Acromegaly: the significance of serum total and free IGF-I and IGF-binding protein-3 in diagnosis

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Abstract

We have studied the physiological and clinical relevance of measurements of serum total and free IGF-I and IGF-binding protein-3 (IGFBP-3) in 57 previously untreated patients with active acromegaly (32 males, 25 females; mean age 47 years) as compared with sex- and age-matched normal healthy controls. Serum total and free IGF-I, but not IGFBP-3, are suitable biochemical parameters for screening for acromegaly. In acromegalics, the mean 24 h serum GH, total IGF-I and IGFBP-3 levels tend to decrease with age. However, in our series of patients, mean 24 h serum GH levels, IGFBP-3, total and free IGF-I do not correlate with disease activity in acromegaly.

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Introduction

In normal healthy adults, spontaneous growth hormone (GH) secretion shows a diurnal pattern and is influenced by age and sex. More than 99% of GH is secreted in a pulsatile fashion. In patients with active acromegaly, only 50% of GH secretion is excreted in a pulsatile manner, but the GH pulse frequency is increased as compared with normal healthy adults (van den Berg *et al.* 1994). Because of the variability in GH secretion over the day and the short half-life of GH (approximately 20 min) in the circulation, random GH measurements have only limited value for the differential diagnosis of acromegaly. Generally, 'undetectable' GH levels measured with a standard commercially available RIA or IRMA in a patient suspected of having acromegaly exclude the diagnosis. However, it should be noted that the actual GH level in this situation may not really be zero, but below the sensitivity level of the assay. In contrast, spontaneous, exercise-induced, or postprandial GH pulses during the day may occasionally result in an abnormally high random GH determination in a normal subject. Using 24 h GH levels, a good separation can be made between patients with active acromegaly and normal subjects, but only after matching for age (Ho & Weissberger 1994). In normal subjects, serum GH levels (measured with a commercially available RIA) are suppressed to <2 μg/l (<4 mU/l) within 1–2 h of the administration of an oral load of 75–100 g glucose after overnight fasting. However, in acromegaly there is a failure to suppress GH levels to this value during oral glucose tolerance testing or they even show a paradoxical rise (Melmed 1990, Melmed *et al.* 1995).

Insulin-like growth factor-I (IGF-I), formerly known as somatomedin-C, mediates many of the actions of GH. GH induces IGF-I synthesis and release, especially from the liver. In contrast with serum GH levels, serum (total) IGF-I levels show little fluctuation during the course of the day. Serum (total) IGF-I levels are invariably elevated in active acromegaly (Clemmons *et al.* 1979). Pregnancy and late puberty are also associated with elevated serum (total) IGF-I levels. A highly significant correlation exists between serum (total) IGF-I levels and the area under the serum GH curve estimated during oral glucose tolerance testing (r=0·66) (Dobrashian *et al.* 1993). A positive correlation has also been demonstrated between the mean 24 h serum GH levels and serum (total) IGF-I levels. The relationship between mean serum GH levels either during oral glucose tolerance testing or in a 24 h profile and serum (total) IGF-I levels shows a curvilinear pattern. A linear relationship exists between GH levels <10 μg/l (<20 mU/l) and serum (total) IGF-I. Serum (total) IGF-I levels reach their maximum at serum GH levels >20 μg/l (>40 mU/l) (Barkan *et al.* 1988, Dobrashian *et al.* 1993).

The majority of IGF-I (and IGF-II) in the serum is complexed with binding proteins (IGFBPs). In the circulation, about 75% of the IGFs are complexed with IGFBP-3 and an acid-labile subunit in a 150–200 kDa ternary complex. The remainder of the circulating IGFs is

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either bound to lower molecular mass IGFBPs or circulates in the free form. IGFBP-3 appears to be the primary regulator of IGF bioavailability in response to changes in circulating GH levels (Baxter 1994). The ternary complex does not cross the vascular endothelium and therefore serves as a reservoir for IGFs. When associated with the ternary complex, the half-life of the IGFs is prolonged. Breakdown of the IGFBPs is regulated by IGFBP proteases. An increase in IGFBP-3 proteolysis will lead to increased formation of lower molecular mass IGF–IGFBP complexes, which are capable of crossing the endothelium. Elevated IGFBP-3 levels have been found in patients with active acromegaly (de Herder *et al.* 1995, Grinspoon *et al.* 1995). The 28 kDa IGFBP-1 appears to be the primary regulator of IGF bioavailability in response to the changes in the circulating insulin level (Baxter 1994). Serum levels of IGFBP-5, but not IGFBP-4, show significant positive correlations with the IGFs. IGFBP-1 and IGFBP-4 in serum have predominant IGF-I inhibitory activities, whereas IGFBP-5 in serum has IGF-I stimulatory activities (Mohan *et al.* 1996).

By analogy with steroid and thyroid hormones, the unbound fraction may be the dominating fraction for the biological activity of IGF-I. Frystyk *et al.* (1994) have shown that fasting serum free IGF-I levels are increased in acromegaly and showed no overlap with levels found in healthy controls.

We have studied the physiological and clinical relevance of measurements of serum total and free IGF-I and IGFBP-3 in acromegaly as compared with normal healthy adults.

Patients and methods

Fifty-seven previously untreated patients (32 males, 25 females; mean age 47 years) with active acromegaly were studied. Disease activity was scored according to five clinical parameters: excessive sweating, headaches, fatigue, paresthesias and arthralgias (see Table 1). Fifty-seven sex- and age-matched adults were studied as controls. In patients with acromegaly, serum GH was sampled from 0800 to 1800 h at hourly intervals, followed by 2 hourly sampling to 0600 h. A final sample was taken at 0700 h. Fasting samples for measurements of IGF-I, IGFBP-3 and free IGF-I were taken from acromegalics and healthy controls.

Serum GH was measured by IRMA supplied by CIS Bio International, Gif-sur-Yvette Cedex, France (intra-assay coefficient of variation (CV) 2·8%, interassay CV 4·4%). Serum total IGF-I was determined by RIA using kits obtained from Medgenix Diagnostics, Fleurus, Belgium (intra-assay CV 6·1%, interassay CV 9·9%). Serum free IGF-I was measured by IRMA supplied by Diagnostic Service Laboratories, Webster, TX, USA (intra-assay CV 10·3%, interassay CV, 10·7%).

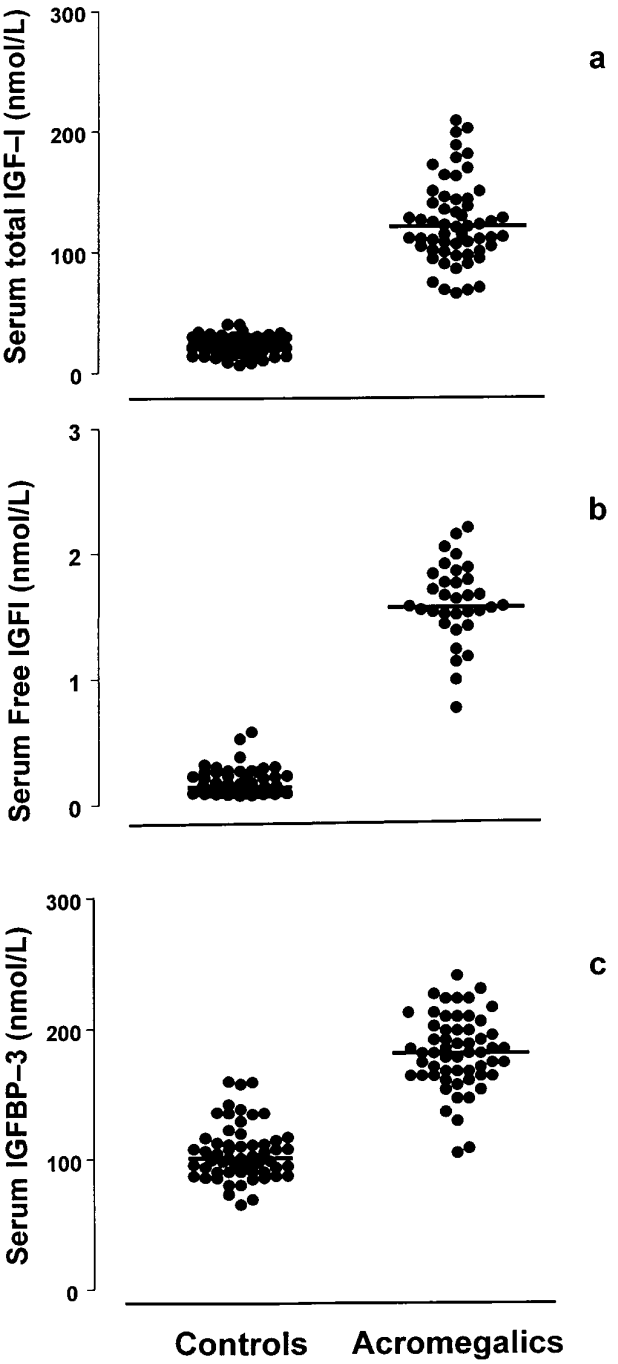

Figure 1 (a) Serum total IGF-I and (c) serum IGFBP-3 in 57 patients with active acromegaly and 57 sex- and age-matched healthy adults. (b) Serum free IGF-I in 33 patients with active acromegaly and 33 sex- and age-matched healthy adults.

Serum IGFBP-3 levels were determined by IRMA supplied by Diagnostic Service Laboratories (intra-assay CV 3·9%, interassay CV 1·9%).

Table 1 Clinical activity score in acromegaly. Total score: <3, mild; 3–5, moderate; >5, severe

	Headaches	Fatigue	Perspiration	Paresthesias	Arthralgia
Score					
0	None	None	None	None	None
1	Mild	Mild	Mild	Mild	Mild
2	Moderate	Moderate	Moderate	Moderate	Moderate
3	Severe	Severe	Severe	Severe/CTS	Severe

CTS, carpal tunnel syndrome.

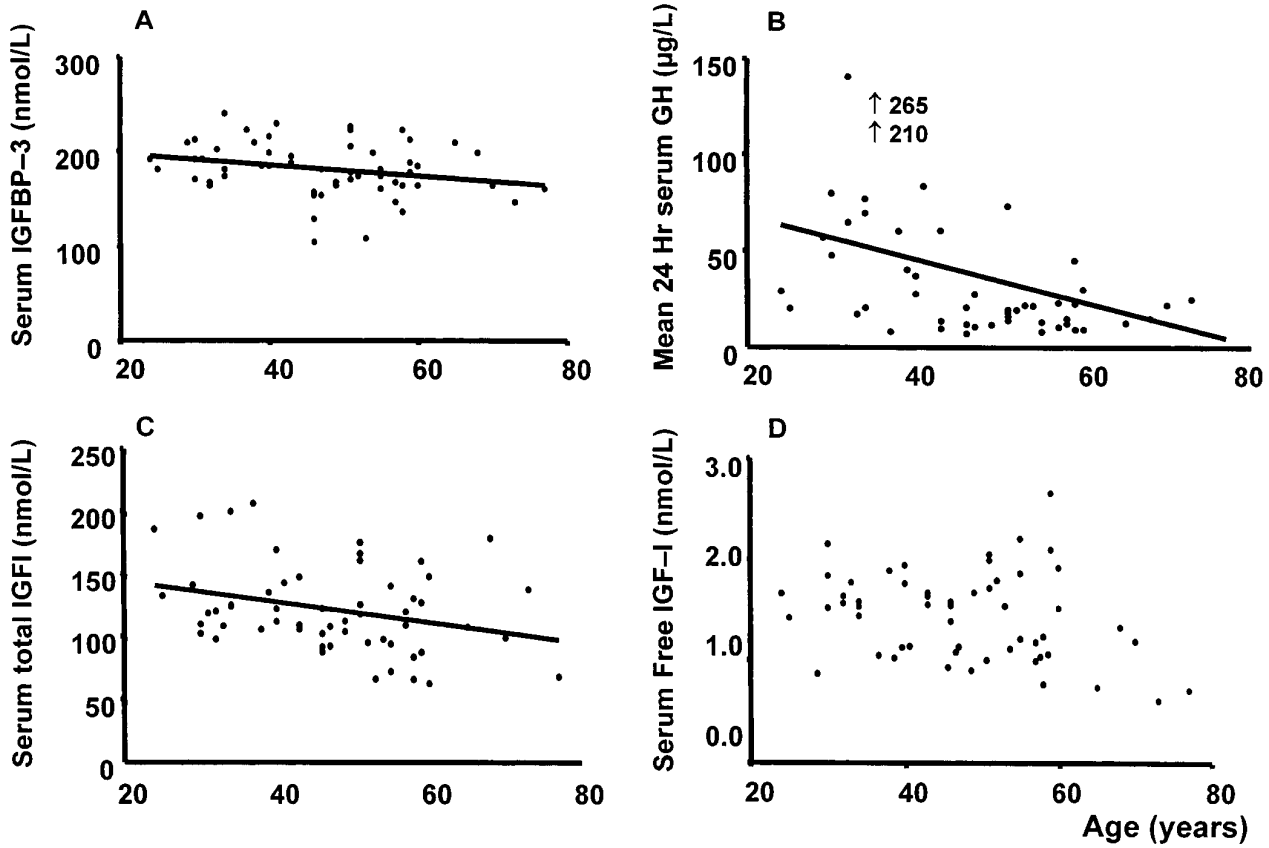

Figure 2 Relations between (A) serum IGFBP-3 and age ($r = -0{\cdot}25$, $P=0{\cdot}02$), (B) mean 24 h serum GH and age ($r = -0{\cdot}29$, $P=0{\cdot}002$), (C) serum total IGF-I and age ($r = -0{\cdot}30$, $P=0{\cdot}02$) and (D) serum free IGF-I and age (not significant) in 57 patients with active acromegaly (non-parametric correlation (Spearman)). Statistical significance was defined as $P<0{\cdot}05$.

Results

In Fig. 1a–c, serum total IGF-I, free IGF-I and IGFBP-3 levels in normal healthy controls and patients with active acromegaly are shown. There was a considerable overlap for serum IGFBP-3 levels between normals and acromegalics, but not for serum total and free IGF-I.

Figure 2A–D shows the relations between age and mean 24 h serum GH, total IGF-I, free IGF-I and IGFBP-3 levels in patients with active acromegaly. Mean 24 h serum GH, serum total IGF-I and IGFBP-3 levels decrease with increasing age. However, there is no relation between serum free IGF-I levels and age in acromegalics.

In Table 2, the correlations between mean 24 h serum GH levels and serum total IGF-I, free IGF-I and IGFBP-3 are shown. There was a significant correlation between mean 24 h serum GH levels and serum total IGF-I levels ($r=0{\cdot}38$, $P=0{\cdot}005$; non-parametric correlation (Spearman)).

Table 2 Correlations between the various clinical and biochemical parameters in 57 patients with active acromegaly (non-parametric correlation (Spearman)). Statistical significance defined as $P<0{\cdot}05$ (NS, not significant)

	Total IGF-I	IGFBP-3	Free IGF-I	Disease activity	Mean 24 h GH
Age	− 0·28 (P=0·04)	− 0·28 (P=0·04)	− 0·13 (NS)	− 0·46 (P<0·001)	− 0·40 (P=0·003)
Total IGF-I		0·55 (P<0·001)	0·12 (NS)	0·18 (NS)	0·38 (P=0·005)
IGFBP-3			0·01 (NS)	0·06 (NS)	0·21 (NS)
Free IGF-I				0·01 (NS)	0·26 (NS)
Disease activity					0·10 (NS)

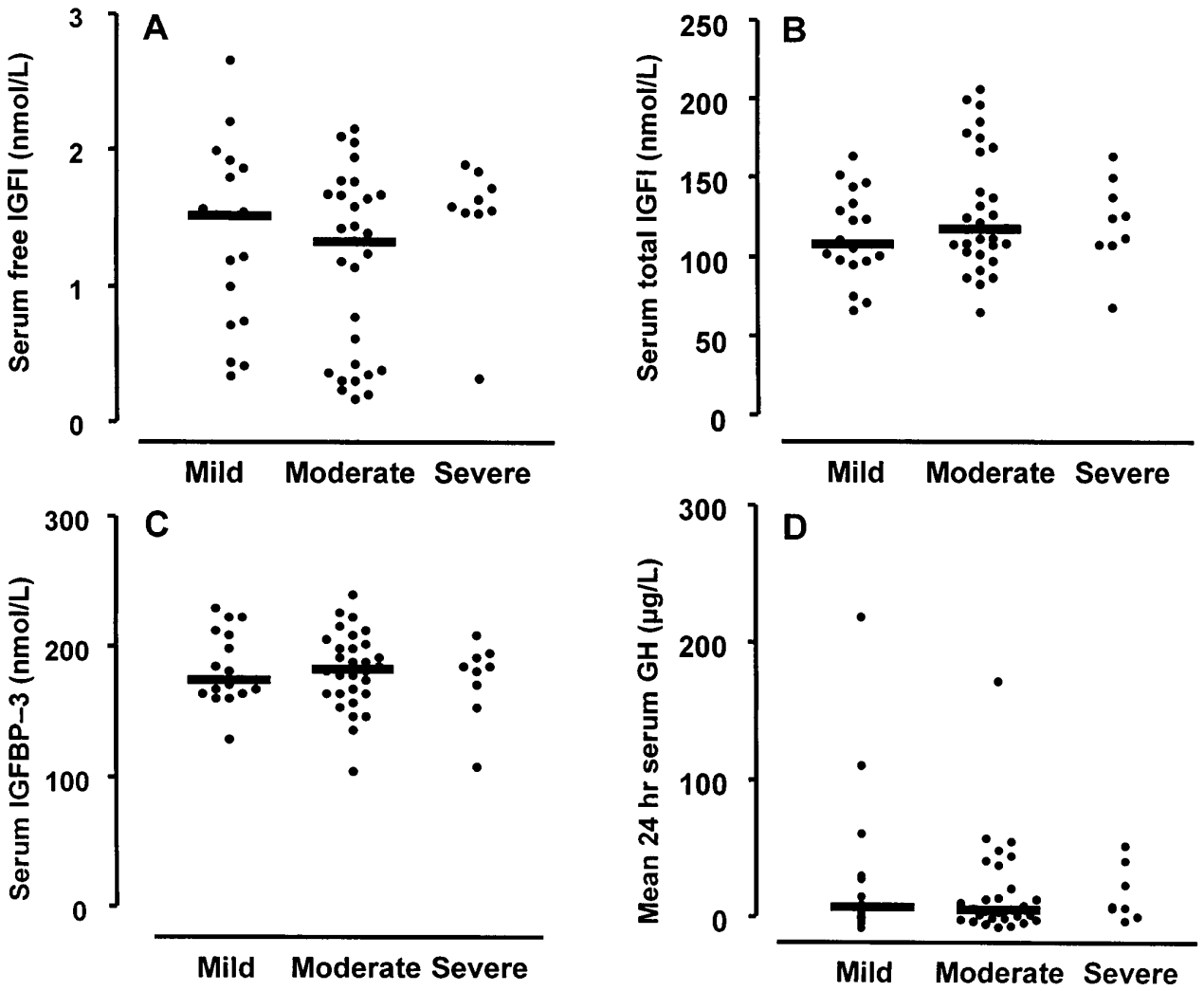

Figure 3 Relations between (A) serum free IGF-I and clinical activity (not significant), (B) serum total IGF-I and clinical activity (not significant), (C) serum IGFBP-3 and clinical activity (not significant) and (D) mean 24 h serum GH and clinical activity (not significant) in 57 patients with active acromegaly (non-parametric correlation (Spearman)). Statistical significance was defined as $P<0{\cdot}05$.

There was no significant correlation between mean 24 h serum GH and serum free IGF-I or serum IGFBP-3 levels. There was a significant correlation between serum total IGF-I and serum IGFBP-3 levels (r=0·55, $P<0{\cdot}001$; non-parametric correlation (Spearman)), but not between serum total and free IGF-I levels. Also, no significant correlation was found between serum free IGF-I and IGFBP-3 levels.

Figure 3A–D shows the correlations between disease activity and mean 24 h serum GH, free and total IGF-I and IGFBP-3 levels. Mean 24 h serum GH, free and total IGF-I and IGFBP-3 levels did not correlate with disease activity.

Discussion

By comparing a large series of patients with untreated acromegaly with sex- and age-matched controls, the present study has shown a considerable overlap of serum IGFBP-3 levels, but not of free and total IGF-I levels between the controls and acromegalics. This is in line with our previous findings in a smaller group of patients with acromegaly (de Herder *et al*. 1995). Therefore IGFBP-3 is not a marker for the assessment of GH excess in adults. Both serum free and total IGF-I measurements can serve this purpose better.

Our results show that in acromegalics, mean 24 h serum GH, total IGF-I and IGFBP-3 levels tend to decrease with age. This is also in line with our previous observations (van der Lely *et al*. 1992, de Herder *et al*. 1995). We could not demonstrate a decrease in serum free IGF-I with age. This might be explained by a parallel decrement in f total IGF-I and IGFBP-3.

We have shown that mean 24 h serum GH levels, IGFBP-3, total and free IGF-I do not correlate with disease activity in acromegaly.

Conclusions

Serum total and free IGF-I, but not IGFBP-3, are suitable biochemical parameters for screening for acromegaly.

In acromegalics, mean 24 h serum GH, total IGF-I and IGFBP-3 levels tend to decrease with age.

Mean 24 h serum GH, total and free IGF-I and IGFBP-3 levels do not correlate with disease activity in acromegaly.

References

Barkan AL, Beitins IZ & Kelch RP 1988 Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. *Journal of Clinical Endocrinology and Metabolism* **67** 69–73.

Baxter RC 1994 Insulin-like growth factor binding proteins in the human circulation: a review. *Hormone Research* **42** 140–144.

van den Berg G, Frolich M, Veldhuis JD & Roelfsema F 1994 Growth hormone secretion in recently operated acromegalic patients. *Journal of Clinical Endocrinology and Metabolism* **79** 1706–1715.

Clemmons DR, Van Wijk JJ, Ridgway EC, Kliman B, Kjellberg RN & Underwood LE 1979 Evaluation of acromegaly by radioimmunoassay of somatostatin-C. *New England Journal of Medicine* **301** 1138–1142.

Dobrashian RD, O'Halloran DJ, Hunt A, Beardwell CG & Shalet SM 1993 Relationships between insulin-like growth factor-1 levels and growth hormone concentrations during diurnal profiles and following oral glucose in acromegaly. *Clinical Endocrinology* **38** 589–593.

Frystyk J, Skjaerbaek C, Dinesen B & Orskov H 1994 Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Letters* **348** 185–191.

Grinspoon S, Clemmons D, Swearingen B & Klibanski A 1995 Serum insulin-like growth factor-binding protein-3 levels in the diagnosis of acromegaly. *Journal of Clinical Endocrinology and Metabolism* **80** 927–932.

de Herder WW, van der Lely AJ, Janssen JA, Uitterlinden P, Hofland LJ & Lamberts SW 1995 IGFBP-3 is a poor parameter for assessment of clinical activity in acromegaly. *Clinical Endocrinology* **43** 501–505.

Ho KY & Weissberger AJ 1994 Characterization of 24-hour growth hormone secretion in acromegaly: implications for diagnosis and therapy. *Clinical Endocrinology* **41** 75–83.

van der Lely AJ, Harris AG & Lamberts SW 1992 The sensitivity of growth hormone secretion to medical treatment in acromegalic patients: influence of age and sex. *Clinical Endocrinology* **37** 181–185.

Melmed S 1990 Acromegaly. *New England Journal of Medicine* **322** 966–977.

Melmed S, Ho K, Klibanski A, Reichlin S & Thorner M 1995 Recent advances in pathogenesis, diagnosis, and management of acromegaly. *Journal of Clinical Endocrinology and Metabolism* **80** 3395–3402.

Mohan S, Baylink DJ & Pettis JL 1996 Insulin-like growth factor (IGF)-binding proteins in serum: Do they have additional roles besides modulating the endocrine IGF actions? *Journal of Clinical Endocrinology and Metabolism* **81** 3817–3820.

Discussion: IGF-I levels and diagnosis

Dr M Besser (London, UK):

I wonder if one has not to be very careful about tautological arguments, in that, as I recall, the GH levels in your patients were really rather high. Did anyone in your group have a mean serum GH level of about 10 mU/l (5 μg/l)? Weren't they all 50 mU/l (25 μg/l) or more?

Dr A J van der Lely (Rotterdam, The Netherlands):

No; the range was quite wide (up to 400 μg/l and down to below 10 μg/l).

Besser:

Did you have anyone down at 10 mU/l (5 μg/l)? We quite often see patients in whom it is clinically very difficult to make the diagnosis, and they never have blood levels below 1 mU/l (0·5 μg/l); those are the patients in whom the IGF-I concentration is not helpful, and yet they improve dramatically with octreotide treatment. I would agree with you that there is a correlation between GH and IGF-I, but this does not help with individual patients who have very mild acromegaly at presentation. In our experience IGF-I is not helpful in low levels at diagnosis, whereas severe acromegaly can be diagnosed on the basis of a single blood sample.

van der Lely:

There are many acromegalics who have a relatively low GH secretion and a considerable amount of IGF-I, and *vice-versa*. Up to now we have not been able to show specific clinical characteristics between such groups.

Dr J A H Wass (Oxford, UK):

To change subject, your interesting data on serum levels of free IGF-I do not appear — rather surprisingly — to go down with age. How do you explain that in terms of the known decline in secretion of GH with age?

van der Lely:

I cannot explain it; that is what we found.

Dr A Barkan (Ann Arbor, USA):

First of all, I completely agree that serum IGF-I level is by far the most sensitive screening test. We have seen many patients with *de novo* untreated acromegaly, with GH fluctuations between 0·5 and 2 μg/l, who were clinically active and

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whose IGF level was extremely high. We have yet to see a patient with acromegaly whose IGF-I level is within the so-called normal range.

Dr H-J Quabbe (Berlin, Germany):

With reference to the negative correlation between clinical score and age of the patient, I tend to believe that older patients probably have a long duration of a very mild case of acromegaly. I think that many of these patients developed tumours at the age of 25–30 years, but that the tumours had very low secretory activity. Have you any information on the estimated duration of disease in these older patients?

van der Lely:

They tend to have longer disease activity than the younger patients but, strangely, tumour volume in older patients is less than that in younger patients, which is inexplicable on the premise that older patients just have the same disease for a longer period.