Growth Hormone Response during Oral Glucose Tolerance Test: The Impact of Assay Method on the Estimation of Reference Values in Patients with Acromegaly and in Healthy Controls, and the Role of Gender, Age, and Body Mass Index

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Context: Besides the measurement of IGF-I, GH suppression during an oral glucose tolerance test is recommended to assess the biochemical status in acromegaly. However, the development of highly sensitive and specific GH assays necessitates a critical reevaluation of criteria for diagnosis and follow-up of disease activity.

Objective: Our objective was to evaluate the between-method discrepancies in GH determinations by different immunoassays considering further confounders like age, gender, and body mass index (BMI).

Design, Subjects, and Methods: We measured GH during a 75-g oral glucose tolerance test in 46 acromegaly patients (18 controlled, 28 uncontrolled; 19 men; 31–63 yr; BMI 26.4 ± 0.4 kg/m^2) and 213 healthy subjects (66 men; 20–76 yr; BMI 30 ± 0.5 kg/m^2), using three different commercially available assays [Immulite (Diagnostic Products Corp., Los Angeles, CA), Nichols (Nichols Institute Diagnostika GmbH, Bad Vilbel, Germany), and Diagnostic Systems Laboratories (Sinsheim, Germany)] that were calibrated against the recently recommended GH standards.

Results: Results from all assays strongly correlated (r = 0.8–0.996; $P < 0.0001$). However, the results obtained with the Immulite assay were, on average, 2.3-fold higher than those obtained with Nichols and 6-fold higher than those obtained with Diagnostic Systems Laboratories. Using cutoff limits of 1 μg/liter (Immulite) and 0.5 μg/liter (Nichols) identified 95% of patients with active disease and 78–80% of patients in remission. Basal and nadir GH levels were significantly higher in females than in males (Immulite 2.2 ± 0.28 μg/liter *vs*. 0.73 ± 0.15 μg/liter and 0.16 ± 0.01 μg/liter *vs*. 0.08 ± 0.01 μg/liter; $P < 0.001$, respectively). In multiple regression analysis, age, BMI, and gender were predictors for basal and nadir GH levels.

Conclusion: Postglucose GH-nadir values are assay, gender, age, and BMI specific, indicating the need of individual cutoff limits for each assay. ***(J Clin Endocrinol Metab* 93: 1254–1262, 2008)**

Acromegaly is a disabling disease that is associated with increased morbidity and reduced life expectancy. However, when adequately treated, the relative mortality risk can be markedly reduced toward that of the normal population (1).

Although the diagnosis is based primarily on clinical features, it is confirmed biochemically by measuring GH levels after oral glucose loading (2) and/or the estimation of IGF-I and IGF binding protein-3 levels (3).

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Abbreviations: BMI, Body mass index; CV, coefficient of variation; IS, international standard; OGTT, oral glucose tolerance test.

Besides its role in establishing the right diagnosis, the results obtained from these measurements have profound effects on the follow-up management of the disease, and are the key criteria for adequate treatment and for monitoring of possible recurrence.

Various studies have been published that addressed the biochemical monitoring of disease activity in acromegaly using baseline or postglucose GH measurements. However, the use of modern highly sensitive immunoassays yielded results that are much lower than those obtained with the older RIAs (4).

As a consequence of the heterogeneity of the results obtained from these studies (5–11), the suggested reference cutoff values for the diagnostic and therapeutic management in acromegaly differed widely and ranged from 0.14–2 μg/liter.

To minimize the discordance between all commercially available immunoassays, it has been recently recommended that the used calibrators should be exclusively unified to the first and second international standard (IS) for recombinant GH (IS 88/624 and IS 98/574). These consist of 22-kDa GH of more than 95% purity instead of the pituitary derived IS 80/505, which contained a mixture of GH isoforms (12). Moreover, it has been recommended to express GH concentrations in mass units rather than international units (13).

In the present study, we determined serum GH concentrations after an oral glucose load (75 g) in a large cohort of apparently healthy subjects and in acromegaly patients with controlled or active disease. GH was measured with three different commercially available assays, whose standards were calibrated against the recommended 22-kDa GH containing ISs (IS 98/574 and 88/624). We studied the impact of the used calibrators, of different matrices, and of the specificity of the respective antibodies on the observed between-assays differences in GH results. Furthermore, we defined individual assay specific cutoff values for diagnosis and remission of acromegaly on the basis of GH concentrations in patients and controls. Finally, we studied the impact of other confounding factors such as age and body mass index (BMI) on baseline and postglucose GH-nadir concentrations in healthy subjects and in patients with acromegaly.

Subjects and Methods

Subjects

We investigated 18 controlled (IGF-I within the age-adjusted normal range) and 28 active acromegaly patients (19 men; age 31–63 yr; BMI 26.4 ± 0.4 kg/m^2), as well as 213 apparently healthy subjects (66 men; age 20–76 yr; BMI 30 ± 0.5 kg/m^2).

The major exclusion criteria for controls included a history of diabetes mellitus, any current inflammatory or malignant disease, and any disorders and/or medications (oral contraceptives or hormone replacement therapy) known to interfere with GH secretion, and pregnancy. Fertile female subjects were tested during the follicular phase of the menstrual cycle (d 7–11).

The study protocol was approved by the Ethical Committee of the Charité University Medicine Berlin, and according to the requirements of the Declaration of Helsinki, written informed consent was obtained from all participants.

Study procedures

Subjects were asked to skip the administration of their current medication (*i.e.* antihypertensive medicine) at the morning of the test. After an overnight fast, an indwelling catheter was inserted at 0800 h into an antecubital vein, and a baseline blood sample was obtained for the determination of blood glucose, GH, and IGF-I. Subjects then ingested 75 g glucose (Gluco-Drink 75; Roche Diagnostics, Mannheim, Germany). Subjects rested in semirecumbent position until the end of the test, and additional blood samples for GH and glucose measurements were collected at 30, 60, 90, 120, and 180 min. Serum samples were stored frozen at −80 C until analysis.

A full medical history was taken in all subjects, followed by a physical examination, and recording of height, weight, and waist and hip circumference for the assessment of BMI and waist to hip ratio.

Hormone assays

Capillary blood glucose concentrations were measured using the glucose oxidase method (Glucometer Biosen 5130; EKF-diagnostic, Magdeburg, Germany).

IGF-I levels were determined in one run by a two-site, solid-phase, chemiluminescent immunometric assay (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA), using murine monoclonal anti-IGF-I antibodies. The standards were calibrated against the World Health Organization second IS 87/518. The assay had a detection range of up to 1600 μg/liter and an analytical sensitivity of 20 μg/liter. The intraassay and interassay coefficients of variation (CVs) were 2.3–3.9% and 3.7–8.1%, respectively.

Serum GH concentrations in healthy subjects were determined in one run and in singlicates by two commercially available automated two-site chemiluminescence immunometric assays (Immulite 2000 assay and Nichols advantage) as well as in duplicates by an ultrasensitive ELISA (DSL-10–19100 ELISA kit; Diagnostic Systems Laboratories, Sinsheim, Germany). In patients with acromegaly, GH concentrations were only estimated using the Immulite and Nichols assays.

The Immulite 2000 human GH (hGH) assay (Diagnostic Products Corp., Biermann GmbH, Bad Nauheim, Germany) uses murine monoclonal antibodies immobilized on a polystyrene bead and an alkaline phosphatase-labeled rabbit polyclonal antibody as tracer. The lower detection limit was 0.05 μg/liter. The intraassay and interassay CVs were 2.9–4.6% and 4.2–6.6%, respectively.

The Nichols Advantage hGH assay (Nichols Institute Diagnostika GmbH, Bad Vilbel, Germany) uses one mouse monoclonal antibody that is coupled to biotin and one goat polyclonal antibody to hGH that is labeled with an acridinium ester as a tracer. Streptavidin-coated magnetic particles are used to allow a highly specific and efficient method of binding the sandwich complex to the solid phase via the high affinity interaction between biotin and avidin. The lower detection limit was 0.02 μg/liter. The intraassay and interassay CVs were 2.8–5.4% and 6.2–8.7%, respectively.

Both assays were calibrated against the National Institute for Biological Standards and Control second IS 98/574 for hGH. For the conversion of mass units to IU, the results should be multiplied by the factor 2.4 and 3 μIU/ng for Immulite and Nichols, respectively.

The DSL-10–19100 ACTIVE Ultra-Sensitive hGH ELISA (Diagnostic Systems Laboratories) is an enzymatically amplified "one-step" sandwich-type immunoassay involving the biotin-streptavidin bridging detection system. The lower detection limit was 0.00066 μg/liter. The upper limit of detection was 0.5 μg/liter. The intraassay and interassay CVs were 3.8–10.4% and 2.8–10.3%, respectively.

The assay was calibrated against the first World Health Organization International Reference Preparation of hGH (IRP 88/624) that also constitutes recombinant 22-kDa GH. For the conversion of mass units to IU, the results should be multiplied by the factor 3.45 μIU/ng.

Statistical analyses

Statistical analyses were performed using SPSS version 14 (SPSS, Inc., Chicago, IL) and MedCalc version 9 (MedCalc Software, Mariakerke, Belgium). All data are expressed as mean ± SEM unless stated otherwise.

For GH assays, values below the limit of detection (as given by the manufacturer) were set to the lowest limit of detection (0.05, 0.02, and

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0.00066 μg/liter for Immulite, Nichols, and Diagnostic Systems Laboratories, respectively). These values were excluded for the comparison among assays. The nadir GH concentration was defined as the lowest value at any time point during the oral glucose tolerance test (OGTT).

To compare the GH assays, we plotted the results as described by Bland and Altman (14), showing the differences (given as percentage of the mean) against the mean of the assays compared. Furthermore, we performed Passing and Bablok regression analysis (15) to obtain the regression equation.

In case of skewed data, the nonparametric Spearman correlation test and the nonparametric Kruskal-Wallis test were used for statistical analysis. The Shapiro-Wilk test was used to test for normal distribution. *P* values less than 0.05 were regarded as statistically significant.

Results

After oral glucose loading, the GH concentrations progressively decreased in all subjects (Fig. 1). Although absolute values of the assays differed substantially, the courses of mean GH concentrations were nearly parallel for the results obtained by different methods.

The impact of the assay method

The results obtained by the Immulite 2000 and Nichols Advantage assays for healthy subjects (n = 983 samples) and for controlled and uncontrolled acromegaly patients (n = 175 samples) correlated strongly (r = 0.98 and 0.996, respectively; $P < 0.001$) (Fig. 2A). Despite good overall correlation, absolute GH concentrations differed dramatically (Fig. 1), and mean GH concentrations measured by the Immulite assay were, on average, 2.3-fold higher than those obtained with the Nichols assay. For each assay, values below the lowest limit of detection were excluded for the comparison among assays. This was the case in 15.2% of all samples measured by Immulite, 25% of samples measured by Nichols, and 27.5% of samples measured by Diagnostic Systems Laboratories.

The results obtained by the Immulite 2000 and DSL-10–19100 assays for healthy subjects at GH concentrations above the upper limit of detection of the Diagnostic Systems Laboratories assay (GH values > 0.5 μg/liter, n = 76) were also strongly correlated (r = 0.94; $P < 0.001$) (Fig. 2B), and were fairly comparable. However, at GH concentrations within the limits of detection of the Diagnostic Systems Laboratories assay (GH values 0.00066–0.5 μg/liter, n = 882), the observed correlation appeared to be somewhat weaker (r = 0.81; $P < 0.001$), and mean GH concentrations measured by the Immulite assay were, on average, 6-fold higher than those obtained with the Diagnostic Systems Laboratories assay (Fig. 2B).

The regression equations based on Passing and Bablok regression (15) were: (Immulite 2000) = 2.042 × (Nichols Advantage) + 0.0183 μg/liter; (Immulite 2000) = 0.74 × (DSL-10–19100) + 0.434 μg/liter (GH > 0.5 μg/liter); and (Immulite 2000) = 5.8 × (DSL-10–19100) + 0.019 μg/liter (GH ≤ 0.5 μg/liter).

These findings were confirmed when analyzing the data by the Bland-Altman plot using the Immulite assay as a reference method. Whereas the results obtained by the Diagnostic Systems Laboratories assay at GH concentrations higher than 0.5 μg/liter were scattered symmetrically on both sides of the x-axis (difference ~0%), values obtained by the Diagnostic Systems Laboratories at GH concentrations lower than 0.5 μg/liter as well as results obtained with the Nichols assay showed a distribution around the mean clearly above the x-axis, with a mean deviation of 145% and 62–76% from the reference method for both as-

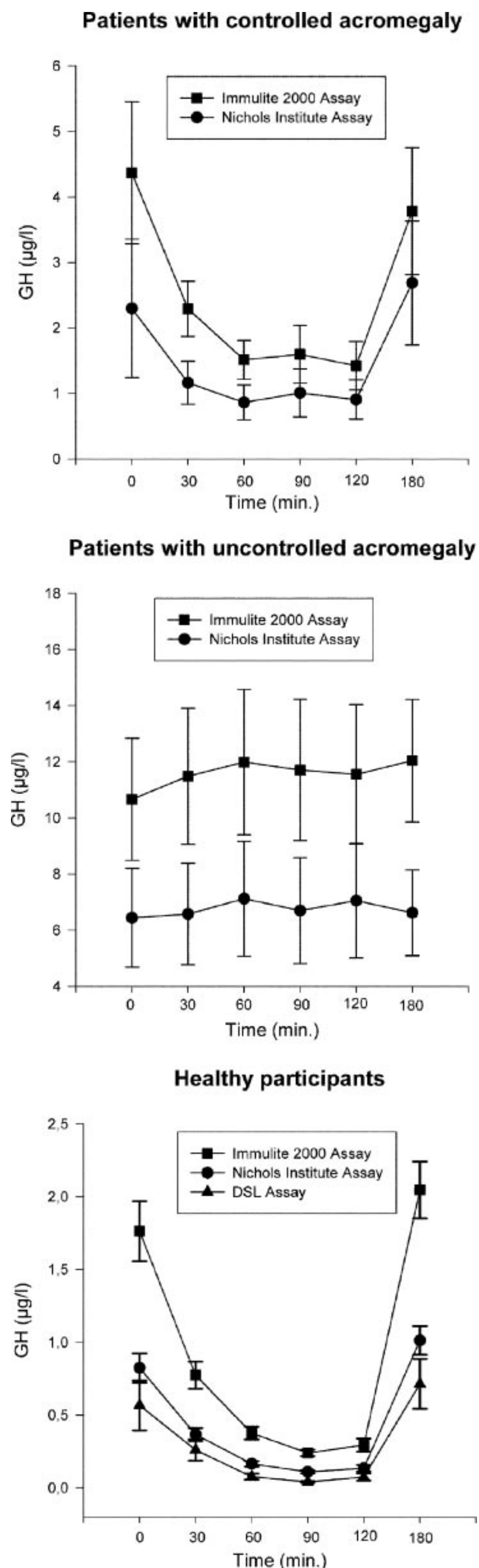

FIG. 1. Mean (±SEM) GH concentrations during an OGTT determined by various assays in patients with active acromegaly, in patients considered to be in remission, and in healthy controls.

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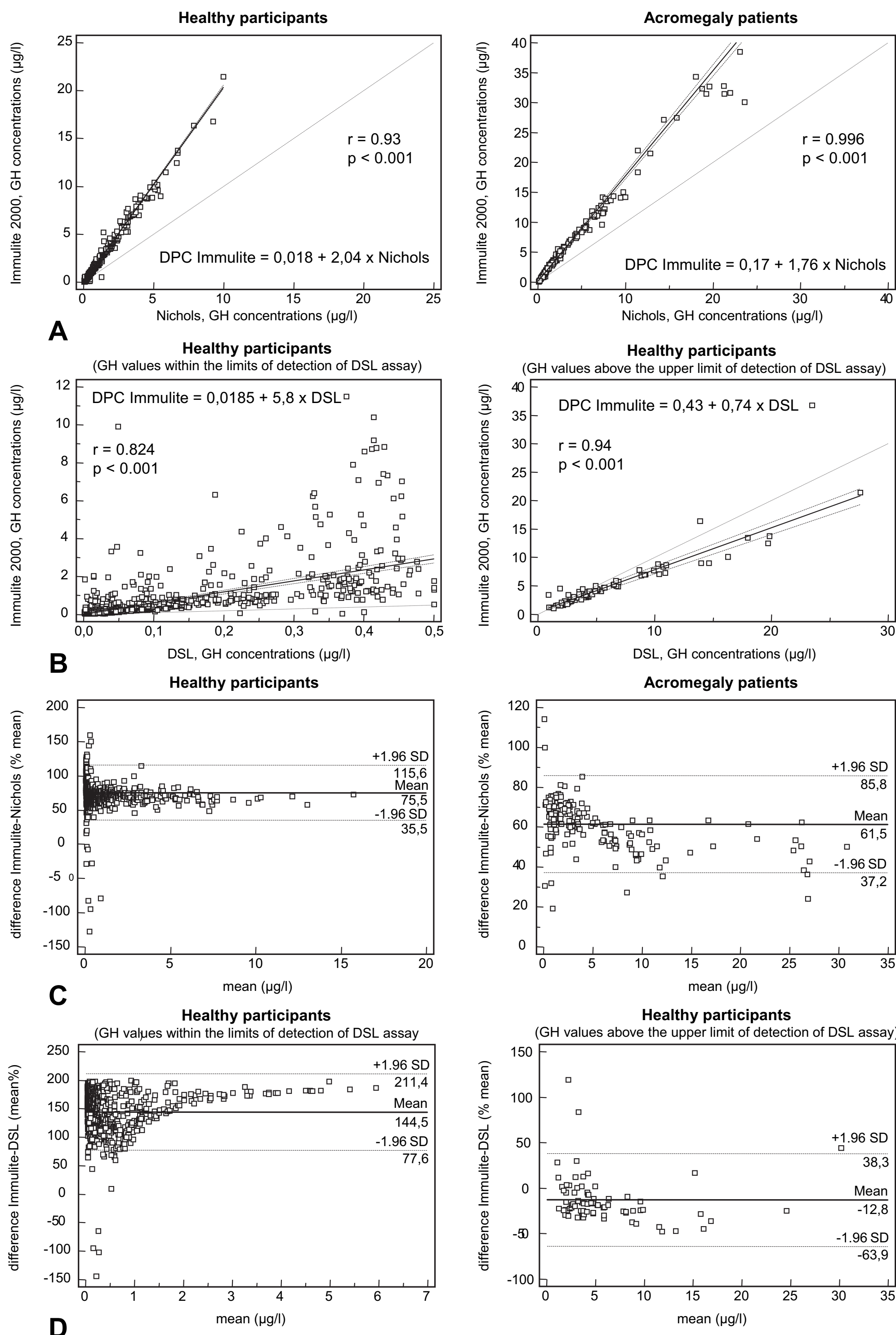

FIG. 2. Comparison of GH concentrations determined by the Immulite 2000 and the Nichols assay in acromegaly patients and in controls, and of GH results determined by the Immulite 2000 and the Diagnostic Systems Laboratories assay in controls at GH concentrations lower than 0.5 μg/liter (*left panel*) or higher than 0.5 μg/liter (*right panel*) using: A and B, Passing and Bablok regression analysis showing correlation coefficients between assays and the regression equations. C and D, Bland and Altman plots of the difference (expressed as a percentage of mean) against the mean of Immulite 2000 (reference method) and each of the other assays (Nichols or Diagnostic Systems Laboratories). DPC, Diagnostic Products Corp.

says, respectively. For both assays absolute deviation from the reference method and the scatter around the mean deviation was most pronounced in the very low range of GH concentrations (Fig. 2, C and D).

The impact of calibration

To evaluate the impact of the calibration method, we measured the calibrators provided by the manufacturer for each assay with all three assays.

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The calibrators of the Immulite assay were underestimated when measured using the Nichols assay, whereas the Nichols calibrators were overestimated as measured using the Immulite assay (Fig. 3A). Using the Diagnostic Systems Laboratories assay, the Immulite calibrators were underestimated at expected GH concentrations less than or equal to 0.5 μg/liter, and overestimated at concentrations more than 2 μg/liter. Similarly, the Diagnostic Systems Laboratories standards at expected concentrations less than or equal to 0.2 μg/liter were clearly overestimated using the Immulite assay (Fig. 3B).

In addition, the recombinant 22-kDa GH (Norditropin; Novo Nordisk GmbH, Mainz, Germany) was serially diluted using the MD2 diluent (Diagnostic Products Corp.) and measured using all three assays. The overall estimation was higher than expected using the Immulite [mean recovery (%) ± SD: 161 ± 31] or the Diagnostic Systems Laboratories assay [mean recovery (%) ± SD: 201 ± 61], whereas expected concentrations were almost found using the Nichols assay [mean recovery (%) ± SD: 102 ± 19] (Fig. 3C).

The role of matrix

To study the role of matrix, the recombinant 22-kDa GH was serially diluted using two different diluents, the MD2 diluent constituting of nonhuman protein/buffer matrix and the Diag-

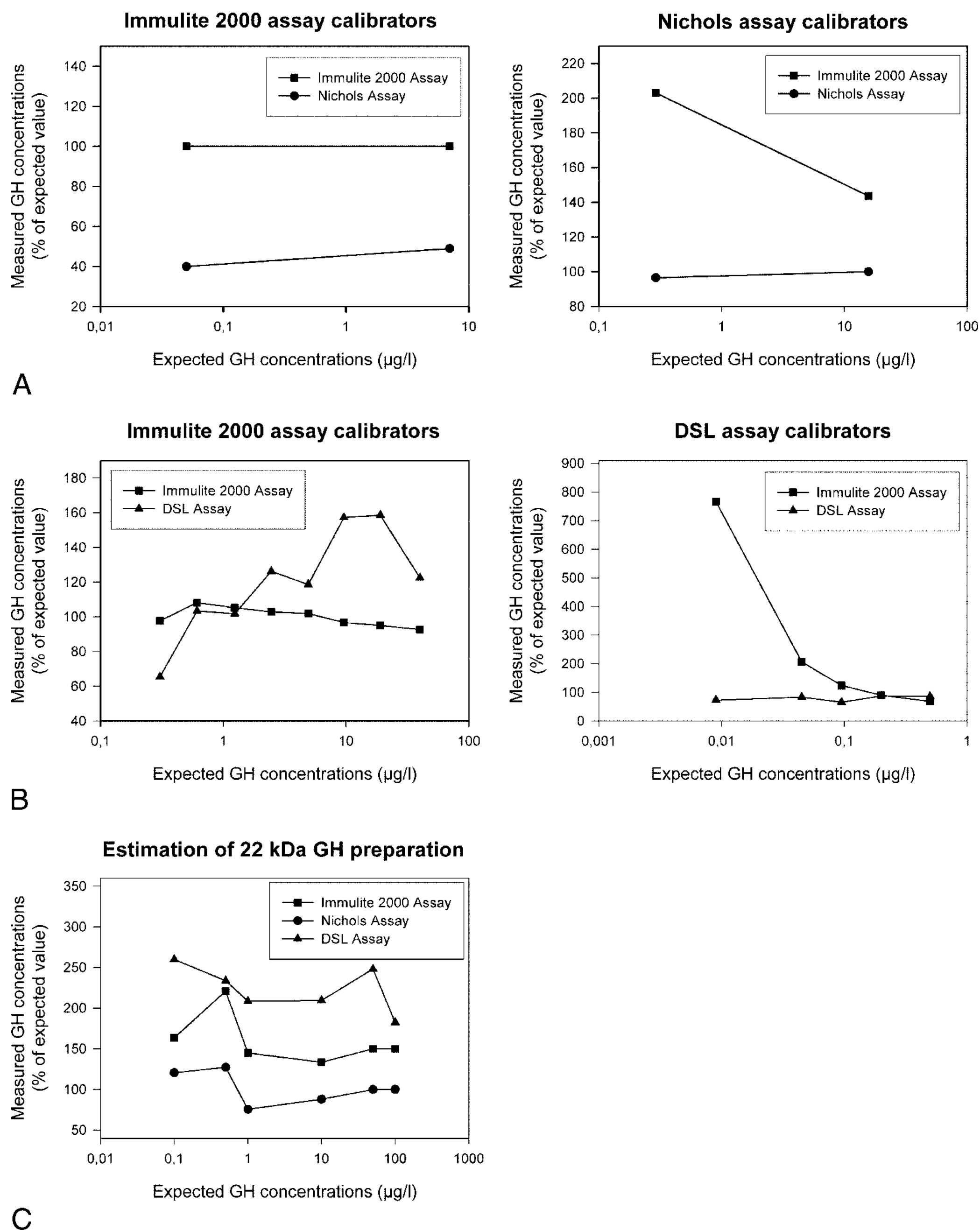

FIG. 3. A, GH concentrations in the Immulite and Nichols calibrants determined using both assays. B, GH concentrations in the Immulite and Diagnostic Systems Laboratories calibrators determined using both assays. C, GH concentrations in the pure 22-kDa GH preparations determined using all three assays. All results (expressed as a percentage of the expected value) are plotted against the expected GH concentrations.

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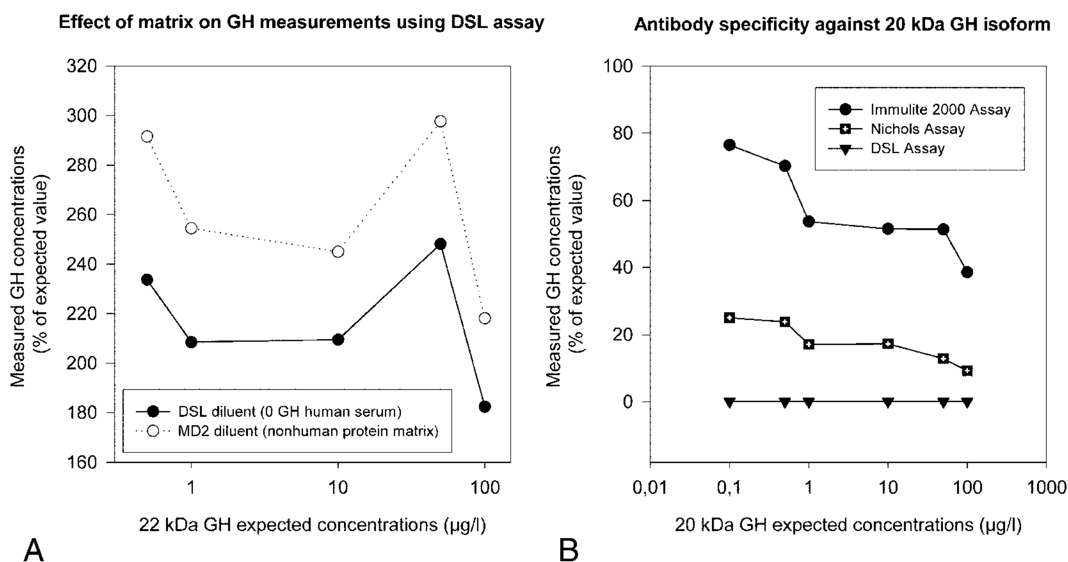

FIG. 4. A, GH concentrations in the purified 22-kDa GH preparations that are diluted using the Diagnostic Products Corp. diluent (*dotted line*) or the Diagnostic Systems Laboratories diluent (*solid line*) and measured using the Diagnostic Systems Laboratories assay. B, GH concentrations in the purified 20-kDa GH preparations that are estimated using all three assays. All results (expressed as a percentage of the expected value) are plotted against the expected GH concentrations.

nostic Systems Laboratories diluent containing zero GH human serum, and measured the samples using the Diagnostic Systems Laboratories assay. The use of zero GH human sera as matrix yielded results that were about 20% lower than those obtained using the nonhuman protein matrix (Fig. 4A).

The impact of antibody specificity against various GH isoforms

The biosynthetic 20-kDa GH was also serially diluted using the MD2 diluent and measured using all three assays. Cross-reactivity of the 20-kDa GH isoform was 30–77% using the Immulite and 9–25% using the Nichols assay, whereas there was no cross-reaction using the Diagnostic Systems Laboratories assay (Fig. 4B).

Nadir GH concentrations in patients with acromegaly and in healthy controls

In healthy subjects, nadir GH concentrations were (mean ± SEM) 0.13 ± 0.01 μg/liter (range 0.05–0.99, 95% percentile: 0.37 μg/liter), 0.06 ± 0.005 μg/liter (range 0.02–0.5, 95% percentile 0.18), and 0.015 ± 0.002 μg/liter (range 0.00066–0.25, 95% percentile: 0.068) for Immulite 2000, Nichols, and Diagnostic Systems Laboratories assays, respectively.

GH nadir in controlled acromegaly patients were (mean ± SEM) 0.98 ± 0.26 μg/liter (Immulite 2000) and 0.5 ± 0.15 μg/liter (Nichols), whereas in those with an active disease, were 7.98 ± 1.7 and 4.5 ± 1.2, respectively (Fig. 5).

Considering cutoff limits of 1 μg/liter (Immulite 2000 assay) and 0.5 μg/liter (Nichols assay), respectively, as based on the highest assay specific nadir GH values observed in controls during an OGTT, appears to be appropriate, identifying 95% of patients with active disease and 78–80% of patients in remission. Comparing patient classifications with respect to gender, these cutoff limits yielded higher sensitivity and specificity in males in comparison to females [100 *vs.* 92% (sensitivity) and 100% *vs.* 71–75% (specificity) for males and females, respectively].

The impact of gender, age, and BMI

Both basal and nadir GH levels were significantly higher in females than in males (Immulite 2000 mean ± SEM: 2.2 ± 0.28 *vs.* 0.73 ± 0.15 μg/liter and 0.16 ± 0.01 *vs.* 0.08 ± 0.01 μg/liter; $P < 0.001$, respectively).

In acromegaly patients, basal GH levels were also significantly higher in females than in males (Immulite 2000: 10.3 ± 2.2 *vs.* 4.9 ± 1.3 μg/liter; $P < 0.05$). Similarly, female acromegaly patients showed higher nadir GH levels than males (7.4 ± 2.2 *vs.* 3.8 ± 0.97 μg/liter), although the variance did not reach the statistical significance.

Age and BMI correlated negatively with both basal and nadir

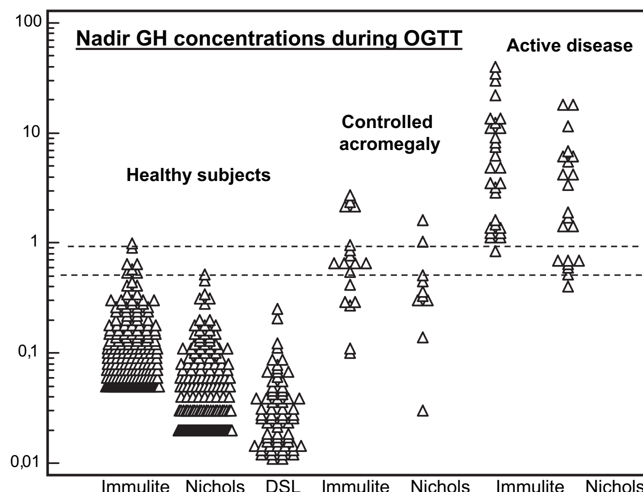

FIG. 5. Nadir GH levels during an OGTT determined by various assays in patients with active acromegaly and in those considered to be in remission shown in comparison to nadir GH levels in healthy subjects. Determination of assay specific cutoff values based on the highest nadir values in healthy subjects.

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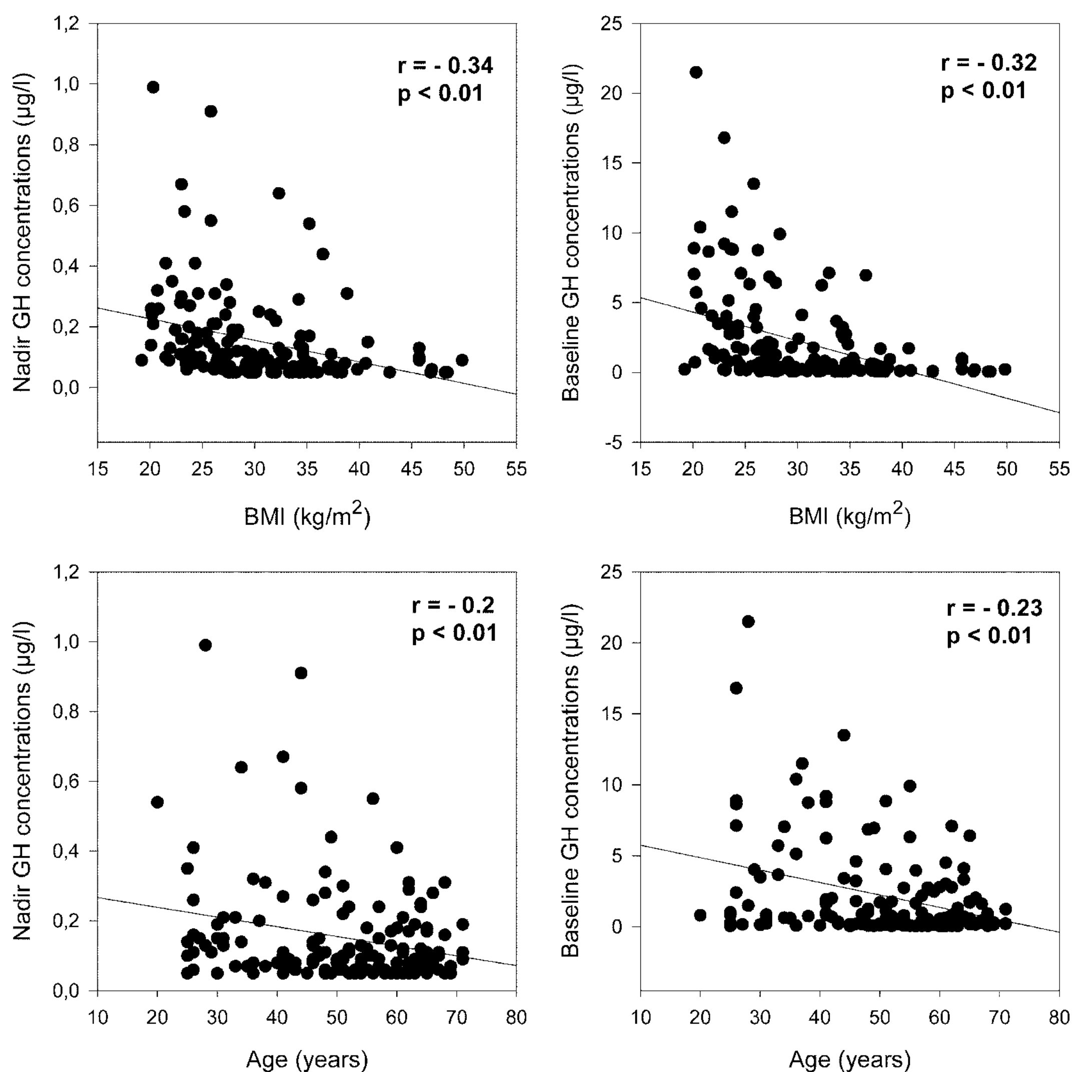

FIG. 6. The impact of age (*bottom panels*) and BMI (*top panels*) on both basal and nadir GH concentrations in 213 healthy subjects.

GH (Fig. 6). In multiple regression analysis, age, BMI, and gender were predictors for both basal and nadir GH levels [standardized β (baseline/nadir GH) = $-0.23/-0.2$, $-0.33/-0.27$, and 0.22/0.26 for age, BMI, and gender, respectively; $R^2 = 0.21$].

To evaluate further the impact of BMI on GH levels, we divided our healthy subjects into lean (BMI 18.4–24.9 kg/m^2; n = 50), overweight (BMI 25–29.9 kg/m^2; n = 72), and obese controls (BMI $\geq$ 30 kg/m^2; n = 91).

Both basal and nadir GH levels were significantly higher in lean subjects (Immulite 2000 mean $\pm$ SEM: 3.7 $\pm$ 0.7 μg/liter and 0.2 $\pm$ 0.03) than in overweight (1.5 $\pm$ 0.3 μg/liter and 0.12 $\pm$ 0.02; $P < 0.001$) or obese subjects (1 $\pm$ 0.17 μg/liter and 0.1 $\pm$ 0.01; $P < 0.001$).

Similarly, we divided our healthy controls into two groups with respect to age: group 1, 20- 52.9 yr (n = 106); and group 2, 53 yr or older (n = 107).

Both basal and nadir GH levels were significantly higher in young (2.3 $\pm$ 0.4 μg/liter and 0.2 $\pm$ 0.02) than in older subjects (1.2 $\pm$ 0.2 μg/liter and 0.1 $\pm$ 0.01; $P < 0.001$).

Discussion

The present study shows that there is a wide between-method discrepancy in GH measurements, using modern highly sensitive immunoassays. This problem exists despite calibration against the recently recommended IS for recombinant somatropin. Despite the good overall correlation between the measured GH concentrations, the Immulite 2000 yielded results about 2.3-fold higher as compared with the Nichols assay, and 6-fold higher compared with the Diagnostic Systems Laboratories assay.

We show that the choice of assay calibrator or reference preparation is one of the most important factors attributing to the discordance in the obtained GH results. Whereas the Immulite calibrators were underestimated using the Nichols assay, the Nichols calibrators were overestimated using the Immulite assay. Similarly, the Immulite calibrators were underestimated at GH concentrations within the measurable range ($\leq$0.5 μg/liter) using the Diagnostic Systems Laboratories assay. We also show that the differences in antibody specificity for the various heterogenous GH isoforms and the matrix differences among the stan-

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dards used for calibration and among the patient samples themselves are strong confounding factors regarding the estimation of GH concentrations. Using the Diagnostic Systems Laboratories assay, the use of nonhuman protein matrix (the one used to lyophilize the Immulite calibrators) yielded results that were 20% higher than those obtained using the zero GH human serum (the matrix used for the Diagnostic Systems Laboratories calibrants). On the other hand, the cross-reactivity of 20-kDa GH isoform was very high using the Immulite followed by the Nichols assay, whereas it did not show any cross-reaction with the Diagnostic Systems Laboratories assay.

Furthermore, we defined a cutoff value for GH nadir of 1 μg/liter (Immulite 2000) or 0.5 μg/liter (Nichols assay) as appropriate for diagnosis and determination of disease activity.

Finally, we show that healthy females present higher baseline and nadir GH levels and lower IGF-I levels than males, and that age and BMI are independent predictors of both baseline and nadir GH concentrations.

Along with the measurement of IGF-I, an OGTT is the recommended test to assess a biochemical status in acromegaly (2). However, the development of highly sensitive and specific GH assays has necessitated a critical reevaluation of the biochemical criteria needed for the diagnosis, the monitoring of disease remission, the disease cure, and for the early recognition of patients with recurrent disease.

Use of these assays has revealed that GH levels after oral glucose loading in healthy subjects and patients with acromegaly are significantly lower than previously recognized with older GH assays. However, the recommended cutoff values in studies that used modern sensitive assays show a wide discrepancy (7–9).

Various factors have been postulated to account for the between-method discrepancies in GH determinations by different immunoassays. The choice of assay calibrator or reference preparation was regarded the most important confounding factor, and recommendations concerning the standardization of the GH assays highlighted the calibration against standards consisting of recombinant GH preparations. Even though most commercially available immunoassays for GH are now calibrated against the international reference preparations of the hormone, the comparative measurement of serum samples by different immunoassays still produces heterogenous results (16). This was confirmed in our study. Despite the fact that all three assays were calibrated against the recommended recombinant 22-kDa GH IS, we still show the wide heterogeneity of the obtained GH results in a direct comparison. We also tested the assays against a pure 22-kDa GH preparation, showing that the recovery of recombinant 22-kDa GH was higher than expected using the Immulite or the Diagnostic Systems Laboratories assay, whereas expected concentrations were found using the Nichols assay. The calibrators of the Immulite assay consisted of pituitary extracts, which may explain the higher recovery of the 22-kDa preparation. On the other hand, the high values obtained by the Diagnostic Systems Laboratories assay can be partly explained by the calibration against a different 22-kDa GH IS.

The impact of the matrix differences was previously described for other assays (17) and can be explained by the interference of the GH-binding protein (18). This problem exists especially when using specific monoclonal antibodies and is less pronounced in assays using polyclonal antibodies. In our study this may explain the lower measured GH concentrations with the Diagnostic Systems Laboratories assay compared with the Immulite assay.

The monomeric 22-kDa GH isoform constitutes only 43–45% of the total GH that is found in our circulation (19). Therefore, the antibody-specificity to the various heterogenous GH isoforms is another potent factor confounding the obtained GH results. The Immulite assay using monoclonal and polyclonal antibodies showed the highest cross-reactivity with the 20-kDa GH isoform. This can be a further explanation for the higher GH results obtained with this assay.

In the present study, the GH results using the Diagnostic Systems Laboratories assay were higher and comparable to those obtained using the Immulite assay at higher GH concentrations (>0.5 μg/liter). Our findings can be explained by the impact of dilution that is decreasing the effect of matrix, and the higher estimation of the 22-kDa GH isoform that may exist to a higher proportion at higher GH concentrations (20).

Recommendations exist regarding the standardization of GH assays. However, discordance in the estimation of disease activity still exists with the current criteria in use because no consensus has been established concerning the definition of normative cutoff limits.

Based on observed GH concentrations in healthy subjects, we were able to define an assay specific cutoff limit of 1 μg/liter for the Immulite and 0.5 μg/liter for the Nichols assay. In our acromegaly patients, these limits proved to be appropriate for confirming the diagnosis and defining remission with a very high sensitivity and specificity, especially in males.

The impact of gender on both basal and nadir GH concentrations after glucose loading is described in some (9, 16, 21), but not all, studies addressing this issue (22). As shown in the present investigation, healthy women have significantly lower IGF-I concentrations and higher GH concentrations than men, both in the basal state and during an OGTT, indicating the need to consider gender in the definition of appropriate cutoff values. However, it should be pointed out that the number of acromegalic patients included in our study was relatively small for a precise definition of the normal range with respect to gender.

In agreement with earlier studies (21, 23, 24), our results confirm the role of age and BMI as predictors for both basal and nadir GH concentrations in healthy subjects. Therefore, the impact of age and BMI differences should be considered to evaluate appropriately the activity of acromegaly.

In conclusion, we demonstrate that postglucose GH-nadir values are assay specific. We further show that the definition of assay methodology is mandatory to define criteria for cured or controlled acromegaly using GH suppression after glucose loading. Finally, because of the impact of age, gender, and BMI on GH secretion in healthy subjects, the estimation of age, gender, and BMI-adjusted reference values for GH during an OGTT appears to be appropriate and highly required.

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