Growth hormone assays: early to latest test generations compared

Mireille L’Hermite-Balériaux, Georges Copinschi, and Eve Van Cauter

We compared the data from four growth hormone (GH) immunoassays for analyzing 24-h GH profiles in four apparently normal subjects and four obese subjects (508 serum samples). The detection limit was 0.02 μg/L for one immunochemiluminometric assay (ICMA), 0.1 μg/L for two IRMAs, and 0.4 μg/L for one RIA. All GH pulses with a peak ICMA value >1 μg/L were detected by each of the other methods. Overall, the correlation coefficient between the values obtained with all four assays exceeded 0.90. However, for GH concentrations ≥0.25 μg/L, acceptable concordance (r² ≥0.80) was reached only between the ICMA and one IRMA; between the ICMA and the RIA, concordance was acceptable only for GH concentrations ≥10 μg/L. In the normal subjects, the percentage of undetectable values was 0% with the ICMA but 29% with one of the IRMAs; in obese subjects, the corresponding values were 12% and 38%.

INDEXING TERMS: intermethod comparison • obesity • immunochemiluminometric assays • immunoradiometric assays • radioimmunoassays • age-related effects

Temporal profiles of growth hormone (GH) secretion have been widely investigated during the past two decades. GH is episodically released into the circulation in short bursts or pulses [1-5]. Although major pulses can be accurately detected and analyzed with conventional assays, GH concentrations between pulses and even during minor pulses are below or near the limit of detection of most analytical procedures in current use. This lack of sensitivity precludes accurate estimations of the characteristics of GH pulsatility under both physiological and pathological conditions.

For ~20 years, GH concentrations have been measured by RIA, for which the detection limit is ~1 μg/L. With the development of IRMAs, this limit was lowered to ~0.1 μg/L. Now, a commercially available immunochemiluminometric assay (ICMA) has further lowered this limit to ~0.02 μg/L, allowing for the detection of very small variations in GH. In a recent study, an in-house modification of this ICMA procedure provided an even lower limit of detection (0.005 μg/L), making it possible to discriminate between normal and insufficient GH secretion by using basal values without having to perform stimulation tests [6].

During the past few years, the growing recognition of the role of GH not only in childhood development but also in the senescence process has stimulated numerous studies on the physiological patterns of GH secretion in a variety of conditions, and as determined with the use of various different assay procedures. To assess the validity of across-studies comparisons, we have examined the performances of two commercially available IRMAs, one commercially available ICMA, and an established RIA procedure, using reagents provided by the National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases (NIADDK)—reagents that have been used in many laboratories, including our own [2].

GH concentrations were measured by all four procedures in samples collected at 20-min intervals over a 24-h span from four healthy young adults with apparently normal GH secretion and in four older overweight patients with relatively low GH secretion.

Subjects and Methods

Eight subjects—four normal young adults (three men, one woman; ages 22–23 years, body mass index 20–25), and four older women (ages 40–59 years), obese (body mass index 27–35) but otherwise normal—were included in the study. A catheter was inserted at 1000 h into an antecubital vein and blood samples were collected at 20-min intervals until 1200 noon on the following day. The intravenous line was kept patent with a slow drip of heparinized saline. To avoid disturbing the subjects’ sleep between 2300 and 0700 h, the intravenous line was
connected to plastic tubing that extended to an adjacent room for sampling, as previously described [2]. Each blood sample was centrifuged at 4 °C without delay, and the resulting plasma samples were frozen and kept at −20 °C until assayed. The protocol was approved by the Ethical Committee of the School of Medicine of the Université Libre de Bruxelles, and written informed consent was obtained from all subjects.

ASSAYS
Four different GH assays were used. The detection limit of each assay was defined as the GH concentration corresponding to a signal 2 SD above or below the zero dose. For statistical purposes, values below the detection limit were assigned the value of the limit. For each method, all samples from the same individual were analyzed in duplicate in the same assay run.

RIA. Polyclonal RIA with the NIADDK reagents was performed as previously described [2]. The hGH-RP1 preparation, which is equivalent in potency to the 1st WHO Reference Preparation 80/505, was used as the standard. The detection limit was 0.4 μg/L. The intraassay CVs were 8.1% for GH concentrations of 0.4–1 μg/L, 6.1% for 1–5 μg/L, and 7.4% for >5 μg/L.

IRMA-M. The Medgenix (Fleurus, Belgium) IRMA is a non-competitive-type IRMA based on the use of antibody-coated tubes and two mouse monoclonal antibodies directed against

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Fig. 1. Individual 24-h profiles of plasma GH in four healthy subjects obtained with the four different immunoassays.
two different epitopes on the GH molecule. The first antibody is fixed on the tube wall and the second antibody is the tracer. This assay, like the IRMA-S and ICMA, is calibrated against the 1st WHO Reference Preparation 80/505; its detection limit is 0.1 µg/L. The assay was performed according to the manufacturer’s instructions, with 50 µL of plasma and a 2-h incubation at room temperature. The intraassay CV was 14.6% for GH values of 0.1–1 µg/L, 4.3% for 1–5 µg/L, and 1.9% for >5 µg/L.

**IRMA-S.** The Sorin (Saluggia, Italy) noncompetitive-type IRMA is also based on the use of antibody-coated tubes as well as two mouse monoclonal antibodies directed against two different epitopes on the GH molecule. The first antibody is fixed on the tube wall and the second antibody is the tracer. The detection limit of this assay is 0.1 µg/L. The assay was performed according to the manufacturer’s instructions with 50 µL of plasma and an overnight incubation at room temperature. The intraassay CV was 7.8% for GH concentrations of 0.1–1 µg/L, 4.3% for 1–5 µg/L, and 4.3% for >5 µg/L.

**ICMA.** The Nichols Institute Diagnostics (San Juan Capistrano, CA) ICMA is an immunochromoluminescence assay utilizing a mouse monoclonal antibody to hGH immobilized on a polystyrene bead and an acridinium ester-labeled goat polyclonal antibody to GH. The acridinium ester emits light when treated with hydrogen peroxide (trigger 1) and an alkaline solution (trigger 2). The luminometer automatically injects the trigger

Fig. 2. Individual 24-h profiles of plasma GH in four obese subjects obtained with the four different immunoassays.
solutions into the assay tube to oxidize the acridinium ester. The oxidized product, which is in an excited state, subsequently returns to ground state, resulting in the emission of light, which is quantified by the luminometer. The detection limit of the assay was 0.02 μg/L for a 50-μL plasma sample and an overnight incubation at room temperature. The intraassay CV was 6.0% for GH concentrations of 0.02–1 μg/L, 5.9% for 1–5 μg/L, and 5.7% for >5 μg/L.

**GH Pulses**

Significant pulses of plasma GH concentrations were identified by using a previously described computer algorithm (ULTRA [7]). A pulse is considered significant if both its increment and its decrement exceed twice the intraassay CV (see above) in the relevant concentration range. The amount of GH secreted in each significant pulse was evaluated by deconvolution analysis [8].

**Statistics**

Statistical analyses (Student’s paired t-test, linear regression, ANOVA for repeated measures, with pairwise contrast tested by the Fisher procedure), were performed by the computer program Statview SE for Macintosh (Abacus Concepts, Berkeley, CA). Unless otherwise stated, all group results are expressed as mean ± SD.

The four GH assays were also compared by “difference plot” analysis [9]: The difference between the results of each assay, expressed as a percentage of the mean of the two measurements for an individual sample, i.e., $y = \frac{(x_1 - x_2)}{(x_1 + x_2)} \times 100$, is plotted against the mean, i.e., $x = (x_1 + x_2)/2$ [9].

**Results**

A total of 508 plasma samples were analyzed. In the normal subjects, 14% of the results were at or below the limit of detection for the RIA, 29% for the IRMA-M, 14% for the IRMA-S, and 0% for the ICMA. In the obese subjects, GH was undetectable in 38% of plasma samples by the RIA, in 48% by the IRMA-M, in 31% by the IRMA-S, and in 12% by the ICMA.

Figure 1 depicts individual 24-h GH profiles for the normal subjects; Fig. 2 illustrates the profiles for the four obese patients. For each subject, largely similar GH profiles were obtained with all four assays. However, the interpulse values as well as the peak values tended to be lower with the most sensitive assay, i.e., the ICMA. This was particularly marked in obese subjects (see Fig. 2, subject 5).

Results were analyzed by matching those from each assay with those from each of the other three methods, to calculate correlation coefficients ($R$) and concordance values ($R^2$) over successive concentration ranges. Each concentration range included all values lower than the upper limit of this range in the more sensitive assay. For each comparison, the pattern of $R^2$ values is illustrated in Fig. 3. Although overall correlations, i.e., including all values, were good ($R^2 \geq 0.87$), correlations for low GH concentrations were generally poor, except between the ICMA and the IRMA-S. An $R^2$ value $\geq 0.80$, arbitrarily chosen to reflect an acceptable concordance between two assays, was reached for low GH concentrations ($\leq 0.25$ μg/L) only between the ICMA and the IRMA-S. For other paired comparisons, this value was reached only for GH concentrations of $\geq 3$ μg/L (ICMA vs IRMA-M) to $\geq 10$ μg/L (IRMA-M vs RIA).

To further analyze relations between the results of the different methods, we constructed difference plots for the lower GH range ($\leq 1$ μg/L; Fig. 4). This analysis allowed demonstration of the true extent of bias at low concentrations. The points were essentially distributed in a broad cloud below the line of equivalence, between 0 and ~50%, thereby indicating for low mean GH concentrations the absence of a consistent quantitative relation between the results of the two assay procedures being compared—except for the ICMA vs IRMA-S comparison at concentrations >0.2 μg/L.
The 24-h GH secretion estimated by the different assays in normal and in obese subjects is shown in Fig. 5. As expected, GH secretion was markedly lower in the obese than in the normal, nonobese subjects by all assays. The highest values were observed with the IRMA-M, the lowest with the ICMA.

The results of the pulse analysis (Table 1) showed that the total number of pulses detected in the profiles from the eight subjects ranged from 65 (IRMA-S) to 82 (ICMA). Only 48 pulses were detected at the same time points by all four methods: 22 pulses in the healthy subjects and 26 in the obese patients. The number of pulses detected by only one assay ranged from 2 to 13, according to the assay used. Surprisingly, 13 pulses were detected only by the RIA, the peak values for these pulses ranging from 0.85 to 2.60 µg/L. All pulses with a peak ICMA value >0.50 µg/L were detected by at least one other method, and all pulses with a peak ICMA value >1 µg/L were detected by all of the other methods.

Considering only the concomitant pulses detected by all four methods, peak ICMA values for the 48 pulses ranged from 0.12 to 34.34 µg/L; moreover, the peak values in these pulses were highly correlated between the assays, with $R^2$ ranging from 0.80 (between ICMA and IRMA-M) to 0.99 (between IRMA-S and IRMA-M). For the concomitant pulses detected by all four methods, pulse increment and amount of GH secreted per pulse measured by each assay were calculated for the group of normal subjects and for the group of obese patients. As expected, GH secretions were markedly lower in obese than in normal subjects, regardless of the assay used (Table 2). In the control
group, the highest values were observed with the IRMA-M. In the obese group, the highest values were observed with the least sensitive assay, i.e., the RIA. In contrast, the lowest values in both groups were observed with the most sensitive assay, i.e., the ICMA.

**Discussion**

As previously shown [10–13], the present data indicate that absolute GH values measured by different assays may vary considerably, even when the same standard and similar methods are used. Thus, in a group of obese subjects, the amount of pulsatile GH secretion was three times higher when measured with an IRMA than when measured with an ICMA. Furthermore, values obtained with one of the IRMAs averaged 200% of the values reported with the other IRMA. Although the between-assay correlations over the entire concentration range were acceptable, no significant relationship was evident in the low-concentration range, except between the IRMA-S and the ICMA. These results confirm that within- and across-subject comparisons of GH secretion definitely need to be performed with a single assay.

All tested methods, including the least sensitive (RIA),

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**Table 1. Number of pulses detected in the eight individual profiles by the four methods.**

<table>
<thead>
<tr>
<th>Method detecting pulse</th>
<th>No. of pulses in subjects</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICMA</td>
<td></td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>14</td>
<td>13</td>
<td>9</td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td>IRMA-S</td>
<td></td>
<td>6</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>65</td>
</tr>
<tr>
<td>IRMA-M</td>
<td></td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>12</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>76</td>
</tr>
<tr>
<td>RIA</td>
<td></td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>14</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>73</td>
</tr>
<tr>
<td>All 4 assays</td>
<td></td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>48</td>
</tr>
<tr>
<td>ICMA and (or) IRMA-S or IRMA-M</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Only one assay</td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

* Subjects 1–4, apparently healthy; subjects 5–8, obese.

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**Table 2. Analysis of concomitant GH pulses (mean ± SE) detected by all four assays in healthy and obese subjects.**

<table>
<thead>
<tr>
<th>Assay</th>
<th>GH, µg/L</th>
<th>Amt. secreted, µg</th>
<th>Assay</th>
<th>GH, µg/L</th>
<th>Amt. secreted, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak</td>
<td>Increment</td>
<td></td>
<td>Peak</td>
<td>Increment</td>
</tr>
<tr>
<td>ICMA</td>
<td>9.0 ± 1.8&lt;sup&gt;a&lt;/sup&gt; &lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.5 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.2 ± 33.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46 ± 0.26&lt;sup&gt;c&lt;/sup&gt; &lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3 ± 0.25&lt;sup&gt;a&lt;/sup&gt; &lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>IRMA-S</td>
<td>10.5 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.9 ± 1.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>159.5 ± 31.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0 ± 0.32&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.83 ± 0.31</td>
</tr>
<tr>
<td>IRMA-M</td>
<td>13.9 ± 2.5</td>
<td>13.1 ± 2.6</td>
<td>235.8 ± 45.2</td>
<td>1.9 ± 0.30&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.75 ± 0.30&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>RIA</td>
<td>11.8 ± 1.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10 ± 2.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>159.7 ± 27.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.1 ± 0.42</td>
<td>2.2 ± 0.45</td>
</tr>
</tbody>
</table>

Significant pairwise contrasts (Fisher test, P < 0.05): * ICMA < IRMA-M, + ICMA < RIA, † IRMA-S < IRMA-M, ‡ RIA < IRMA-M, § ICMA < IRMA-S, ‡ IRMA-S < RIA, ≠ IRMA-M < RIA.
allowed for the detection of GH pulses with peak values in the ICMA of >1 µg/L, and the peak values recorded in the different assays were closely related to one another. All techniques were able to grossly discriminate subjects with low GH concentrations (obese patients) from subjects with normal GH secretion. Therefore, all of the assays appear to be suitable for estimating the results of stimulation tests in clinical practice. In contrast, particularly because of the differences in assay sensitivity, interassay comparisons in the low-concentration range showed major discrepancies. In 24-h profiles of the normal subjects, only the ICMA allowed measurements of GH in all of the plasma samples. In the obese patients, only 50% of the values could be estimated with IRMA-M, whereas almost 90% could be evaluated with the most sensitive assay, the ICMA. Paradoxically, several low-amplitude pulses were detected only by the RIA, and the percentage of undetectable values was lower with the RIA than with the IRMA-M, even though the latter method was four times more sensitive. These findings suggest that some of the low concentrations measured by the RIA may be artifactual, as is also suggested by the wide dispersion of the values on the corresponding difference plots involving the RIA. Another possibility is that the polyclonal antibodies used in the RIA could have detected molecular forms of GH not recognized by the monoclonal antibodies used in the other three assays [14].

In conclusion, these data indicate that any currently available GH assay can be used to accurately evaluate relatively high concentrations of plasma GH, but only the most sensitive methods (preferably the latest-generation methods, with a detection limit ≤0.02 µg/L) are suitable to investigate basal secretion. In clinical conditions characterized by low GH secretion, e.g., obesity and senescence, even the most sensitive commercially available assay is unable to detect all GH values.

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References


