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ORIGINAL ARTICLE

Growth hormone nadir during oral glucose load depends on waist circumference, gender and age: normative data in 231 healthy subjects

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Summary

Objective (i) To analyse the predictors of GH suppression after standard glucose load (oGTT) in the healthy population and (ii) to establish the 97th percentile of GH nadir post-oGTT according to these variables.

Design Analytical, retrospective.

Measurements GH nadir after oGTT.

Subjects Two hundred and thirty-one healthy subjects (113 women, 118 men 15–80 years) were studied.

Results The GH nadir after glucose load ranged from 0·01 (<assay detection limit) to 0·65 μg/l was higher in women and was inversely correlated with age, BMI, waist circumference, waist/hip, total cholesterol, triglycerides, basal and maximal glucose and basal insulin levels and directly correlated with basal GH levels, IGF-I SDS and HDL-cholesterol (P values ranging 0·004–<0·0001). On multistep regression analysis, the best predictors of nadir GH levels were waist circumference ($t = -9{\cdot}64$, $P < 0{\cdot}0001$), gender ($t = -3{\cdot}86$, $P = 0{\cdot}0001$) and age ($t = -3{\cdot}63$, $P = 0{\cdot}0003$). The results of comparative analysis among subjects grouped according to these variable showed different results in GH nadir in premenopausal women with waist circumference ≤88 cm (97th percentile 0·65 μg/l), in premenopausal women with waist circumference ≤88 cm and in men of any age with waist circumference ≤102 cm (97th percentile 0·33 μg/l) and in subjects of either gender and any age with waist circumference >88 cm in women and 102 cm in men (97th percentile 0·16 μg/l).

Conclusions The results of this study show that GH nadir after oGTT should be analysed according to gender, menopausal status and waist circumference. The GH cut-off should be limited to the assay used.

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Introduction

In the presence of elevated age-normalized IGF-I levels, the currently used biochemical criteria to diagnose active acromegaly include a mean 24 h GH level >2·5 μg/l and/or a GH level of >1 μg/l after oral glucose load (oGTT).[1] Although the GH response to oGTT was suggested to have limited additional diagnostic value over a single fasting GH level when IGF-I levels are elevated,[2] this test is used worldwide to confirm the diagnosis of acromegaly, and to evaluate the activity of the disease, after surgery.

There is a discussion nowadays concerning the optimal nadir GH postglucose to diagnose active acromegaly as some recent studies have indicated that the old cut-off of 1 μg/l is probably inaccurate.[3] In fact, GH nadir after oGTT in acromegaly is reported to be correlated with gender,[4–8] age,[5–9] BMI[7,8] and, more importantly, the GH assay used.[8] These data are in analogy with the GH cut-off after stimulation test in GH deficiency currently considered in a different way in different age and BMI categories.[10–15]

Nevertheless, limited data are currently available on the GH nadir postglucose in the healthy population, so it is difficult to draw from the literature the optimal GH cut-off postglucose to use in acromegaly. In the study from Freda *et al.*,[16,17] who used an ultrasensitive GH assay, it was reported that GH nadir postglucose was well below the threshold of 1 μg/l, representing the cut-off currently used. Similarly, Arafat *et al.*[8] demonstrated a threefold difference among different assays in nadir GH levels, supporting the need of specific assay-related cut-off. Normative data are, thus, essential to propose the use of nadir GH after oGTT to establish the activity of acromegaly when a reliable IGF-I assay is available.

This study has a twofold aim: (i) to study the GH response after oGTT in a large control population to understand the most important predictors of such response (ii) to establish the 97th percentile of GH response to oGTT in physiological conditions to propose reliable cut-off values, at least limited to the assay we have used.

Subjects and methods

Study design

This is an analytical study to evaluate GH cut-off during oGTT in the general population. This study takes advantage from data collected in a large, prospective database to investigate the effects of GH and IGF-I on the cardiovascular system (no. 63/97) approved by the Ethical Committee of the 'Federico II' University of Naples.

Subjects

Two hundred and thirty-one subjects (113 women, 118 men 15–80 years), recruited among the medical and paramedical personnel of our Department and their relatives, and the patients' relatives from 1 January 2002 to 31 December 2006 were enrolled in the study. During this period, GH and IGF-I assays did not change in our laboratory. The exclusion criteria for controls were (i) personal history of cardiovascular or pituitary disease as reported in interviews with individual subjects; (ii) previous or current treatments with drugs known to interfere with glucose or lipid metabolism or to influence blood pressure; (iii) previous treatment with corticosteroids for longer than 2 weeks; (iv) previous or current treatment with estrogens or testosterone for longer than 12 weeks; (v) smoking of more than 15 cigarettes/day and alcohol abuse (more than three glasses of wine/day).

All patients and controls signed an informed consent to participate in the study that was conducted in accordance with the II Declaration of Helsinki on human experimentations. The subjects' profile at the time of testing is shown in Table 1.

Study protocol

At study entry, all the subjects underwent complete metabolic and endocrine screening. After an overnight fast, serum IGF-I levels were assayed in duplicate in a single sample at the time 0 of the GH profile; GH levels were measured every 30 min over a period of two hours, and the mean value of 5 samples (named 'mean GH') was used for statistics. The oGTT was performed within one week of the spontaneous profile by measuring GH, glucose and insulin levels, every 30 min for 2 hours after oral ingestion of 75 g of glucose in 250 ml of water. The lowest GH value after glucose load (named 'nadir GH') was considered for statistical analysis (Table 1). As reference for diagnosis of diabetes and classification of waist circumference, we used modern guidelines.[18,19]

Table 1. Characteristics of the study subjects

	All	Females	Males	*P*
No.	231	113	118	1·000
Age (years)	44 ± 20	45 ± 20	44 ± 21	0·72
Body mass index (kg/m^2)	28·2 ± 6·6	28·7 ± 7·9	27·8 ± 5·1	0·19
Waist (cm)	89·4 ± 16·8	85·9 ± 17·6	92·7 ± 15·4	0·041
Waist/hip	0·86 ± 0·14	0·83 ± 0·15	0·90 ± 0·12	0·006
IGF-I levels (SDS)	0·10 ± 0·76	0·23 ± 0·85	−0·02 ± 0·64	0·065
GH levels (μg/l)				
Mean	0·47 ± 0·38	0·56 ± 0·40	0·36 ± 0·31	<0·0001
Nadir	0·13 ± 0·13	0·17 ± 0·15	0·10 ± 0·08	<0·0001
GH suppression (%)	63·5 ± 21·8	62·6 ± 22·7	64·4 ± 21·0	0·041

P values refer to comparison between women and men by Mann–Whitney *U*-test.

Assays

GH levels were assayed by immunoradiometric assays (IRMA) manufactured by Diagnostic System Laboratories Inc. (Webster, TX, USA). The sensitivity of the assay was 0·01 μg/l. The standard curve was calibrated against WHO 1st IRP 80/505 (1 mg = 2·6 IU). The intra- and inter-assay coefficients of variation (CVs) were, respectively, 4·1% and 8·7%. All values measured as below the detection limit were arbitrary assigned to 0·01 μg/l. Serum IGF-I was measured by IRMA after ethanol extraction using Diagnostic System Laboratories Inc. In our laboratory,[20] the normal ranges in men aged ≤20, 21–30, 31–40, 41–50, 51–60, 61–70 and >70 years were 180–625, 118–475, 102–400, 100–306, 95–270, 88–250, 78–200 μg/l, respectively, whereas in women, they were 151–530, 118–450, 100–390, 96–288, 90–250, 82–200, 68–188 μg/l, respectively. The sensitivity of the assay was 0·8 μg/l. The intra-assay CVs were 3·4%, 3·0% and 1·5% for the low, medium and high points of the standard curve, respectively. The inter-assay CVs were 8·2%, 1·5% and 3·7% for the low, medium and high points of the standard curve. The standard curve was calibrated against WHO 1st IRR 87/518 and performance verified by immunoassay.

Statistical analysis

In the text, results were expressed as mean ± SD unless otherwise specified, in the figures they are expressed as mean ± SEM or interquartile range to improve reading. The statistical analysis was performed by MedCalc Software for Windows (MedCalc, Mariakerke, Belgium) package using nonparametric tests after the Shapiro and Wilk normality test. Analysis of continuous variables between two groups was compared with the Mann–Whitney *U*-test while among three of more groups by the Kruskal–Wallis test followed by the all pair comparison Dwass–Steel–Chritchlow–Fligner test. Analysis of repeated variables was performed with the Freidman test followed by the all pair comparison Dwass–Steel–Chritchlow–Fligner test.

To analyse the best predictors of nadir GH postglucose in healthy subjects, we first performed a correlation study by calculating the Spearman's rho with the 95% confidence interval: in this analysis, we included patients' gender (female = 1, males = 2), age, BMI, waist circumference, waist to hip ratio, mean GH, IGF-I levels as standard deviation score (SDS), serum total cholesterol, HDL-cholesterol and triglycerides levels, glucose and insulin levels as fasting sample and as peak response after glucose and glucose tolerance (normal glucose tolerance = 0, impaired glucose tolerance = 1, diabetes mellitus = 2) (Table 2).

The stepwise multiple linear regression then assessed which parameters better predicted nadir GH postglucose: in this analysis

Table 2. Results of the univariate analysis by calculating the Spearman's rank correlation coefficient

Nadir GH levels (μg/l) *vs*	*r*	95%CI		*P*
Gender	−0·30	−0·41	−0·18	<0·0001
Age	−0·24	−0·36	−0·12	0·0002
BMI	−0·46	−0·55	−0·35	<0·0001
Waist	−0·57	−0·65	−0·49	<0·0001
Waist/hip ratio	−0·53	−0·62	−0·43	<0·0001
Basal mean GH levels (μg/l)	0·76	0·70	0·80	<0·0001
IGF-I SDS	0·19	0·06	0·31	0·004
Basal glucose levels (mmol/l)	−0·44	−0·54	−0·33	<0·0001
Maximal glucose increase during oGTT (mmol/l)	−0·31	−0·42	−0·19	<0·0001
Basal insulin levels (mUI/l)	−0·36	−0·47	−0·25	<0·0001
Maximal insulin increase during oGTT (mUI/l)	−0·26	−0·38	−0·14	<0·0001
Total cholesterol levels (mmol/l)	−0·33	−0·44	−0·21	<0·0001
HDL-cholesterol levels (mmol/l)	0·22	0·09	0·33	0·009
Triglycerides levels (mmol/l)	−0·28	−0·39	−0·16	<0·0001
Glucose tolerance	−0·39	−0·50	−0·28	<0·0001

oGTT, oral glucose tolerance test.

were entered only the variables with a P value <0·01 in the univariate analysis. The subjects were then grouped according to the results of multistep regression analysis to calculate the 97th percentile of GH nadir in each individual group.

Results

The GH nadir after glucose load ranged from 0·01 (below the detection limit of the assay) to 0·65 μg/l and was significantly correlated with several variables (Table 2). On multistep regression analysis, the best predictors of nadir GH levels after oGTT were waist circumference ($t = -9{\cdot}64$, $P < 0{\cdot}0001$), gender ($t = -3{\cdot}86$, $P = 0{\cdot}0001$) and age ($t = -3{\cdot}63$, $P = 0{\cdot}0003$).

Waist circumference

The GH curve after oGTT in the subjects with waist circumference below 88 cm (in women) and 102 cm (in men) had significantly higher GH levels than those with levels above this threshold during the entire curve (Fig. 1, top panel), and nadir GH level was also significantly lower in the latter group (Fig. 2, top panel). The 97th percentile of GH nadir in the subjects with waist circumference below or above the threshold were respectively 0·47 and 0·17 μg/l.

Gender

Women had significantly higher GH levels than men during the entire curve, as well as nadir GH level (Table 1). When the female group was divided into the women in pre- and those in postmenopausal stage, the former had significantly higher GH nadir than the latter (Figs 1 and 2, middle panel). No difference was found between postmenopausal women and men.

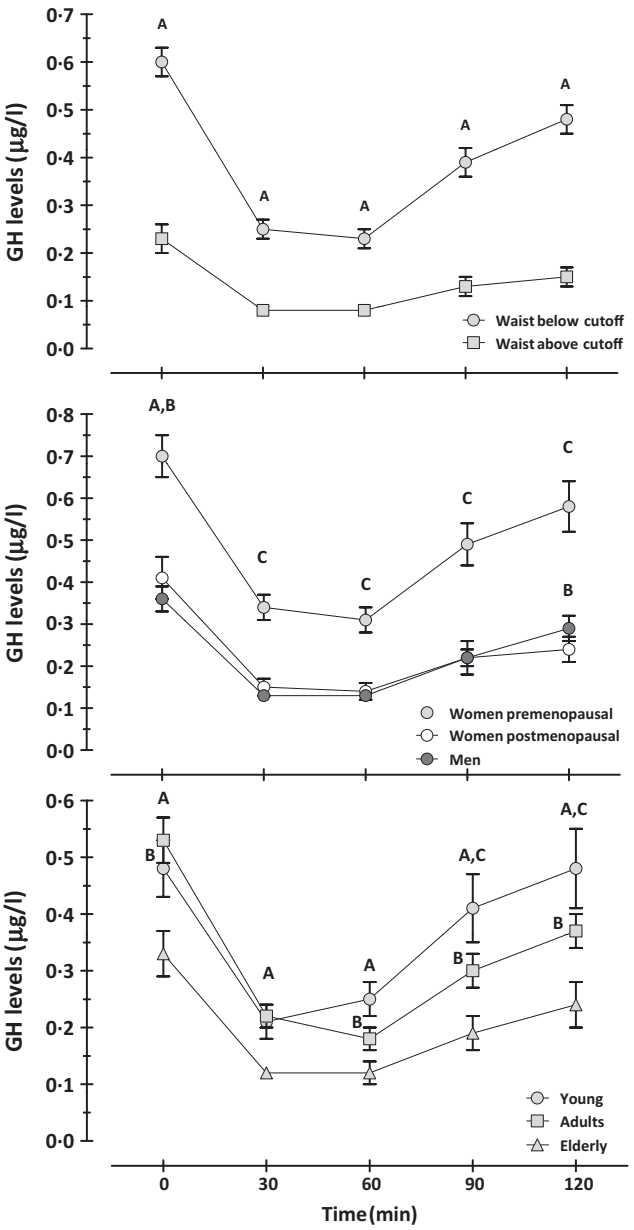

Fig. 1 The GH profile after oral ingestion of 75 g glucose in subjects grouped according to waist circumference (top), gender (middle) or age (bottom). The analysis was made by the Kruskal–Wallis test followed by the all pair comparison Dwass–Steel–Chritchlow–Fligner test in individual groups. Top panel: A = $P < 0{\cdot}0001$ *vs* waist circumference above the threshold. Middle panel: A = $P < 0{\cdot}0001$ *vs* men; B = $P < 0{\cdot}05$ *vs* postmenopausal women; C = $P < 0{\cdot}0001$ *vs* postmenopausal women and men. Bottom panel: A = $P < 0{\cdot}001$ *vs* elderly; B = $P < 0{\cdot}01$ *vs* elderly; C = $P < 0{\cdot}01$ *vs* adults. Data are shown as mean ± SEM.

The 97th percentile of GH nadir in premenopausal women, postmenopausal women and men were, respectively, 0·55, 0·31 and 0·31 μg/l.

Age

The subjects were arbitrarily divided according to their age as follows: young from 15–25 years, adults from 26–65 years and elderly

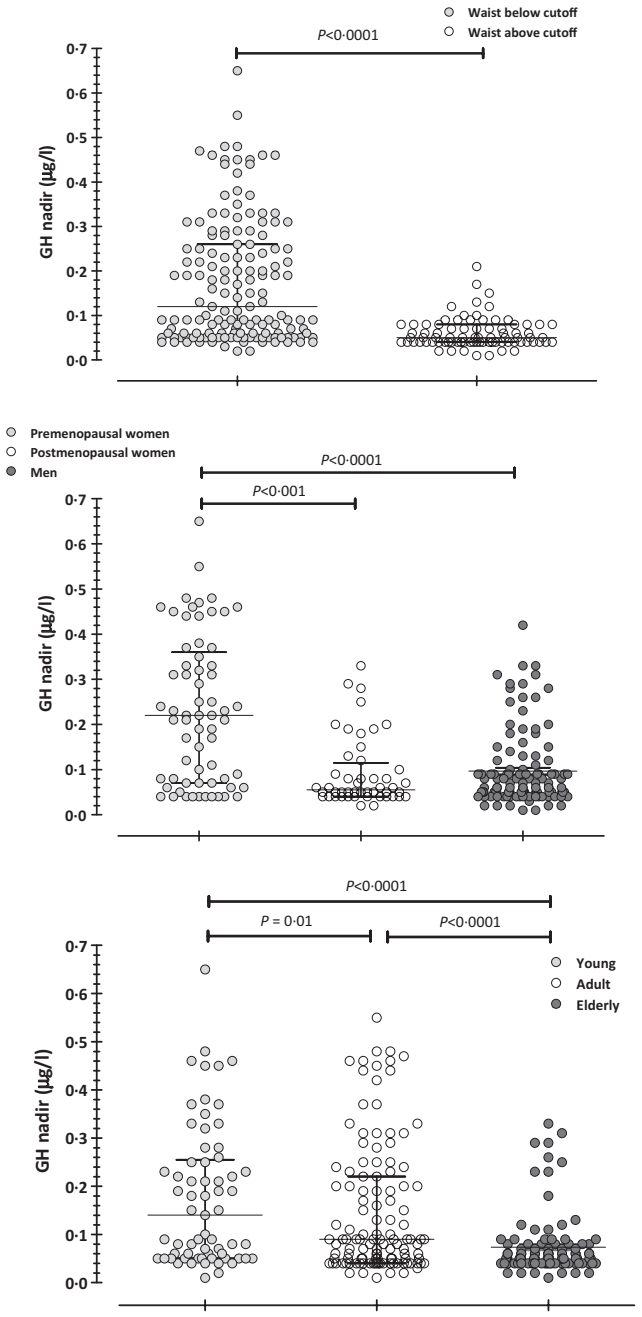

Fig. 2 GH nadir after standard glucose load in the subjects grouped according to waist circumference (top), gender and menopausal status (middle) or age (bottom). Statistical analysis was performed by Mann–Whitney (top panel) and Kruskal–Wallis test followed by the all pair comparison Dwass–Steel–Chritchlow–Fligner test (middle and bottom panel). Data are shown as individual data and median and interquartile range.

from 66 to 80 years. The young and adult patients had similar baseline GH levels as well as GH levels 30 min after glucose ingestion (Fig. 1, bottom panel). In the remaining time course, adult patients had significantly lower GH levels than the young. The elderly had significantly lower GH levels than the young and the adults during oGTT (Fig. 1, bottom panel). Nadir GH level was significantly lower in the adults and elderly than in the young and in the elderly than in the adults (Fig. 2, bottom panel).

The 97th percentile of GH nadir in the young, adult and elderly subjects were, respectively, 0·48, 0·47 and 0·29 µg/l.

Nadir GH after oGTT according to waist circumference, gender and age

The subjects were, thus, subdivided according to waist circumference (≤ or >88 cm in women, ≤ or >102 cm in men), gender (females, males), menopausal stage (pre- or postmenopause) and age [15–25 years (young), 26–65 years (adults) and >65 years (elderly)] (Fig. 3a,b). The results of comparative analysis among these groups showed that premenopausal women with waist circumference below 88 cm had the highest higher GH nadir in the population; postmenopausal women and men with waist circumference below 88 cm or 102 cm, respectively, had a similar GH nadir as well as women and men of any age with waist circumference above 88 cm or 102 cm, respectively (Fig. 3a). The 97th percentile of GH nadir was 0·60 µg/l in premenopausal women with a waist circumference ≤88 cm, 0·33 µg/l in in men of any age with waist circumference ≤102 cm and in premenopausal women with a waist circumference ≤88 cm, and 0·17 µg/l in men and women of any age with a waist circumference >88 cm in women and 102 cm in men (Table 3).

Discussion

The oral glucose tolerance test is used worldwide to establish the status of acromegaly either on first diagnosis and after treatment. The GH nadir cut-off currently used is 1 µg/l[1] even if some criticisms exist on the validity of this cut-off[4–9,16,17] and of the test itself in the diagnosis and follow-up of acromegaly.[2,3,21,22]

Most criticisms are based on the well-known influence of gender, age and BMI on GH and IGF-I secretion, as demonstrated consistently in GH deficiency.[10–15] In a study including a large series of hypopituitary patients, we recently demonstrated that waist circumference was the strongest predictor of GH peak after GHRH plus arginine test and was superior to BMI.[15] No data are currently available on the role of waist circumference on GH suppression after glucose load neither in the healthy population nor in acromegaly.

A limitation of several previous studies concerning the use of nadir GH after oGTT in acromegaly is also the lack of standardization of the GH assay used. As clearly demonstrated by Arafat *et al.*,[8] the use of different GH assay kits produced results up to three times different each other, indicating that GH cut-off should be gender, age, BMI (or waist circumference) and assay related.

Because only limited data are available concerning the GH response to glucose load in the healthy population (Table 4), we designed this retrospective study to investigate the major determinants of such a response and to clarify the best GH cut-off values, limited to the assay we used. We had the opportunity to study a large population of healthy subjects in a prospective database that had the main outcome measures in the cardiovascular impact of GH and IGF-I in physiology and diseases associated with alterations of this axis. A rather large number of control subjects have been enrolled, and GH and IGF-I have been assayed with the same

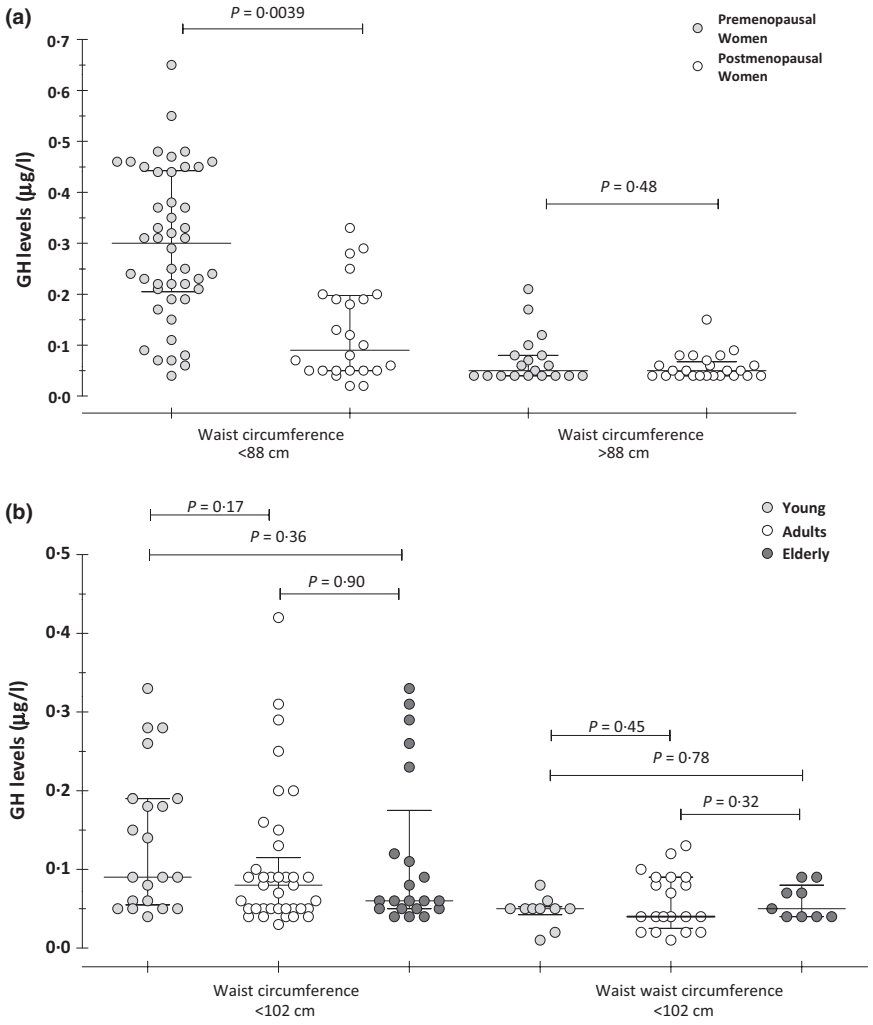

Fig. 3 GH nadir after standard glucose load in the women (a) and men (b) grouped according to waist circumference, menopausal status and age. Significance refers to Kruskal–Wallis test followed by the all pair comparison Dwass–Steel–Chritchlow–Fligner test. Data are shown as individual data and median and interquartile range.

Table 3. GH nadir after glucose load (75 g) in the controls grouped according to waist circumference, gender, age and menopausal stage. Data are shown as Mean ± SD, 95%CI, 97th percentiles

	No.	Mean ± SD	95%CI	97th percentile (µg/l)
Premenopausal women with waist circumference <88 cm	46	0·30 ± 0·15	0·25–0·34	0·65
Postmenopausal women with waist circumference <88 cm	24	0·13 ± 0·09	0·09–0·17	0·33
All men with waist circumference <102 cm	79	0·12 ± 0·09	0·09–0·14	0·33
All subjects with waist circumference >88 or 102 cm (according to gender)	82	0·06 ± 0·03	0·05–0·07	0·16

kits during the study period. One limitation of our study is that the study population was recruited with the intention to exclude major cardiovascular disease and thus might represent a selected population though diabetes or hypertension discovered at study entry were not exclusion criteria for receiving the test. Anyhow, subjects of both gender, with a very large age range and with different BMI, have been included, so that the variables known to display the greatest influence on GH secretion are well represented in our series. Another drawback of our study is in the use of only one GH and IGF-I assay: in accordance with Arafat *et al.*,[8] our results are best restricted to this assay method.

The role of body weight and body composition

A few studies have investigated the role of BMI on nadir GH post-glucose. Both Vierhapper *et al.*[7] and Arafat *et al.*[8] have shown that obese or overweight subjects have a significant reduction in GH nadir after glucose when compared to lean subjects. In contrast, Freda *et al.*[16,17] did not find any difference with BMI in their series. In our series, there was a strong correlation between BMI and nadir GH, in line with previous studies. However, on stepwise regression analysis, waist circumference (as indirect indicator of central obesity) was a stronger predictor than BMI. None of the previous

Table 4. Literature data on GH response to oral glucose tolerance test (oGTT) in healthy subjects

Publication				
Authors	No. cases	Men/women	Glucose dose	Notes
Chapman *et al.*[4]	15	9/6	100 g	Gender related
Freda *et al.*[16]	25	14/11	100 g	No gender, age or BMI difference
Freda *et al.*[17]	46	26/20	100 g	No gender or age difference.
Costa *et al.*[5]	56	26/30	75 g	Gender related. Age related only in women
Vierhapper *et al.*[7]	196	69/127	Not known	Age and BMI related
Arafat *et al.*[8]	213	66/147	75 g	Gender, age, BMI and assay related
Colao *et al.* (current study)	231	118/113	75 g	Waist circumference, gender, menopausal status and age related

studies has reported on waist circumference, so our results are unique in this respect. A waist circumference above the currently accepted threshold[19] is associated with an approximately threefold lower nadir GH levels than in premenopausal females and twofold lower than in postmenopausal women and in men with lower waist circumference.

The role of gender

With the exception of the studies by Freda *et al.*,[16,17] in all the others, women reportedly had higher nadir GH levels than men.[4,5,7,8] There is a well-defined gender difference in GH and IGF-I secretion, likely played by the action of estrogens on the liver.[23] Basal and mean 24 h GH levels have been reported to be higher in women than men and their patterns of GH secretion may differ.[24–26] In our study, we could confirm the existence of a gender difference in the GH response to oGTT and demonstrated that premenopausal women had higher levels than postmenopausal ones, in whom results were similar to men. These data reinforce the physiological role of estrogens in modulating the relationships between GH and IGF-I. In our setting, premenopausal women with waist circumference below 88 cm had the highest GH nadir of the entire series (0·65 μg/l), while no difference was found between postmenopausal women and men within the same waist circumference group. No other data are available on the role of menopausal status of GH response to glucose. One limitation of our study is that we could not calculate whether time from menopause could have influenced the response, as this was not in the scope of the current study. The role of gender has also been reported in patients with acromegaly, with no data related to menopausal status.[6,9]

The role of age

There is a well-known reduction of GH and IGF-I secretion with age. An age-related response after glucose load has been reported by Chapman *et al.*,[4] Vierhapper *et al.*[7] and Arafat *et al.*,[8] while Costa *et al.*[5] found an age-dependent decrease in GH response only in women and Freda *et al.*[16,17] did not find any age effect in her series. We found that age entered into the regression model, but difference into GH nadir was not significant across age groups when subjects were stratified for waist circumference and gender. In any case, if the other variables were not considered, the subjects aged <65 years had a nadir GH levels 1·6 times higher than elderly ones.

Conclusion

With the limitation of the assay kit used, we have demonstrated that waist circumference and gender (and menopausal status in particular) are the major determinants of the GH response after oGTT, followed by age. Subjects with waist circumference above 88 cm in women and 102 cm in men independently from age had the lowest GH nadir postglucose (97th percentile value 0·16 μg/l), while premenopausal women with a waist circumference below 88 cm had the highest GH nadir (97th percentile value 0·65 μg/l). Postmenopausal women and men with a waist circumference below the safe threshold had GH nadir in between (97th percentile value of 0·33 μg/l). Thus, GH nadir postglucose cut-off should be limited to single GH assays and should consider waist circumference, gender and menopausal status as they influence this response. These cut-off values should then be validated in the acromegalic population to analyse their diagnostic power.

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Disclosure statement

During the study period, A. Colao was the recipient of unrestricted grants by Ipsen, Italfarmaco, Novartis, Pfizer for research programs in acromegaly, received lectures fees by Ipsen, Italfarmaco, Novartis, Pfizer and has been member of the Scientific Boards of Novartis and Ipsen. G. Lombardi received lecture fees for lectures on acromegaly from Ipsen, Italfarmaco, Novartis. R. Pivonello received lecture fees by Novartis. None of the other authors have conflict of interest to disclose.

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