Intraindividual Variation of Glycohemoglobin: Implications for Interpretation and Analytical Goals

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Intraindividual variation (CV) for glycohemoglobin (GHB) was estimated from serial measurements in patients with diabetes in either stable or variable clinical control. GHB determinations were performed by an affinity column procedure with an analytical imprecision of 4.9% (weighted average; GHB 8.2–14.7%). Within the groups of patients, both a short- (28–32 days) and long-term (85 days) sampling protocol was used. The derived CV, for each category was 4.2% (n = 16, stable, short-term), 7.1% (n = 23, stable, long-term), 5.1% (n = 13, variable, short-term), and 9.8% (n = 21, variable, long-term). The mean GHB within each category was similar (~11%), and there was no statistically significant difference in GHB values between categories. The results establish that the CV, for GHB is affected by both clinical control and the sampling time interval. These findings have important implications for the estimation of significant differences between serial GHB measurements and the setting of appropriate analytical precision goals.

Indexing Terms: diabetes mellitus - assay imprecision

Measurement of glycohemoglobin (GHB) in diabetic patients is currently acknowledged as the most reliable indicator for assessment of retrospective glycemic control and the planning of clinical management (1–3). Interpretation of changes in patients’ results, however, depends on the analytical imprecision and biological variability. Furthermore, there is increasing consensus that performance standards for analytical methods, such as those based on estimations of GHB, are best derived from data on biological variation (4–7) rather than the judgment of clinicians or health professionals (8, 9).

In this paper, we estimate biological variability and discuss appropriate analytical goals based on serial GHB data, measured by an affinity column method, from diabetic patients attending an outpatient clinic.

Materials and Methods

Materials. The GlycoTest-100 affinity mini-columns were purchased from Pierce Chemical Co., Rockford, IL.

Preparation of controls. Hemolysates from patients’ samples were prepared from unwashed packed cells by lysing one volume of cells with 20 volumes of distilled water. After centrifugation (1000 x g, 10 min), the clear supernate was separated, and 200-μL aliquots were stored in sealed glass ampoules at –70 °C.

Assay procedure. The method was substantially as previously described (10), except that duplicate aliquots (300 μL) of the bound and unbound eluates were pipetted into the wells of a microtiter plate, and the absorbance at 415 nm, corrected for background at 630 nm, was determined for each well with a Bio-Tek EL311 microplate reader (Bio-Tek Instruments, Winooeki, VT) interfaced to a microcomputer for immediate data reduction.

Subjects. The first group of subjects (n = 26), insulin-dependent and noninsulin-dependent diabetic patients, was participating in a double-blind, placebo-controlled, randomized multicenter trial conducted over 48 weeks to evaluate the effect of an angiotensin-converting enzyme inhibitor on microalbuninuria. Specific inclusion criteria were GHB ≤13.5%; mean sitting diastolic blood pressure ≤95 mmHg on two of three occasions; body mass index ≤32 kg/m². Exclusion criteria included cardiovascular or other medical diseases; medication affecting blood pressure; women of childbearing age; and lack of cooperation. All patients were seen monthly and any adverse events, either clinical or laboratory, were noted. The study was approved by the Queen Elizabeth Hospital Ethics Committee and written informed consent was obtained from all patients. These patients were categorized as being in stable clinical control. The short-term intraindividual variation was estimated for GHB by sampling at 4-week intervals during the 12-week run-in phase. Within the short-term sampling regime, eight patients only had three GHB measurements performed and were excluded from this part of the study. The long-term GHB variation was based on five GHB measurements determined at about 12-week intervals.

The second group comprised patients (n = 34) regularly attending an outpatients diabetic clinic and who had had at least four GHB measurements at ~28- or 84-day intervals. These patients were categorized as being in variable clinical control because of numerous complicating and coexisting medical problems that affected overall management of their diabetes.

The basic difference between the categories of stable and variable clinical control therefore relates to the absence or presence of ongoing and coexisting medical disorders, frequency of hospitalization, and (or) the necessity to regularly attend the Outpatients Clinic for clinical management.

Results

Imprecision. The intra- and interassay CVs for the affinity column method were calculated as described by

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© Nonstandard abbreviations: GHB, glycohemoglobin; CV, intraindividual variation; CV, total assay imprecision; and HbA1c, hemoglobin A1c.

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Linnet (11) from the analysis of duplicate hemolysates (see Materials and Methods) within each assay set. During the overall study time span of 19 months, 11 different controls (median 11.7%, range 8.2–14.7%) were used. The estimate of total assay imprecision (CVt) was based on the weighted average of all the CVs so obtained and calculated to be 4.9%.

Intraindividual variation. Subjects whose serial GHB values had a statistically significant trend (P ≤0.01) were excluded from the study (12), resulting in omission of GHB data for two and three patients from the short- and long-term categories, respectively. Within each of the four categories, the series of individual mean GHB values and their variance were tested for the presence of outliers with the Grubbs (13) and Cochran (d) tests, respectively. The separate categories of patients had homogenous intraindividual variances (P > 0.1), as determined by Bartlett’s procedure (14). The groups of patients and the respective statistical analysis of GHB data are summarized in Table 1. Figures 1–4 show the mean and range of GHB levels within the four categories. There was no statistically significant difference (P > 0.1, Mann–Whitney U test) among the four categories with respect to the GHB values. It may therefore be assumed that the overall degree of blood glucose control was similar between the categories. Analysis of variance of the log value of the GHB variances between the four groups of patients established a significant intergroup effect (F8.69 = 3.7, P = 0.016), and Bartlett’s test for equality of variances was not statistically significant (P > 0.05). The true intraindividual variance was constant for each category, based on the calculated index of heterogeneity, whereas the estimate of the common true variances (σ2) was negative for all groups except for long-term sampling and variable clinical control (Table 1). The index of heterogeneity and σ2 calculated for all patients as one group were 1.08 and 0.054, respectively, indicating a general increase in heterogeneity of intra-subject variances. The derived median intraindividual variation (Table 1) was used to determine a critical difference between two successive measurements in a patient (d).

Discussion

Traditionally, clinicians interpret serial measurements of GHB by knowing the total variance of the measurement and setting guidelines for significant changes in terms of this variance. Laboratory scientists may set targets for analytical imprecision to allow changes considered clinically significant to be detect-

| Table 1. Estimated CV, in Subjects with Diabetes Mellitus |
|---------------------------------|-----------------|-----------------|
|                                | Clinically stable | Clinically variable |
| n (M/F)                        | 16 (3/13)        | 13 (6/7)        |
| Median age (range), years      | 48 (22–63)       | 62 (53–80)      |
| Sampling interval, days        | 28              | 32              |
| Mean samples per subject       | 4.1             | 4.4             |
| Mean GHB, %                    | 0.83            | 0.97            |
| Index of heterogeneity(1)      | 0.0417          | 0.0110          |
| Estimated variance of individual true variances(2) | -0.0961        | 0.0716          |
| Median CV, ± %                 | 6.5             | 7.1             |
| Median CV, %                   | 4.2             | 5.1             |

- Calculated as ratio of CV of (SD2 + SD2) to \(\sqrt{2(n-1)}\), where n is the mean number of samples per subject.
- (Var(var) – 32/(n – 1) [mean var2]/(n - 1)/(n + 1)).
- CV, estimated at 4.9%.

Fig. 1. Mean, maximum, and minimum GHB values for 16 diabetics in stable clinical control obtained by repeat sampling (n = 4.1) at 28-day intervals.

Fig. 2. Mean, maximum, and minimum GHB values for 23 diabetics in stable clinical control obtained by repeat sampling (n = 5) at 84-day intervals.
The level of clinical control may affect estimates of CV, because low values of GHB will produce higher CVs for a common intraindividual variance. Moreover, low GHB levels may be associated with aggressive therapeutic management, which itself may be associated with greater intraindividual variance because of recurrent hypoglycemia and subsequent swings in glycemic control. The stability of clinical control will also affect intraindividual variation—for example, clinical conditions such as illness, periods of hypoglycemia, or hospitalization will cause variation in glycemic control.

Because the GHB level has been shown to represent a weighted average of daily mean glucose values during the preceding 28 days (15), sampling at shorter time intervals following adjustments to medication or subsequent to episodes of variable clinical control is unlikely to yield a GHB level representative of any particular equilibrium set point.

Two previous studies investigated the physiological variation of hemoglobin A1c (HbA1c) in patients with diabetes (5, 6). The results of Howey et al. (6) cannot be generalized, however, because of the sampling frequency (3 weeks) and restricted population of eight patients (rehabilitation after amputation). Petersen et al. (5) reported CV1 values of 8.9%, 4.1%, and 5.2% for groups of diabetic patients classified in excellent, fair, and poor control, respectively. However, these patients were categorized by their initial HbA1c level rather than by assessment of the clinical stability of their diabetes. The 6 patients in fair metabolic control gave a CV1 of 4.1%, almost identical to the 4.2% found in our study. The value of 7.3% reported by Howey et al. (6), with a sampling interval of ~3 days, is considerably greater than the CV1 of 5.1% found in our outpatient diabetic population sampled at 32-day intervals.

Cembrowski (9) recently claimed that intensive insulin treatment schedules may achieve CV1 <4.1%. However, the glycemic control target of 6–7.5% HbA1c is paradoxically the level at which Petersen et al. (5) recorded their highest CV1 of 8.9%. This higher value may reflect the lower mean HbA1c and also wider swings in glycemic control in association with an aggressive treatment schedule. This highlights an erroneous but common supposition that the lowest mean HbA1c values are associated with the minimum CV1. Our results show an association between CV1 and clinical stability of the patient, and the sampling interval (Table 1).

The maximum allowable analytical imprecision has recently been defined by Fraser et al. (7), using data on physiological variation and physician-based judgment. The proposed formula is $\text{CV}_w < \sqrt{\frac{(\Delta/2Z)^2}{Z^2 - \text{CV}_r^2}}$, where $\Delta$ is the clinically perceived important percentage change and Z is the Z-statistic. At discrete values of Z and $\Delta$, $\text{CV}_w$ must decrease as $\text{CV}_r$ increases. The relation between $\text{CV}_1$, $\text{CV}_w$, and the critical percentage difference between successive GHB values is summarised in Table 2. The results highlight that the stability of clinical control is important in any interpretation of significant changes between measurements. Table 2 also demonstrates that if the GHB value exceeds 7.8%, an absolute
The interpretation of serial measurements and the setting of analytical goals is not straightforward for GHb. The traditional use of a median intrasubject variation to estimate significant changes between successive values or to establish optimal analytical goals is not appropriate. This unusual situation results from the dependence of CVs on the degree of clinical control and the sampling interval. For clinically stable patients and a monthly sampling frequency, a CV of 4–5% can be expected, whereas for patients in whom episodes of clinical variability occur, CVs as high as 10% are probable when a 3-month sampling interval is used. Given the uncertainty of the proper CV to use for individual patients, time-series analysis of the individual patient's data may be a more appropriate strategy to detect significant changes (18).

Table 2. Critical Differences (%) between Serial GHb Measurements at Different Probability Levels and CVs. (*

<table>
<thead>
<tr>
<th>Short-term (4–6 weeks)</th>
<th>Long-term (11–13 weeks)</th>
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<tbody>
<tr>
<td></td>
<td>4.2%, Stable</td>
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<tr>
<td>CVs, %</td>
<td>0.05*</td>
</tr>
<tr>
<td>0</td>
<td>11.6</td>
</tr>
<tr>
<td>2</td>
<td>12.9</td>
</tr>
<tr>
<td>3</td>
<td>14.3</td>
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<tr>
<td>5</td>
<td>18.1</td>
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* Based on Z = 2(1/2 CV^2 + CV_s^2) (see ref 4).
  * Sampling interval.
  * CV_s, Clinical control.
  * Probability of false result.

change of 1% (i.e., a 12.9% relative change) cannot be deemed significant at \( P = 0.05 \) when the analytical imprecision exceeds 2%, and is unrealizable at any analytical precision when a longer sampling interval is used.

An alternative might be to set the optimum analytical imprecision at one-half of the physiological variation (CV_s = ½CV_i), as suggested by Fraser and Harris (4). This would require a CV_s between 2.1% and 4.9%, depending on the group of patients studied, and a practical “working” CV_s of ~3%.

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References