give false-positive results in S. pneumoniae cases because only the amount of CDT (a- to d- or trisialotransferrin) and/or the CDT/transferrin ratio is determined (19). HUS associated with S. pneumoniae therefore has to be considered in the diagnosis of primary and secondary defects in the glycan part of N-glycoproteins.

The non-S. pneumoniae-associated HUS cases showed normal isof orm distribution in both glycoproteins investigated in this study. This means that IEF of plasma transferrin distinguishes between S. pneumoniae-associated HUS and the two other HUS forms. This technique may therefore be complementary to the conventional bacteriologic techniques that are available for this purpose and may help to avoid the use of blood transfusions which would be life-threatening in cases of HUS associated with a S. pneumoniae infection.

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References
10. Stibler H. CDT in serum: a new marker of potentially harmful alcohol pose and may help to avoid the use of blood transfusions which would be life-threatening in cases of HUS associated with a S. pneumoniae infection.

Can Glycohemoglobin Be Used to Assess Glycemic Control in Patients with Chronic Renal Failure? Randie R. Little,1,2* Alethea L. Tennill,1 Curt Rohlfing,1 Hsiao-Mei Wiedmeyer,1 Ramesh Khanna,3 Sharad Goel,4 Richard Madson,5 and David E. Goldstein1,2,3 (Departments of 1 Child Health, 2 Pathology and Anatomical Sciences, and 3 Internal Medicine, University of Missouri School of Medicine, 1 Hospital Dr., Columbia, MO 65212; 4 Kidney & Hypertension Center, 1210 Hicks Blvd., Fairfield, OH 45014; 5 Renal Physicians, Inc., 4700 Springboro Pike, Dayton, OH 45439; 6 Department of Statistics/Biostatistics, 223 Math Sciences Bldg., University of Missouri, Columbia, MO 65211; * address correspondence to this author at: Department of Child Health, M767, University of Missouri School of Medicine, 1 Hospital Dr., Columbia, MO 65212; fax 573-884-8823, e-mail LittleR@health.missouri.edu)

Many factors can affect interpretation of glycohemoglobin (GHB/HbA1c) measurements in patients with chronic renal failure (CRF). Several reports have suggested that erythrocyte survival is substantially lowered in most patients with CRF; this would be expected to lower GHB results (1–6). Several reports have also suggested that GHB methods, especially those based on charge separation (e.g., ion-exchange HPLC), may have interference by carboxylated hemoglobin that would be expected to falsely increase GHB results (7–17). Many of these reports evaluated older assay methods; newer ion-exchange methods may show improved separation of the HbA1c fraction from other hemoglobin adducts (15, 17).

Because renal failure is common in patients with diabetes and GHB is widely used as an index of mean blood glucose in these patients, we examined GHB results in patients with CRF by several different GHB assay methods. We also investigated the impact of shortened erythrocyte lifespan by comparing the GHB results obtained for nondiabetic patients with and without CRF.

Fifty-five patients with CRF (blood urea nitrogen >400 mg/L) were recruited for this study. Twenty-nine were not receiving dialysis and were seen at the University of Missouri Nephrology Clinic; the remaining 26 had end stage renal disease and were receiving hemodialysis at the Dialysis Clinic. Patients with renal failure included those with and without diabetes (n = 28 and 27, respectively). The GHB concentration (reported as percentage of HbA1c or equivalent) ranged from 4.4% to 11.2%. Informed consent was obtained from all participants according to
Routine clinical specimens from the University of Missouri Hospital and Clinics sent for analysis of GHB as well as specimens from nondiabetic volunteers served as non-CRF control specimens. Control specimens (n = 55) were selected to approximate the GHB range of the CRF samples, and all were confirmed to have blood urea nitrogen <200 mg/L.

All samples were analyzed for GHB with the following methods: Primus CLC330 HPLC (Primus Corporation), Diamat and Variant HPLC (Bio-Rad Laboratories), 2.2 Plus HPLC (Tosoh Medics), and Unimate/Cobas Mira (Roche Diagnostics). A subset of each group of specimens (n = 38) was also analyzed using Variant II HPLC (Bio-Rad Laboratories). The Primus CLC330 was used as the comparative method because carbamylated hemoglobin has been shown not to interfere with affinity chromatography methods (12–14). Our data from in vitro carbamylated specimens supported this finding; whole blood samples incubated in 5 mmol/L sodium cyanate for 2 h did not show any increase in GHB with the Primus method (R. R. Little, A. L. Tennill, C. Rohlfing, H. M. Wiedmeyer, D. E. Goldstein, unpublished data).

Linear regression analyses were performed between each test method and the comparative method (Primus) for each group of specimens (with or without CRF). An overall test of coincidence was used to determine the statistical significance of CRF on the relationship between each test method and the comparative method, i.e., whether the two regression lines (CRF, test vs comparative method; non-CRF, test vs comparative method) were significantly different. In addition, the slope and intercept for each line were examined separately.

Linear regression analyses were also used to determine whether the presence of carbamylated hemoglobin produced clinically significant interference. Given the Diabetes Control and Complications Trial Reference Method upper limit of normal of ~6% and the American Diabetes Association goal and action limits of 7% and 8%, respectively, GHB (HbA1c) concentrations of 6% and 9% were chosen as important evaluation limits. Given the need to clearly distinguish the difference among 6%, 7%, and 8% GHB, a clinically significant difference was defined as ≥0.5% GHB. Both a t-test and a Mann-Whitney test were used to compare GHB results for nondiabetic individuals with and without CRF (measured using the comparative method) to assess whether GHB results for CRF patients were significantly affected by altered erythrocyte lifespan.

Although there was a statistically significant difference (P < 0.05) between the regression lines (CRF vs non-CRF) for all methods except the Variant II (Tosoh, P = 0.0058; Diamat, P = 0.0026; Unimate, P = 0.0183; Variant, P = 0.0001; Variant II, P = 0.2338), only the Variant HPLC method showed a clinically significant positive bias (0.59% at 6% GHB, 0.88% at 9% GHB) attributable to CRF (Table 1). Although it is possible that the smaller sample size used for the Variant II analysis could have provided insufficient power to detect a statistical difference, it is unlikely given the small bias at both 6% and 9% GHB. The difference in results between the Variant HPLC and the comparative method was significantly correlated with blood urea nitrogen (n = 110; r = 0.54; P < 0.0001).

For nondiabetic individuals, the mean ± SD was 5.11% ± 0.44% GHB for the group with CRF (n = 27) and 5.06% ± 0.36% GHB for the group without CRF (n = 30). There was no significant difference in the percentage of GHB between the means of the two groups of nondiabetic individuals (P = 0.63, t-test). Because the distribution of data appeared to be somewhat nonGaussian, we also performed a Mann–Whitney test, which showed no significant difference between the medians of the two groups (5.00% vs 4.95%; P = 0.79).

Of the four ion-exchange methods evaluated, only the Variant HPLC showed a clinically significant positive bias in CRF. Unfortunately, there is no simple way of determining which samples have high concentrations of carbamylated hemoglobin during routine measurement of GHB. Each new GHB method should be evaluated for interference from carbamylated hemoglobin; interference cannot be determined based on the method type (e.g., ion-exchange chromatography).

We did not see any evidence of shortened erythrocyte survival in the present group of nondiabetic patients with renal failure. It is possible that, contrary to some reports, most patients with CRF do not have shortened erythrocyte survival. Alternatively, impaired glucose control in CRF may have offset the effect of shortened erythrocyte survival.

We conclude that GHB can provide valid results for most patients with CRF if an appropriate methodology is used.

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References

Table 1. Carbamylated hemoglobin interference by method.

<table>
<thead>
<tr>
<th>Method</th>
<th>At 6% GHB</th>
<th>At 9% GHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diamat (HPLC)</td>
<td>0.23</td>
<td>0.42</td>
</tr>
<tr>
<td>Variant HbA1c (HPLC)</td>
<td>0.59*</td>
<td>0.88*</td>
</tr>
<tr>
<td>Tosoh 2.2 Plus (HPLC)</td>
<td>-0.15</td>
<td>0.23</td>
</tr>
<tr>
<td>Unimate immunoassay</td>
<td>-0.01</td>
<td>0.26</td>
</tr>
<tr>
<td>Variant II HbA1c (HPLC)</td>
<td>0.00</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Clinically significant.


Estimation of True Calcium Absorption in Men, Robert P. Heaney,* Mary Susan Dowell,1 and Randi L. Wolf2 (1 Creighton University, Omaha, NE 68131; 2 Teachers College, Columbia University, New York, NY 10027; * address correspondence to this author at: Creighton University, 601 N. 30th St., Suite 4841, Omaha, NE 68131; fax 402-280-4751, e-mail rheaney@creighton.edu)

When both calcium intake and calcium absorption are measured under controlled conditions, variation in absorption efficiency explains more of the interindividual differences in balance than does actual calcium intake (1). Small wonder, therefore, that interest in measuring absorption has remained high for nearly 40 years.

True calcium absorption is defined as the quantitative, unidirectional flux of calcium from intestinal lumen into the blood. It is most accurately measured by a dual-tracer method, with one tracer labeling the oral calcium load and the other labeling the miscible calcium pool into which the absorbed calcium is introduced. This approach was first developed into a practicable human test by deGrazia et al. (2). As described, it is usually time-consuming and expensive. To reduce these barriers for widespread use, Heaney and Recker (3, 4) developed a single-tracer variant for women, requiring only a single blood sample, and calibrated it against a simultaneously performed double-tracer method. The single-tracer method has been used efficiently in thousands of women (5). However, because the calibration is empirical and based on body-size variables, it is not directly suitable for use in men who, with a typically higher proportion of fat-free mass than women, would be expected to distribute absorbed tracer in a larger mass of calcium.

To fill this methodologic gap, we performed a small set of parallel measurements in adult men, using the female-based algorithm together with a modified double-tracer approach.

Participants in the study were 30 Caucasian men (age range, 20–60 years; weight range, 63.5–104 kg; height range, 1.67–1.93 m). All participants were free of known diseases affecting bone remodeling or calcium homeostasis, and tests were not performed if the individual had experienced any gastrointestinal disturbance in the preceding 5 days. Each gave informed consent after the procedures of the study were explained. Both the project and the consent were approved by the Creighton Institutional Review Board. Each volunteer was tested twice. We performed the first test for several unrelated projects, using the standard, single-dose protocol, giving a 45Ca-labeled oral load and obtaining the usual 5-h serum sample for measurement of serum calcium specific activity. The volunteers abstained from all food after the test breakfast until the 5-h blood sample was drawn. The test calcium load (depending on the individual projects) was 300 mg in 25 individuals and 500 mg in 5. Sources were calcium-fortified orange juice in 20 volunteers, skim milk in 5, and precipitated calcium carbonate in 5. The second test, performed 6.2 (± 3) days later, used an intravenous (i.v.) dose of high-specific activity 45Ca, given 2 h after an identical test breakfast that contained the same oral calcium source and the same calcium load as on the first test day. With the second test, serum was obtained 3 h after the i.v. dose for measurement of serum calcium specific activity. This timing reproduces accurately the dosing scheme of a simultaneous double-tracer experiment in which, as originally described (2), the i.v. tracer is given 2 h after the oral tracer. (The 2-h lag introduces the i.v. tracer at the approximate midpoint of absorption of the oral tracer.) Because the same tracer was used to label both the oral load and then subsequently the miscible calcium pool, a baseline serum sample was obtained at the second test to determine the concentration of residual tracer from the earlier oral test. (Mean correction was −9.6% of the total counts in the 3-h blood sample, with the maximum being −25% for the shortest interval and the minimum being −3.1% for the longest.)

Absorption fraction was calculated in two ways. The first consisted of the female-derived algorithm:

\[
\text{AbsFx} = (\text{SA}_5^{0.9237}) \times [0.3537 \times (\text{Ht}^{0.52847}) \times (\text{Wt}^{0.37213})]
\]

in which AbsFx, SA, Ht, and Wt represent absorption fraction, 5-h serum calcium specific activity (fraction of oral tracer dose/g calcium), height (m), and weight (kg),