CLINICAL STUDY

Ghrelin test for the assessment of GH status in successfully treated patients with acromegaly

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Abstract

Objective: Posttreatment assessment of disease activity and definition of cure of acromegaly, using measurement of GH secretion, remains problematic. Furthermore, with our efforts to achieve tight biochemical control of the disease it is foreseeable that a proportion of patients may be rendered GH deficient, thus requiring testing for GH deficiency. The aim of our study was to evaluate residual GH secretion in cured patients with acromegaly.

Design and methods: At baseline, circulating GH, IGF-I, IGFBP-3, leptin and lipid (cholesterol and triglycerides) levels were measured in 33 acromegalic patients nine years after treatment with surgery of whom 6 were additionally irradiated. Two tests were performed: the GH suppression test - oral glucose tolerance test (OGTT) and the GH provocation test - ghrelin test (1 µg/kg i.v. bolus) and the results were compared with 11 age- and sex-matched control subjects.

Results: According to the consensus criteria (normal IGF-I levels and post-OGTT GH nadir < 1 µg/l), 21 treated acromegalic patients were cured, 6 had discordant IGF-I and GH nadir values during OGTT, while 6 had persistent acromegaly. After the GH provocative test with ghrelin (cut-off for severe GH deficiency is GH < 3 µg/l), we detected 9 severely GH deficient patients (GHD) among 21 cured acromegalic patients. Mean GH peak (±S.E.M.) response to the ghrelin test in GHD acromegalics was significantly lower compared with acromegalics with sufficient GH secretory capacity and control subjects (1.2±0.2 µg/l vs 20.1±2.4 µg/l vs 31.1±2.5 µg/l respectively, $P<0.0001$). Mean IGF-I and IGFBP-3 levels were not different between GHD and GH-sufficient cured acromegalics. Leptin levels and body mass index (BMI) were significantly higher in GHD male acromegalics compared with GH-sufficient male acromegalics. GHD female acromegalics tended to have higher BMIs while leptin levels were not different.

Conclusions: The assessment of residual GH secretory capacity by the GH provocation test is necessary in the long-term follow-up of successfully treated acromegalics since a large proportion of these patients are rendered GH deficient.

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Introduction

Acromegaly is a disfiguring and disabling illness in which, by definition, the disorder is caused by a pituitary growth hormone (GH)-secreting adenoma resulting in high circulating levels of GH and insulin-like growth factor-I (IGF-I). The condition develops insidiously over many years. If untreated, life expectancy is reduced by an average of 10 years (1). Overall mortality in patients with acromegaly is increased compared with the general population and is due to cardiovascular, cerebrovascular, respiratory and malignant disease (2–6). In a recent report on prognostic factors relating to mortality, if serum GH is reduced to

less than 1 µg/l and/or IGF-I levels are reduced to normal levels, the mortality rate becomes indistinguishable from that expected for the age-matched general community (7).

Assessment of GH status in treated acromegalics is difficult since there is no single cut-off value for GH with perfect discrimination between acromegaly and normality. Furthermore, some acromegalics may have subtle abnormalities in GH secretion resulting in a post glucose GH nadir value in the designated 'normal' range together with elevated IGF-I levels (8). Thus the recommended post glucose GH nadir value of 1 µg/l is now considered to be inappropriately high, and measurement of IGF-I levels although extremely valuable has its limitations

(9–11). Some studies have recently proposed the lowering of post glucose GH nadir levels to $<0.3 \mu\text{g/l}$ in order to improve identification of biochemical remission of acromegaly as well as better to recognise residual disease during therapy (12). With our efforts to achieve tight biochemical control of the disease it is foreseeable that a proportion of patients may be rendered GH deficient, once more increasing the mortality risk (13). The diagnosis of GH deficiency (GHD) is based on an inadequate response of GH to provocation. The consensus has recommended that in patients with defined pathology in the hypothalamic–pituitary region, one provocation test will suffice. The gold standard test is the insulin-induced hypoglycaemia test (ITT) and an alternative one is the growth hormone releasing hormone (GHRH) + arginine test (14, 15). According to current consensus statements for GH deficiency, most normal subjects respond to any provocative stimuli with a peak GH concentration $>5 \mu\text{g/l}$, while those severely GH deficient are defined as those with a peak GH response to any provocative stimuli of less than $3 \mu\text{g/l}$ (14). Among the many provocative tests available, we have used the GH-releasing effect of the synthetic GH secretagogue GH releasing peptide-6 (GHRP-6) coadministered with GHRH (GHRP-6 + GHRH) to diagnose adult GH deficiency (16). Synthetic growth hormone secretagogues (GHS) were developed for the GH-releasing effect and they release GH by stimulating GHS-receptor (GHS-R) shown to be present at the level of both the pituitary and the hypothalamus (17). GHS-Rs have also been shown to be present on pituitary adenomas of various cell types including somatotrophinomas (18). We and others have shown that GHS retain their GH-releasing effect in active acromegaly (19). Ghrelin, the natural ligand of the GHS-R, has potent GH-releasing activity and specificity. It was found to increase GH release in newly diagnosed acromegalic patients, a response that is positively correlated with basal IGF-I concentrations (20, 21). Aimaretti *et al.* have administered ghrelin to patients with isolated severe GH deficiency and have clearly shown that the GH response to ghrelin is severely impaired (22). Having in mind that in treated acromegalics, one provocative test will suffice to assess GH status and that ghrelin administration is a powerful specific stimulus for GH release, we challenged acromegalics post surgery with ghrelin, using a stringent criterion for diagnosing severe growth hormone deficiency (peak GH $<3 \mu\text{g/l}$). The reason for choosing this cutoff was to avoid any influence of age and obesity on the GH response to ghrelin administration.

Patients and methods

Subjects

Thirty-three patients (11 males and 22 females) aged 49.6 ± 1.9 years (range, 29–67 years) with a mean body mass index (BMI) of $29.2 \pm 0.9 \text{ kg/m}^2$

(range, $21.2\text{--}42.9 \text{ kg/m}^2$) were included in the study (Table 1). They all underwent transphenoidal surgery for a GH-secreting pituitary tumour 8.8 ± 1.1 years ago (range, 1–23 years). Acromegaly was diagnosed on the basis of clinical features, non-suppressible GH during an oral glucose tolerance test (OGTT) with elevated age-, gender- and BMI-adjusted serum IGF-I levels, and with evidence of pituitary adenoma by magnetic resonance imaging. Only six underwent additional conventional ($n = 5$) and gamma-knife ($n = 1$) irradiation 11.0 ± 3.0 years ago. Anterior pituitary function was assessed in all and five patients showed different kinds of anterior pituitary hormone deficiencies that were adequately replaced.

Eleven control (5 males and 6 females) age- and sex-matched subjects were also studied. The study was approved by the Hospital Ethical Committee and after informed consent, blood samples were obtained.

Hormonal investigations and lipids

At baseline (0800 h), after an overnight fast, samples for lipid levels (cholesterol and triglyceride), GH, IGF-I, IGF binding protein-3 (IGFBP-3) and leptin (in 29 out of 33 acromegalic patients) were taken and all samples were stored at -80°C until assayed.

The OGTT

After an overnight fast, an i.v. cannula was inserted at 0800 h and acromegalic patients and control subjects underwent a standard OGTT (75 g). Samples for GH were taken at 0, 30, 60, 90 and 120 min after OGTT. The OGTT was performed in all acromegalic patients except one female who had diabetes mellitus type 2 (patient no. 12 in Table 1).

The ghrelin test

A GH provocative test in all treated acromegalic patients and control subjects was performed using the ghrelin test. After an overnight fast, an i.v. cannula was inserted at 0800 h and acromegalic patients and control subjects underwent a ghrelin test ($1 \mu\text{g/kg}$ i.v. bolus). This dose of ghrelin has previously been shown to be effective in stimulating GH release in humans (20). Samples for GH were taken at 0, 15, 30 and 45 min after ghrelin administration. According to current consensus statements for GH deficiency, most normal subjects respond to any provocative stimuli with a peak GH concentration $>5 \mu\text{g/l}$, while those severely GH deficient have a peak GH response to any provocative stimuli of less than $3 \mu\text{g/l}$ (14). Thus, in our study a peak GH of less than $3 \mu\text{g/l}$ cut-off defined severe GHD, between 3 and $10 \mu\text{g/l}$ defined GH insufficiency

Table 1 Clinical characteristics and hormonal data in 33 treated acromegalic patients.

Patient no.	Sex (M/F)	Age (yrs)	BMI (kg/m ²)	No. of hormone deficits	IGF-I (ng/ml)	Nadir GH _{OGTT} (μg/l)	Peak GH _{ghrelin} (μg/l)	Leptin (ng/ml)
1	F	54	25.9	0	270.0	0.48	5.77	33.87
2	F	30	26.1	0	144.0	0.07	1.73	3.70
3	F	47	27.7	0	68.5	0.05	3.40	18.90
4	M	55	30.2	0	242.0	0.49	0.69	16.60
5	M	51	32.1	0	118.0	0.10	1.23	13.90
6	F	29	27.0	0	334.0	0.30	1.37	13.20
7	F	52	35.7	3	93.0	0.21	1.67	15.20
8	F	53	32.1	0	146.0	0.11	0.37	44.60
9	F	56	27.0	2	165.0	0.38	3.53	7.40
10	F	67	25.4	0	108.0	0.45	1.03	10.60
11	M	41	42.9	3	45.6	0.01	1.20	34.63
12	F	62	31.8	0	84.9	DM	1.71	46.76
13	F	56	36.5	0	148.0	0.33	8.70	59.50
14	F	40	25.0	0	210.0	1.03	8.10	35.93
15	M	44	26.3	0	124.0	0.23	20.60	2.60
16	M	49	29.0	3	282.0	0.75	11.45	7.80
17	F	47	23.9	0	174.0	0.51	30.33	13.78
18	F	57	35.8	0	281.0	0.29	19.50	32.49
19	F	67	23.6	0	60.9	0.18	25.70	5.54
20	F	34	22.1	0	207.0	0.10	16.87	25.75
21	M	65	28.7	0	138.5	0.56	16.57	—
22	F	34	21.2	0	288.0	1.29	31.99	8.80
23	M	50	28.3	0	305.0	0.91	15.30	7.30
24	M	47	33.6	0	267.0	1.67	12.75	66.10
25	F	67	23.5	0	403.0	0.70	16.37	25.20
26	F	58	31.6	0	208.1	2.71	4.43	—
27	F	62	31.2	0	400.0	0.26	8.84	—
28	F	40	37.2	0	449.0	2.20	6.13	44.80
29	M	36	27.9	0	452.0	15.67	40.14	8.78
30	M	46	32.6	0	543.0	2.13	34.35	10.66
31	F	62	24.7	0	765.0	2.96	39.12	5.70
32	F	35	22.4	0	398.0	1.80	19.6	27.28
33	M	44	35.4	1	426.0	1.24	5.82	—

DM, diabetes mellitus type 2.

(GHI) and more than 10 μg/l defined acromegalics with sufficient GH (GH-sufficient).

MO, USA) with a detection limit of 0.5 ng/ml, intra-assay CV of 6%, and interassay CV of 8%.

Methods

GH was assayed with an immunofluorimetric assay (Wallac Oy, Turku, Finland) with a GH sensitivity of 0.01 μg/l, an interassay coefficient of variation (CV) of 6.3% and an intra-assay CV of 4.2%.

IGF-I levels were measured using a chemiluminescent enzyme immunoassay with the Immulite Analyser (Diagnostic Product Corporation, Los Angeles, CA, USA); the limit of detection was 20.0 ng/ml, the intra-assay CV was 2.3–3.9% and the interassay CV was 3.7–8.1%.

IGFBP-3 levels were measured using a chemiluminescent enzyme immunoassay with the Immulite Analyser (Diagnostic Product Corporation). The intra-assay CV was 4.6–6%, and the interassay CV was 6.8–9.5%.

Leptin was measured in pooled serum from three samples taken at 15-min intervals at 0800 h after an overnight fast by commercial RIA (Linco, St Charles,

Statistical analysis

Hormone concentrations are presented and analysed as absolute values, as the nadir GH (the lowest value at any time after OGTT) value, or as the GH peak value after ghrelin administration (the maximum value of GH measured after stimulus). All results are expressed as means ± S.E.M. The integrated areas of secretion (area under the curve, AUC) were calculated using the trapezoidal method. IGF-I levels were compared with their age- and sex-appropriate absolute values normal range.

Statistical analysis was performed using one-way ANOVA and the Mann–Whitney test. Correlation between various parameters in patients was analysed using Pearson correlation. Analyses were performed using SPSS software (SPSS for Windows, 10.0; SPSS, Chicago, IL, USA). *P* values of less than 0.05 were regarded as indicating statistical significance.

Results

IGF-I levels and GH suppression after an oral glucose tolerance test

Table 1 represents the clinical characteristics and hormonal data for each individual treated acromegalic patient (basal serum IGF-I levels, post glucose GH nadir, peak GH response to ghrelin test and leptin levels).

All treated acromegalics ($n = 33$) were divided into 3 groups: cured, persistent and discordant according to the IGF-I levels and post glucose GH nadir: cured patients, $n = 21$ (63.6%) had normal IGF-I levels for age/sex (164.0 ± 17.7 ng/ml) and post glucose GH nadir < 1 μ g/l (0.33 ± 0.06 μ g/l); persistent acromegalics, $n = 6$ (18.2%) had elevated IGF-I levels for age/sex (505.5 ± 55.6 ng/ml), high basal GH levels (9.2 ± 5.1 μ g/l) and post glucose GH nadir > 1 μ g/l (4.3 ± 2.3 μ g/l); discordant acromegalic patients, $n = 6$ (18.2%) had discordant IGF-I and post glucose GH nadir values – 3 had normal IGF-I levels (254.4 ± 23.9 ng/ml) with post glucose GH nadir > 1 μ g/l (1.9 ± 0.4 μ g/l), and another 3 had elevated IGF-I levels (369.3 ± 32.2 ng/ml) and post glucose GH nadir < 1 μ g/l (0.6 ± 0.2 μ g/l).

A significant positive correlation between IGF-I levels and post glucose GH nadir ($r = 0.417$, $P = 0.02$) in all treated acromegalic patients was found.

The ghrelin test

In all cured acromegalic patients ($n = 21$) the mean GH peak in response to the ghrelin test was 8.6 ± 2.0 μ g/l, significantly lower compared with patients with persistent acromegaly (24.2 ± 6.5 , $P < 0.01$), and control

subjects (31.1 ± 2.5 μ g/l, $P < 0.001$). According to the peak GH response to the ghrelin test, cured acromegalic patients were further divided into three subgroups (Table 2, Fig. 1). (1) Those with severe GH deficiency (GHD, $n = 9$), (2) those with GH insufficiency (GHI, $n = 5$), and (3) those with sufficient GH reserve (GH-sufficient, $n = 7$). In the GHD subgroup only 2 acromegalic patients were treated with irradiation after the operation, in the GHI subgroup one patient was irradiated and in the GH-sufficient subgroup two patients were treated with irradiation after surgery.

Mean peak GH responses ($\pm$ S.E.M.) to the ghrelin test in GHD patients (1.2 ± 0.2 μ g/l) were significantly lower than in the GHI group (5.9 ± 1.1 μ g/l, $P < 0.001$), the GH-sufficient group (20.1 ± 2.4 μ g/l, $P < 0.0001$), the discordant patients (14.9 ± 3.9 , $P < 0.001$), patients with persistent acromegaly (24.2 ± 6.5 , $P < 0.0001$) and control subjects (31.1 ± 2.5 μ g/l, $P < 0.0001$; Table 2, Fig. 2). Mean AUC_{GH} ($\pm$ S.E.M.) values during the ghrelin test were significantly lower in GHD patients (38 ± 5 μ g/l per 45 min), compared with GHI patients (187 ± 39 μ g/l per 45 min, $P < 0.001$), GH-sufficient patients (676 ± 114 μ g/l per 45 min, $P < 0.0001$), discordant patients (443 ± 121 μ g/l per 45 min, $P < 0.0001$), patients with persistent acromegaly (849 ± 276 μ g/l per 45 min, $P < 0.0001$) and control subjects (1041 ± 180 μ g/l, $P < 0.0001$, Table 2).

Comparative clinical characteristics and hormonal data in 33 treated acromegalic patients and 11 control subjects are summarised in Table 2.

There was a significant negative correlation between the peak GH response to the ghrelin test in treated acromegalic patients and BMI ($r = -0.402$, $P = 0.023$), while no correlations between the GH peak in response to the ghrelin test and age and sex were observed.

Table 2 Clinical characteristics and hormonal data in 33 treated acromegalic patients and 11 control subjects.

	GHD	GHI	GH-suff	Discordant	Persistent	Control subjects
Number	9	5	7	6	6	11
Male/female	3/6	0/5	1/6	2/4	3/3	5/6
Age (yrs)	48.9 ± 4.4	50.6 ± 3.1	51.9 ± 4.5	53.0 ± 4.9	43.8 ± 4.0	40.2 ± 3.4
BMI (kg/m ²)	31.5 ± 1.8^j	28.4 ± 2.1^p	27.1 ± 1.8	28.2 ± 2.0	$30.0 \pm 2.4^{**}$	23.8 ± 0.8
GH at baseline (μ g/l)	$0.50 \pm 0.10^{e,g}$	1.10 ± 0.50^n	$2.81 \pm 0.62^{r,s}$	1.72 ± 0.41	$9.2 \pm 5.1^*$	3.8 ± 1.6
Nadir _{GH} (OGTT, μ g/l)	$0.22 \pm 0.06^{e,g}$	0.45 ± 0.16^m	$0.37 \pm 0.08^{q,s,u}$	$1.26 \pm 0.35^{w,x}$	4.3 ± 2.3^z	0.17 ± 0.07
AUC _{GH} (OGTT, μ g/l/120 min)	$47 \pm 12^{e,g}$	83 ± 38^m	$70 \pm 10^{r,s}$	$182 \pm 44^{w,y}$	860 ± 502^z	75.4 ± 25.5
Peak _{GH} (ghrelin test, μ g/l)	$1.2 \pm 0.2^{a,b,d,f,i}$	$5.9 \pm 1.1^{k,n,o}$	20.1 ± 2.4^t	14.9 ± 3.9^x	24.2 ± 6.5	31.1 ± 2.5
AUC _{GH} (ghrelin test μ g/l/45 min)	$38 \pm 5^{a,b,d,f,i}$	$187 \pm 39^{k,o}$	676 ± 114^t	443 ± 121^x	849 ± 276	1041 ± 180
IGF-I (ng/ml)	$146.2 \pm 29.7^{e,f}$	$172.3 \pm 33.5^{l,m}$	$181.1 \pm 31.0^{q,s}$	311.8 ± 31.3^v	505.5 ± 55.6^z	151.8 ± 24.1
IGFBP-3 (μ g/l)	3.7 ± 0.3^h	4.5 ± 0.5	4.1 ± 0.6	4.6 ± 0.7	$6.7 \pm 0.5^{**}$	3.9 ± 0.2
Leptin – male (ng/ml)	21.7 ± 6.5^c	—	5.2 ± 2.6	36.7 ± 29.4	9.7 ± 0.9	10.8 ± 0.8
Leptin – female (ng/ml)	20.0 ± 5.8	43.1 ± 8.2	19.4 ± 6.0	17.0 ± 8.2	25.9 ± 11.3	20.1 ± 4.0

GHD, GH deficient; GHI, GH insufficient; GH-suff, normal GH response.

^aGHD vs GHI ($P < 0.001$); ^bGHD vs GH-suff ($P < 0.0001$); ^cGHD vs GH-suff ($P < 0.05$); ^dGHD vs discordant ($P < 0.0001$); ^eGHD vs discordant ($P < 0.0001$); ^fGHD vs persistent ($P < 0.0001$); ^gGHD vs persistent ($P < 0.001$); ^hGHD vs persistent ($P < 0.001$); ⁱGHD vs control ($P < 0.0001$); ^jGHD vs control ($P < 0.05$); ^kGHI vs GH-suff ($P < 0.01$); ^lGHI vs discordant ($P < 0.05$); ^mGHI vs persistent ($P < 0.01$); ⁿGHI vs persistent ($P < 0.05$); ^oGHI vs control ($P < 0.0001$); ^pGHI vs control ($P < 0.05$); ^qGH-suff vs discordant ($P < 0.01$); ^rGH-suff vs discordant ($P < 0.05$); ^sGH-suff vs persistent ($P < 0.001$); ^tGH-suff vs control ($P < 0.01$); ^uGH-suff vs control ($P < 0.05$); ^vdiscordant vs persistent ($P < 0.01$); ^wdiscordant vs persistent ($P < 0.05$); ^xdiscordant vs control ($P < 0.001$); ^ydiscordant vs control ($P < 0.05$); ^zpersistent vs control ($P < 0.0001$); ^{**}persistent vs control ($P < 0.01$); ^{***}persistent vs control ($P < 0.05$).

Figure 1 Mean GH ($\pm$ S.E.M.) response to the ghrelin stimulation test in 21 treated acromegalic patients with normal IGF-I levels. GHD, GH deficiency; GHI, GH insufficiency; GH-suff, normal GH response. ^aGHD vs GHI ($P < 0.001$) and GH-suff ($P < 0.0001$); ^bGHI vs GH-suff ($P < 0.01$).

A significant positive correlation between the peak GH response to the ghrelin test in all acromegalic patients and the post glucose GH nadir ($r = 0.510$, $P = 0.003$) was found.

IGF-I levels

Mean IGF-I levels ($\pm$ S.E.M.) in all cured acromegalic patients ($n = 21$) were 164.0 ± 17.7 ng/ml, not different from control subjects (151.8 ± 24.1 ng/ml, $P > 0.05$). There were no differences in mean IGF-I levels between the patients with or without GHD diagnosed by the ghrelin stimulation test (GHD: 146.2 ± 29.7 ng/ml, GHI: 172.3 ± 33.5 ng/ml, GH-sufficient: 181.1 ± 31.0 ng/ml, $P > 0.05$; Table 2, Fig. 3). Three out of nine GHD patients had IGF-I concentrations below the limit of the normal range for age and two of them had multiple pituitary hormone deficiencies.

Figure 2 Mean GH peak response ($\pm$ S.E.M.) to the ghrelin stimulation test in treated acromegalic patients. GHD, GH deficiency; GHI, GH insufficiency; GH-suff, normal GH response. ^aGHD vs GHI ($P < 0.001$), GH-suff ($P < 0.0001$), discordant ($P < 0.0001$), persistent ($P < 0.0001$) and control subjects ($P < 0.0001$); ^bGHI vs GH-suff ($P < 0.01$), discordant ($P < 0.05$), persistent ($P < 0.05$) and control subjects ($P < 0.0001$); ^cGH-suff vs control subjects ($P < 0.01$); ^ddiscordant vs control subjects ($P < 0.001$).

Figure 3 IGF-I levels (mean $\pm$ S.E.M.) in three subgroups of treated acromegalic patients in biochemical remission ($n = 21$), divided according to the GH response to the ghrelin stimulation test. GHD, GH deficiency; GHI, GH insufficiency; GH-suff, normal GH response.

A significant positive correlation between the peak GH response to the ghrelin test in all acromegalic patients and IGF-I levels ($r = 0.545$, $P = 0.001$ Fig. 4) was found.

IGFBP-3 levels

Mean IGFBP-3 levels ($\pm$ S.E.M.) in all cured acromegalic patients ($n = 21$) were not different from control subjects (4.07 ± 0.3 µg/ml vs 3.9 ± 0.2 ng/ml, $P > 0.05$), but were significantly lower in comparison with patients with persistent acromegaly (6.7 ± 0.5 ng/ml, $P < 0.05$). Mean IGFBP-3 levels were not different between the patients with and without GHD diagnosed by the ghrelin stimulation test (GHD: 3.7 ± 0.3 µg/ml, GHI: 4.5 ± 0.4 µg/ml, GH-sufficient: 4.1 ± 0.6 µg/ml, $P > 0.05$; Table 2).

Leptin and lipid levels

In cured acromegalic patients ($n = 21$), mean leptin levels were 24.5 ± 4.4 ng/ml in females and 15.1 ± 5.5 ng/ml in males. Mean BMI values and leptin levels in females were not significantly different but BMI tended to be higher in GHD female patients in comparison with female GH-sufficient patients

Figure 4 Relationship between the peak GH level to the ghrelin test and IGF-I levels in treated acromegalic patients.

(BMI: $29.1 \pm 1.3 \text{ kg/m}^2$ vs $26.3 \pm 3.2 \text{ kg/m}^2$, leptin: $20.0 \pm 5.8 \text{ ng/ml}$ vs $19.4 \pm 6.0 \text{ ng/ml}$, $P > 0.05$; Table 2, Fig. 5a,b). Mean BMI values and mean leptin levels in GHD males were significantly higher compared with GH-sufficient males (BMI: $35.1 \pm 4.0 \text{ kg/m}^2$ vs $28.0 \pm 0.9 \text{ kg/m}^2$, leptin: $21.7 \pm 6.5 \text{ ng/ml}$ vs $5.2 \pm 2.6 \text{ ng/ml}$, $P < 0.05$; Table 2, Fig. 5a,b).

There were no differences in mean cholesterol levels between acromegalics with or without GHD diagnosed by the ghrelin stimulation test (GHD: $6.2 \pm 0.4 \text{ mmol/l}$, GHI: $6.6 \pm 0.7 \text{ mmol/l}$, GH-sufficient: $6.1 \pm 0.4 \text{ mmol/l}$, $P > 0.05$).

There were no differences in mean triglyceride levels between the patients with or without GHD diagnosed by the ghrelin stimulation test (GHD: $1.9 \pm 0.3 \text{ mmol/l}$, GHI: $1.9 \pm 0.1 \text{ mmol/l}$, GH-sufficient: $1.5 \pm 0.3 \text{ mmol/l}$, $P > 0.05$).

Neuroradiological imaging

On magnetic resonance imaging, pituitary morphology was reported to be normal in 7 GHD patients, 3 GHI patients, 5 GH-sufficient patients, 2 discordant patients and 1 patient with persistent acromegaly. Secondary

empty sella was reported in 2 GHD patients, 2 GHI patients, 2 GH-sufficient acromegalics, 1 discordant patient and 1 patient with persistent acromegaly. Residual pituitary adenoma was reported in 3 discordant patients and 4 patients with persistent acromegaly.

Discussion

Our data show for the first time that 9 out of 21 successfully treated acromegalic patients (by the present consensus criteria for cure of acromegaly - suppression of GH during OGTT $< 1 \mu\text{g/l}$ and normal IGF-I matched for age and sex and BMI (9)) are growth hormone deficient. This is particularly important because introducing more rigorous criteria for the interpretation of GH suppression during an OGTT (nadir GH $< 0.3 \mu\text{g/l}$) as suggested by some recent studies (23), could create even more GHD acromegalic patients. Moreover, in our study a post glucose GH nadir value of less than $1 \mu\text{g/l}$ correlated with GH peak levels after the ghrelin test and thus our results suggest caution when recommending more rigorous criteria for defining biochemical remission of acromegaly.

Assessment of GH status in the long term post-operative follow-up of acromegalics is difficult but with the use of very potent and highly specific GH secretagogues it is possible to assess the residual GH secretory capacity. A few studies have addressed this issue (24). As previously mentioned, synthetic GH secretagogues (GHS) were developed for their GH-releasing effects. GHS release GH by stimulating GHS-receptor (GHS-R) shown to be present at the level of both the pituitary and the hypothalamus (17). GHS-Rs have also been shown to be present on pituitary adenomas of various cell types including somatotrophinomas (18). We and others have shown that GHS retain their GH-releasing effect in active acromegaly (19). Ghrelin, the natural ligand of the GHS-R with more potent GH-releasing activity and specificity was found to elicit an increase in GH in newly diagnosed acromegalic patients, and the response was positively correlated with basal IGF-I concentrations (20, 21).

On the other hand, as mentioned before, the diagnosis of growth hormone deficiency is based on an inadequate response of GH to provocation (25). In the case of acromegalic patients who are operated on for a defined pathology in the pituitary region, one test will suffice. Aimaretti *et al.* have recently shown that the ghrelin test can be used for the diagnosis of severe isolated GH deficiency. They have demonstrated an impaired GH response to ghrelin administration with a higher GH response to the ghrelin test with respect to ITT and GHRH + arginine (22). In the present study, ghrelin administration resulted in significantly lower GH responses when compared with

Figure 5 (a) Mean BMI values ($\pm$ S.E.M.) in two subgroups of treated acromegalic patients in biochemical remission ($n=21$), divided according to the GH response to the ghrelin stimulation test. GHD, severe GH deficiency; GH-suff, normal GH response. $*P < 0.05$ GHD vs GH-suff. (b) Mean leptin levels ($\pm$ S.E.M.) in two subgroups of treated acromegalic patients in biochemical remission ($n=21$), divided according to the GH response to the ghrelin stimulation test. GHD, severe GH deficiency; GH-suff, normal GH response. $*P < 0.05$ GHD vs GH-suff.

healthy subjects. The cut-off value for defining severe isolated GHD in acromegalic patients, $\text{GH} < 3 \mu\text{g/l}$, was chosen in order to avoid the influence of obesity on the GH response to ghrelin administration. We demonstrated a peak $\text{GH} < 3 \mu\text{g/l}$ in 9/21 (43%) cured acromegalics. Only 2 GHD cured patients received conventional irradiation to the hypothalamo–pituitary axis. Thus the majority had sufficient GHRH release from the hypothalamus required for the action of growth hormone secretagogues. Only 3 patients had other anterior pituitary hormone deficiencies. The degree of severity of GH deficiency is usually based on the presence of additional anterior pituitary hormone deficits (26). Most of our acromegalic patients thus had severe isolated GH deficiency. It has been hypothesised that isolated GHD mostly reflects impaired activity of the hypothalamic GHRH-secreting neurons (27). However, the fact that the ghrelin-induced GH response in isolated GHD has been shown to be higher than the GH elicited by GHRH + arginine in the study of Aimaretti *et al.* (22), and the fact that 5 out of 21 cured acromegalics received radiotherapy which could have affected hypothalamic GHRH secreting neurons, points towards a primary somatotroph defect in our operated acromegalics.

In another study with the use of the GHRH + arginine test in cured acromegalics, a high incidence of GH deficiency has also been reported (28).

It is recommended that glucose-suppressed GH levels be interpreted in conjunction with those of IGF-I since IGF-I is a GH-regulated peptide. The validity of serum IGF-I levels as the best predictor of overall disease status in acromegaly has been recommended by several studies (23). Seeking the optimal target range for IGF-I during treatment of adult growth hormone disorders seems to be difficult and there has been little agreement as to the levels of IGF-I that constitute appropriate safe targets of therapy (29). The normal ranges for IGF-I values in adult populations have recently been re-evaluated showing intervals that are narrower than previously considered (30). Although low plasma concentrations of IGF-I might help in the diagnosis of GH deficiency, the specificity and sensitivity of the test is poor and most studies show that about one third of patients with GHD diagnosed by stimulated GH levels have IGF-I levels in the normal range (29, 31). Not a lot of GH is needed to get normal IGF-I levels. Thus, which GH level is truly normal or abnormal with respect to IGF-I or other aspects of GH action is not clear.

Assessment of residual disease activity in patients with acromegaly following treatment is a problem because no sensitive clinical parameters are available and there is no well-defined clinical endpoint that defines cure. The difficulties of assessing residual clinical activity in acromegaly are due to differences in GH sensitivity, height of GH and variability of IGF-I concentrations, and limited cohort sizes due to the

low prevalence of the disorder. Evolving clinical strategies for optimising control of acromegaly are necessary, and one of them could be the assessment of changes in body composition after treatment. Growth hormone deficient adults have increased abdominal and visceral fat, decreased lean body mass and decreased total body water compared with age-matched healthy controls. Changes in body composition are well recognised effects of GH replacement therapy and are more prominent in males than in females (32). Leptin, the adipocyte-derived hormone is produced in proportion to fat stores. We have previously shown that leptin concentrations are higher in operated patients with acromegaly than in naive disease after adjustment for age, BMI and gender, suggesting that a rise in leptin levels might precede changes in body composition after successful transphenoidal surgery (33, 34). Leptin concentrations in these studies were significantly higher in patients with cured acromegaly who were diagnosed as GH deficient compared with those cured but with normal GH secretory capacity. This is particularly true for male acromegalic patients, consistent with that observed in GH-deficient hypopituitary patients (35). Lipid abnormalities in GHD acromegalics have not been found in our study possibly due to a small number of GHD acromegalics. It would also be useful to know how skeletal health and quality of life are affected in these patients.

In conclusion, very low residual GH secretory capacity assessed by the ghrelin provocation test in acromegalic patients in remission together with high leptin levels confirms the diagnosis of GH deficiency.

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References

- 1 Rajasoorya C, Holdaway J, Wrightson P, Scott D & Ibbertson H. Determinants of clinical outcome and survival in acromegaly. *Clinical Endocrinology* 1994 **41** 95–102.
- 2 Bates A, Van't Hoff W, Jones J & Clayton R. An audit of outcome of treatment in acromegaly. *Quarterly Journal of Medicine* 1993 **86** 293–299.
- 3 Lombardi G, Colao A, Ferone D, Marzullo P, Landi ML, Longobardi S, Iervolino E, Cuocolo A, Fazio S, Merola B & Sacca L. Cardiovascular aspects in acromegaly: effects of treatment. *Metabolism* 1996 **45** 57–60.
- 4 Melmed S. Acromegaly and cancer: not a problem. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2929–2934.
- 5 Jenkins P & Besser M. Acromegaly and cancer: a problem. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 2935–2941.
- 6 Orme S, McNally R, Cartwright R & Belchetz P. Mortality and cancer incidence in acromegaly: a retrospective cohort study. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3411–3418.
- 7 Holdaway I, Rajasoorya R & Gamble G. Factors influencing mortality in acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 667–674.

- 8 Dimaraki E, Jaffe C, De Mott-Friberg R, Chandler W & Barkan A. Acromegaly with apparently normal GH secretion: implications for diagnosis and follow-up. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 3537–3542.
- 9 Giustina A, Barkan A, Casanueva F, Cavignini F, Frohman L, Ho K, Veldhuis J, Wass J, Von Werder K & Melmed S. Criteria for cure of acromegaly: a consensus statement. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 526–529.
- 10 Melmed S, Casanueva F, Cavignini F, Chanson P, Frohman L, Grossman A, Ho K, Kleinberg D, Lamberts S, Laws E, Lombardi G, Vance ML, Werder KV, Wass J, Giustina A & Acromegaly Treatment Consensus Workshop Participants. Guidelines for acromegaly management. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4054–4058.
- 11 Consensus Statement. Biochemical assessment and long-term monitoring in patients with acromegaly: statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 3099–3102.
- 12 Freda P. Current concepts in the biochemical assessment of the patient with acromegaly. *Growth Hormone and IGF Research* 2003 **13** 171–184.
- 13 Murray R & Shalet S. Adult growth hormone replacement: lessons learned and future directions. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 4427–4428.
- 14 Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 379–381.
- 15 Ghigo E, Aimaretti G, Arvat E & Cammani F. Growth hormone-releasing hormone combined with arginine or growth hormone secretagogues for the diagnosis of growth hormone deficiency in adults. *Endocrine* 2001 **15** 29–38.
- 16 Popovic V, Leal A, Micic D, Koppeschaar H, Torres E, Paramo C, Obradovic S, Dieguez C & Casanueva FF. GH-releasing hormone and GH-releasing peptide-6 for diagnostic testing in GH-deficient adults. *Lancet* 2000 **356** 1137–1142.
- 17 Muccioli G, Ghe C, Ghigo MC, Papotti M, Arvat E, Boghen MF, Nilsson MH, Deghenghi R, Ong H & Ghigo E. Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland. *Journal of Endocrinology* 1998 **157** 99–106.
- 18 Korbonits M, Jacobs RA, Aylwin SJ, Burrin JM, Dahia PL, Monson JP, Honegger J, Fahlbush R, Trainer PJ, Chew SL, Besser GM & Grossman AB. Expression of the growth hormone secretagogue receptor in pituitary adenomas and other neuroendocrine tumors. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3624–3630.
- 19 Popovic V, Damjanovic S, Micic D, Petakov M, Dieguez C & Casanueva F. Growth hormone (GH) secretion in active acromegaly after the combined administration of GH-releasing hormone and GH-releasing peptide-6. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 456–460.
- 20 Popovic V, Miljic D, Micic D, Damjanovic S, Arvat E, Ghigo E, Dieguez C & Casanueva FF. Ghrelin main action on the regulation of growth hormone release is exerted at hypothalamic level. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 3450–3453.
- 21 Arosio M, Ronchi C, Gebbia C, Pizzinelli S, Conte D, Capiello V, Epaminonda P, Cesana BM, Beck-Peccoz P & Peracchi M. Ghrelin administration affects circulating pituitary and gastro-enteropancreatic hormone in acromegaly. *European Journal of Endocrinology* 2004 **150** 27–32.
- 22 Aimaretti G, Baffoni C, Broglio F, Janssen J, Corneli G, Deghenghi R, van der Lely A, Ghigo E & Arvat E. Endocrine responses to ghrelin in adult patients with isolated childhood-onset growth hormone deficiency. *Clinical Endocrinology* 2002 **56** 765–771.
- 23 Puder J, Nilavar S, Post K & Freda P. Relationship between disease-related morbidity and biochemical markers of activity in patients with acromegaly. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1972–1978.
- 24 Murray R, Peacey S, Rahim A, Toogood A, Thorner M & Shalet S. The diagnosis of growth hormone deficiency (GHD) in successfully treated acromegalic patients. *Clinical Endocrinology* 2001 **54** 37–44.
- 25 Dattani M & Preece M. Growth hormone deficiency and related disorders: insights into causation, diagnosis and treatment. *Lancet* 2004 **363** 1977–1987.
- 26 Toogood AA, Beardwell CG & Shalet SM. The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. *Clinical Endocrinology* 1994 **41** 511–516.
- 27 Shalet SM, Toogood A, Rahim A & Brennan BM. The diagnosis of growth hormone deficiency in children and in adults. *Endocrine Reviews* 1998 **19** 203–223.
- 28 Conceicao F, Fisker S, Andersen M, Kaal A, Jorgensen J, Vaisman M & Christiansen JS. Evaluation of growth hormone stimulation tests in cured acromegalic patients. *Growth Hormone and IGF Research* 2003 **13** 347–352.
- 29 Mukherjee A, Monson J, Jonsson P, Trainer P & Shalet S on behalf of the KIMS International Board. Seeking the optimal target range for insulin-like growth factor I during the treatment of adult growth hormone disorders. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5865–5870.
- 30 Brabant G, von zur Muhlen A, Wuster C, Ranke MB, Kratzsch J, Keiss W, Ketelslegers J, Wilhelmens L, Halten L, Saller B, Mattson A, Wilde J, Schemer R & Kann P, on behalf of the German KIMS board. Serum insulin-like growth factor-I reference values for an automated chemiluminescence immunoassay system: results from a multicenter study. *Hormone Research* 2003 **60** 53–60.
- 31 Molitch M. Diagnosis of GH deficiency in adults - how good do the criteria need to be? *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 473–476.
- 32 Drake WM, Howell SJ, Monson JP & Shalet SM. Optimizing GH therapy in adults and children. *Endocrine Reviews* 2001 **22** 425–450.
- 33 Damjanovic S, Neskovic A, Petakov M, Popovic V, Macut D, Vukojevic P & Joksimovic MM. Clinical indicators of biochemical remission in acromegaly: does incomplete disease control always mean therapeutic failure? *Clinical Endocrinology* 2005 **62** 410–417.
- 34 Damjanovic S, Petakov M, Raicevic S, Micic D, Marinkovic J, Dieguez C, Casanueva FF & Popovic V. Serum leptin concentrations in patients with acromegaly before and after correction of hypersomatotropism by transphenoidal surgery. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 147–154.
- 35 Johansson AG, Engstrom B, Ljunghall S, Karlsson FA & Burman P. Gender differences in the effects of long term growth hormone (GH) treatment on bone in adults with GH deficiency. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 2002–2007.

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