Evaluation of suppression of growth hormone levels following a 75g oral glucose tolerance test

W M Wan Nazaimoon, PhD
*M L Ng, PhD
N Satgunasingam, PhD
**B A K Khalid, FRACP

*Radiochemistry Division, Institute for Medical Research, Jln Pahang, 50588 Kuala Lumpur. Departments of *Biochemistry and **Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jln Raja Abd Aziz, 50300 Kuala Lumpur*

Summary

Growth hormone (GH) levels were measured after a 75g oral glucose load (OGTT) in normal adults, patients with impaired glucose tolerance (IGT), insulin-dependent diabetes mellitus (IDDM) and acromegaly. Nadir GH levels at 2-hour post-OGTT in normal subjects ranged from 0.4 to 8.4 mIU/L, the 95% confidence interval being 0.4–4.4 mIU/L. In IGT and IDDM subjects basal fasting GH levels were not significantly different from normal and did not alter during OGTT. The high fasting GH level measured in one each of the IGT and IDDM patients was suppressable at 1-hour after glucose intake. In contrast, acromegalic patients had elevated fasting GH levels (11.8–178 mIU/L) although in 3 patients, the levels were mildly elevated and overlapped with normal. OGTT failed or only partially suppressed GH secretion in all acromegalics. Therefore, elevated fasting GH levels are not diagnostic and OGTT is required for accurate diagnosis and assessment of treatment of acromegalic patients.

Key words: Human growth hormone (GH), radioimmunoassay (RIA), oral-glucose tolerance test (OGTT), acromegaly, impaired glucose tolerance (IGT).

Introduction

Secretion of growth hormone (GH) is regulated by two hypothalamic hormones namely, growth hormone releasing hormone (GRF) and growth hormone release-inhibiting hormone (GHR–IH) or somatostatin. In normal individuals, GH secretion is episodic, remaining low or undetectable during much of the day but at other times is released in intermittent, discrete bursts with increased amplitude[1–4]. GH secretion is stimulated by a number of physiological factors such as sleep[5], exercise[6], protein ingestion[7] and stress induced by acute hypoglycaemia[8]. Hence, measurement of single random basal levels of GH has very limited clinical value. It is therefore essential to perform appropriate tests in order to assess GH status in patients found to have high GH levels or suspected to have acromegaly. Since GH is a counter-regulatory hormone to insulin with respect to blood glucose control, hyperglycaemia in normal subjects will suppress GH secretion[9]. The most commonly used laboratory method to confirm pathological increase are GH levels at 1 and 2-hours following ingestion of 75g glucose (OGTT). Failure of suppression of high GH levels during OGTT is diagnostic of active acromegaly[10], whilst a low, suppressed level at 2-hour post-OGTT is considered a criterion for remission[11]. However,

as reviewed by Holly *et al*[12], excessive GH secretion antagonizes insulin hormone action with consequent adverse effects on glycaemic control and hyperinsulinaemia. Its lack of suppression or paradoxical early release during OGTT in patients with impaired glucose tolerance (IGT) has been reported[13]. A proportion of acromegalics have diabetes or IGT[14], and all have raised insulin[15]. Conversely, many diabetics or patients with IGT have been suspected of being acromegalics. It is therefore important to define the specificity of the OGTT in suppressing GH at 1-hour or 2-hours in diagnosing acromegaly.

Recently, Stewart *et al*[16] evaluated normal GH response to OGTT using a sensitive immunoradiometric assay (IRMA) and confirmed that an upper limit of normal of 2 mIU/L (using the GH standard IRP 66/217) used by the majority of laboratories in United Kingdom is appropriate. Nevertheless, establishing a cut-off value for GH levels in OGTT using a variety of commercial reagents or kits can be a problem not only because there will be discrepancy in results, thereby making interpretation and comparison difficult[17–19], but also because these kits are expensive and their supply is sometimes erratic. To overcome these problems, we set up an in-house radioimmunoassay (RIA) for GH[20]. The objective of our present study thus is to use this assay to evaluate the specificity of OGTT to suppress GH levels at 1-hour and 2-hour in normal, IGT, diabetic and acromegalic patients. In addition, we need to establish our own cut-off values, in the hope that a single blood sample at a specific time after 75g glucose drink will be sufficient to confirm the diagnosis of acromegaly.

Materials and methods

Subjects

The subjects comprised of normal adults, twelve men and thirteen women of mean age 32.0 ± 6.7 years (range 20–42), 10 IGT patients (six men and four women, age range 28–50 years), 10 insulin-dependent diabetes mellitus (IDDM) patients (two men, eight women, age range 12–52 years), and twelve acromegalic patients (five men and seven women, age range 20–58 years, diagnosed clinically and radiologically) from the Endocrine Clinic, Faculty of Medicine, Universiti Kebangsaan Malaysia. Informed consent was obtained from the subjects prior to the study.

Experimental procedure

Subjects were fasted overnight, and at 08.00h, 5 ml of blood was withdrawn from the forearm vein. Venous blood samples were further collected at 1 hour intervals for 2 hours after drinking a freshly prepared solution of 75g glucose in 200 ml lukewarm water. A small aliquot of all blood samples was immediately tested for glucose using Ames Glucostix Reagent Strips (Miles Australia, Pty Ltd.) Serum samples were stored at -20 C before analysis.

Growth hormone assay

Growth hormone levels were determined by our in-house RIA[20], which had been validated in an external quality assurance scheme (UK–EQAS, Immunoassay Section, Bristo Place, Edinburgh) ($r = 0.975$, $y = 1.02x - 0.77$, $p<0.001$, $n = 26$) (Figure 1). All samples from an individual subject were analysed in the same assay and all results were calibrated against GH standard I.S. 80/505. The intra-assay coefficients of variation (CVs) at doses 3.4, 19.1 and 55.7 mIU/L were 6.2, 5.3 and 7.9% respectively and the corresponding inter-assay CVs were 6.5, 5.8 and 8.7% respectively. Since the lower limit of sensitivity of the assay is 0.4 mIU/L, GH values of 0.4 mIU/L or less were assigned the value of 0.4 mIU/L.

Statistical analysis

Data were analysed by the Statistical Analysis Software (SAS). Comparison within and between groups were by the nonparametric Wilcoxon's signed rank and Wilcoxon's rank sum tests respectively. A value of $p<0.05$ was considered significant.

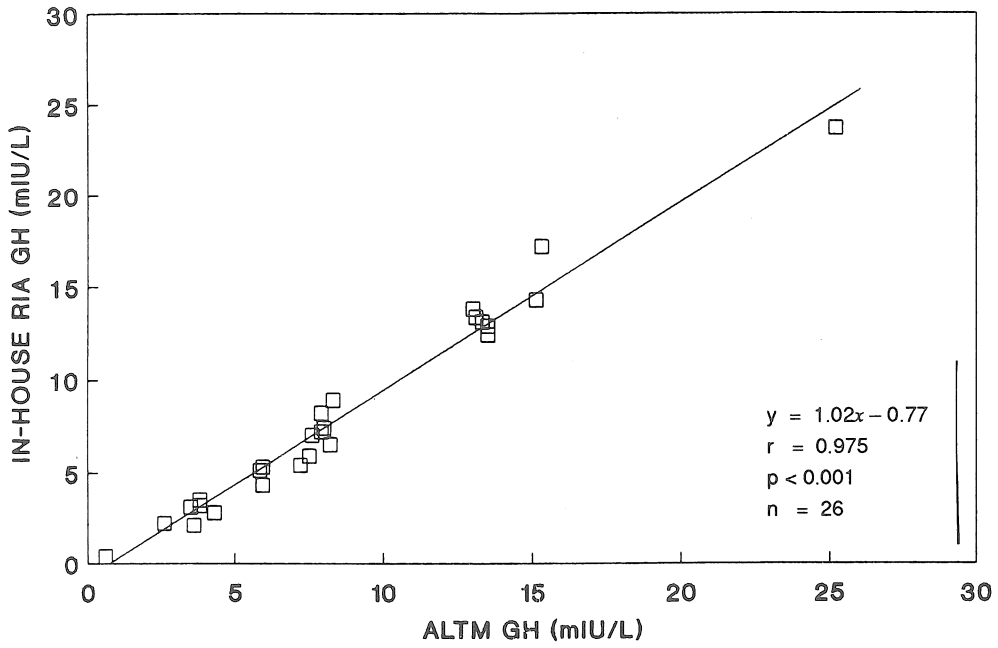

Fig. 1: Performance of in-house GH RIA in the UK-EQAS

Results

The mean (± SD) blood glucose levels of the twenty-five normal subjects at 1-hour and 2-hour post-OGTT were 5.8 ± 1.8 mM and 4.7 ± 1.2 mM respectively. All IGT patients exhibited impaired glucose tolerance; their blood glucose levels at 2-hour post-OGTT were greater than 7.8 mM. Serum GH levels of fasting, 1-hour and 2-hour post-OGTT samples from all normal subjects are shown in Figure 2. Although the majority of the normal subjects had fasting serum GH levels below our established upper normal limit of 10 mIU/L[20], elevated fasting levels were observed in 5(20%) cases. Their individual results are shown in Figure 3. Normal suppression by glucose was observed in all five subjects with nadir occurring at 2-hour post-OGTT. At 1-hour post-OGTT, serum GH levels were suppressed in all normal subjects with one exception of raised GH at 18.7 mIU/L. Nadir suppression of GH levels was only achieved in all normal subjects after 2 hours.

The range, median and 95% confidence interval calculated for normal, IGT, IDDM and acromegalic subjects are tabulated in Table I. As shown, the upper limit of suppressed GH level at the 95% confidence interval in the normal subjects was found to be 2.9 and 4.4 mIU/L at 1-hour and 2-hour respectively. GH levels at fasting and at 1-hour and 2-hour post 75g glucose in patients with IGT and IDDM were not significantly different from those in the normal subjects. Two of these patients, one with IGT and another with IDDM, had high fasting GH level of 16 and 17.8 mIU/L respectively, which was suppressed after IGT and IDDM patients, there was no significant rise in GH levels during the study.

In contrast, fasting serum GH levels of acromegalic patients were all raised and remained elevated throughout OGTT (Figure 2). Fasting serum GH levels ranged from 11.8 to 178 mIU/L and were 22.2 to 188 mIU/L and 14.7 to 230 mIU/L at 1-hour and 2-hour post-OGTT respectively. The fasting serum GH concentrations of three patients were only moderately elevated with values that overlapped those of some normal subjects (Figure 3). However, raised fasting GH levels in acromegalic patients were

Table I
Growth hormone levels in normal subjects, IGT, diabetic and acromegalic patients during OGTT

	GH (mIU/L)		
	Fasting	**OGTT-1h**	**OGTT-2h**
Normals (n = 25)			
Median	1.5	0.9[a]	0.8[a]
Range	(0.4 – 32.3)	(0.4 – 18.7)	(0.4 – 8.4)
95% CI	(0.4 – 26.4)	(0.4 – 2.9)	(0.4 – 4.4)
IGT (n = 10)			
Median	0.4	0.4	0.4
Range	(0.4 – 16)	(0.4 – 2.7)	(0.4 – 3.8)
IDDM (n = 10)			
Median	41.6[b]	44.3[b]	32.9[b]
Range	(11.8 – 178)	(22.2 – 188)	(14.7 – 230)

[a] $p < 0.01$ *vs.* fasting level, Wilcoxon's signed rank test.
[b] $p << 0.001$ *vs.* respective normal levels, Wilcoxon's signed rank test.

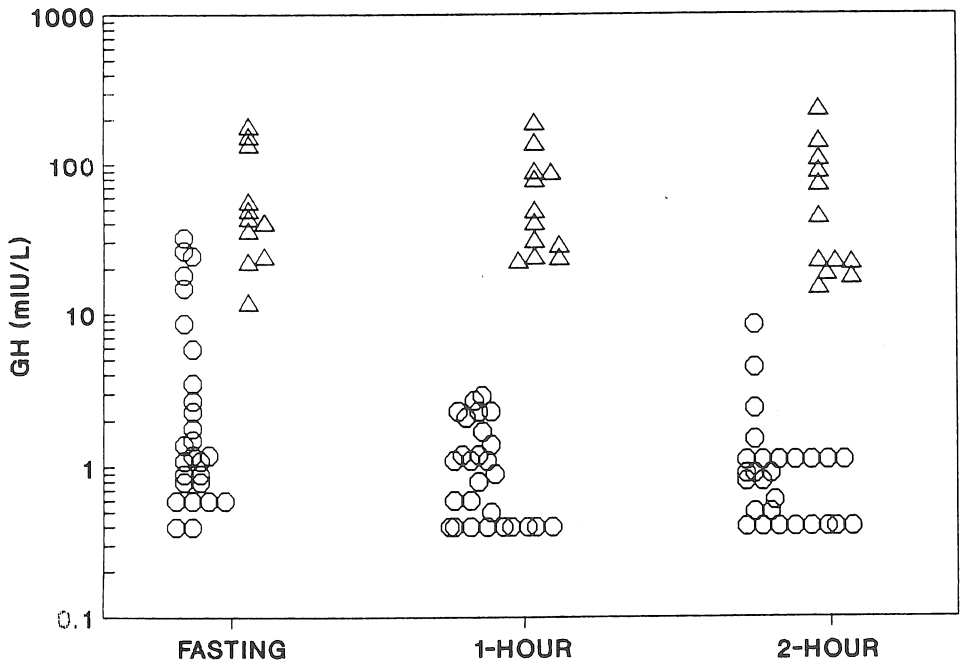

Fig. 2 : Serum GH concentrations in normal adults.

○ Normal
△ Acromegaly

either unchanged or only partially suppressed in six cases and showed paradoxical increases of 140% to 230% of fasting level in the remainder six cases (Figure 3).

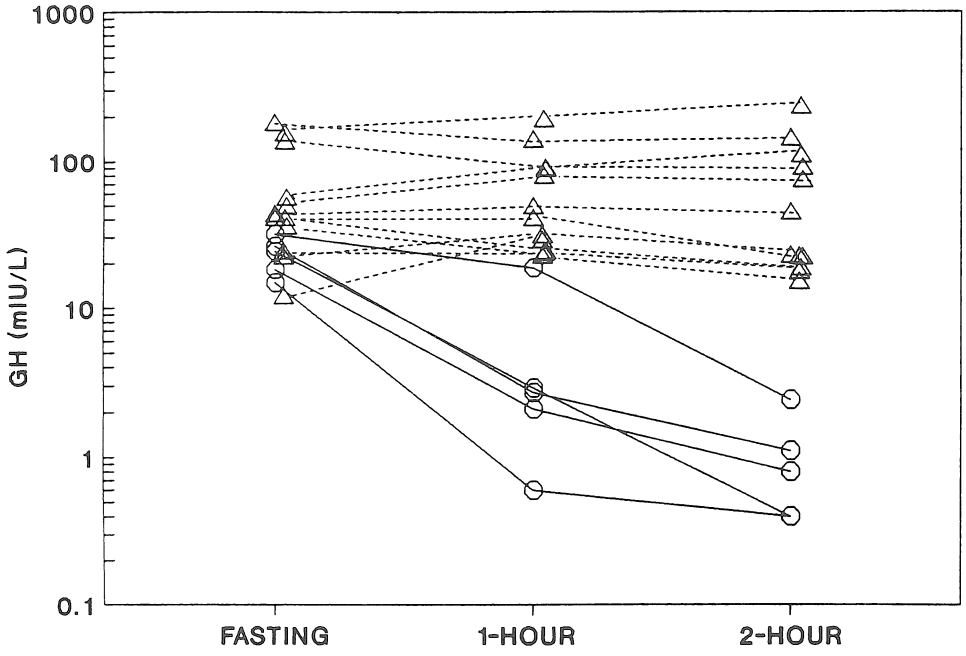

Fig. 3 : Effect of glucose load on elevated GH level of some normal adults (O) and acromegalic patients (△)

Discussion

The response of normal subjects to OGTT is as expected and in agreement with a number of previous studies[9,16]. The upper limit of 4.4 mIU/L for nadir GH level after glucose suppression in the normal control subjects as measured by in-house RIA is within the cut-off level of 1 to 10 mIU/L (GH standard IRP 66/217) reported in the United Kingdom External Quality Assessment Scheme[16]. The small paradoxical increase in GH secretion in two of our controls at 2-hour post-glucose load was also observed in two of their normal volunteers. The high fasting but suppressable GH levels during OGTT seen in some of the controls have also been reported by Roth *et al*[8] and Hunter and Greenwood[21]. If the diagnosis of acromegaly is based on a single measurement of fasting GH concentration, false positive results of up to 20% would be encountered. Contrary to the report by Grecu *et al*[13], who found that 44% of their IGT subjects showed paradoxical GH release during the first 2 hour of OGTT, there was no significant rise in GH levels in any of our IGT and IDDM subjects. This is comparable to the reports by Hunter *et al*[22] and Hattori *et al*[23].

Measurement of GH levels in serum or plasma during OGTT is an acceptable and widely used test in the investigation of suspected acromegaly and in assessing the efficacy of its treatment. All of our patients showed elevated fasting GH but three of whom had values which overlapped with the normals, and hyperglycemia failed to lower the levels when compared to the responses exhibited by normal subjects. The paradoxical rise in GH secretion seen in six of our acromegalic patients during OGTT agrees well with the results from a number of previous studies[8,9,21,24]. The partial suppression by glucose in the other three patients have also been reported by others[25,26]. Nevertheless, as in our study, the levels were still higher than those measured in normal subjects.

Our findings that acromegalics may have only moderately raised basal fasting GH levels that overlap with values of some normal subjects whilst a normal fasting level may be as high as 32 mIU/L, clearly underlines the importance of oral glucose tolerance test in confirming the diagnosis of GH hypersecretion as well as in monitoring its treatment. Relying on a single measurement of basal fasting or random blood sample in either case would have obviously resulted in misleading interpretation and diagnosis. Based on the present study, 75g glucose at 2-hour post-ingestion is effective in totally suppressing the high basal GH level seen in normal, IGT and IDDM subjects to a nadir level which is distinctly different from that obtained in acromegalic patients. However, since total suppression of GH levels in all normal subjects was achieved only at 2-hours post-OGTT, we therefore concluded that, if for any reason or just to reduce laboratory cost, only a single sample is to be taken, then it should be taken at 2 hours after the glucose load.

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