Measurement of Hemoglobin A_{1c}

A new twist on the path to harmony

DAVID B. SACKS, MB, CHB, FRCPATH

Evaluation of glycemia is used for the diagnosis and management of patients with diabetes. Glucose and hemoglobin A_{1c} (HbA_{1c}) provide complementary information and both are used to assess an individual's glycemic status. The concentration of glucose in the blood indicates the subject's glycemia at the time of blood sampling. However, blood glucose concentrations are modified by numerous factors, ranging from food ingestion and exercise to stress and medication (1). By contrast, the concentration of HbA_{1c} in the blood reflects the average glucose over the preceding 8–12 weeks. Thus, HbA_{1c} provides an additional criterion for assessing glucose control that is free of the wide diurnal fluctuations that occur with blood glucose. HbA_{1c} has several additional attributes, which render it valuable in the setting of diabetes. These include, but are not limited to, the following: the subject does not need to be fasting, blood can be sampled any time of the day, the sample is stable, and there is very little biological variability (1). These factors, in conjunction with the documentation that HbA_{1c} predicts the development of microvascular (2,3) and macrovascular (4) complications of diabetes, have led to the widespread adoption of HbA_{1c} as integral to the management of patients with diabetes. Guidelines from several prominent clinical organizations recommend that HbA_{1c} be measured at regular intervals in all patients with diabetes (5,6).

The quality of analytical methods for HbA_{1c} initially lagged considerably behind the evidence of its clinical value. Early assays lacked standardization, substantially limiting the use of HbA_{1c} in patient care. Considerable effort was invested to effect standardization, with schemes developed in the 1990s in Japan (7), Sweden (8), and the U.S. (9). All HbA_{1c} results were reported as a percentage of hemoglobin. The subsequent development, almost a decade later, of a reference method for HbA_{1c} (10,11) led to different units (12). This situation has generated considerable controversy as to how HbA_{1c} should be reported. In this review, the background leading up to HbA_{1c} standardization and the development of different units are summarized. The formula for converting patient results from one set of units to the other is provided, and the current state of HbA_{1c} reporting in several countries is indicated.

Identification of HbA_{1c}—In normal adults, hemoglobin usually contains HbA (~97% of the total) (Table 1), HbA_2 (~2.5%), and HbF (~0.5%). HbA is made up of four polypeptide chains, two α- and two β-chains. Several posttranslational modifications of hemoglobin have been observed. These include carbamylation, acetylation, sulfation, and glycation. Glycation is the nonenzymatic attachment of a sugar to amino groups of proteins. The phenomenon of glycation, also termed "browning" or the "Maillard reaction," has been known for over 100 years (13). Early evidence documenting that hemoglobin is glycated was the demonstration in 1955 that small amounts of hemoglobin could be separated from HbA by their migration on electrophoresis in a starch slab (14). Three years later, three minor heme proteins, termed HbA_{1a}, HbA_{1b}, and HbA_{1c} on the basis of their elution, were observed to be resolved when normal human adult hemoglobin was subjected to cation-exchange chromatography (15). (In this review, the term "glycated hemoglobin" is used to refer to the set of all glycated hemoglobins, and "HbA_{1c}" is used to refer to a specific molecular form as described in the text.) The clinical significance of this finding remained obscure for 10 years until Rahbar (16) detected an unusual hemoglobin on electrophoresis of blood from patients with diabetes. This hemoglobin, which was identified as HbA_{1c}, was found to be increased twofold in patients with diabetes compared with healthy individuals (17). At essentially the same time, analysis revealed that HbA_{1c} has a hexose attached covalently to the NH_2-terminal valine residue of the β-chain of HbA (Table 2) (18). Several years later, HbA_{1c} was defined by the International Union of Pure and Applied Chemistry as the fraction of the β-chains of hemoglobin that has a stable hexose adduct on the NH_2-terminal amino acid valine (19).

It is important to emphasize that glycation of hemoglobin may also occur at sites other than the end of the β-chain, such as the NH_2-terminal valine residue of the α-chain as well as lysine residues on the α-chain or β-chain (20). These glycated hemoglobins are referred to as glycated HbA_0 or total glycated hemoglobin (GHb) (Table 1). The components of other forms of HbA have been identified. HbA_{1a1} and HbA_{1a2}, which make up HbA_{1a}, have fructose 1,6-diphosphate and glucose 6-phosphate, respectively, attached to the NH_2 terminus of the β-chain (Table 2). The structure of HbA_{1b}, solved by mass spectrometry, contains pyruvic acid linked to the NH_2-terminal valine of the β-chain, probably by a ketimine bond (21).

Measurement of HbA_{1c}—Commercial assays to measure HbA_{1c} became available in 1978 (22,23), and the test gained popularity during the 1980s. The first mention of glycated hemoglobin by the World Health Organization was in 1985 when the potential value of its measurement in diabetes was indicated (24). In 1988, the American Diabetes Association (ADA) recommended in its Standards of Medical Care that HbA_{1c}

From the Department of Laboratory Medicine, National Institutes of Health, Bethesda, Maryland.
Corresponding author: David B. Sacks, sacksdb@mail.nih.gov.
Received 9 July 2012 and accepted 1 August 2012.
DOI: 10.2337/dc12-1348

See accompanying articles, pp. 2415, 2421, 2429, and 2447.

Table 1—*Nomenclature of selected hemoglobins*

Name	Components
HbA	Contains two α- and two β-chains; constitutes ~97% of HbA
HbA_0	Nonglycated hemoglobin; usually synonymous with HbA
HbA_1	Comprises HbA_{1a}, HbA_{1b}, and HbA_{1c}
Total GHb	Comprises HbA_1 and other hemoglobin-carbohydrate adducts

In this review, the term "glycated hemoglobin" is used to refer to the set of all glycated hemoglobins, and "HbA_{1c}" is used to refer to a specific molecular form as described in the text.

determination should be performed at least semiannually for routine monitoring of patients with diabetes (25). The commercialization of HbA_{1c} assays led to the development of a plethora of methods to measure glycated hemoglobin. The general concept underlying these methods is to separate the glycated from the nonglycated hemoglobin and quantify the amount of each. Techniques that have been used to achieve this separation include those based on charge differences (ion-exchange chromatography, high-performance liquid chromatography [HPLC], electrophoresis, and isoelectric focusing), structural differences (affinity chromatography and immunoassay), or chemical analysis (photometry and spectrophotometry) (26). Analysis by electrophoresis or chemical techniques has become obsolete in most countries. The methods most commonly used today are HPLC and immunoassays (27).

Unfortunately, the early glycated hemoglobin assays suffered from several deficiencies, most notably a lack of standardization. The diverse methods, coupled with the different forms of glycated hemoglobin that were measured, produced a very wide variation in results. For example, in 1993 only 50% of clinical laboratories in the U.S. were reporting glycated hemoglobin as HbA_{1c} (28). The remaining laboratories were measuring, and reporting, HbA_1 (21%) or total GHb (29%). One study compared seven glycated hemoglobin methods and observed that results for a single sample varied from 4.0 to 8.1% among the different methods (29). Many people in the diabetes community were unaware of these differences in reporting. The publication of the Diabetes Control and Complications Trial (DCCT) (2) in 1993 provided the impetus necessary to initiate a resolution to this problem.

Documentation of the clinical value of HbA_{1c}—The DCCT evaluated the effect of intensive insulin therapy (compared with conventional insulin therapy) in patients with type 1 diabetes (2). The study documented that maintaining lower blood glucose concentrations (assessed by HbA_{1c}) resulted in a delayed onset and reduced the rate of progression of microvascular complications. (All of the glycated hemoglobin measurements in the DCCT were performed in a single laboratory by an HPLC assay that measured HbA_{1c}. This approach obviated the issue of test variability and established HbA_{1c} as the species of glycated hemoglobin that should be reported.) The risk of retinopathy increased continuously with increasing HbA_{1c}, and a single measure of HbA_{1c} predicted the progression of retinopathy 4 years later. Further analysis of the DCCT data revealed that the mean HbA_{1c} was the dominant predictor of retinopathy progression, and a 10% lower HbA_{1c} concentration (e.g., from 9 to 8.1%) was associated with a 45% lower risk (30). Extended follow-up demonstrated that the incidence of cardiovascular disease was reduced by 42% in patients with lower HbA_{1c} (4). Thus, the DCCT unequivocally established the value of measuring HbA_{1c} in patients with diabetes.

Table 2—*Species of modified HbA*

Name	Site	Modification
A_{1a1}	NH_2 terminus of the β-chain	Fructose 1,6-diphosphate
A_{1a2}	NH_2 terminus of the β-chain	Glucose 6-phosphate
A_{1b}	NH_2 terminus of the β-chain	Pyruvic acid
A_{1c}	NH_2 terminus of the β-chain	Glucose

Evidence that lowering HbA_{1c} in patients with type 2 diabetes reduces complications was provided in 1998 with the publication of the UK Prospective Diabetes Study (UKPDS) (3). To ensure that HbA_{1c} results in the UKPDS were comparable to those in the DCCT, an ion-exchange HPLC method calibrated to the DCCT was used. Mean HbA_{1c} was 7.0% in the intensive group compared with 7.9% in the conventional group (3). Notwithstanding the seemingly small difference in HbA_{1c} concentrations between the two groups, significant differences in the rate of complications were found. Analogous to the DCCT, the UKPDS showed that intensive blood glucose control reduced the risk of microvascular complications. Risk reductions of 37% for microvascular disease, 21% for deaths related to diabetes, and 14% for myocardial infarction were observed for each 1% reduction in HbA_{1c} (e.g., from 9 to 8%) (31). Ten-year follow-up demonstrated that the risk of myocardial infarction was significantly lower in patients who had lower HbA_{1c} at the end of the UKPDS (32). Thus, both the UKPDS and DCCT (large, prospective, multicenter, clinical studies) documented that a small change in HbA_{1c} values translates into a large alteration in the risk of diabetes complications in patients with type 1 or type 2 diabetes. It was evident that the state of measurement of HbA_{1c} in routine patient samples was untenable, and accurate, standardized HbA_{1c} testing was essential.

Standardization to DCCT/NGSP numbers—There are >100 methods currently available to measure glycated hemoglobin, and it is vital that they are standardized to report the same (or at least a very similar) result for a single blood sample. Moreover, as mentioned earlier, both the DCCT and UKPDS measured exclusively HbA_{1c} and not other forms of glycated hemoglobin. As a result of the DCCT, the American Association for Clinical Chemistry (AACC) established a committee in 1993 to standardize glycated hemoglobin testing (9). The NGSP (previously called the National Glycohemoglobin Standardization Program) was created 3 years later to execute the protocol developed by the AACC committee. The goal of the NGSP is to standardize glycated hemoglobin test results to those of the DCCT and UKPDS, which established direct relationships between HbA_{1c} concentrations and outcome risks

in patients with diabetes. The concept is that all clinical laboratories that measure patient samples should report an HbA_{1c} value equivalent to that reported in the DCCT and UKPDS. A network of laboratories, located in the U.S., Europe, and Japan, has been established to achieve this standardization (28). A brief description of the process follows.

The Central Primary Reference Laboratory (CPRL) measures HbA_{1c} with a Bio-Rex 70 cation-exchange HPLC, which is the method used in the DCCT (28). Three primary reference laboratories (PRLs), which use the same method, serve as backup to the CPRL. The eight second reference laboratories (SRLs) assist manufacturers with calibrating their assays so they will report a value equivalent to that measured in the DCCT. (A calibrator is a material of known concentration that is used to adjust a measurement procedure.) Thus, an HbA_{1c} result of 7.0% in a patient's blood performed by that assay in a routine clinical laboratory would be essentially identical to a result of 7.0% in the DCCT or UKPDS. If an HbA_{1c} method meets strict accuracy criteria, the manufacturer receives a certificate that is valid for 1 year (28). In 2011, there were 112 methods that had NGSP certification. The ADA recommends that laboratories use only HbA_{1c} assays that are certified by NGSP as traceable to the DCCT reference (33). These assays are listed on the NGSP website (http://www.ngsp.org) and are updated at least annually. The HPLC method used in the CPRL and PRLs is not suitable for routine measurement of patient samples. By contract, the SRLs all use commercially available methods, identical to those used in clinical laboratories. National standardization schemes were developed in Japan and Sweden (7,8). The Japanese and Swedish values were rarely adopted by other countries. Due to its link to the DCCT and UKPDS, the NGSP system was, by an overwhelming margin, the most popular global HbA_{1c} standardization system. NGSP-certified methods are currently used worldwide in the clinical laboratories that measure patient samples, and these results are directly traceable to the DCCT and UKPDS.

The efforts of the NGSP resulted in a considerable improvement in the performance of HbA_{1c} measurement by routine clinical laboratories (28). The fraction of laboratories reporting glycated hemoglobin measurements as HbA_{1c} increased from 50% in 1993 to 80% in 1996 to ~99% in 2004. This was accompanied by a concurrent improvement in accuracy and reduced variability among laboratories (28).

IFCC standardization—A working group on HbA_{1c} standardization was established by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1995. The strategy adopted by this committee was different from that of the NGSP. Instead of standardizing to a comparison method, the primary objective of the IFCC committee was to develop a true reference method for HbA_{1c}. This goal was achieved (10). In brief, the approach is to digest hemoglobin with an enzyme (termed endoproteinase Glu-C) that cleaves a hexapeptide off the NH_2 terminus of the β-chain of hemoglobin. The glycated hexapeptides are separated from the nonglycated hexapeptides by reverse-phase HPLC. The peptides are then quantified by either mass spectrometry or capillary electrophoresis. HbA_{1c} is measured as the ratio of glycated to nonglycated hexapeptide. Results were initially reported as a percentage. The IFCC Working Group also developed primary reference materials of pure HbA_{1c} and HbA_0 (10). These are purified from human whole blood, blended together, and used to calibrate the primary reference measurement system. Secondary reference materials are also produced, and manufacturers use these to calibrate their instruments. A network of reference laboratories (15 at the time of this report) works together to form an IFCC reference system (34). The IFCC reference method is technically demanding, time consuming, and very expensive and is not designed for routine analysis of patient samples. It serves as a reference measurement procedure with well-defined metrologic traceability to a "higher-order method."

The NGSP and IFCC networks have complementary roles in the HbA_{1c} standardization process. The IFCC provides manufacturers with traceability to a standard of higher metrologic order, and the NGSP defines the limits of acceptability for method performance. The two networks together form a solid basis to establish the accuracy and reliability of HbA_{1c} measurement in a patient's sample performed in a clinical laboratory anywhere in the world.

Comparison between networks—Analyses using pooled whole-blood samples were conducted to compare the values of HbA_{1c} obtained by the IFCC and NGSP networks. A linear relationship was observed between HbA_{1c} results of the IFCC reference method and the NGSP network (11). The calculated regression equation is NGSP = 0.09148(IFCC) + 2.152. This equation is termed the "master equation" and permits conversion between the two sets of values. Importantly, the HbA_{1c} values measured by the IFCC method are significantly lower than the NGSP values. Moreover, the difference is not constant. For example, an NGSP value of 6.0% is 4.2% in IFCC numbers (difference of 1.8), and 10.0% NGSP is 8.6% IFCC (difference of 1.4). Comparisons of the IFCC network with the Swedish and Japanese standardization schemes revealed a unique regression equation for each network, with lower values obtained by the IFCC method in all cases (11,34). The most likely explanation for the higher values with the HPLC-based standardization schemes is that the HbA_{1c} peak on the chromatogram contains other substances in addition to HbA_{1c}. These observations introduced a conundrum and have generated considerable controversy as to how HbA_{1c} should be reported. The major arguments promulgated for and against each set of units are summarized in Table 3.

Reporting HbA_{1c}—Three preeminent clinical diabetes organizations, the ADA, the European Association for the Study of Diabetes (EASD), and the International Diabetes Federation (IDF), attempted to resolve the dispute by agreeing to consider reporting HbA_{1c} with estimated average glucose (eAG) (35,36). A prospective multinational study documented a linear relationship between HbA_{1c} and mean blood glucose (37). Nevertheless, many experts concluded that the large variability between HbA_{1c} and mean blood glucose precludes the use of eAG (38–42). By contrast, others believe that eAG is useful in communicating the extent of glycemic control with patients (43–45). In response to a questionnaire included in the College of American Pathologists HbA_{1c} proficiency survey in April 2012, 1,153 (35.7%) of 3,233 participating clinical laboratories (~90% of which are located in the U.S.) indicated that they report eAG together with HbA_{1c} results.

Another factor has exerted considerably more influence on how HbA_{1c} is reported. A decision was made in 2007 to

Table 3—*Comparison of IFCC and NGSP units for reporting HbA_{1c}*

Units	Advantages	Disadvantages
IFCC	Values are scientifically accurate	High cost and prolonged timeline for education are necessary to avoid confusion
	Opportunity to re-educate professionals and people with diabetes about the meaning and value of the HbA_{1c} test	Partial implementation is likely to worsen existing differences among laboratories
	Opportunity to redefine HbA_{1c}	May be confused with glucose concentrations in countries where mg/dL used
	SI units are used for reporting other blood tests in most countries	Question whether values should be rounded up or down to simplify
NGSP	Familiar to clinicians, other health care professionals, and patients	Not the "pure" result
	Directly relates HbA_{1c} values to existing evidence base, e.g., DCCT and UKPDS	Frequently confused with glucose concentrations in countries where mmol/L used
		Missed opportunity to reinforce the importance of the test

Adapted from Sacks (36).

Table 4—*Comparison of HbA_{1c} values*

NGSP (%)	IFCC (mmol/mol)
4.0	20
5.0	31
6.0	42
6.5	48
7.0	53
8.0	64
9.0	75
10.0	86
11.0	97
12.0	108

NGSP values should be reported to one decimal; IFCC values should be without a decimal.

report IFCC results in the International System of Units (SI) rather than percent (12,19). Thus, IFCC values are now expressed as millimoles of HbA_{1c} per mole of HbA_0. The Committee on Nomenclature, Properties, and Units of the IFCC proposed a new term for HbA_{1c}, namely Haemoglobin beta chain(Blood-N-(1-deoxyfructos-1-yl)haemoglobin beta chain; substance fraction) (19). This term has not gained wide acceptance. However, the SI units have (see "Current reporting" section below). A considerable advantage of reporting IFCC values in SI units (as opposed to reporting IFCC values as percent) is that it will avoid the confusion that would almost certainly have ensued from using the same units (namely percent) but different reference intervals. For example, NGSP-certified HbA_{1c} concentrations of 6.5 and 7.0% (previously 4.8 and 5.3% in the original IFCC reporting system) now correspond to 48 and 53 mmol/mol, respectively (Table 4).

In 2004, a working group with representatives from the ADA, EASD, and IDF was established to harmonize HbA_{1c} reporting (35,36). There was unanimous agreement among the members of the group, termed the ADA/EASD/IDF Working Group of the HbA_{1c} Assay, that the same HbA_{1c} values should be reported throughout the world. The initial report was followed in 2007 by a consensus statement on the worldwide standardization of HbA_{1c} (46). The publication called for HbA_{1c} results to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation. A subsequent publication appeared 3 years later (47) and reiterated that HbA_{1c} results should be reported by clinical laboratories worldwide in SI and derived NGSP units. Regrettably, this ideal has not been realized.

Current reporting—In 1998, the European Union introduced a directive on in vitro diagnostics requiring that laboratory tests be traceable to a "higher-order method" (48). This factor, in conjunction with the use of SI units for reporting almost all other laboratory tests in many countries, has resulted in the adoption of SI units for HbA_{1c} by some countries in Europe and the Antipodes (Table 5). Most of the countries that have decided to convert to SI units have had a period of dual reporting where both NGSP and IFCC units have been used before switching to single reporting of SI units. The two countries that had their own national standardization schemes, namely Sweden and Japan, both chose to discontinue reporting results using these numbers. Sweden elected to adopt SI units exclusively, whereas Japan is currently reporting both NGSP and Japan Diabetes Society numbers, before switching to only NGSP numbers in 2013 (Table 5). After considerable deliberation, Canada has recently decided to continue using NGSP values. Although no formal statement has been issued, the U.S., which continues to use "traditional" (non-SI) units for all blood tests, is extremely unlikely to switch to SI units for HbA_{1c}. Most countries have not yet made a decision whether to adopt SI units, but it is likely that at least some will convert. A concern has been expressed by some experts from both less-developed countries and newly industrialized countries that efforts, and limited resources, should be directed toward adopting higher-quality (and standardized) analytical methods, expanding the use of HbA_{1c} in patient care, and educating health care providers. The concern is that introducing SI units will both divert resources from these necessary tasks and generate confusion.

Implications of different HbA_{1c} units—The hope expressed by members of the ADA/EASD/IDF Working Group of the HbA_{1c} Assay that the same HbA_{1c} values be reported globally (36) has clearly not been realized (Table 5). This situation is most unfortunate and has consequences for all those in the field of diabetes. It is essential that countries that choose to report HbA_{1c} in SI units introduce extensive education programs that will adequately explain the new units to all health care providers. Because management of diabetes requires the active participation of the patient (49), the change in reporting must also be clearly communicated to patients. It is important that if HbA_{1c} units are altered, the modification should be coordinated so that it is instituted throughout the entire country to avoid having different units used in a single country. Medical and scientific journals should require that authors provide both SI and DCCT units for all HbA_{1c} results. This dual reporting will enable

Table 5—*Units for reporting HbA_{1c} in selected countries*

Country	Original units†	Dual reporting initiated	Single reporting	
			Units	Date
Germany	NGSP	1 January 2009	SI	1 January 2010
Italy	NGSP	1 January 2011	SI	1 October 2011
The Netherlands	NGSP	1 January 2010	SI	1 January 2011
Sweden	Mono-S	1 September 2010	SI	1 January 2011
U.K.	NGSP	1 June 2009	SI	1 October 2011
Australia	NGSP	1 July 2011	SI	1 July 2013‡
New Zealand	NGSP	3 August 2009	SI	1 October 2011
Canada	NGSP	NA	NGSP	NA
Japan	JDS	1 April 2012	NGSP	1 April 2013‡
U.S.	NGSP	NA	NGSP	NA

JDS, Japan Diabetes Society; NA, not applicable. †All original units for reporting HbA_{1c} were %. ‡Anticipated date for conversion to single units.

readers to evaluate results and compare them to prior publications.

Physicians and investigators need to be aware that the conversion of HbA_{1c} results between DCCT/NGSP (%) and IFCC units is not as simple as changing glucose values from traditional to SI units or vice versa. The glucose conversion is based on the molecular mass of glucose ($C_6H_{12}O_6$), which is 180.16 g/mol. Therefore, to change glucose from mg/dL to mmol/L, one divides by 18.016 (usually rounded off to 18), and values are multiplied by 18 to switch from mmol/L to mg/dL. For example, 126 mg/dL is equivalent to 7.0 mmol/L, and 40 mg/dL is 2.2 mmol/L. By contrast, the conversion of HbA_{1c} results is more complex, partly because it is the conversion of a ratio of two measurements made in different assays rather than of a single concentration. At an NGSP HbA_{1c} of 4%, the IFCC values are fivefold higher (20 mmol/mol), whereas at 12%, the IFCC results are ninefold greater (108 mmol/mol) (Table 4), precluding the use of a simple multiplication or division factor to transform values. Inspection of Fig. 1 reveals that although the relationship between HbA_{1c} measured in NGSP units and IFCC units is a straight line, that line has a slope that differs significantly from 1 and an intercept that differs from 0. Therefore, conversion between NGSP and IFCC units requires a simple linear equation, the master equation (NGSP = 0.09148(IFCC) + 2.152 or IFCC = 10.93(NGSP) − 23.50). It is reassuring that the master equation has been shown to be stable for >11 years (34), and comparisons between the NGSP and IFCC networks continue to be conducted twice a year. These ongoing assessments, which validate the stability and reliability of the networks, will ensure that results can be converted from DCCT/NGSP units to SI units and vice versa. Tables and/or calculators that convert units should be readily accessible to health care providers and patients. To assist in this endeavor, the NGSP has posted both a table and a calculator on its website (http://www.ngsp.org/convert1.asp). A calculator is also available at http://www.hba1c.nu/eng2.html. For countries that select SI units for reporting HbA_{1c}, it is imperative that the tenet "primum non nocere" (first do no harm) is adhered to and that care of patients with diabetes is not compromised.

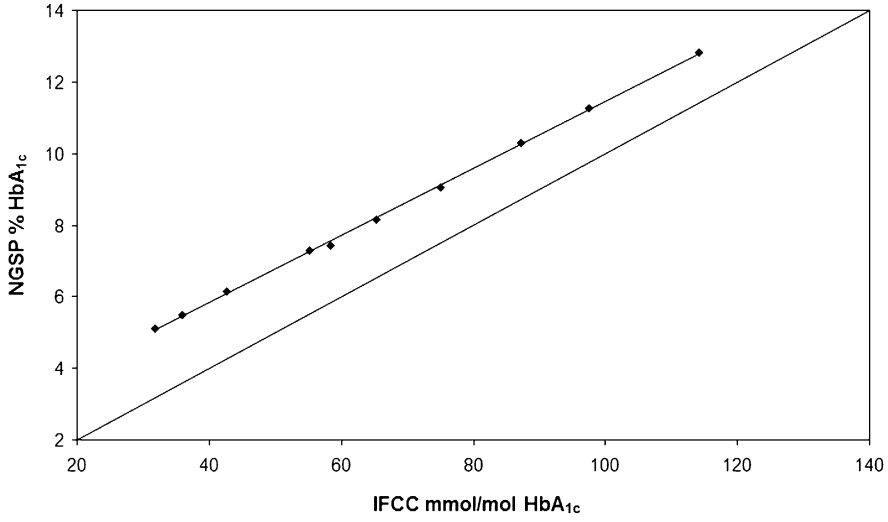

Figure 1—*Comparison of HbA_{1c} values between the NGSP and IFCC networks. HbA_{1c} was measured in 10 pooled blood samples by the NGSP (mean value of 7 network laboratories) and IFCC (mean value of 13 network laboratories) networks. ◆ is the regression line, and the solid black line is the* y = x *line (line of identity).*

Acknowledgments—This work was supported by the Intramural Research Program of the National Institutes of Health.

No potential conflicts of interest relevant to this article were reported.

The author thanks Drs. Carla Siebelder and Cas Weykamp (Queen Beatrix Hospital, Winterswijk, the Netherlands) for kindly providing the figure. This article is dedicated to the memory of Mervyn C. Berman (1934–2012), scholar, mentor, role model, and friend.

References

1. Sacks DB. A1C versus glucose testing: a comparison. Diabetes Care 2011;34:518–523
2. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986
3. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–853
4. Nathan DM, Cleary PA, Backlund JY, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular

disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–2653
5. American Diabetes Association. Standards of medical care in diabetes—2012. Diabetes Care 2012;35(Suppl. 1):S11–S63
6. International Diabetes Federation Task Force. *Global Guideline for Type 2 Diabetes*. Brussels, International Diabetes Federation, 2005, p. 1–11
7. Shima K, Endo J, Oimomi M, Oshima I, Omori Y, Katayama Y. Inter-laboratory difference in HBA1c measurement in Japan. A report of the Committee on an Inter-laboratory Standarization of HbA1c Determination, the Japan Diabetes Society. J Jpn Diabetes Soc 1994;37:855–864
8. Eckerbom S, Bergqvist Y, Jeppsson JO. Improved method for analysis of glycated haemoglobin by ion exchange chromatography. Ann Clin Biochem 1994;31:355–360
9. Little RR, Rohlfing CL, Wiedmeyer HM, Myers GL, Sacks DB, Goldstein DE; NGSP Steering Committee. The national glycohemoglobin standardization program: a five-year progress report. Clin Chem 2001;47:1985–1992
10. Jeppsson JO, Kobold U, Barr J, et al.; International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002;40:78–89
11. Hoelzel W, Weykamp C, Jeppsson JO, et al.; IFCC Working Group on HbA1c Standardization. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin Chem 2004;50:166–174
12. Mosca A, Goodall I, Hoshino T, et al.; International Federation of Clinical Chemistry and Laboratory Medicine, IFCC Scientific Division. Global standardization of glycated hemoglobin measurement: the position of the IFCC Working Group. Clin Chem Lab Med 2007;45:1077–1080
13. Maillard LC. The action of amino acids on sugar. The formation of melanoidin by a methodic route. Cr Hebd Acad Sci 1912;154:66–68
14. Kunkel HG, Wallenius G. New hemoglobin in normal adult blood. Science 1955;122:288
15. Allen DW, Schroeder WA, Balog J. Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin - a study of the effects of crystallization and chromatography on the heterogeneity and isoleucine content. J Am Chem Soc 1958;80:1628–1634
16. Rahbar S. An abnormal hemoglobin in red cells of diabetics. Clin Chim Acta 1968;22:296–298
17. Rahbar S, Blumenfeld O, Ranney HM. Studies of an unusual hemoglobin in patients with diabetes mellitus. Biochem Biophys Res Commun 1969;36:838–843
18. Bookchin RM, Gallop PM. Structure of hemoglobin AIc: nature of the N-terminal beta chain blocking group. Biochem Biophys Res Commun 1968;32:86–93
19. Nordin G, Dybkaer R; International Federation Of Clinical Chemistry And Laboratory Medicine, IFCC Scientific Division. Recommendation for term and measurement unit for "HbA1c". Clin Chem Lab Med 2007;45:1081–1082
20. Shapiro R, McManus MJ, Zalut C, Bunn HF. Sites of nonenzymatic glycosylation of human hemoglobin A. J Biol Chem 1980;255:3120–3127
21. Prome D, Blouquit Y, Ponthus C, Prome JC, Rosa J. Structure of the human adult hemoglobin minor fraction A1b by electrospray and secondary ion mass spectrometry. Pyruvic acid as amino-terminal blocking group. J Biol Chem 1991;266:13050–13054
22. Davis JE, McDonald JM, Jarett L. A high-performance liquid chromatography method for hemoglobin A1c. Diabetes 1978;27:102–107
23. Beccaria L, Chiumello G, Gianazza E, Luppis B, Righetti PG. Hemoglobin A1C separation by isoelectric focusing. Am J Hematol 1978;4:367–374
24. World Health Organization. Diabetes mellitus. Report of a WHO Study Group. World Health Organ Tech Rep Ser 1985;727:1–113
25. American Diabetes Association. Standards of medical care for patients with diabetes mellitus. Diabetes Care 1989;12:365–368
26. Sacks DB. Carbohydrates. In *Tietz Textbook of Clinical Chemistry*. 3rd ed. Burtis C, Ashwood E, Eds. Philadelphia, Saunders, 1994, p. 928–1001
27. Sacks DB. Diabetes mellitus. In *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 5th ed. Burtis CA, Ashwood ER, Bruns DE, Eds. St. Louis, Elsevier Saunders, 2012, p. 1415–1456
28. Little RR, Rohlfing CL, Sacks DB; National Glycohemoglobin Standardization Program (NGSP) Steering Committee. Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care. Clin Chem 2011;57:205–214
29. Little RR, Wiedmeyer HM, England JD, et al. Interlaboratory standardization of measurements of glycohemoglobins. Clin Chem 1992;38:2472–2478
30. DCCT. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the Diabetes Control and Complications Trial. Diabetes 1995;44:968–983
31. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405–412
32. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–1589
33. Goldstein DE, Little RR, Lorenz RA, et al. Tests of glycemia in diabetes. Diabetes Care 2004;27:1761–1773
34. Weykamp C, John WG, Mosca A, et al. The IFCC Reference Measurement System for HbA1c: a 6-year progress report. Clin Chem 2008;54:240–248
35. Report of the ADA/EASD/IDF Working Group of the HbA1c Assay, London, UK, January 2004. Diabetologia 2004;47:R53–R54
36. Sacks DB; ADA/EASD/IDF Working Group of the HbA1c Assay. Global harmonization of hemoglobin A1c. Clin Chem 2005;51:681–683
37. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ; A1c-Derived Average Glucose Study Group. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008;31:1473–1478
38. Young IS. Counterpoint: the reporting of estimated glucose with hemoglobin A1c. Clin Chem 2010;56:547–549
39. Barth JH, Marshall SM, Watson ID. Consensus meeting on reporting glycated haemoglobin and estimated average glucose in the UK: report to the National Director for Diabetes, Department of Health. Ann Clin Biochem 2008;45:343–344
40. Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. Is haemoglobin A1c a step forward for diagnosing diabetes? BMJ 2009;339:b4432
41. Bloomgarden ZT, Inzucchi SE, Karnieli E, Le Roith D. The proposed terminology 'A (1c)-derived average glucose' is inherently imprecise and should not be adopted. Diabetologia 2008;51:1111–1114
42. Herman WH, Cohen RM. Racial and ethnic differences in the relationship between HbA1c and blood glucose: implications for the diagnosis of diabetes. J Clin Endocrinol Metab 2012;97:1067–1072
43. Iqbal N, Morgan C, Maksoud H, Idris I. Improving patients' knowledge on the relationship between HbA1c and mean plasma glucose improves glycaemic control among persons with poorly controlled diabetes. Ann Clin Biochem 2008;45:504–507
44. Sacks DB, Bergenstal RM, McLaughlin S. Point: the reporting of estimated glucose with hemoglobin A1c. Clin Chem 2010;56:545–546

45. Kahn R, Fonseca V. Translating the A1C assay. Diabetes Care 2008;31:1704–1707
46. Consensus Committee. Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. Diabetes Care 2007;30:2399–2400
47. Hanas R, John G; International HbA(1c) Consensus Committee. 2010 consensus statement on the worldwide standardization of the hemoglobin A1c measurement. Clin Chem 2010;56:1362–1364
48. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. OJ L, 1998, 331:1–37
49. Heisler M, Vijan S, Anderson RM, Ubel PA, Bernstein SJ, Hofer TP. When do patients and their physicians agree on diabetes treatment goals and strategies, and what difference does it make? J Gen Intern Med 2003;18:893–902