presence but also the severity of GHD is related to the

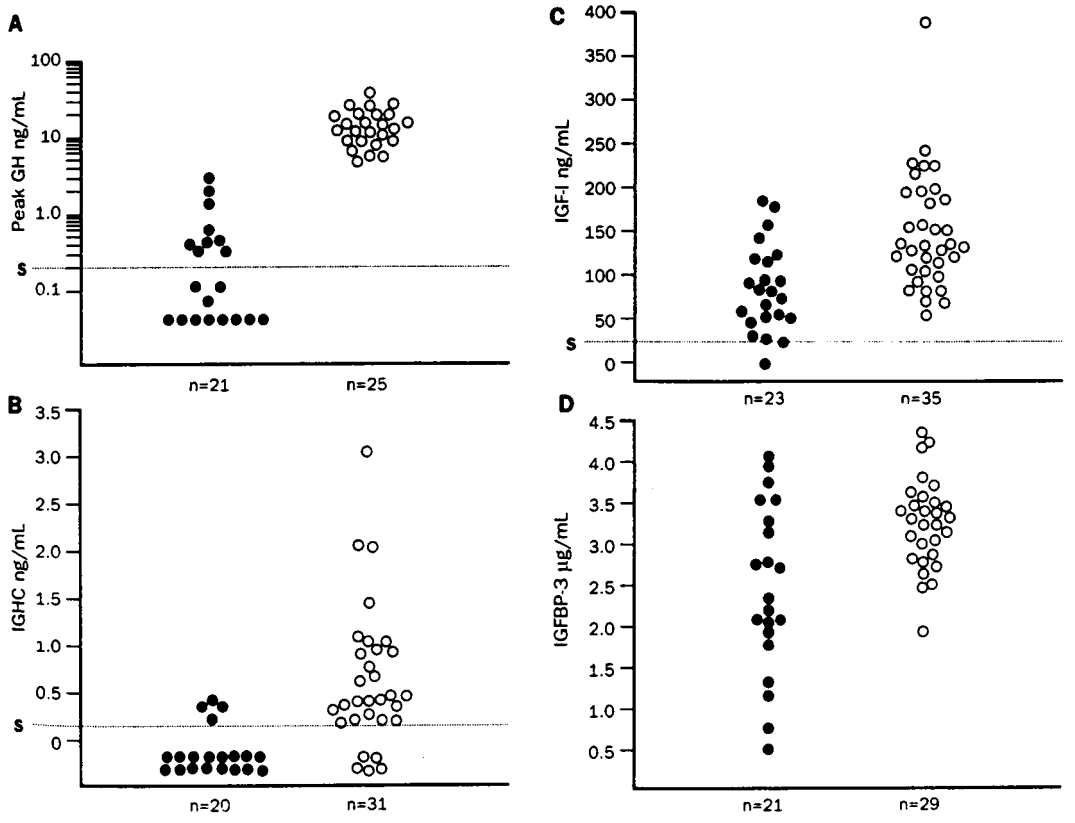

FIG. 4. Results of tests of GH deficiency in normal (○) and hypopituitary (●) subjects. A, Peak GH response to ITT; B, mean 24-h GH (IGHC); C, IGF-I concentration; D, IGFBP-3 concentration. S, Assay sensitivity for GH (0.2 ng/ml) and IGF-I (25 ng/ml). [Reproduced with permission from D. M. Hoffman *et al.*: *Lancet* 343:1064–1068, 1994 (173).]

number of additional pituitary hormone deficits (4, 176). Thus, Toogood *et al.* (4) showed that the median peak GH response to an ITT in four age-matched groups of adult patients with isolated GHD (GHDO) and GHD plus one (GHD1), two (GHD2), and three (GHD3) additional pituitary hormone deficits was 3.8, 1.5, 0.8, and 0.7 ng/ml, respectively (Fig. 5). There was a significant downward trend in the median peak GH responses of the four groups with the differences between GHDO and GHD1, and GHD1 and GHD2, being highly significant; however, there was no difference between the GHD2 and GHD3 groups. Ninety one percent of patients in combined groups GHD2 and GHD3, 55% of GHD1, and 24% in GHDO had a peak GH response less than 1.9 ng/ml. Similar conclusions regarding the relationships between GHD and additional pituitary hormone deficits have been drawn irrespective of whether GH status has been assessed by ITT (176), urinary GH measurement (177), or 24-h spontaneous GH profile (178).

C. Physiological assessment of GH secretion

Measurements of 24-h spontaneous GH profiles by Hoffman *et al.* (173) demonstrated a significant overlap of IGHC values between normal and GH-deficient adults. One third of the matched young normal subjects aged less than 50 yr had IGHC values within the range of the GH-deficient patients. Thus, estimation of IGHC failed to discriminate satisfactorily between GHD and normality, and at least part of the failure was due to the number of normal and hypopituitary subjects with values that were below the limit of detection of the GH RIA (173).

Using a newer generation enzyme-linked immunoabsorbent assay to enhance sensitivity (1 ng/liter), the same group reassessed the discriminatory capacity of IGHC (179). Twenty six percent of hypopituitary subjects had IGHC values within the normal range (179). Age stratification improved the separation, but an overlap between normal and GHD remained in those adults aged less than 50 *vs.* those over 50 yr of age. All samples, including nadirs, from GH-deficient subjects were well within the GH assay detection limit (1 ng/liter) (179). Peak 24-h GH levels in GH-deficient subjects were lower and did not overlap those in the normal subjects (180). Nadir GH concentrations were significantly lower in GH-deficient subjects but the range overlapped that of normal subjects (180). Total daily GH production in GHD was approximately 5% of the production in matched normal subjects (180). This difference resulted from a greater reduction in the pulsatile (by 96%) than in the tonic (by 47%) component, so that the fractional daily contribution by tonic GH release in GH-deficient subjects was markedly greater (180).

Thus, the peak 24-h GH level but not the IGHC or the nadir GH level discriminated satisfactorily between GH-deficient subjects and controls; however, the series contained only 10 GH-deficient patients and 10 controls (180). Furthermore, one could only determine the peak spontaneous GH level by

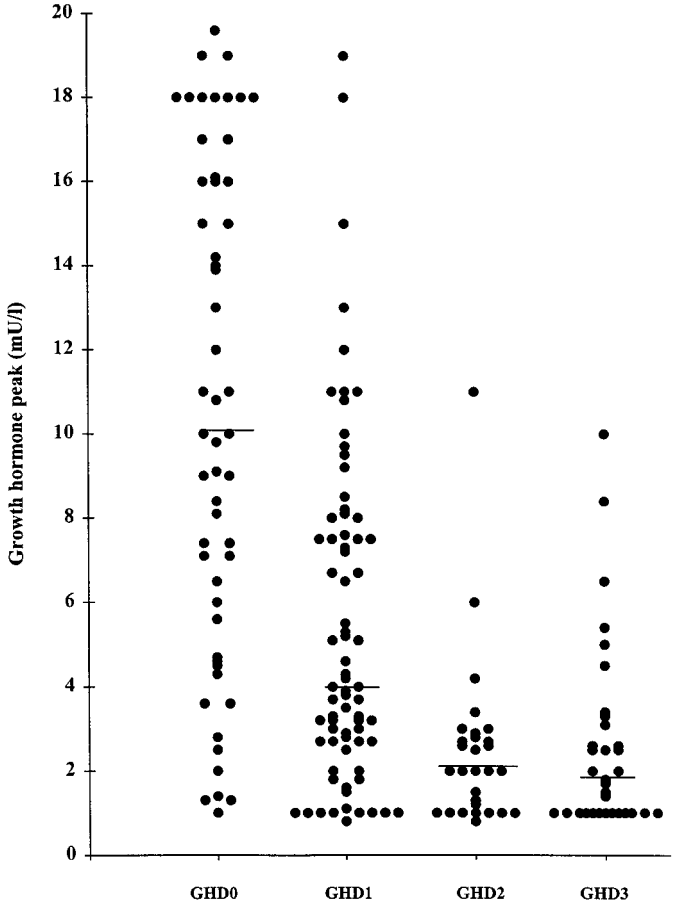

FIG. 5. The distribution of the peak serum GH levels in response to an ITT in 190 patients divided into groups according to the degree of hypopituitarism present, *i.e.*, number of anterior pituitary hormone deficiencies, in each patient. *Horizontal bars* represent medians. a, GHDO; b, GHD1; c, GHD2; d, GHD3. 1 ng/ml = 2.6 mU/liter. [Reproduced from A. A. Toogood *et al.*: *Clin Endocrinol (Oxf)* 41: 511–516, 1994 (4).]

performing a full 24-h GH profile! These physiological studies utilizing highly sensitive GH assays have the potential to advance our understanding of the factors that control tonic and pulsatile GH secretion. Nonetheless, they are unlikely to contribute significantly to the pragmatic need to define GHD biochemically in an individual patient.

There is less information from alternative methods of assessment of physiological GH secretion. Urinary GH excretion showed reasonable separation from normal controls in GH-deficient patients defined by the presence of known pituitary disease and a peak GH response of less than 5 ng/ml to an ITT (177). At a sensitivity of above 90%, the specificity for diagnosing GHD was 79% in adult patients below 40 yr, 67% between 40 and 60 yr, and an unimpressive 36% above the age of 60 yr (177).

D. IGF-I and IGFBP-3

The changes in IGF-I levels throughout life are similar to those of GH. With the onset of puberty there is a 2 to 3-fold rise in serum IGF-I concentrations followed by a decline such that average adult levels are reached by the early twenties (86). There follows a gradual decline with advancing age (86). Similar to IGF-I but less age-dependent, serum IGFBP-3 levels rise to a peak during the pubertal years and then slowly decline in adulthood (95). Thus, in the adult patient with potential GHD, serum IGF-I and IGFBP-3 measurements can only be interpreted if decade-based normative data are available.

The usefulness of an IGF-I estimation in the diagnosis of adult GHD is a matter of contention although it has become clear that at least some of the disparity between studies is explained by the timing of the onset of GHD. Hoffman *et al*. (173) found that 70% of IGF-I and 72% of IGFBP-3 values in adult-onset GHD patients, mean age 45 yr, were within the range of normal subjects even allowing for the effects of age. Holmes (181), in a subsequent study of 65 adults, mean age 35 yr, with GHD defined by a peak GH response of less than 3.8 ng/ml to a provocative test, and associated with other evidence of pituitary disease, found that 70% had an IGF-I SDS below −2. In the latter study, however, the 65 adults consisted of a mixture of childhood-onset and adult-onset GHD (181).

DeBoer *et al*. (11) focused on childhood-onset GHD and reevaluated GH status in 89 young adult males who had previously received GH replacement in childhood. Approximately 93% of the patients had an IGF-I level below the normal range with a similar number of subnormal IGFBP-3 levels among the patients (Fig. 6). Attanasio *et al*. (150) pursued the same theme in a large study of 74 childhood-onset and 99 adult-onset GHD patients. They concluded that there are profound differences between these two forms of GHD (150). With group data they observed that the serum IGF-I levels were below normal in both groups of GH-deficient patients but were significantly lower in childhood-onset than adult-onset GHD patients (150). Other authors have made similar observations (182, 183). In these studies, however, it is not clear whether sufficient decade-based normative data were available or whether the severity of GHD was equal in the childhood-onset and adult-onset groups. Thus, apart from the potential impact of the timing of onset of GHD, it is possible that the severity of GHD influences the interpretation of IGF-I results.

To pursue this question further, we have analyzed the IGF-I SDS results of the mixed population of 65 adults with childhood- and adult-onset GHD studied by Holmes (181) in the context of the severity of GHD. A direct comparison of the IGF-I SDS with peak GH response is impossible due to the multiplicity of GH provocative tests used in that study (181). Therefore, the IGF-I SDS data have been stratified, in the context of additional pituitary hormone deficits, into those with GHDO, GHD1, GHD2, and GHD3 (Fig. 7). In each of the latter categories, there is a mix of adult-onset and childhood-onset GHD patients, thereby allowing a comparison of IGF-I status among groups with tightly defined degrees of GHD. The numbers are sufficient for statistical comparison in the GHDO, GHD1, and GHD3 groups and, in each category, the median childhood-onset IGF-I SDS is lower than the median adult-onset IGF-I SDS, reaching significance in two of the three groups (GHDO, −3.83 *vs.* −2.69, $P = 0.239$; GHDI, −5.75 *vs.* −2.03, $P = 0.022$; GHD3, −6.28 *vs.* −2.09, $P = 0.0003$) (181). These observations suggest that for a given

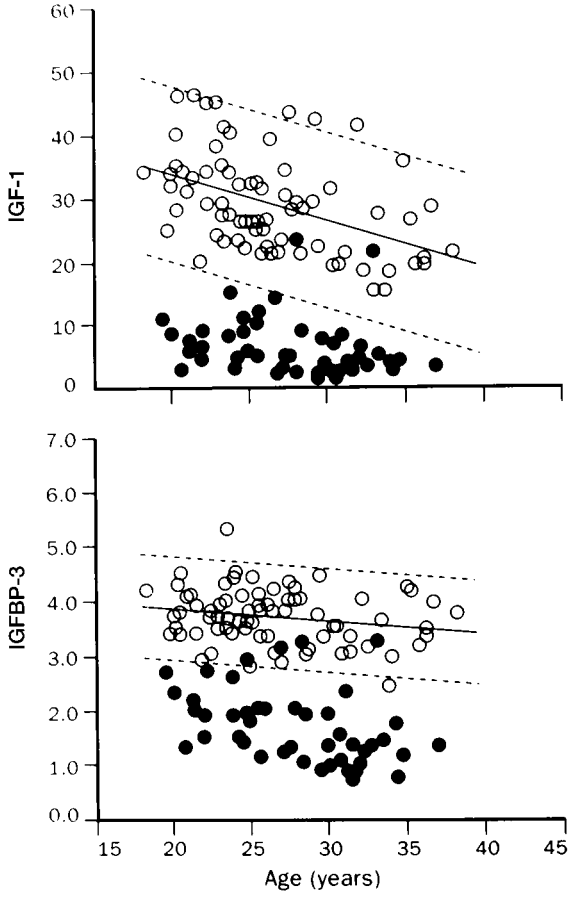

FIG. 6. Serum IGF-I and IGFBP-3 in patients with established GH deficiency (●) and healthy controls (○). Lines represent mean (± 2SD) of values in controls. [Reproduced with permission from H. De Boer, G. J. Blok, C. Popp-Snijders, E. A. van der Veen, Diagnosis of growth hormone deficiency in adults (letter). *Lancet* 343:1645–1646, 1994.]

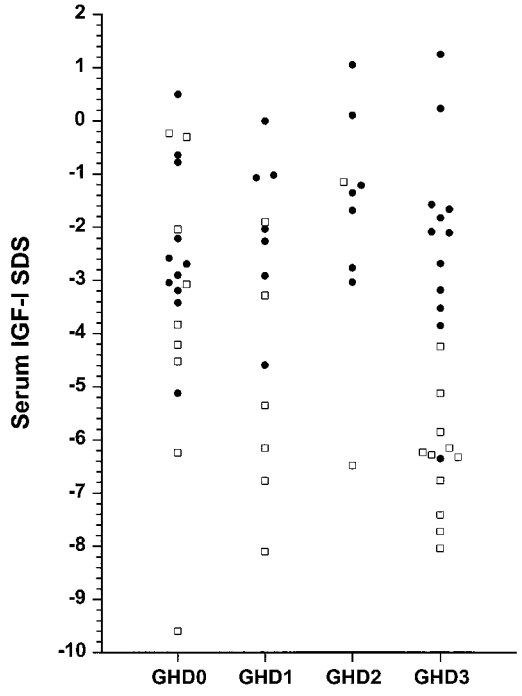

FIG. 7. IGF-I SDS in 65 adults with either childhood-onset (□) GHD or adult-onset (●) GHD stratified into four groups: GHDO, GHD1, GHD2, and GHD3.

degree of GHD, the serum IGF-I concentration is lower in adults with childhood-onset compared with adult-onset disease. The explanation for this latter observation is unclear; however, it supports the notion that an IGF-I estimation is more likely to be of diagnostic use in childhood-onset than in adult-onset GHD.

An additional observation needs to be made in the patients with adult-onset isolated GHD. Toogood *et al*. (4) had shown previously that 24% of GHDO patients had a peak GH response of less than 1.9 ng/ml to an ITT. In the data of Holmes (181), there are 11 patients in the GHDO group with adult-onset disease, eight of whom have an IGF-I SDS below −2. Therefore, any therapeutic strategy to decide which GH-deficient patients warrant a trial of GH therapy must allow for patients with isolated GHD, who on an individual basis may show as severe a degree of GHD as a patient with panhypopituitarism.

E. Obesity

Obesity is one pathophysiological state that may be difficult to distinguish from organic GHD in the adult. There is substantial evidence that morbid obesity is accompanied by suppression of GH release and that substantial weight loss may restore spontaneous (184, 185) and stimulated GH secretion (184). Veldhuis *et al*. (186) observed mean 24-h GH levels in obese men (BMI 41–58 kg/m^2) to be 25% that of an age-matched control group (BMI 23–33 kg/m^2). Even in subjects of near normal weight, relative obesity as determined by BMI is negatively correlated with GH secretion (160, 187, 188) due both to reduced GH production and increased clearance (186). Each unit increase in BMI, at a given age, reduced daily GH secretion by 6% (187). Furthermore, in clinically nonobese healthy adults, relative adiposity in the abdominal region in particular proved to be a major negative determinant of stimulated GH secretion (160).

Changes in IGF-I levels in obesity are disputed. There are data showing decreased (187, 189–191) or normal (186, 192) IGF-I levels in adults and even elevated (193) IGF-I levels in obese children. In the largest population-based study of IGF-I levels in adults, an apparent decline in IGF-I levels attributable to adiposity disappeared when age was accounted for in a multivariate analysis (194).

Exactly how obesity reduces GH secretion has not been clarified; however, there is increasing evidence that FFA play a significant role (195, 196). Recent studies with acipimox, a nicotinic acid analog that blocks lipolysis and is devoid of side effects, indicate that FFA reduction enhanced the GH response to a variety of GH secretagogues, but it did not increase spontaneous GH secretion in obese individuals (197–199). The possible site of action of FFA is at the pituitary somatotrope.

A recent study in age and sex-matched subjects suggests that there is a much more profound reduction in total GH secretion in a group of individuals with organic GHD compared with a group of obese subjects (200). In the obese

individual with pituitary disease, however, and no other pituitary hormone deficit, a reduced GH response to any of the standard provocative tests may reflect organic GHD or obesity itself. In this clinical situation, distinction between the two possibilities is not possible with any degree of certainty at the present time.

F. Elderly

GH secretion in healthy elderly adults is reduced compared with that in young adults (186, 187, 189, 201–204). GH secretion declines by approximately 14% per decade from young adult life (187), and an early study even suggested that GH secretion may eventually cease in certain elderly subjects (201). Normal aging is associated with changes in body composition similar to those seen in patients with GH deficiency, *i.e.*, a reduction in bone mass, osteoporosis in severe cases, and a reduction in renal and cardiac function. The similarity of the changes in adults with GHD and those that are part of the normal aging process has led some researchers to suggest that aging may be due to GHD (205). The decline in GH secretion and the physiological changes seen with aging have been grouped together and termed the somatopause.

In the clinical setting this raised a number of questions. Can the GH status of elderly patients with organic pituitary disease be distinguished from that of the normal elderly? Do middle-aged adults with GHD need to continue with GH replacement beyond the age of 60 yr?

The prevalence of nonfunctioning pituitary adenomas increases with age (206) and thus pituitary disease is common in the elderly. Toogood *et al*. (207) have now established that GH secretion is significantly reduced in the elderly with pituitary disease compared with normal controls of similar age. They studied GH secretion in 24 patients (age 61–85 yr) with hypothalamic-pituitary disease and in 24 controls (age 60–87 yr) matched for BMI. The median (range) area under the curve of the 24-h GH profile [<9.6 (<9.6–20) *vs.* 18.5 (10.7–74.4) ng/ml], the median stimulated peak GH response to arginine [<0.4 (<0.4–7.7) *vs.* 8.0 (1.6–37.0) ng/ml], and the median serum IGF-I concentration [102 (<14–162) *vs.* 147 (65–255)ng/ml] were significantly lower in the patients than in the controls (207). For each of the three latter indices of GH status, there was overlap between the two groups. Fifteen of the 24 patients showed no evidence of spontaneous or stimulated GH secretion (using a standard immunoradiometric assay with a sensitivity of 0.4 ng/ml) whereas all controls showed evidence of both.

Under circumstances in which GH secretion is known to be reduced, however, most values during a 24-h GH profile cannot be estimated with conventional RIAs. Thus, a limitation of the original study by Toogood *et al*. (207) was that 93% and 61% of the GH estimations in the 24-h profiles of patients and controls (72 samples), respectively, fell below the sensitivity of the conventional assay. Subsequently, the latter GH profiles were reexamined using an ultrasensitive chemiluminescent assay (sensitivity 2 ng/liter). GH secretion was detectable throughout the 24-h profile in all patients and controls (178). Furthermore, the pattern of GH secretion remained pulsatile in all but one patient, despite previous pituitary pathology, surgery, and irradiation. GH pulse frequency was unaffected in the patients studied by Toogood *et al*. (178) and, similar to the findings of Reutens *et al*. (180) in middle-aged adults with organic GHD, absolute peak GH levels were reduced to a greater extent than absolute nadir GH levels. Nonetheless, overlap between patients' and controls' values remained unless the analysis was restricted to those patients with the most severe degree of GHD, *i.e.*, those with two or more additional pituitary hormone deficits in whom the absolute peak GH concentration was distinctly separate from control values (178).

In addition, utilizing the ultrasensitive GH assay on the samples obtained during the provocative GH test revealed a highly significant correlation (Fig. 8) between the peak GH response to arginine and the area under the 24-h GH profile, which was more pronounced in the patients (r = 0.9) than in the controls (r = 0.5) (178).

IGF-I estimation appears to offer relatively little to the diagnosis of GHD in the elderly. Despite the group differences, overlap between patients with organic GHD and controls is considerable, with only 21% of elderly GH-deficient patients having a serum IGF-I level below the range found in the elderly controls (207). Serum IGFBP-3 measurement proved even less rewarding, and there is absolutely no difference between the values of the GH-deficient patients and controls (208).

Thus, despite the fact that GH secretion in the elderly with pituitary disease may be reduced to about 13% of age-matched controls (178), confirming GH deficiency in an individual patient remains a problem. Twenty four-hour GH profiles and IGF-I and IGFBP-3 measurements are unsatisfactory either because of impracticality or lack of discriminatory power. The excellent correlation seen between the peak GH response to arginine and spontaneous GH secretion supports the view that the arginine stimulation test is a reasonable choice to assess GH status in patients with GHD2 or GHD3 disease, particularly in an age group in whom an ITT may carry an increased risk of morbidity or mortality. In an elderly patient with pituitary disease and either one or no

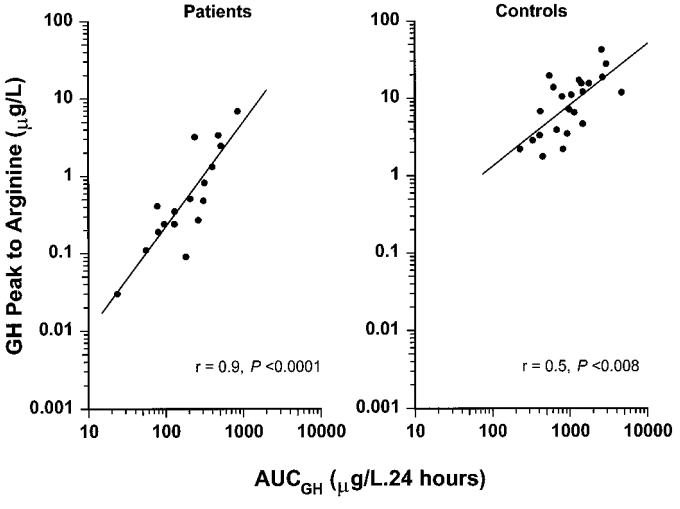

FIG. 8. Relationship between the peak GH response to arginine stimulation and the area under the 24-h GH profile in 17 elderly patients with organic GHD and 24 elderly BMI-matched controls.

additional pituitary hormone deficits, the distinction between GHD and normality remains a challenge.

G. Childhood onset

Currently, GH replacement in childhood is continued until growth is completed or there is an agreement between the family and the endocrinologist that a satisfactory height has been achieved. Over the last 10 yr there have been a number of studies in which the GH status of children and young adults, who had received GH replacement during childhood, was reassessed after completion of growth and puberty (11, 209–214).

The purpose of the studies was at least 2-fold: first, to establish how many individuals no longer appeared GH-deficient at reevaluation, and second, to determine how many individuals still had severe GHD requiring consideration of GH replacement during adult life. All individuals were documented biochemically to be GH-deficient in childhood but at reassessment GH status was considered to be normal in 20–87% (209–215). The etiological classification of the childhood diagnosis in the vast majority of the latter subjects was isolated idiopathic GHD. This raises interesting questions regarding the nature of their defect in GH secretion during childhood. The diagnostic threshold for GHD is arbitrarily defined, and the reproducibility of the GH response to provocative testing within individuals is not high. On these grounds alone, it would be anticipated that a percentage of those considered GH-deficient at one point in time might be considered normal at reevaluation. Furthermore, it is likely that in a proportion of these patients, the childhood diagnosis was CDGP and not isolated idiopathic GHD, but the initial GH provocative tests carried out in the "unprimed" state failed to make the distinction. Finally, it remains possible that transient GHD in childhood is a real entity although longitudinally obtained proof is lacking.

In contrast to the findings in the isolated idiopathic GHD population, those young adults diagnosed as having organic GHD in childhood, as a consequence of either a mass lesion, pituitary surgery, or irradiation damage to the hypothalamic-pituitary axis, rarely revert to normal GH status (213).

In addition to influencing the incidence of reversal of GH status, the etiology of the childhood diagnosis of GHD also affects the strategy of how to reevaluate. In our center (213), two provocative tests were carried out in two-thirds of 88 young adults undergoing reassessment of GH status, and one GH provocative test was performed in the remainder. By far, the most commonly used GH provocative tests were the ITT and the arginine stimulation test. Of those undergoing two tests, 55 patients had a peak GH less than 3.5 ng/ml in response to one test, of whom 26 had a peak GH greater than 3.5 ng/ml in response to the second test; similarly, of the 41 patients with a GH peak less than 1.9 ng/ml in response to one test, 26 of them had a GH peak greater than 1.9 ng/ml in response to the second test (213). Although there is a considerable degree of disagreement between the results of the two tests in individuals, the discordance was not explained by weighting associated with a persistently greater or lesser GH response to one particular test (213). Furthermore, discordance between test results is not distributed randomly. Of the 58 patients undergoing two tests at reassessment, 15 had additional anterior pituitary hormone deficits; all of them had a GH peak response below 3.5 ng/ml to both tests. At a diagnostic threshold of 3.5 ng/ml, discordance between the two GH test results only occurred among those patients with a diagnosis of isolated GHD (213).

The results from these studies emphasize that all children who have received GH replacement therapy in childhood should undergo reassessment of GH status in young adult life. The percentage of such patients who merit consideration for GH replacement in adult life will vary depending on the definition of severe GHD in use and the etiological mixture of GH-deficient patient populations from different centers. Patients with isolated GHD should undergo two tests of GH status, but those with additional anterior pituitary hormone deficiencies require only one test at reassessment.

H. Newer strategies for assessment of GH status

Earlier in this review the use of a combination of GHRH with pyridostigmine as a diagnostic test of childhood GHD was not recommended (52), the main reason being that in most children with GHD, the site of abnormal pathophysiology is believed to be hypothalamic rather than pituitary. In adults the different epidemiology of the pathophysiology of GHD makes the combined GHRH-pyridostigmine test more attractive. The most common cause of adult-onset GHD is a pituitary adenoma or treatment with pituitary surgery and/or irradiation. It is likely, but not proven, that the majority of patients with a pituitary mass lesion who are GH-deficient before or after pituitary surgery have sustained a pituitary insult; in contrast, GHD as a consequence of irradiation to the hypothalamic-pituitary axis is more likely to be hypothalamic in origin (216). Thus, provided that the endocrinologist is comfortable in the knowledge that the patient's defect is pituitary-related rather than hypothalamic, the GHRH-pyridostigmine test can be used to assess GH status.

Anderson *et al*. (217) have performed a GHRH-pyridostiguine dose response study to determine the optimal dose of pyridostigmine and found it to be 120 mg combined with 1 μg GHRH/kg. They observed a lower reference limit of 21 ng/ml for the peak GH response to the combined test in 40 normal adults, aged between 20 and 60 yr (217); there was no gender difference.

Using the threshold of 21 ng/ml for the peak GH response to the GHRH-pyridostigmine test and an arbitrarily defined level of 10 ng/ml for the ITT, they found consistent classification of normal and subnormal GH responses in 44 of 47 patients with pituitary disease (217).

Perhaps even more promising than GHRH plus pyridostigmine is the combination of GHRH and arginine. Unlike the former combination, the GH response to GHRH and arginine is independent of age; furthermore, the GH response is pronounced and shows much less inter- and intraindividual variability than the more conventional GH provocative tests (104). No overlap was found between GH peak responses in 24 adults with organic GHD and normal subjects (218).

Thus far, the requirement has been only to diagnose severe GHD in adult life. There is no question, however, that just as

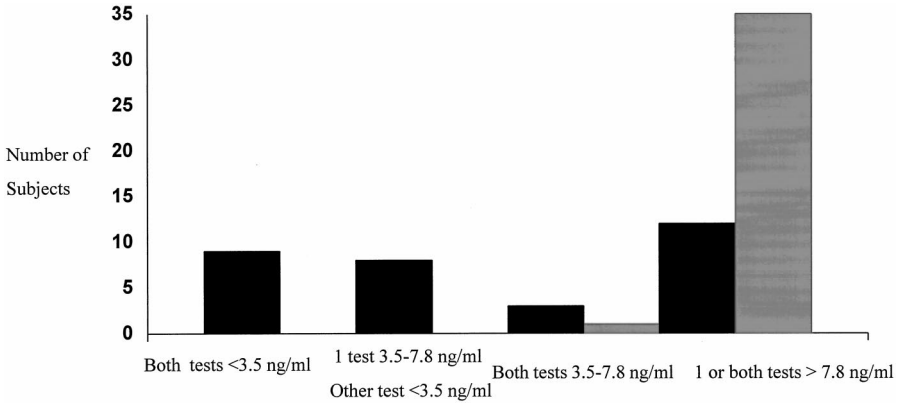

FIG. 9. Peak GH response to an ITT and to arginine stimulation in 32 young adults, who previously received cranial irradiation in childhood for ALL (■), and 35 age-matched normals (■). 1 ng/ml = 2.6 mU/liter. [Reproduced with permission from B. M. D. Brennan *et al.*: *Clin Endocrinol (Oxf)*, in press (219).]

in childhood, GH secretion is a continuum in adult life. Therefore, the concept of GH insufficiency is a reality. In a current study, GH status was evaluated in 32 young adults, aged 18 to 33 yr, who previously received cranial irradiation in childhood as part of their treatment for acute lymphoblastic leukemia, and 35 age-matched controls (219). Each subject underwent an ITT and an arginine stimulation test. The median peak GH responses to both tests were significantly greater in the control group than in the patient group. Nine patients had a peak GH response to both tests less than 3.5 ng/ml; 11 patients had a peak GH response less than 7.8 ng/ml to both tests but a peak GH response to one or both tests greater than 3.5 ng/ml; and the remaining 12 patients had a peak GH response to one or both tests in excess of 7.8 ng/ml. In contrast, all the controls bar one (peak GH, 7.4 ng/ml) had a peak GH response greater than 7.8 ng/ml to one or both tests (219) (Fig. 9).

Thus, many of these patients show subnormal GH responses but do not fulfil the recommended criteria for the diagnosis of severe GHD. The impact of these milder degrees of GHD on biological endpoints, such as body composition and bone, are unknown in adult life.

In the future it is likely that the strategy for evaluation of GH status may take into account the degree of GHD, the etiology of the hypothalamic-pituitary disease, and probable site of the defect within the hypothalamic-pituitary region.

I. Practical approach to the diagnosis of GHD

Thus far, the main suggestion in the literature has been that the peak GH response to an ITT be the gold standard for the biochemical diagnosis of severe GHD. The ITT does provoke a pronounced GH response in normals, it allows the pituitary-adrenal axis to be tested at the same time, and morbidity associated with the performance of the test is low in experienced endocrine units. A diagnostic cut-off of either 3 ng/ml or 5 ng/ml has been evaluated by pooling the ITT data available from the literature, albeit with the necessary assumptions required when GH values from different centers are considered together (175). Nonetheless, based on a cut-off of 5 ng/ml, the ITT provides a specificity of 97%, a sensitivity of 100%, a positive predictive value of 99%, and a negative predictive value of 100% (175)!

It is not possible to rely on the ITT alone. Under specific circumstances, the ITT is contraindicated and on other occasions two provocative tests are required. Furthermore, the lack of standardization of GH assays means that each laboratory must establish its own diagnostic threshold values rather than simply accepting the recommended cut-off level of 3 ng/ml or 5 ng/ml.

With the knowledge that they need to gather their own data, endocrine centers should concentrate on establishing the credentials of the test that they favor by acquiring GH data in normal subjects and in patients with hypothalamic-pituitary disease. The patients with additional pituitary hormone deficiencies could provide the gold standard for the diagnosis of severe GHD. Thus, in our center approximately 90% of patients in groups GHD2 and GHD3 had a peak GH response of 1.9 ng/ml to an ITT, and in 100% the peak GH response was less than 4.2 ng/ml (4). This model can be applied to any single test of GH status. The performance requirements for the test in the normal subjects are a pronounced GH response with very few individual "failures." Provided that these criteria are satisfied, glucagon, arginine, or even urinary GH estimation may be suitable. Clonidine is unsuitable and the GHRH-pyridostigmine test would be more attractive if the physician could be certain that he was dealing with a pituitary rather than a hypothalamic defect. The combined GHRH-arginine test appears promising but, thus far, few adult patients with hypothalamic-pituitary disease have been studied.

To discriminate between GHD and normality, an IGF-I SDS estimation is extremely useful for retesting the young adult with a diagnosis of childhood-onset GHD, moderately helpful (~30–50% positive predictive value) in the middle-aged adult (25–55 yr), and rarely helpful in the elderly (over 60 yr).

Within the limitations of the tests, which we have already discussed, it would be reasonable to perform only one provocative test of GH release in patients with two or three additional pituitary hormone deficits. In the patient with pituitary disease and the possible diagnosis of adult-onset isolated GHD or GHD plus one additional pituitary hormone deficit, two provocative tests of GH release would be appropriate.

The same strategy can be applied for reassessing GH status in young adults, who received GH replacement for childhood GHD, as has been recommended for establishing the diagnosis of adult-onset GHD. However, IGF-I SDS estima-

tion by itself should be considered adequate in those with multiple pituitary hormone deficiencies and should serve as one of two tests of GH status in the much larger cohort of individuals with a diagnosis of isolated GHD, all of whom require to be retested.

Finally, in those patients with pituitary disease, who are either morbidly obese or elderly, establishing the diagnosis of isolated GHD remains a challenge.

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