Commercial kits give different measurements for concentrations of growth hormone (GH, somatotropin) in serum. Most notably, a two-site monoclonal-antibody-based immunoradiometric assay (IRMA) from Hybritech routinely yields lower values than do conventional RIAs in which polyclonal antibodies are used. We used purified dimeric biosynthetic human GH as a model compound to investigate the specificity of five commercial immunoassays for size variants of GH. In all five assays, biosynthetic monomeric GH was significantly more potent than pituitary-derived standard GH supplied with the kits. Dimeric GH was significantly less potent than monomer in four of the five assays, and cross-reactivities varied more than fivefold, from 15% to 84%. Using three commercial kits selected for their specificity for dimeric GH, we measured GH in serum samples from 18 normal adults. The mean GH concentrations in serum—0.7 (Hybritech, IRA), 1.8 (Diagnostic Products, RIA), and 3.1 (Cambridge, RIA) μg/L—differed significantly, but in the same rank order as that obtained in the experiments on dimer cross-reactivity.

We used dimeric GH purified from production lots of biosynthetic human GH as a model compound to investigate the cross-reactivity of GH size variants in commercial diagnostic kits. These kits differ substantially in their ability to detect the monomeric and dimeric forms of biosynthetic GH and, presumably, in their ability to cope with the molecular heterogeneity of circulating GH.

Materials and Methods

Growth hormone. The purified recombinant DNA-derived human GH used in this study was prepared at Eli Lilly & Co., Indianapolis, IN. This biosynthetic GH is structurally, physically, antigenically, and biologically equivalent to pituitary-derived GH (21–23). The preparation and the characterization of a GH reference standard have been described elsewhere (24), as have purification and characterization of a dimeric form of biosynthetic GH (21). In brief, dimeric GH, purified by size-exclusion chromatography and anion-exchange chromatography, is a noncovalent form that dissociates into monomer in the presence of detergents or acetonitrile (300 mL/L). In bioassays, purified dimer displays less activity than monomeric GH, and it was characterized as the dominant dimeric species present in production lots of both biosynthetic and pituitary-derived GH (21).

GH immunoassays. The cross-reactivity of monomeric and dimeric biosynthetic GH was evaluated in commercial immunoassays obtained from the following vendors: Assay 1, Cambridge Medical Diagnostics (Billerica, MA); Assay 2, Diagnostic Products Corp. (Los Angeles, CA); Assay 3, Kallestad Diagnostics (Austin, TX); Assay 4, Serono Diagnostics (Brantree, MA); and Assay 5, Hybritech Inc. (San Diego, CA). Assays 1 through 4 are conventional polyclonal-antibody-based RIA methods, whereas Assay 5 is a two-site IRMA involving monoclonal antibodies. In each commercial kit, pituitary-derived GH is the reference standard used for estimating concentrations of GH in serum. Assays 2 through 4 include GH standards calibrated against the WHO reference preparation 66/217, whereas the kit standards in Assays 1 and 5 are calibrated against the NIH reference preparations AFP-4793B and HS-2243E, respectively. In each RIA kit the bound fraction of labeled GH is quantified after separation by a second-antibody precipitation method. The IRMA makes use of antibody-coated plastic beads as a separation method.

For cross-reactivity experiments with biosynthetic GH, we used reference preparations of monomer (lot no. RS0038) and purified dimer (lot no. P45-J67-168D-1). Fresh stock solutions (1 mg/mL) of monomer and dimer were prepared daily in 25 mmol/L sodium phosphate, pH 7.5, and their protein concentrations were verified spectrophotometrically. To prepare standard curves, we diluted each stock solution in the assay matrix supplied for the kit standard GH. We analyzed each standard concentration of monomer, dimer, or kit-supplied GH in triplicate and performed all assays in accordance with the vendors’ instructions. Bound radioactivity was measured for 2 min in an automatic

Additional Keyphrases: radioimmunoassay, immunoradiometric assay compared • polyclonal, monoclonal antibody assays compared • somatotropin • “kit” methods • reference values

The diagnosis of growth hormone (GH, somatotropin) deficiency has traditionally been based on radioimmunoassay (RIA) of GH in serum during stimulation-based tests for secretion of pituitary GH (1, 2). However, different commercial immunoassays have detected different GH concentrations (3–9). Most notably, a two-site monoclonal-antibody-based immunoradiometric assay (IRMA) from Hybritech routinely yields lower values for GH in serum than do conventional RIAs with polyclonal antibodies. Presumably this discordance results from differences in the specificity of commercial kits for variant forms of GH.

Both in the pituitary and serum, GH is present as a heterogeneous mixture of genetically related proteins, varying both in size and charge (10–12). GH size variants have been characterized as an oligomeric series of GH monomers (13), which can be released from the pituitary by physiological or pharmacological stimuli (14–16). Both noncovalent and covalent forms of dimeric GH have been described in serum and in extracts of pituitary gland (13, 17–19). GH fragments may constitute a major fraction of the total GH immunoreactivity present in serum during nonstimulated periods (15). Additionally, a 20-kDa variant form that lacks residues 32–46 constitutes about 5% of circulating GH (20).

1 Lilly Laboratory for Clinical Research, Eli Lilly & Co., Wishard Memorial Hospital, 1001 West 10th Street, Indianapolis, IN 46202.
2 Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN 46285.

Received October 10, 1989; accepted November 20, 1989.
gamma counter (Apex 10/600; ICN Micromedic Systems, Inc., Horsham, PA). Commercial immunoassay controls (Lyphocheck I-III, master lot no. 2000; Bio-Rad Labs., Richmond, CA) were used to validate the accuracy of each immunoassay.

Data analysis. RIA data were plotted log–linear, fitted with a four-parameter logistic model algorithm, and analyzed statistically with the computer program ALLFIT (25). In this program, the ED$_{50}$ is defined as the concentration of GH necessary to displace 50% of bound labeled GH. Relative potency estimates were then calculated from the ratios of the ED$_{50}$ values for monomeric and dimeric GH. The value for the slope estimated for each displacement curve by ALLFIT corresponds to the slope estimate derived from a logit–log transformation. IRMA data were fitted using a linear–linear, as recommended by the kit's vendor, and then fitted by use of a linear-regression equation. Potency estimates for monomeric and dimeric GH in the IRMA were calculated from the ratios of the slopes.

We calculated GH concentrations in serum for the commercial RIAs by using a weighted four-parameter logistic model curve-fitting program, "RIASYS," developed at Eli Lilly & Co. For the IRMA they were estimated by using an IBM-PC-based immunoassay data-reduction system (Isolab, Inc., Rolling Meadows, IL) with a French curve algorithm. We compared the concentrations obtained with each commercial kit statistically by using a nonparametric binomial sign test for paired observations (26). Differences were defined to be statistically significant at $P < 0.05$.

Results

Figure 1 shows the displacement curves for monomeric and dimeric biosynthetic GH in each commercial RIA kit; in all cases, monomeric and dimeric biosynthetic GH displaced labeled GH in a manner parallel to kit-supplied standard GH. Figure 2 shows the cross-reactivity of monomer and dimer in the IRMA. In all five assays, biosynthetic monomeric GH was significantly more potent than pituitary-derived kit-supplied standard GH, with cross-reactivities ranging from 133% to 220% (Table 1). In all assays except Assay 1, dimeric GH was significantly less potent than monomer, with cross-reactivities ranging from 15% to 84% (Table 2). The potency estimates for dimeric GH in the commercial kits were also more variable than those for the monomer.

Because of the fivefold difference we found in dimer cross-reactivity, we measured the GH concentrations in serum samples obtained from 18 unselected, ostensibly normal adults, using three different commercial kits. Assays 1, 2, and 5 were selected because of their wide specificity for dimeric GH. The mean serum GH concentrations found were 3.1, 1.8, and 0.7 μg/L, respectively (Figure 3). GH concentrations as measured with Assay 5 were significantly lower than those measured with Assay 1.

Discussion

Differences in concentrations of GH measured in serum have been detected with different commercial immunoassay kits (3–9). GH deficiency has been defined traditionally by conventional RIA methodology as maximum stimulated concentrations in serum of <10 μg/L (1). Recently, IRMA kits for serum GH have become available commercially. The Hybritech IRMA (Assay 5) reportedly yields lower values for GH than do conventional RIA methods (5, 9). The

![Fig. 1. Standard curves for monomeric (●) and dimeric (▲) biosynthetic human GH and kit standard GH (●) in four RIAs.
Top left, Assay 1; top right, Assay 2; bottom left, Assay 3; bottom right, Assay 4. Ordinate: percent binding of labeled GH relative to its maximum binding](image-url)
Table 1. Potency of Monomeric Biosynthetic Human 
GH in Commercial Assays

<table>
<thead>
<tr>
<th>Assays</th>
<th>Monomeric</th>
<th>Kit standard</th>
<th>Cross-reactivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1</td>
<td>2.8</td>
<td>133&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>3.7</td>
<td>6.1</td>
<td>165&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>4.4</td>
<td>8.4</td>
<td>191&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>2.2</td>
<td>220&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>665</td>
<td>148&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Significant at <sup>a</sup> P <0.05 and <sup>b</sup> P <0.001.

Table 2. Potency of Dimeric Biosynthetic Human GH
In Commercial Assays

<table>
<thead>
<tr>
<th>Assays</th>
<th>Monomeric</th>
<th>Dimeric</th>
<th>Cross-reactivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1</td>
<td>2.5</td>
<td>84</td>
</tr>
<tr>
<td>2</td>
<td>3.7</td>
<td>11.8</td>
<td>31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>4.4</td>
<td>11.7</td>
<td>38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>4.0</td>
<td>25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
<td>142</td>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant at P <0.001.

basis for this discrepancy remains unresolved. Furthermore, these analytical differences have raised concerns regarding the applicability of a 10 ng/mL cutoff value for GH deficiency to all immunoassays (8).

The molecular heterogeneity of circulating GH may be one factor affecting measurement of GH by immunoassays. However, the lack of availability of purified GH size variants has precluded investigating the hypothesis in detail. We used dimeric GH purified from production lots of recombinant GH as a model compound to investigate the specificity of commercial immunoassays for size variants. We performed preliminary experiments to define the approximate potencies of biosynthetic monomeric and dimeric GH in the commercial kits. For each commercial immunoassay kit we optimized the range of GH concentrations to permit analysis of the data by ALLFIT (25). In all cases except Assay 3, the displacement curves produced by the kit standards were sigmoidal, based on analysis with ALLFIT. The concentration range for the standards in Assay 3 was too limited to accurately define the zero-dose parameter (A) and the infinite-dose parameter (D), so these variables were fixed by using the estimates obtained with monomeric GH. The coefficients of variation for multiple determinations of the values for the ED<sub>50</sub> and the slope in the RIA kits ranged from 9.5% to 16.0%. In each case we obtained the same rank order for the potencies of monomeric, dimeric, and kit-supplied standard GH. In each RIA, monomeric and dimeric biosynthetic GH displaced labeled GH parallel to pituitary-derived kit standard GH. As shown in Figures 1 and 2 and summarized in Table 1, biosynthetic monomeric GH was significantly more potent than kit standard GH in all five of the commercial assays. Monomer cross-reactivities ranged from 133% with Assay 1 to 220% obtained with Assay 4. The differences in the cross-reactivity of monomeric GH suggest that differences in pituitary-derived GH reference standard may contribute to the discordance in values seen with commercial kits. However, major differences in the kit standard matrices precluded performing an inter-assay comparison of kit standard GH. Size variant forms of GH are present in clinical-grade preparations of pituitary GH (26). Biosynthetic monomeric GH also has greater potency on a weight basis than does standard pituitary-derived GH in bioassays (21, 23). The greater potency of biosynthetic GH in commercial assays may result from its higher purity (22, 23). Thus, biosynthetic GH may be superior to pituitary-derived GH as a reference material in commercial immunoassays. The use of biosynthetic GH as an immunoassay standard would result in lower values for apparent GH in serum by a factor of two, owing to its higher potency (4).

The potency of biosynthetic dimeric GH varied more than fivefold in the commercial kits (Table 2). Dimer cross-reactivity could be grouped roughly into one of three categories. In Assay 1, dimer potency was not significantly different from monomer. In Assays 2–4, dimer cross-reacted about one-third as well as did monomer. In Assay 5, dimer only cross-reacted 15% as well as monomer. Thus, the Hybritech IRMA is a relatively specific assay for monomeric GH. The decreased dimer cross-reactivity in the IRMA may reflect a superior analytical specificity resulting from the
use of monoclonal antibodies. Size variants of pituitary GH have decreased potency relative to monomeric GH with some monoclonal antibodies (22, 29). Presumably, certain epitopes are sterically masked in size-variant forms of GH (29). Alternatively, dimer formation could produce conformational changes in GH epitopes that decrease cross-reactivity with monoclonal antibodies (29). Several IRMA methods give higher estimates for GH than does the Hybritech kit, but values comparable to those values found with polyclonal-based RIAs (5, 6, 9). Additionally, the 20-kDa variant form of GH, which does not cross-react in the Hybritech IRMA, displays varying cross-reactivity in other IRMAs (7, 9). Thus, all IRMAs are not equivalent, which suggests that the selection of GH monoclonal antibodies is of pivotal importance for maximizing assay specificity for monomeric GH.

Using three different commercial kits, we measured the GH concentrations in serum samples obtained randomly from 18 normal adults. Assays 1, 2, and 5 were selected on the basis of their wide range of specificity for dimeric GH. The GH concentrations obtained with Assay 5 were significantly lower than the concentrations found with Assay 1 (Figure 3). Thus, we demonstrated a difference in apparent concentrations in serum as a result of analytical differences in commercial GH immunoassays. These data also support the results obtained in the cross-reactivity experiments. The highest mean concentration of GH in serum was found with Assay 1, the assay having the greatest dimer cross-reactivity. The assay displaying the lowest dimer cross-reactivity, Assay 5, also yielded the lowest mean value for GH in serum.

In summary, we have examined the specificity of five commercial GH immunoassays with recombinant DNA-derived monomeric and dimeric GH, the objective being to investigate a potential mechanism for the discordance in GH concentrations measured with commercial kits. A better understanding of these analytical differences will permit better clinical application of these methods in the diagnosis of disorders of GH secretion. We found that commercial kits differed substantially in their ability to detect both the monomeric and dimeric forms of biosynthetic GH. Thus, we recommend that biosynthetic monomeric GH be used as the reference material in commercial kits to assess GH deficiency. This should improve calibration and decrease kit-to-kit variability. The Hybritech kit was unique in that it was relatively specific for monomeric GH, the predominant biologically active form in vivo. The lower values for serum GH obtained with the Hybritech IRMA suggest that the molecular heterogeneity of circulating GH contributes to the discrepancies among commercial diagnostic kits.

References
27. Wallis M, Surowy TK, Daniels M, Harteas AS, Fosten A. An investigation of the heterogeneity of clinical grade human growth
Effects of Lyophilization of Serum on the Measurement of Apolipoproteins A-I and B

Santica M. Marcovina, Janet L. Adolphson, Mariella Partavecchia, and John J. Albers

A common accuracy-based standardization program is indispensable for establishing reference intervals for the clinical use of apolipoproteins. The development and distribution of reference materials and quality-control materials that do not exhibit matrix effects between methods is essential to the standardization process. We examined the suitability of lyophilized material as a common reference material for the measurement of apolipoproteins A-I and B. We determined values for apolipoproteins A-I and B in frozen and lyophilized serum pools, using different immunochemical approaches. We found little or no differences in apolipoprotein A-I values between frozen and lyophilized pools as determined by the different methods. In contrast, values for apolipoprotein B in lyophilized samples were consistently lower than those obtained for frozen samples. After adjusting for the effect of dilution due to reconstitution, the difference in the apolipoprotein B values for lyophilized as compared with frozen samples ranged from −26% to 4%, depending upon the assay method. Evidently, serum pools in lyophilized form are not a suitable matrix for reference materials for apolipoprotein B measurements but can be used for apolipoprotein A-I measurements.

Additional Keyphrases: standardization · reference materials · calibration materials

Measurements of apolipoproteins (apo) A-I and B are increasingly used in defining a person's risk of cardiovascular disease and for assessing therapeutic response. Between-laboratory comparability of these measurements and a common accuracy-based standardization program are essential for establishing reference values for clinical use. Appropriate reference materials are needed to assign target values to calibration materials and to minimize differences between methods.

Historically, serum pools in lyophilized form have been used as reference materials and as external quality-assurance materials for cholesterol and apolipoproteins (1, 2). These materials are stable during long-term storage and shipment and are relatively inexpensive to mail. Recently, in a study on the effect of lyophilization of serum pools on the determination of cholesterol, the measured concentration of cholesterol was shown to be less for lyophilized serum than for frozen serum, and the decrease in the original cholesterol value varied depending on the cholesterol assay method (3).

In our experience, if a pooled specimen of serum was stored frozen and used as a common calibrator for methods differing in principles and antibody source, essentially identical apo A-I and B values were obtained for fresh samples of normolipidemic sera. Despite agreement on patients’ samples, however, we found that with a serum pool in lyophilized form prepared as candidate Reference Material by the Centers for Disease Control (CDC) (pool 1883), there was about a 30% difference between apo B values obtained by radial immunodiffusion (RID) and those obtained by nephelometry. Therefore, one aim of the present study was to verify whether the differences obtained with CDC material were unique to the CDC material or common to other lyophilized materials. Furthermore, we examined the suitability of lyophilized material as a common reference material for use in measurements of apo A-I and B.

Materials and Methods

Serum Pools

Lyophilized pools used as calibrators of commercially available kits for apo A-I and B were obtained from seven different companies. Candidate serum reference pool for apo A-I and B was obtained from the CDC (pool 1883). Nine fresh-frozen and lyophilized serum pools were prepared as follows. For each serum pool, 2 mL of serum were obtained from each of 50 patients who had been pre-screened for cholesterol and triglyceride concentrations. All serum pools were made from subjects whose triglyceride concentration was <1.4 g/L. Serum with low triglyceride concentrations was selected because artifacts in apo B measurements can occur with hypertriglyceremic samples (4). The low-, normal-, and high-concentration pools were obtained by pooling specimens from patients whose cholesterol concentrations were, respectively, <2.0 g/L, between 2.0 and 2.4 g/L, and >2.4 g/L. Each pool was divided into 1-mL aliquots by a Micromedic dispenser and quick-frozen in individual vials at −78 °C. Half of the vials were kept frozen at

1 Northwest Lipid Research Center, Harborview Medical Center, and Department of Medicine, University of Washington, Seattle, WA 98104–2499.

Address for correspondence: S. M. Marcovina, NWLRC, ZA-36 Harborview Medical Center, 326 Ninth Avenue, Seattle, WA 98104–2499.

2 Laboratories of Immunochemistry and Lipoproteins, Scientific Institute San Raffaele, Milano, Italy.

3 Nonstandard abbreviations: apo, apolipoprotein; CDC, Centers for Disease Control; HDL, high-density lipoprotein; and RID, radial immunodiffusion.

Received September 28, 1989; accepted November 17, 1989.