CLINICAL STUDY

The GHRH/GHRP-6 test for the diagnosis of GH deficiency in elderly or severely obese men

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Abstract

Objective and design: Ageing and obesity result in decreased activity of the GH/IGF-I axis and concomitant impaired GH responses to secretory stimuli. We therefore determined the validity of the GH cut-off value of 15.0 µg/l in the GH-releasing hormone (GHRH)/GH releasing peptide-6 (GHRP-6) test for the diagnosis of GH deficiency in elderly or severely obese men.

Methods: We performed a combined GHRH/GHRP-6 test in ten elderly men (mean age 74 years; mean body mass index (BMI) 24.6 kg/m²), nine obese men (mean age 47 years; mean BMI 40.6 kg/m²) and seven healthy male controls (mean age 51 years, mean BMI 24.3 kg/m²). After assessment of fasting plasma GH, IGF-I and IGF-binding protein-3 (IGFBP-3), GHRH (100 µg) and GHRP-6 (93 µg) were given intravenously as a bolus injection. Repeated GH measurements were performed for two hours.

Results: Both peak GH levels and areas under the curve (AUC) were significantly lower in the obese than in the controls (peak 13.2 vs 53.4 µg/l, $P = 0.001$; AUC 707 vs 3250 µg/l × 120 min; $P = 0.001$). Mean GH response in the elderly was lower than in the controls (peak 35.0 µg/l; AUC 2274 µg/l × 120 min), but this was not statistically significant. In contrast, GH peak levels in seven obese men remained below the cut-off level of 15.0 µg/l associated with severe GH deficiency. All others had GH peak levels exceeding this threshold. IGFBP-3 levels were significantly lower in the elderly than in the controls (1.35 vs 2.05 mg/l, $P = 0.001$). Baseline GH or IGF-I did not differ significantly between groups.

Conclusions: GH responses following GHRH/GHRP-6 administration were significantly reduced in severely obese men, but were not significantly reduced in elderly men, despite a negative trend. Our data indicate that the cut-off GH level of 15.0 µg/l after GHRH + GHRP-6 administration for the diagnosis of severe GH deficiency cannot be used in severely obese men.

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Introduction

Both ageing and obesity are associated with low plasma growth hormone (GH) levels, reduced spontaneous GH secretion and, in particular in obesity, a blunted GH response to all known GH secretory stimuli (1–5). In addition, plasma insulin-like growth factor I (IGF-I) levels are attenuated in the elderly, whereas IGF-I levels in the obese are usually reported in the low to normal range (6, 7). Although normal ageing is associated with signs and symptoms which resemble GH deficiency, such as diminished lean body mass, increased body fat mass, decreased protein synthesis and reduced bone mineral density (8, 9), Toogood *et al.* (10) have demonstrated that elderly subjects with pituitary disease and organic GH deficiency have

specific alterations in body composition, in particular characterised by an increase in fat mass. In obesity, the low levels of the lipolytic hormone GH may contribute to perpetuation of the obese status, although alterations in circulating IGF-I-binding proteins (IGFBPs) could also account for a state of pseudo-hyposomatotropism (5, 11). The underlying pathophysiological mechanisms responsible for the changes in activity of the somatotrophic axis in ageing and obesity have not yet been elucidated, but alterations in the balance between GH-releasing hormone (GHRH) and somatostatin, possibly as a consequence of changes in circulating free fatty acids, insulin or ghrelin, are likely to play a role.

At present, the insulin tolerance test (ITT) is considered to be the test of choice for diagnosing GH

deficiency in adults with hypothalamic or pituitary disease (12). Unfortunately, this test has several major drawbacks: the reproducibility is relatively poor (13) while the test is contra-indicated in several clinical conditions, including ischaemic heart disease, cerebrovascular disease and epilepsy. Moreover, the test is inconvenient for both patient and hospital staff because the induced hypoglycaemia often leads to unpleasant symptoms and necessitates the permanent presence of a physician. Finally, besides an ITT a second stimulation test is recommended for the diagnosis of isolated GH deficiency (12).

An alternative for the ITT is therefore desirable and recently the GHRH/GH-releasing peptide-6 (GHRP-6) test has been proposed as such (14). GHRP-6 is a synthetic GH secretagogue that elicits a dose-dependent and almost specific GH release by binding to the GH secretagogue receptor, for which ghrelin has been discovered to be the natural ligand (15, 16). The GHRH/GHRP-6 test has been shown to be highly sensitive and specific in diagnosing GH deficiency at a threshold peak GH level of 13–15 µg/l (14, 17). Furthermore, this test does not share the aforementioned drawbacks of the ITT. However, although the control group in these studies was characterised by a wide range in age (15–92 years) and body mass index (BMI; 17.8–32.0 kg/m²), the validity of the GHRH/GHRP-6 test has not been assessed separately in subjects with morbid obesity and only to a limited extent in the elderly or less severely obese (18–20).

To fill this gap, we studied GH responses following combined GHRH and GHRP-6 administration in elderly or severely obese (BMI > 35 kg/m²) and healthy control male subjects. In addition, we related the outcome of the GH release following GHRH and GHRP-6 stimulation to baseline GH, IGF-I and IGFBP-3, which may be involved in the underlying mechanisms of decreased activity of the GH/IGF-I axis in ageing and obesity.

Subjects and methods

Study subjects

Ten healthy elderly Caucasian men and nine otherwise healthy obese Caucasian men participated in this study together with seven healthy male Caucasian controls who were matched for age with the obese group and matched for BMI with the elderly group (see Table 1

for further clinical characteristics). For the elderly group, the age inclusion criterion was between 65 and 85 years, for the other groups between 30 and 60 years. For the obese group, the BMI inclusion criterion was set higher than or equal to 35 kg/m², for the other groups between 20 and 28 kg/m². Other inclusion criteria were an absent history of recent medical illness (including diabetes, hyperlipidaemia, hypertension and pituitary disease), and that they were taking no medication known to influence the somatotropic axis. Written informed consent was obtained from each subject. The protocol was approved by our institutional ethical committee.

Test procedures

On the test day, the subjects were fasting and were asked not to smoke or to perform strenuous exercise before the test. All tests started between 0830 and 0900 h. After height and weight measurements, an intravenous catheter was placed in the forearm for blood sampling and drug administration. Initial blood samples were drawn at *t* = 0 for measurement of basal levels of GH, IGF-I and IGFBP-3 and subsequently GHRH (100 µg; GHRH Ferring; Ferring Pharmaceuticals Ltd, Hoofddorp, The Netherlands) and GHRP-6 (93.1 µg; His-DTrp-Ala-DPhe-Lys-NH₂; Clinalfa, Laufelfinger, Switzerland) were administered simultaneously i.v. as a bolus injection. Blood was then sampled at 15, 30, 45, 60, 90 and 120 min after injection of GHRH and GHRP-6 for assessment of GH responses. The subjects remained fasting, non-smoking and sitting during the tests.

Biochemical analyses

GH was measured using an immunometric technique on an Immulite Analyser (Diagnostic Products, Los Angeles, CA, USA). The lower limit of detection was 0.01 µg/l; the interassay variations were 9.7, 5.6, 4.4 and 5.2% at 0.13, 0.80, 4.2 and 15.4 µg/l respectively (*n* = 69). One microgram per litre corresponds to 2.6 mIU/l (WHO International Reference Preparation 80/505). IGF-I was measured using a immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was 6.0 ng/ml and interassay variations were 8.7, 5.8 and

Table 1 Clinical characteristics.

	Elderly (<i>n</i> = 10)	Obese (<i>n</i> = 9)	Controls (<i>n</i> = 7)
Age (years)	74 ± 1.4 (67–81)	47 ± 3.8 (30–60)	51 ± 2.3 (41–58)
Weight (kg)	78 ± 2.2 (69–85)	138 ± 7.1 (115–167)	79 ± 4.9 (60–93)
Height (cm)	178 ± 1.6 (168–186)	184 ± 2.1 (174–193)	179 ± 3.4 (171–192)
Body mass index (kg/m ²)	24.6 ± 0.77 (20.6–28.0)	40.6 ± 1.7 (35.0–47.4)	24.3 ± 1.0 (20.8–28.1)

All data are mean ± S.E.M. Ranges are in parentheses.

6.5% at mean IGF-I plasma levels of 33, 174 and 445 ng/ml respectively ($n = 115$). IGFBP-3 was measured by specific RIA, as described previously (21). The lower limit of detection was 0.002 mg/l (absolute concentration) and interassay variations amounted to 9.3, 6.9 and 10.2% at mean plasma IGFBP-3 levels of 0.97, 2.0 and 3.0 mg/l respectively ($n = 50$).

Statistical analyses

GH responses to GHRH and GHRP-6 were evaluated by calculating the area under the curve (AUC) over a time-period of 120 min, as well as by the peak responses. For between-group comparisons, a non-parametric Kruskal–Wallis test was used. When a significant difference was found, two sample Mann–Whitney tests were performed to specify the differences. Statistical significance was accepted at $P < 0.05$ (two-tailed). Pearson's correlation coefficients were used to determine the associations between the GH responses and age, BMI and baseline levels of GH, IGF-I and IGFBP-3. All data are expressed as means $\pm$ S.E.M.

Results

Mean baseline GH and IGF-I levels did not differ significantly between groups (Table 2), while significantly lower IGFBP-3 levels were observed in the elderly in comparison with the controls.

Peak GH responses (Fig. 1) were blunted in the obese group as compared with the controls (13.2 ± 2.3 vs 53.4 ± 11.7 μ g/l, $P = 0.001$). There was no significant difference in peak GH levels between the elderly and control groups (35.0 ± 7.0 vs 53.4 ± 11.7 μ g/l). Mean calculated AUC was significantly lower in the obese than in the

Figure 1 GH responses to the simultaneous administration of GHRH and GHRP-6 in the elderly, obese and control groups. All data represent mean values $\pm$ S.E.M. The mean AUC was significantly lower in the obese than in the control group ($P < 0.001$, Mann–Whitney).

controls (707 ± 145 vs 3250 ± 651 μ g/l $\times$ 120 min, $P = 0.001$), but no significant difference in AUC was found between the elderly and the controls (2274 ± 443 vs 3250 ± 651 μ g/l $\times$ 120 min).

We observed a significant negative correlation between BMI and GH response when the obese and control groups were combined, in terms of both peak GH level ($r = -0.80$, $P < 0.001$; Fig. 2) and AUC ($r = -0.83$, $P < 0.001$; Fig. 2). Age and GH response were not significantly correlated (Fig. 2). Baseline GH levels (Table 2) were positively correlated with GH responses when all three groups were combined, in terms of both peak GH level ($r = 0.51$, $P = 0.008$) and AUC ($r = 0.47$, $P = 0.014$). For the elderly and control groups together, a negative correlation was found between age and IGFBP-3 levels ($r = -0.74$, $P = 0.001$). Finally, a positive correlation was found between IGFBP-3 and IGF-I levels for all groups together ($r = 0.47$, $P = 0.015$). GH peak levels in seven out of nine obese subjects remained below the cut-off level of 15.0 μ g/l as described by Popovic *et al.* (14). All other subjects had peak GH levels above this value.

Discussion

The primary objective of the present study was to assess the validity of the previously described cut-off peak GH level of 15.0 μ g/l for the GHRH/GHRP-6 test in diagnosing GH deficiency in elderly or severely obese men (14). Our findings indicated that the reported threshold value is valid in elderly men, as all elderly subjects had peak GH levels above 15.0 μ g/l, as did all control subjects. In contrast, our data clearly demonstrated that the cut-off level of 15 μ g/l should not be used in subjects with a BMI exceeding 35 kg/m², as seven out of nine subjects in this severely obese group had peak GH levels below 15.0 μ g/l after GHRH and GHRP-6 stimulation, and would therefore be falsely classified as GH deficient. Judging by the lowest GH peak level that we observed in this specific obese population, the threshold level for the diagnosis of GH deficiency in severely obese men should be set below 6.1 μ g/l. These findings are in line with a previous report by Ozata *et al.* (19), who observed peak GH levels following a GHRH/GHRP-6 test ranging from 6.2 to 7.3 μ g/l in three subjects with a mean BMI of 50.3 ± 2.0 kg/m². As no other data have been reported regarding GH

Table 2 Baseline hormone concentrations.

	Elderly ($n = 10$)	Obese ($n = 9$)	Controls ($n = 7$)
GH (μ g/l)	0.74 ± 0.35	0.36 ± 0.17	3.00 ± 1.60
IGF-1 (ng/ml)	109 ± 10	126 ± 18	132 ± 9
IGF-BP3 (mg/l)	$1.35 \pm 0.12^*$	1.76 ± 0.19	2.05 ± 0.07

All data are mean $\pm$ S.E.M. IGFBP-3 was significantly lower in elderly than in controls ($*P = 0.001$, Mann–Whitney).

Figure 2 Correlations (Pearson) between GH response to GHRH/GHRP-6, represented as GH peak vs BMI (upper panel), GH AUC vs BMI (middle panel) in obese and control groups and between age and AUC in elderly and control groups (lower panel).

peak levels after GHRH and GHRP-6 stimulation in severely obese (BMI > 35 kg/m²) healthy subjects or in severely obese GH-deficient patients, the specificity

and sensitivity of this threshold level cannot be determined exactly.

Previous studies regarding the reliability of other GH stimulation tests in obese subjects indicate that validated GH cut-off levels in a non-obese population may lack validity in obesity. However, the number of published studies is limited, and BMI levels vary considerably between studies (18, 22, 23). All obese men who participated in our study had BMI values exceeding 35 kg/m², a condition which has not been studied separately on previous occasions using the GHRH/GHRP-6 or other GH stimulation tests. According to the BMI distribution in the control group in the study by Popovic *et al.* (14), the GH cut-off level of 15 µg/l can probably be safely used in subjects with a BMI up to 32.0 kg/m². However, the authors indicate that GH peak levels between 10 and 20 µg/l represent a grey zone, without 100% sensitivity and specificity; additional (circumstantial) evidence is needed for the confirmation or rejection of GH deficiency. As four of our obese subjects had peak GH levels below 10 µg/l, we have concluded that this grey zone should be adjusted in subjects with a BMI above 35 kg/m². As we also observed a negative correlation between BMI and GH response, BMI should always be taken into consideration when interpreting the outcome of the GHRH/GHRP-6 test if the peak GH level is within this grey zone and the patient's BMI exceeds 35 kg/m². It was recently suggested that GH levels after GHRH/GHRP-6 in a group of less obese subjects (mean BMI 34.2 kg/m²) did rise to 14.7 µg/l and higher (maximum 86 µg/l), indicating that the cut-off point of 15 µg/l can probably be used safely in subjects with a lower BMI for the rejection of the diagnosis of GH deficiency (18). This observation is complementary to the present study, as we did not evaluate GH response in subjects with a BMI between 28 and 35 kg/m². Our findings may seem to disagree with previous reports that the GH response of the GHRH/GHRP-6 test is independent of the degree of obesity (14, 18). However, these studies were all performed in less obese subjects (BMI < 35 kg/m²). Our conclusion that obesity can be an important confounder when interpreting the outcome of the GHRH/GHRP-6 test for the diagnosis of GH deficiency is therefore limited to subjects with a BMI that exceeds 35 kg/m².

We used the cut-off GH level reported by Popovic *et al.* (14). It should be noted that this multicentre study was performed using different GH assays according to local experience. Cut-off levels for different GH stimulation tests may also vary somewhat according to the assay used, and therefore should be validated in the local setting. However, large numbers of both control subjects and GH-deficient patients would be needed to define specific cut-off GH levels for each individual clinic, which has major practical implications.

In the present study, we used slightly lower GHRH and GHRP-6 doses than in the study by Popovic *et al.* (14) for the obese group, as we used fixed doses of

GHRH and GHRP-6 instead of weight-adjusted doses, similar to those used by Cordido *et al.* (18). As the agents used exert their GH-releasing effect quickly, and as their distribution volume is small and relatively independent of fat mass, it is unlikely that peak pituitary concentrations within 30 min after injection vary to a large extent between obese and non-obese subjects.

When searching for alternatives for the ITT, it should be noted that the use of the conventional cut-off level of 3.0 µg/l for the diagnosis of GH deficiency has not been validated properly in obese subjects. In the study performed by Cordido *et al.* (18), GH peaks during the ITT varied from 2.3 to 16.6 µg/l in subjects with a mean BMI of 34.2 kg/m², and three subjects would have been classified as GH deficient (of which one severely GH deficient) according to the ITT, while the response was normal during the GHRH/GHRP-6 test. Separate data regarding GH responses during the ITT in subjects with a BMI exceeding 35 kg/m² have not been published. This may be partly explained by the fact that the performance of an appropriate ITT with an adequate hypoglycaemia is relatively difficult in this population.

As expected, the GH response to GHRH and GHRP-6 was significantly impaired in the obese subjects as compared with the controls, in terms of both peak GH level and AUC. The GH response of the elderly subjects was decreased compared with that of the controls, but this did not reach the level of significance, possibly as a consequence of the small group sizes. This finding confirms that when interpreting the outcome of a GHRH/GHRP-6 test in an elderly person, age should also be considered, in particular when the GH peak level is within the grey zone between 10 and 20 µg/l. It has previously been shown that elderly subjects with organic GH deficiency are characterised by changes in body fat mass, plasma lipids and bone metabolism, and may benefit from GH substitution therapy (10, 24). Previously, the arginine stimulation test was shown to be a reliable test for the diagnosis of GH deficiency in elderly subjects (25). From the present and previously published data, we conclude that the GHRH/GHRP-6 test is a useful and validated tool for the diagnosis of GH deficiency in the elderly, as it is not affected by age and can be performed without risk in this patient group.

The exact mechanism of the impaired GH response in the obese is still largely unknown. Increased circulating free fatty acid levels, hyperinsulinaemia and/or decreased plasma ghrelin levels, all associated with obesity, may contribute to an attenuated GH response in the obese (26–28). Probably as a consequence of these and other contributing factors, the balance between GHRH, ghrelin and somatostatin at the hypothalamic–pituitary axis is altered in obesity (29, 30). An enhanced somatostatinergic tone has been demonstrated in obesity as pyridostigmine, a somatostatin tone-lowering agent, potentiates the GH release to GHRH (31). Nevertheless,

after pyridostigmine the GHRH-mediated GH response in obese subjects remains attenuated in comparison with non-obese controls, suggesting that other mechanisms are also involved.

Taken together, the results in this study show that the somatotroph response to the GHRH/GHRP-6 test is independent of age, but dependent on BMI. In particular, our data indicate that the cut-off level of 15.0 µg/l in the GHRH/GHRP-6 test as previously described (14) should not be used in severe obesity, i.e. in subjects with a BMI exceeding 35 kg/m². If the concept of a grey zone (for which additional circumstantial evidence is required for the diagnosis of GH deficiency) is considered when using the GHRH/GHRP-6 test, the lower limit should be set at or below 6.1 µg/l in obese subjects with a BMI > 35 kg/m², whereas the cut-off GH level of 15.0 µg/l can be used safely in elderly subjects.

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References

- 1 Cordido F, Penalva A, Peino R, Casanueva FF & Dieguez C. Effect of combined administration of growth hormone (GH)-releasing hormone, GH-releasing peptide-6, and pyridostigmine in normal and obese subjects. *Metabolism* 1995 **44** 745–748.
- 2 Arvat E, Gianotti L, Grottoli S, Imbimbo BP, Lenaerts V, Deghenghi R, Camanni F & Ghigo E. Arginine and growth hormone-releasing hormone restore the blunted growth hormone-releasing activity of hexarelin in elderly subjects. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 1440–1443.
- 3 Veldhuis JD, Iranmanesh A, Ho KK, Waters MJ, Johnson ML & Lizarralde G. Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 51–59.
- 4 Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA & Bain RP. Impaired growth hormone secretion in the adult population: relation to age and adiposity. *Journal of Clinical Investigation* 1981 **67** 1361–1369.
- 5 Frystyk J, Vestbo E, Skjaerbaek C, Mogensen CE & Orskov H. Free insulin-like growth factors in human obesity. *Metabolism* 1995 **44** S37–S44.
- 6 Cordido F, Casanueva FF, Vidal JJ & Dieguez C. Study of insulin-like growth factor I in human obesity. *Hormone Research* 1991 **36** 187–191.
- 7 Copeland KC, Colletti RB, Devlin JT & McAuliffe TL. The relationship between insulin-like growth factor-I, adiposity, and aging. *Metabolism* 1990 **39** 584–587.
- 8 Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Hintz R, Ho K, Laron Z, Sizonenko P, Sonksen PH, Tanaka T & Thorne M. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 382–395.
- 9 Corpas E, Harman SM & Blackman MR. Human growth hormone and human aging. *Endocrine Reviews* 1993 **14** 20–39.
- 10 Toogood AA, Adams JE, O'Neill PA & Shalet SM. Body composition in growth hormone deficient adults over the age of 60 years. *Clinical Endocrinology* 1996 **45** 399–405.
- 11 Frystyk J, Skjaerbaek C, Vestbo E, Fisker S & Orskov H. Circulating levels of free insulin-like growth factors in obese subjects: the

- impact of type 2 diabetes. *Diabetes Metabolism Research and Reviews* 1999 **15** 314–322.
- 12 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.
 - 13 Hoeck HC, Vestergaard P, Jakobsen PE & Laurberg P. Test of growth hormone secretion in adults: poor reproducibility of the insulin tolerance test. *European Journal of Endocrinology* 1995 **133** 305–312.
 - 14 Popovic V, Leal A, Micic D, Koppeschaar HPE, 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.
 - 15 Bowers CY. Synergistic release of growth hormone by GHRP and GHRH: scope and implication. In *Growth Hormone Secretagogues in Clinical Practice*, pp 1–25. Eds BB Bercu & RF Walker. New York: Marcel Dekker, 1998.
 - 16 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo M & Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999 **402** 656–660.
 - 17 Petersenn S, Jung R & Beil FU. Diagnosis of growth hormone deficiency in adults by testing with GHRP-6 alone or in combination with GHRH: comparison with the insulin tolerance test. *European Journal of Endocrinology* 2002 **146** 667–672.
 - 18 Cordido F, Alvarez-Castro P, Isidro ML, Casanueva FF & Dieguez C. Comparison between insulin tolerance test, growth hormone (GH)-releasing hormone (GHRH), GHRH plus acipimox and GHRH plus GH-releasing peptide-6 for the diagnosis of adult GH deficiency in normal subjects, obese and hypopituitary patients. *European Journal of Endocrinology* 2003 **149** 117–122.
 - 19 Ozata M, Dieguez C & Casanueva FF. The inhibition of growth hormone secretion presented in obesity is not mediated by the high leptin levels: a study in human leptin deficiency patients. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 312–316.
 - 20 Popovic V, Pekic S, Doknic M, Micic D, Damjanovic S, Zarkovic M, Aimaretti G, Corneli G, Ghigo E, Dieguez C & Casanueva FF. The effectiveness of arginine+GHRH test compared with GHRH + GHRP-6 test in diagnosing growth hormone deficiency in adults. *Clinical Endocrinology* 2003 **59** 251–257.
 - 21 de Vries BB, Robinson H, Stolte-Dijkstra I, Tjon Pian Gi CV, Dijkstra PF, van Doorn J, Halley DJ, Oostra BA, Turner G & Niermeijer ME. General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation. *Journal of Medical Genetics* 1995 **32** 764–769.
 - 22 Cordido F, Dieguez C & Casanueva FF. Effect of central cholinergic neurotransmission enhancement by pyridostigmine on the growth hormone secretion elicited by clonidine, arginine, or hypoglycemia in normal and obese subjects. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 1361–1370.
 - 23 Cordido F, Fernandez T, Martinez T, Penalva A, Peino R, Casanueva FF & Dieguez C. Effect of acute pharmacological reduction of plasma free fatty acids on growth hormone (GH) releasing hormone-induced GH secretion in obese adults with and without hypopituitarism. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 4350–4354.
 - 24 Toogood AA, Adams JE, O'Neill PA & Shalet SM. Elderly patients with adult-onset growth hormone deficiency are not osteopenic. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 1462–1466.
 - 25 Toogood AA, Jones J, O'Neill PA, Thorner MO & Shalet SM. The diagnosis of severe growth hormone deficiency in elderly patients with hypothalamic–pituitary disease. *Clinical Endocrinology* 1998 **48** 569–576.
 - 26 Lanzi R, Manzoni MF, Andreotti AC, Malighetti ME, Bianchi E, Sereni LP, Caumo A, Luzi L & Pontiroli AE. Evidence for an inhibitory effect of physiological levels of insulin on the growth hormone (GH) response to GH-releasing hormone in healthy subjects. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 2239–2243.
 - 27 Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E & Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001 **50** 707–709.
 - 28 Casanueva FF, Villanueva L, Dieguez C, Diaz Y, Cabranes JA, Szoke B, Scanlon MF, Schally AV & Fernandez Cruz A. Free fatty acids block growth hormone (GH) releasing hormone-stimulated GH secretion in man directly at the pituitary. *Journal of Clinical Endocrinology and Metabolism* 1987 **65** 634–642.
 - 29 Tannenbaum GS, Lapointe M, Gurd W & Finkelstein JA. Mechanisms of impaired growth hormone secretion in genetically obese Zucker rats: roles of growth hormone-releasing factor and somatostatin. *Endocrinology* 1990 **127** 3087–3095.
 - 30 Ghigo E, Procopio M, Maccario M, Bellone J, Arvat E, Campana S, Boghen MF & Camanni F. Repetitive GHRH administration fails to increase the response to GHRH in obese subjects. Evidence for a somatotrope defect in obesity? *Hormone and Metabolic Research* 1993 **25** 305–308.
 - 31 Cordido F, Casanueva FF & Dieguez C. Cholinergic receptor activation by pyridostigmine restores growth hormone (GH) responsiveness to GH-releasing hormone administration in obese subjects: evidence for hypothalamic somatostatinergic participation in the blunted GH release of obesity. *Journal of Clinical Endocrinology and Metabolism* 1989 **68** 290–293.

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