say CVs. The high precision of the results may enable single measurements instead of multiple determination, thereby reducing costs.

The significantly lower values for ghrelin in citrate plasma may only partially (~12%) be explained by dilution with the anticoagulating liquid in the tubes (118 ± 15 µL). The discrepancy between citrate plasma and serum was ~25%, a discrepancy that is too high for a recommendation for citrate plasma. In contrast, results for lithium-heparin plasma were in only ~7% lower than serum results, which we consider to be acceptable.

It should be kept in mind that the magnitude of the difference between values from matched sample matrices might be influenced by the assay system used, as has been shown for the determination of cardiac troponin T and I (14, 15). Our findings are based on the use of a direct RIA that is commercially available and is currently widely used for research.

As we have determined, storage of serum under cooled conditions allows stable results for up to 3 days. Storage at warm temperatures for >1 day should be avoided. This is very important when samples are transported by mail. Because no significant decrease in the ghrelin values was observed after repeated freezing and thawing, there should be no problems if sample tubes are used several times, e.g., for repeating an assay or using material after determination of other analytes.

In conclusion, ghrelin is relatively stable when stored under cooled conditions. This, as well as the fact that several sample matrices can be used as alternatives, is a good precondition for further studies on this interesting peptide hormone.

References


Biological Variation of Glycohemoglobin, Curt Rohlfing,1 Hsiao-Mei Wiedmeyer,1 Randie Little,1 V. Lee Grotz,2 Alethea Tennill,1 Jack England,1 Richard Madson,1 and David Goldstein1 (1 University of Missouri School of Medicine, Columbia, MO 65212; 2 McNeil Specialty Products Company, New Brunswick, NJ 08903; * address correspondence to this author at: Department of Child Health, University of Missouri–Columbia, 1 Hospital Dr., M772, Columbia, MO 65212; fax 573-884-4748, e-mail RohlfingC@health.missouri.edu)

Glycohemoglobin (GHb) is a measure of long-term mean glycemia that predicts risks for the development and/or progression of diabetic complications in patients with type 1 and type 2 diabetes (1, 2). Several reports have suggested, however, that although the within-subject variation in GHb unrelated to glycemia is minimal, there is substantial between-subject variation in GHb, e.g., “low glycators” and “high glycators” (3–5). These reports have suggested that because of this large between-subject variation, GHb may not be useful for diabetes screening or diagnosis and that when GHb is used for routine management of patients with diabetes, different patients may require very different GHb target values to achieve the same overall glycemic status. We therefore examined the biological variation of GHb and fasting plasma glucose (FPG) in nondiabetic individuals.

Individuals without diabetes (n = 48) participated in a study of an artificial sweetener that has no effect on GHb or plasma glucose concentrations [Submission to Food and Drug Administration. McNeil Specialty Products Company food additive petition 7A3987 (Sucralose), 1987–1997]. Because the study was designed to detect minimal changes in plasma glucose concentrations, all participants were men to avoid the effects of cyclic hormonal changes on insulin (and therefore, plasma glucose) concentrations. At the presurvey screening, all individuals were healthy on the basis of a medical history, physical examination, and electrocardiography results; results of hematologic and blood chemistry studies, urine examination, and measures of blood glucose control (FPG, insulin, C-peptide, and hemoglobin A1c) were all within their respective reference intervals. Participants who failed a baseline oral glucose tolerance test [fasting >7.8 mmol/L (140 mg/dL), 1 h >11.1 mmol/L (200 mg/dL), and/or 2 h >7.8 mmol/L (140 mg/dL)] were excluded. Those who took medications that could affect glucose
metabolism or who failed a drugs-of-abuse screen, had a history of a gastrointestinal disorder, or had a history of consuming more than two alcoholic drinks per day were also excluded. Serial samples for FPG and GHb analysis were collected by venipuncture after a minimum 8-h overnight fast on a weekly basis for a total of 12 visits. Three men with <10 data points for either FPG or GHb were excluded from the analysis. The study received approval from the institutional review boards of all participating study centers, and all individuals gave informed consent before their participation.

GHb values were measured by HPLC (Primus boronate affinity; interassay CV <3%) (6). FPG values were measured by a hexokinase assay (Roche Cobas Mira; interassay CV <3%) (7). SAS software was used to perform all statistical analyses; linear regression analysis examined the correlation between initial FPG and GHb values. We estimated the between-subject ($S_g^2$), within-subject ($S_i^2$), and assay ($S_a^2$) components of the total variance by a nested ANOVA (8) using the SAS Proc VarComp software; restricted maximum likelihood was the method of estimation. We calculated the within-day component of assay variance (within-day $S_a^2$) by combined within-run variance estimates for quality-control specimens analyzed two to five times in each analytic assay.

The mean, minimum, and maximum GHb values for each participant are shown in Fig. 1. The correlation between initial GHb and FPG values was weak but statistically significant ($r^2 = 0.102; P < 0.05$). Table 1 shows the estimated variance components for GHb and FPG. The $S_g^2$ component for GHb was much larger than the $S_i^2$ component. The mean GHb value was 4.9%; the between-subject SD ($S_g$) was 0.20% GHb (CV$_g$ = 4.0%). Thus, the between-subject mean ± 2 SD interval (95% confidence interval) was 4.5–5.3% GHb. The within-subject SD ($S_i$) was 0.08% GHb (CV$_i$ = 1.7%), and the between-day assay SD (between-day $S_a$) was 0.11% GHb (CV$_a$ = 2.3%). The $S_i^2$ component of variation included the within-day $S_a^2$ and $S_i^2$ because we were unable to directly separate the two components (the specimens were not analyzed in duplicate). However, the estimated within-day analytic SD (within-day $S_d$), based on quality-control data, was 0.07% GHb (within-day CV$_d$ = 1.5%), which indicates that most of the estimated CV$_i$ was attributable to the within-day $S_a^2$ rather than $S_i^2$.

For FPG, $S_g^2$ and $S_i^2$ were comparable. The mean FPG value was 5.3 mmol/L; the estimated $S_{g'}$, $S_{g''}$, and between-day $S_{g''}$ SDs were 0.31 mmol/L (CV$_g$ = 5.8%), 0.30 mmol/L (CV$_i$ = 5.7%), and 0.09 mmol/L (between-day CV$_a$ = 1.7%), respectively. The estimated within-day $S_{a'}$ based on quality-control data, was 0.04 mmol/L (within-day CV$_a$ = 0.8%), which indicates that most of the estimated CV$_i$ was attributable to $S_{a''}^2$.

Our data show that for GHb, $S_d^2$ in nondiabetic individuals is minimal. Although we were unable to obtain a precise estimate of $S_d^2$ separate from $S_g^2$, the CV$_g$ was likely <1%. Although the between-subject component was the largest component of the total variation in GHb, the between-subject mean ± 2 SD range in GHb for nondiabetic men was <1% GHb after accounting for $S_g^2$.

A previous large-scale study has shown that GHb reliably categorizes glycemic control in nondiabetic individuals (9), and several studies have shown correlations between GHb concentrations and outcome risks in both diabetic (1, 2) and nondiabetic (10, 11) persons. Such findings suggest that the between-subject differences in GHb are mainly attributable to differences in mean glycemia rather than other factors.

Several factors may explain the relatively poor correlations observed between FPG and GHb values in nondiabetic individuals (3, 12). Both $S_d^2$ and $S_g^2$ were higher for FPG than for GHb, and the intervals for these variables in nondiabetic individuals are relatively narrow. We also note that GHb is a more comprehensive measure of mean glycemia than FPG, as evidenced by recent studies showing that, in diabetic individuals, postmeal plasma glucose correlates better with GHb than does FPG (13, 14).

Biological variation has generally been defined as “random fluctuations around a homeostatic set-point” (9, 15). Several studies have examined biological variation in diabetic individuals and have concluded that there is a significant biological variation in GHb values that must be considered when test results are interpreted (5, 15). It is important to note that in diabetes patients, fluctuations in GHb concentrations are not random but are “pathologic”, i.e., caused by changes in mean glycemia. It is also unclear how a homeostatic setpoint can be determined for an

![Fig. 1. Mean, minimum, and maximum GHb for study participants. HbA1c, hemoglobin A1c.](image-url)

**Table 1. Variance components for GHb and FPG.**

<table>
<thead>
<tr>
<th>Variance component</th>
<th>GHb, %</th>
<th>FPG, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between-subject $S_g$ (CV$_g$)</td>
<td>0.20 (4.0%)</td>
<td>0.31 (5.8%)</td>
</tr>
<tr>
<td>Within-subject $S_i$ (CV$_i$)*</td>
<td>0.08 (1.7%)</td>
<td>0.30 (5.7%)</td>
</tr>
<tr>
<td>Analytic $S_a$ (CV$_a$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between day</td>
<td>0.11 (2.3%)</td>
<td>0.09 (1.7%)</td>
</tr>
<tr>
<td>Within day$^a$</td>
<td>0.07 (1.5%)</td>
<td>0.04 (0.8%)</td>
</tr>
</tbody>
</table>

* Also includes within-day analytical variation.

$^a$ Estimated from quality-control data.
individual with diabetes because the setpoint itself can (and often does) change over time.

In summary, these data suggest that between-subject variation in GHb is minimal and is therefore not a major consideration when GHb is used for routine clinical care. Assay quality, however, is an important factor when interpreting GHb results (16–18), and imprecise assays may compromise the clinical utility of the test.

References


6. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin–assay quality, however, is an important factor when interpreting GHb results (16–18), and imprecise assays may compromise the clinical utility of the test.

Soluble Transferrin Receptor (sTfR), Ferritin, and sTfR/Log Ferritin Index in Anemic Patients with Nonhematologic Malignancy and Chronic Inflammation, Eun Jung Lee,1 Eun-Jee Oh,2 Yeon-Joon Park,2 Hae Kyung Lee,1 and Byung Kee Kim1 (1 Department of Clinical Pathology, College of Medicine, the Catholic University of Korea, Uijongbu St. Mary’s Hospital, 65-1 Kumoh-Dong, Uijongbucity, Kyunggi-Do 480-130, Korea; 2 Department of Clinical Pathology, College of Medicine, the Catholic University of Korea, Kangnam St. Mary’s Hospital, 505 Banpo-dong Seocho-ku, Seoul 137-040, Korea; * author for correspondence: fax 82-2-592-4190, e-mail ejoh@catholic.ac.kr)

The soluble transferrin receptor (sTfR) has been introduced as a promising new diagnostic tool for differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) (1–3). The circulating sTfR concentration is proportional to cellular expression of the membrane-associated TfR and increases with increased cellular iron needs and cellular proliferation (4). Furthermore, because serum ferritin reflects the storage iron compartment and sTfR reflects the functional iron compartment, the sTfR/log ferritin index (sTfR-F index), based on these two values, has been suggested as a good estimate of body iron compared with the sTfR/ferritin ratio (5). However, whether they could be useful in evaluating the iron deficiency in various malignancies has not been reported. In addition, some data have demonstrated that sTfR offers little advantage over conventional laboratory indicators of iron status (6) and might not assess the iron status of patients with ACD. A potential explanation for these differences may be the ACD patient population studied. Because several studies of patients with solid malignancies have reported that the erythropoietin concentrations are inappropriate for the degree of anemia (7) and chemotherapy-induced bone marrow (BM) suppression may also decrease sTfR concentration, the sTfR concentrations in the ACD population may depend on the proportion of patients with malignancy in the study group.

In the present study, we assessed the diagnostic performance of sTfR, ferritin, and sTfR-F index for detecting iron depletion in several groups of patients (IDA, chronic inflammation or infection, and nonhematologic malignancy) according to the guidelines (8). The diagnostic classification of all patients was based on an examination of the BM iron stain as the gold standard for iron depletion.

The patient population consisted of 120 (58 men and 62 women; age range, 21–85 years; mean, 54 years) anemic adult patients who underwent a BM examination for anemia study and 81 nonanemic controls. The 120 anemic patients were divided into five populations on the basis of the BM examination and clinical data: IDA (n = 31), which included patients who had no stainable iron in the BM because of an uncomplicated iron deficiency (i.e., simple blood loss); I-IDA (n = 15), which included patients who had chronic inflammatory disease (chronic