in buffered phosphate–citrate with 20 g/L bovine serum albumin. After incubation, samples were stored at −70 °C. Tissue samples were homogenized, and CK, CKMB\textsubscript{act}, CKMB\textsubscript{mass}, and HBD were assayed in the homogenates as well as in the incubation buffers. CKMB\textsubscript{mass} completely disappeared within 2 h at pH 5.0 and 5.5 (Fig. 1). In contrast, HBD remained stable at all pH values, and CK remained stable for pH values down to 5.5, but was inactivated at pH 5.0. Because of the stability of total CK (predominantly CKMM) at pH 5.5, total inactivation of CKMB\textsubscript{act} at this pH may seem surprising. The Isomune assay, however, measures CK activity before and after blocking of M subunits and then subtracts the CK activity measured after the removal of all M units, including CKMB, from the sample. A CKMB molecule with an inactivated B subunit will not contribute to the activity in either fraction and thus will not be detected. We do not know if the M subunit activity remains.

The transmural ratios (endocardial/epicardial) of HBD and CKMB\textsubscript{mass} were computed in only four of the first nine non-AMI hearts and in four of the six AMI hearts because of the very low CKMB\textsubscript{mass} content (<0.2 μg/mg) in the remaining hearts. Because of the short time (<6 h) between onset of symptoms and death, infarcted areas in the AMI hearts had physiologic HBD content (>0.4 U/mg dw) and endocardial/epicardial ratios of HBD were equal in non-AMI and AMI hearts (1.08 ± 0.04 and 0.98 ± 0.05, respectively; mean ± 95% confidence interval). For CKMB\textsubscript{mass}, however, these values were 0.92 ± 0.12 and 0.53 ± 0.16, respectively (P <0.005; Student t-test). Apparently, endocardial CKMB\textsubscript{mass} disappears from AMI hearts even before significant leakage of proteins from the infarcted tissue has occurred.

In conclusion, our study shows that the low and highly variable CKMB content of myocardial autopsies is not related to the influence of sample location or autopsy delay, but may be caused by perimortal tissue acidosis. The narrow range of pH values at which inactivation occurs, between 5.5 and 6.0, could explain the noted heterogeneity of the GH molecule in human serum (c) the interference of endogenous GH binding protein (d); (c) the various GH epitope specificities of anti-GH antibodies; and (e) the addition of serum to GH calibrators. Because all of these reasons are closely interrelated, their interactions may also accentuate interlaboratory differences in results. The use of methods able to quantify the 22-kDa form of GH exclusively has been proposed as a means of approaching uniformity in results (5). The 22-kDa form is the major circulating fraction and carries the dominant bioactivity.

We analyzed serum GH cutoff values, using different immunoassays. We defined the commercial assay SER 66/217 as the Reference Method, and 10 μg/L as the reference cutoff value for this test, based on several years of clinical experience. In the present study, we analyzed 80 samples to compare the Reference Method with nine other commercially available assays. In addition, results of all assays were compared with those of the Delfia

References

Wallac assay (DELFIA 80/505), which utilizes a monoclonal antibody specific for 22-kDa GH.

Blood samples (n = 80) were from 42 different individuals (26 males and 16 females; age range, 3.2–16 years). All samples were selected from arginine and clonidine pharmacologic tests (6). Patients had either idiopathic short stature or GH deficiency, and basal samples were also selected to include both spontaneous and provoked GH secretion. Seventy percent of the collected samples had values close to 10 µg/L as measured by the SER 66/217 assay. Samples were fractionated once, and aliquots were kept frozen until assayed, up to 3 years.

The types of assay, antibodies, reference standards (2), assay specificities for the GH 22-kDa molecule as claimed by the manufacturers, and CVs are shown in Table 1. Serum GH was measured in every sample by 10 different commercial assays. In the case of the Serono immunoradiometric assays (Bio Chem Immuno Systems, Rome, Italy), the assay (SER 66/217) was used with WHO International Reference Preparation (IRP) for human GH for immunoassay 66/217, as provided by the manufacturer. However, it was also modified to generate two additional assays. Both modified assays used the original reagents, except for the reference standard: one assay used the WHO IRP for human GH for bioassay 80/505 (SER 80/505), and the other used the WHO IRP international standard for somatotropin 88/624 (SER 88/624). The other seven commercial assays were as follows (abbreviation is followed by type of IRP): four other IRMAs, IMM 80/505 (Immunotech, Marseilles, France), DSL 80/505 (Diagnostic System Laboratories, Webster, TX), NI 80/505 (Nichols Institute Diagnostics, San Juan Capistrano, CA), and DSL 88/624, a time-resolved fluoroimmunoassay; DELFIA 80/505 (Wallac Oy, Turku, Finland); a chemiluminescent enzyme immunoassay, DPC Q 80/505 (Diagnostic Products Corporation, Los Angeles, CA); and a RIA, DPC RIA 66/217. Assay CVs were calculated from duplicates.

To compare cutoff values, linear regression analyses between the 80 samples measured with the SER 66/217 (initial Reference Method) and with each of nine different commercially available assays were performed. The y value for 10 µg/L in the SER 66/217 assay was calculated from each equation line.

The influence of specific determination of 22-kDa GH in the assays was studied by choosing the 22-kDa GH-specific DELFIA 80/505 as our 22-kDa GH Reference Method. Ratio plots were used for between-assay comparisons (7). Ratios were calculated by dividing individual GH results determined by the DELFIA 80/505 assay by individual GH results determined by each of the other assays. A 95% prediction interval was calculated as proposed by Andersen et al. (7). Acceptability criteria were based on inherent analytical imprecision (8) and on analytical quality specifications for imprecision. The allowable CV was calculated from CVwithin-subject as proposed by Cotlove et al. (8): CVallowable = 1.96 × [(CVwithin-subject × 0.5)² + (CVwithin-subject × 0.5)]¹/₂. Bland–Altman difference plots (9) were also used: the difference between two methods (y axis) was plotted as a function of their mean GH concentration (x axis). To study possible effects of GH dose, linear regression was calculated for every difference plot.

The results of linear regression analyses between SER 66/217 (Reference Method) and the other nine assays are shown in Table 1. y values for a fixed x value of 10 µg/L are shown. Comparisons between the DELFIA 80/505 (22-kDa GH Reference Method) and the SER 88/624, IMM 80/505, and DSL 80/505 assays using ratio plots (individual GH results by DELFIA 80/505 divided by results of other assays) are shown in Fig. 1, which also shows both 95% prediction intervals and allowable limits. The mean ratio was 1.07 ± 0.16, 0.98 ± 0.20, and 1.31 ± 0.31 for the SER 88/624, IMM 80/505, and DSL 80/505 assays, respectively, whereas the within-subject biologic variation was 44%, 50%, and 49%, respectively. Additional figures showing the ratio plots for the remaining six assays are supplied in the data supplement for this report, available at Clinical Chemistry Online (http://www.clinchem.org/content/vol47/issue9).

### Table 1. Serum GH assay compared in this study: Assay characteristics.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Isotopic/Nonisotopic</th>
<th>Antibodies</th>
<th>Reference preparation (WHO IRP)</th>
<th>Specificity for 22-kDa GH</th>
<th>CVwithin-subject %</th>
<th>CVallowable %</th>
<th>y value for x = 10 µg/L in terms of SER 66/217</th>
</tr>
</thead>
<tbody>
<tr>
<td>SER 66/217</td>
<td>I</td>
<td>M/M</td>
<td>IRP 66/217</td>
<td>No*</td>
<td>5.0</td>
<td>7.0</td>
<td>10.11</td>
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<td>SER 88/505</td>
<td>I</td>
<td>M/M</td>
<td>IRP 80/505</td>
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<td>11.7</td>
<td>10.11</td>
</tr>
<tr>
<td>SER 88/624</td>
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<td>M/M</td>
<td>IRP 80/505</td>
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<td>5.9</td>
<td>4.39</td>
</tr>
<tr>
<td>IMM 80/505</td>
<td>I</td>
<td>M/M</td>
<td>IRP 80/505</td>
<td>Yes</td>
<td>6.6</td>
<td>9.3</td>
<td>5.04</td>
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<tr>
<td>DELFIA 80/505</td>
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<td>M/M</td>
<td>IRP 80/505</td>
<td>Yes</td>
<td>3.3</td>
<td>4.7</td>
<td>4.81</td>
</tr>
<tr>
<td>DSL 80/505</td>
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<td>M/M</td>
<td>IRP 80/505</td>
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<td>7.8</td>
<td>11</td>
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<td>M/M</td>
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<td>P</td>
<td>IRP 66/217</td>
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<td>13</td>
<td>18</td>
<td>7.21</td>
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<tr>
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<td>M/M</td>
<td>IRP 88/624</td>
<td>Yes</td>
<td>7.8</td>
<td>11</td>
<td>2.95</td>
</tr>
</tbody>
</table>

*CVwithin-subject and CVallowable: within- and between-assay CV, respectively.

b I, isotopic; N, nonisotopic; M, monoclonal antibody; P, polyclonal antibody.

c "No" indicates either nonspecific or information not supplied by the manufacturer.
Bland–Altman difference plots between the DELFIA 80/505 and three other assays were also constructed (data not shown). The mean bias for SER 88/624 (−0.41 μg/L), IMM 80/505 (0.22 μg/L), and DSL 80/505 (−0.64 μg/L) was similar and relatively small, and no concentration effect was observed.

In summary, our sample universe included patients with and without GH deficiency, both before and after two different provocative tests of GH secretion, in an effort to include samples with an ample variety of GH circulating forms. Differences among cutoff values of the 10 assays were striking. However, differences were smaller among the four assays specific for 22-kDa GH. Therefore, the information provided by our study could be useful in clinical practice to assess provocative tests when different assays are used. However, it is advisable that cutoff equivalents be checked in individual laboratories before being accepted.

The use of an assay system that exclusively quantifies 22-kDa GH has been recommended as part of an ideal GH assay (8). We therefore chose the DELFIA 80/505 as our Reference Method for 22-kDa GH and applied both the ratio plot, as recommended by Andersen et al. (7), and Bland–Altman difference plots (9) to compare differences among assays. With the ratio plots, only three other assays showed values with ratios close to 1 and variations within acceptable limits: SER 88/624, IMM 80/505, and DSL 80/505. Mean ratios were close to 1, and most of the 80 points were within the 95% prediction interval. This interval was narrower than the biologic variation of the sample. The last two assays share the same reference standard with the DELFIA 80/505, and the three are specific for 22-kDa GH. The explanation for the similarity with SER 88/624 is not apparent. When we compared these methods, we also found that there was no dose effect in the Bland–Altman difference plots, making them more reliable, at least in the concentration ranges studied. We conclude that the DELFIA 80/505, IMM 80/505, DSL 80/505, and SER 88/624 could be used interchangeably, but with a cutoff between 4.2 and 5 μg/L, to assess provocative tests of GH secretion in children.

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References
Plasma Total Homocysteine in Hyper- and Hypothyroid Patients before and during 12 Months of Treatment, Bjørn G. Nedrebø,* Ottar Nygård, Per M. Ueland, and Ernst A. Lien (LOCUS for Homocysteine and Related Vitamins, University of Bergen, 5021 Bergen, Norway; * address correspondence to this author at: Department of Internal Medicine, Haukeland University Hospital, N-5021 Bergen, Norway; fax 47-55975814, e-mail bjoern.gunnar.nedrebo@haukeland.no)

There are consistent reports demonstrating that thyroid status is an important determinant of the plasma/serum concentration of total homocysteine (tHcy) (1–7), which has been established as an independent risk factor of vascular occlusive disease (8). In previous studies, we showed that plasma tHcy was higher in hypothyroid than in hyperthyroid patients (1, 2). Prospective studies have demonstrated normalization of tHcy after thyroid hormone replacement therapy in hypothyroid patients (3, 4), and we recently observed a transient increase in tHcy during short-term iatrogenic hypothyroidism in thyroidectomized patients (5).

To our knowledge, there has been no longitudinal study on tHcy in hyper- and hypothyroid patients across normalization of their thyroid status during treatment. In the present study, we investigated such patients before and during the first 12 months of treatment.

Forty consecutive patients with hyperthyroidism and 12 with hypothyroidism were enrolled. The study protocol was approved by the ethics committee of Haukeland University Hospital, and informed consent was obtained from all participants.

The diagnosis of hyperthyroidism was based on basal serum thyrotropin (TSH) values <0.3 mIU/L, and hypothyroidism on TSH >15 mIU/L. All hyperthyroid patients except one were diagnosed with Graves disease.

Thirty-one of the patients with hyperthyroidism were treated with carbimazole or propylthiouracil. Nine of the patients with hyperthyroidism received radioiodine treatment as primary treatment. All patients with hypothyroidism received thyroxine as the only thyroid hormone replacement therapy.

Blood specimens were collected at inclusion and 1, 6, and 12 months after initiation of treatment. Fasting EDTA-blood samples for tHcy determination were centrifuged within 30 min. The EDTA-plasma was stored at −20°C until analysis. Plasma tHcy, serum cobalamin, folate, creatinine, and cholesterol were determined according to established methods (5, 9). TSH and free thyroxine (FT₄) in serum were measured with the AutoDELFIA hTSH Ultra reagent set and the AutoDELFIA FT₄ reagent set from Wallac Oy. Thyroid receptor anti-bodies (TRAbs) were determined by radioimmunoassay from DLD Diagnostika GMBH.

Baseline characteristics of the hyper- and hypothyroid patient groups were compared by Mann-Whitney U-test. To investigate changes in analyte concentrations within each group, we applied analyses of covariance using an unbalanced repeated-measure design (5V module in BMDP) (10). The covariance between any pairs of repeated measurements was assumed to be equal (compound symmetry). In case of missing data in the response variable (unbalanced data), the program computes and substitutes for the missing value an imputed value that is predicted from the covariate information. In the present study, missing values in the response variables varied from 0% at baseline up to 32% for tHcy in hyperthyroid patients at 12 months. The maximum likelihood of regression and covariance parameters were calculated.

The change in parameters over time was represented by a linear trend model. The time trend was coded as 0, 1, 2, and 3. Thus, the regression coefficients for time trend represent the change relative to the previous visit and are not a function of the length of follow-up. The intercept in this model represents the estimated concentration before treatment, which is close to the mean concentration at baseline. Analyses or graphic presentations based on log-transformed data, percentage of change from baseline, median values, or individual raw data gave similar results and are not presented. Essentially the same results were obtained when the analyses were restricted to patients with complete data sets (25 hyperthyroid, 9 hypothyroid). We also investigated the change in tHcy by treatment after adjustment for sex, age (years), and one additional covariate.

For patients with hyperthyroidism (n = 40) and hypothyroidism (n = 12), the median ages were, respectively, 44 years (range, 19–89 years) and 55 years (range, 35–78 years). In both groups, 75% of the patients were women. In patients with hyperthyroidism, median FT₄ was 57.4 pmol/L (24.2 to >77 pmol/L), and except for one patient with a TSH value of 0.1 mIU/L, all remaining patients had TSH <0.05 mIU/L. In hypothyroid patients, median FT₄ was 5.4 pmol/L (<5 to 8.6 pmol/L). Three hyperthyroid patients had TSH values of 21.0, 28.2, and 33.5 mIU/L, respectively; all remaining patients had TSH >50 mIU/L.

At baseline, tHcy in plasma and folate, total cholesterol, HDL-cholesterol, and creatinine in serum were significantly higher in patients with hypothyroidism than in patients with hyperthyroidism, whereas cobalamin and triglycerides did not differ between the patient groups (data not shown).

In hyperthyroid patients, tHcy, creatinine, and cholesterol increased during treatment. Serum folate decreased significantly, whereas there was no significant change in cobalamin (Fig. 1 and Table 1). In hypothyroid patients, significant decreases were observed for tHcy, creatinine, and cholesterol. There were no significant changes in cobalamin and folate (Fig. 1 and Table 1). After 12 months