Comparisons of Studies on Diabetic Complications Hampered by Differences in GHb Measurements

CARIN E. KULLBERG, PHD
ANDERS BERGSTRÖM, MD
BO DINESEN, MSC
LASSE LARSSON, MD
RANDIE R. LITTLE, PHD
DAVID E. GOLDSTEIN, MD
HANS J. ARNQVIST, MD

OBJECTIVE — To compare glycated hemoglobin (GHb) values of the relationship between glycemic control and complications of diabetes from laboratories involved in long-term studies (Steno, Oslo, Stockholm, Diabetes Control and Complications Trial, and Linköping).

RESEARCH DESIGN AND METHODS — Blood samples were collected from 25 subjects selected to represent the clinically relevant measurement range. Fresh whole-blood samples were distributed and analyzed within 4 days of sample collection. Pretreatment of samples and analyses of GHb were performed according to the routine method of each study's central or reference laboratory. Results from each laboratory were compared with the group mean, i.e., the mean of all results for each sample.

RESULTS — Regression analyses with the group mean values as independent variables and results from each laboratory as dependent variables showed that Oslo's results had a slope significantly different from the group mean. Laboratories used by the DCCT, Oslo, and Steno studies gave, on average, 0.4, 0.4, and 0.7% higher HbA_{1c} readings than the group mean, respectively, while HbA_{1c} results from Linköping and Stockholm were, on average, 0.6 and 1.0% lower, respectively.

CONCLUSIONS — There were large differences in GHb values among laboratories participating in studies of diabetic complications. The present data offer a guide to the comparison of results from the studies and underscores the need for standardization of GHb measurements.

The relationship between glycemic control and the development and progression of diabetic complications has been a matter of debate for decades. Studies were hampered by the inadequacy of methods used to assess long-term glycemic control. With the introduction of glycated hemoglobin (GHb) measurements, an accurate objective estimate of average blood glucose control for a period of several weeks became available (1). Several methods have been developed to measure GHb. Because these methods may assess different components of GHb (e.g., HbA_1, HbA_{1c}, or total GHb), and measure them in different ways, and because there is no universal agreement of a reference method, it is difficult to compare GHb values among laboratories (2–5).

Recently, several long-term studies (6–12) have shown that poor glycemic control, assessed by GHb, is the major cause of diabetic microangiopathy and neuropathy. However, it is difficult to compare results of these studies because different methods for GHb analysis were used.

The present study is not intended to be a comprehensive comparison of commonly used GHb methodology. Instead, the aim is to compare GHb values, as assessed in long-term studies of glycemic control and complications of diabetes, i.e., Diabetes Control and Complications Trial (DCCT) (6), Steno studies 1 and 2 (7), Oslo Study (8), Stockholm Diabetes Intervention Study (9), and studies from Linköping (10–12), thereby allowing the comparison of results from these studies.

RESEARCH DESIGN AND METHODS

— Blood samples from 25 subjects were collected at the diabetic outpatient clinic at the University Hospital, Linköping. Participants, diabetic patients and healthy nondiabetic individuals, were selected to represent a large span of the clinically relevant HbA_{1c} range. EDTA-treated fresh (chilled but not frozen) whole-blood samples were distributed by courier post to participating laboratories. All samples were analyzed within 4 days of collection.

Samples were pretreated and analyzed for GHb according to the current methods of each laboratory. Participating laboratories and the studies in which they were involved are shown in Table 1. For the present study, the Linköping, DCCT, Steno, and Stockholm laboratories all used ion-exchange high-performance liquid chromatography (HPLC) methods measuring HbA_{1c} (Pharmacia Mono-S HR, Pharmacia-LKB Technology, Uppsala, Sweden; Bio-Rad Diamat and Bio-Rad Variant, Bio-Rad laboratories, Hercules, CA). The Oslo laboratory used an immunochemical technique that measures HbA_{1c} (Tina Quant, Boehringer Mannheim, Mannheim, Germany).

Because of the introduction of new methods during or after long-term studies, some of the laboratories adjusted values obtained with the newer methods, by direct calibration or conversion equations, to match the values of the original methods. In this way, all data presented are consistent with the original data obtained during these long-term studies of diabetic complications. Also indicated in Table 1 are the methods used during each study and the current reference ranges for nondiabetic subjects.

In Linköping and Steno, a change

From the Department of Internal Medicine (C.E.K., A.B., H.J.A.), Faculty of Health Sciences, and the Department of Clinical Chemistry (L.L.), University Hospital, Linköping, Sweden; the Department of Clinical Chemistry (B.D.), Steno Diabetes Center, Gentofte, Denmark; and the Department of Pathology and Child Health (R.R.L., D.E.G.), University of Missouri, School of Medicine, Columbia, Missouri.

Address correspondence and reprint requests to Carin E. Kullberg, PhD, Department of Internal Medicine, Faculty of Health Sciences, S-581 85 Linköping, Sweden.

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CV, coefficient of variation; DCCT, Diabetes Control and Complications Trial; HPLC, high-performance liquid chromatography.

Table 1—*Methods used in five long-term studies of glycemic control and diabetic complications*

Long-term study and analyzing laboratory	Original study method	Current method	Current reference interval	Current interassay CV (high and low control)	Current intra-assay CV (high and low control)	Conversion to original method
DCCT; University of MO-Columbia	Bio-Rex 70 resin HPLC	Diamat HPLC	4.0–6.0	1.0; 2.0	0.6; 1.1	Calibration
Linköping; Laboratoriet för klinisk kemi, Universitets sjukhuset	Auto A1c HA 8110 HPLC	Mono-S HR HPLC	3.6–5.4	2.5; 3.9	1.3; 2.0	No adjustment required
Oslo; Sentral-laboratoriet, Aker sykehus	Diamat HPLC	Tina Quant immunoassay	3.9–5.7	2.8; 3.1	3.3; 3.3	0.86 × (Tina Q.) + 1.70
Steno; Klinisk Kemisk Afdeling, Steno Diabetes Center	Diamat HPLC	Variant HPLC	4.1–6.4	1.9; 2.5	0.5	No adjustment required
Stockholm; Kemikliniken, Södersjukhuset	Mono-S HPLC	Variant HPLC	3.9–5.7	1.5; 1.9	2.0; 2.2	1.05 × (Variant) − 1.55

Steno intra-assay CV refers to HbA_{1c} samples in the 5.3–16.1% range (mean 8.6%). Current reference interval and current inter- and intra-assay are expressed as percent.

in method was made, but the new and old methods produced the same numerical results. At the University of Missouri, Columbia, MO, the Bio-Rex 70 HPLC method remains the back-up/reference system for the newer Diamat HPLC. Inter- and intra-assay coefficient of variation (CV) for the currently used methods are given in Table 1. The procedure to determine CV differs among laboratories, e.g., the CV estimates are based on different number of samples. Total assay imprecision for control subjects with low HbA_{1c} levels (4.8–6.0%) ranged between 1.6 and 4.5% CV and for high HbA_{1c} (8.0–12.7%), between 1.2–4.3% CV for the currently used assays and according to the standard quality controls of each laboratory.

Data analysis

Since at present no internationally accepted standard exists, and a true HbA_{1c} or GHb concentration therefore cannot be determined, all data for the present study were compared with a calculated group mean, which was defined as the mean value for each sample analyzed in all five laboratories. Results from the separate laboratories were evaluated by bivariate linear regression analyses, with the group mean as independent variable and each laboratory as the dependent variable. The SPSS statistical package (SPSS for Macintosh, version 4.02, and SPSS for Windows, release 6.1, SPSS, Chicago, IL) was used for the statistical analysis.

RESULTS— The group mean values, i.e., the mean value for each sample, ranged from 4.3 to 11.4% HbA_{1c}. The difference between the highest and lowest result of the sample was, on average, 1.7% HbA_{1c} (range 1.2–2.4% HbA_{1c}). The interlaboratory variability for the five laboratories ranged between 6–22% (CV), with samples with higher concentrations of HbA_{1c} giving lower CVs. The values obtained by each laboratory are given separately in Fig. 1 and 2.

Regression analyses showed that the Oslo results had a slope significantly different from the group mean (Fig. 3) and a larger CI for the slope (interval range 0.13, compared with 0.05–0.06 for the other laboratories; Table 2), i.e., the variation in the deviation from the group mean values was greater than for the other laboratories. The DCCT, Oslo, and Steno laboratories gave, on average, 0.4, 0.4, and 0.7% higher HbA_{1c} readings than the group mean, respectively, while Linköping and Stockholm reported, on average, 0.6 and 1.0% lower HbA_{1c} readings, respectively. In Table 3, comparative values of HbA_{1c}, as calculated from the regression analyses, are given.

Note that, though Stockholm and Steno labs used the same method to ana-

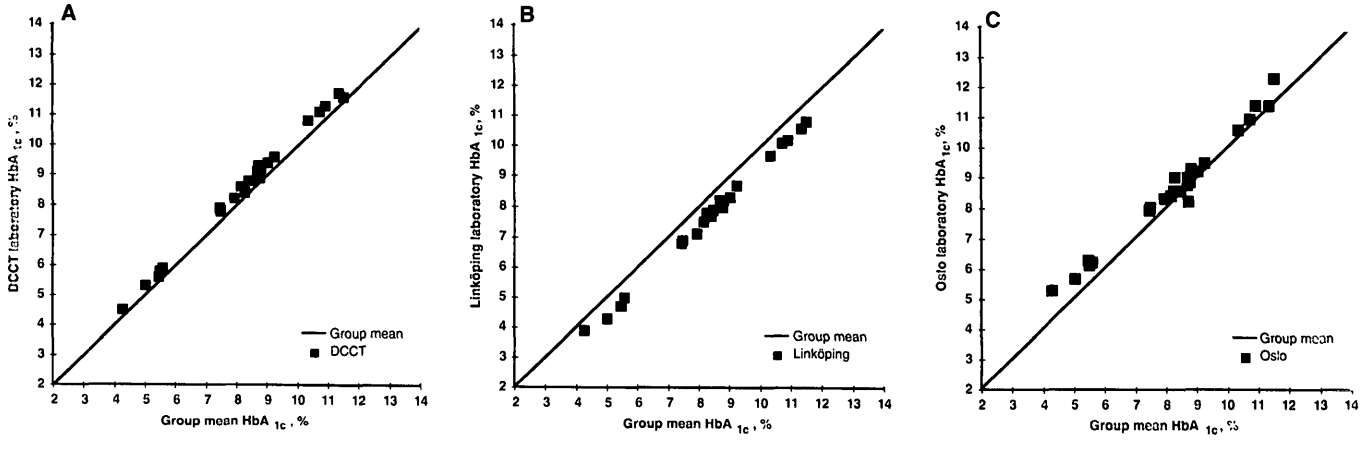

Figure 1—*Relation between group mean values (mean of each sample of all methods) and HbA_{1c} analyzed by study methods from DCCT (A), Linköping (B), and Oslo (C).*

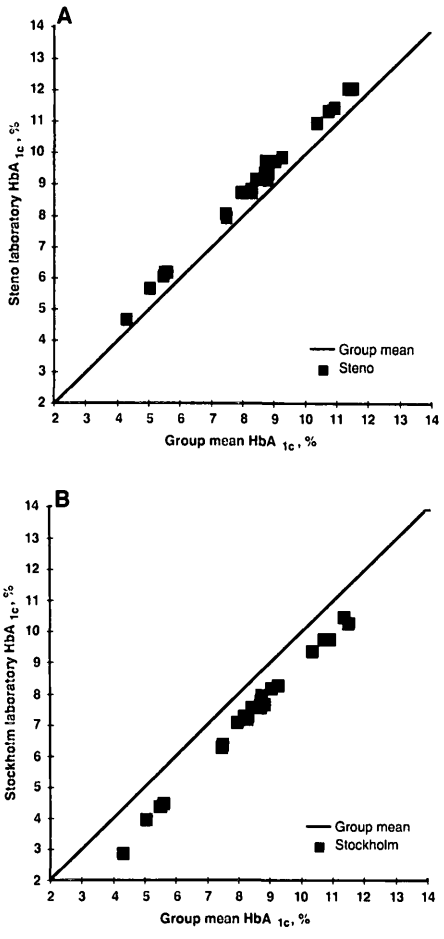

Figure 2—*Relation between group mean values (mean of each sample of all methods) and HbA_{1c} analyzed by study methods from Steno (A) and Stockholm (B).*

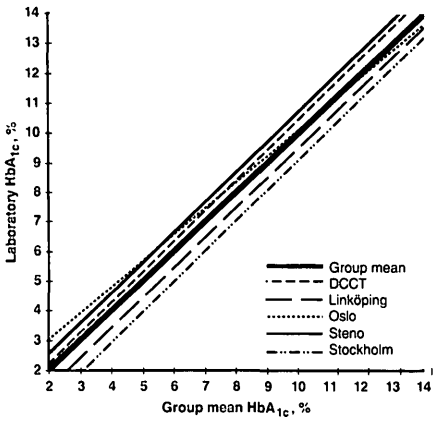

Figure 3—*Regression lines for the separate laboratories compared with the group mean (mean for each sample of all methods).*

Table 2—*Regression coefficients (i.e., slopes) and intercepts from bivariate regression analyses, with each laboratory as the dependent variable and the calculated group mean values as the independent variable*

Laboratory	Slope (95% CI)	Intercept (95% CI)
DCCT	1.03 (1.00–1.05)	0.16 (−0.04–0.37)
Linköping	1.01 (0.98–1.03)	−0.63 (−0.84–−0.42)
Oslo	0.89 (0.82–0.95)	1.25 (0.72–1.77)
Steno	1.02 (1.00–1.06)	0.51 (0.26–0.75)
Stockholm	1.04 (1.01–1.07)	−1.28 (−1.54–−1.03)

The group mean was calculated as the mean of all five readings for each sample.

lyze the samples, the Stockholm laboratory adjusted their results to match their original method (see Table 1). Similarly, the Oslo laboratory used a conversion factor to match their previously used Diamat method.

CONCLUSIONS — This study shows that there are large discrepancies in GHb results from different laboratories used in studies of diabetic complications, even though the laboratories measured the same component of GHb, i.e., HbA_{1c}. In the DCCT, the average difference in HbA_{1c} among the groups receiving conventional and intensified therapy was <2% HbA_{1c} (6), and in the Stockholm study, the difference was 1.5% HbA_{1c} (9). In the present comparison, the difference between the highest and lowest reading for the same sample was, on average, 1.7% HbA_{1c}. Such a difference is of major clinical importance, and the interpretation of the numerical values presented in each study must be carried out with great care. Though the correlation coefficients are excellent (as high as $r = 0.99$), agreement in the absolute HbA_{1c} values between laboratories is poor. Furthermore, the difference in slope shown for the Oslo study indicates not only a simple shift in the absolute value for HbA_{1c} compared with other studies, but a difference in the actual magnitude of change in glycemia for each 1% HbA_{1c}. For example, if a 1% change in HbA_{1c} in the DCCT corresponds to a difference in average glucose of ~36 mg/dl (6), a 1% change in the Oslo study would correspond to ~42 mg/dl change in average glucose.

The lack of GHb standardization makes it exceedingly difficult to compare results among laboratories and to set uniform goals for diabetes care. The present data offers a guide to the comparison of glycemic control in some studies regarding the relationship between long-term HbA_{1c} and diabetic complications.

Though conversion factors between laboratories and methods are necessary tools when comparing data from the early 1980s and onward, the need for standardization of GHb analysis in the near future is obvious. Recently, the American Association for Clinical Chemistry (AACC) and International Federation of Clinical Chemistry (IFCC) committees on GHb standardization have agreed to follow a protocol that would facilitate the standardization to achieve the end result of comparability of fresh sample results with the DCCT reference. Processes for standardization of results from many different methods, based on studies performed in the U.S. and The Nether-

Table 3—*Comparison of results between studies at selected levels of glycemic control, as calculated from the regression analyses*

	Group mean HbA_{1c} (%)					
	4.0	6.0	8.0	10.0	12.0	14.0
DCCT	4.3	6.3	8.4	10.5	12.5	14.6
Linköping	3.4	5.4	7.4	9.5	11.5	13.5
Oslo	4.8	6.6	8.3	10.1	11.9	13.7
Steno	4.6	6.7	8.7	10.8	12.8	14.9
Stockholm	2.9	5.0	7.1	9.2	11.2	13.3

lands, have been described in detail (2–5). In each case, a single group mean method was chosen, and all other method values were adjusted accordingly. These studies show that, using a variety of calibrator materials and calibration formats, different types of methods can report the same GHb values for a given specimen.

The data from the present report show that both national and international standardization of GHb is imperative.

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