

Translating the A1C Assay

In the clinical management of diabetes, the A1C assay has become indispensable. Used worldwide to monitor chronic glycemia, the assay is an essential tool to determine whether a patient has achieved the core goal of therapy for diabetes: a marked and sustained reduction in plasma glucose to achieve as close to a normal level as can be safely attained. With the publication of the A1c-Derived Average Glucose (ADAG) study in this issue of *Diabetes Care* (1), the evolution of the A1C assay continues and an important milestone has been reached. To better appreciate this recent report, a brief and admittedly incomplete historical perspective may be useful.

It was 60 years ago that Allen et al. (2) showed that hemoglobin A (which makes up about 97% of total hemoglobin) contains three minor components, designated HbA_{1a}, HbA_{1b}, and HbA_{1c} (A1C). In the decades that followed, we learned that a hexose molecule is attached to these components (3) and that hemoglobin A actually has two more minor glycosylated derivatives. The five altogether comprise ~5–7% of the HbA molecule (4).

In the early course of the biochemical dissection of hemoglobin, Huisman and Dozy (5) noted, virtually in passing, that the level of glycosylated hemoglobin components was increased in a few individuals they studied who happened to have diabetes. It took 4 more years, however, for Rahbar and colleagues (6,7) to document that diabetes is clearly associated with an elevation in glycosylated hemoglobin. The Rahbar reports stimulated other investigators to confirm these initial findings and to seek an explanation for how glucose binds to hemoglobin. It was not for another few years, in 1972, that Bunn et al. (8) elegantly showed that the cause of the increased glycosylated hemoglobin in diabetes, which was predominantly the A1C component, was a result of excess nonenzymatic glycation that occurred throughout the lifespan of red cells and in an essentially irreversible process.

The A1C-diabetes story then shifted from clinical chemistry to clinical medicine. Koenig et al. (9) were the first to show that A1C levels correlated well with fasting blood glucose, and they concluded that A1C levels “probably reflect

... the mean daily blood glucose concentration ... and may provide a better index of control of the diabetic patient.” Indeed, soon after their report, many other investigators confirmed a strong association between A1C and glycemic control and that the measurement had clinical utility (10–15), clearly surpassing in utility what was then the conventional assessment of metabolic control over time (e.g., signs, symptoms, urine, and blood glucose levels) (15).

The thorough biochemical experiments performed in the 1970s and 1980s, most notably by Mortensen and Christophersen (16), demonstrated that the fraction of A1C in a sample depends on the glucose levels over a previous period, along with red cell turnover, reaching a steady state sometime between 4 and 12 weeks. Such kinetics were supported by many clinical studies in both type 1 and type 2 diabetic patients where the A1C level was found to correlate well with glucose regulation (17) or the mean blood glucose derived over time from multiple fingersticks (9,15,18–24).

As the use of the A1C test gained traction, dozens of different analytical methods based on different assay principles (e.g., ion-exchange chromatography, affinity chromatography, immunoassay, and electrophoresis) were used to measure glycosylated hemoglobin. Without a common reference method and in the absence of a standardized assay, results varied considerably when the same sample was tested by different laboratories or methods or even when the same sample was tested repeatedly by one methodology. It was quite common, for example, to have values ranging from 4.0 to 8.1% on the same blood sample (25). In addition, the assays used then (and even now) in clinical medicine not only measured A1C itself but also more or lesser amounts of the other glycosylated hemoglobin components, and results were reported as A1C, HbA₁, or total glycosylated hemoglobin. The results were also influenced by other interfering substances in the sample.

The Diabetes Control and Complications Trial (DCCT) Study Group, recognizing these problems, centralized the measurement of A1C from the onset of the study so as to avoid confounding re-

sults if such a key analyte were to be measured at many sites (26). Also, in anticipation of the DCCT results, the American Association for Clinical Chemistry (AACC) established, in 1993, an A1C standardization workgroup to bring consistency to the measurement of A1C and to facilitate the traceability of results back to the DCCT such that these results could be directly related to the risk or progression of diabetes complications.

After the standardization protocol was developed, the American Association for Clinical Chemistry group was dissolved and the National Glycohemoglobin Standardization Program (NGSP) began in 1996 (27). Briefly, in the NGSP, the reference method is the measurement of A1C by ion-exchange high-performance liquid chromatography, as was used in the DCCT. Manufacturers of testing equipment can receive NGSP certification if their instruments are calibrated to match the results obtained by the NGSP. Laboratories can also be certified by the same protocol and thereby document optimal performance in their setting.

All this has led to a dramatic reduction in interlaboratory variability and a marked improvement in the precision and comparability of values (28). In 2007, ~99% of all A1C test results in the U.S. were traceable to those obtained in the DCCT, with similar percentages in test results throughout the U.K. and in Canada (D. Sacks, personal communication). Although comparable data are not readily available from other countries, it appears that much of the world's A1C testing is traceable to the DCCT numbers.

Still, issues remain. First, the high-performance liquid chromatography reference method used by the NGSP is somewhat nonspecific in that the methodology, like many others, measures more than just A1C in a sample. Although this problem is obviated by the consistent use of one reference method, in the world of clinical chemistry, this situation is “metrologically unsound.” Second, although most methods used worldwide are NGSP certified, there are other standardization programs, most notably in Japan (29) and in Sweden (30). Thus,

there is no truly international standardization program.

Both of these issues led the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1995 to embark on the development of a reference method that would be very specific, i.e., only measures A1C, and that could lead to worldwide standardization based on a metrologically sound international measurement system (31). Not only did the IFCC succeed (32,33) in developing such an assay but the reference method has been approved by all of their member societies, and a global network of reference laboratories has been established (33).

But progress often brings other difficulties and problems. First, the IFCC method is very complicated, requires costly equipment (a mass spectrometer), and is very expensive. Thus, as with many other reference methods, it cannot be used by a clinical laboratory to measure A1C in routine samples. That means it can only be used to calibrate laboratory instruments that measure A1C as before, i.e., by any one of a wide variety of methods. Although disappointing, this does not diminish the virtue of now having a much more robust standardization program.

Second, and much more important, since the new reference method measures A1C itself, and thus non-A1C components are no longer detected, the normal range for A1C is reduced—by about two percentage points lower than that currently reported. Moreover, the IFCC recommended (to be metrologically correct) that A1C be expressed in millimoles A1C per mole of total hemoglobin; this would result in a normal range of around 29–43 mmol A1C/mol hemoglobin (34).

A shift to lower A1C percentages would no doubt be intolerably confusing and likely lead to a deterioration in glycemic control (35), but a wholesale shift to the IFCC units would surely create mayhem. Although one could clearly program a laboratory instrument to convert the new IFCC values to DCCT-derived values, the IFCC maintained that the expression of an analyte as a percentage is not metrologically sound and thus should not be used. In response to the direction proposed by the IFCC, an American Diabetes Association/European Association for the Study of Diabetes/International Diabetes Federation workgroup was formed (including some of the IFCC leadership) to

make recommendations on how this impending crisis could be avoided (36).

What emerged was not only the recommendation that DCCT-derived numbers should be maintained if possible but also that an international study should commence to look more closely at the relationship between A1C and mean blood glucose. If the study was “successful,” at least we could adopt an A1C-derived unit (e.g., “estimated average glucose” in milligrams per deciliter or millimoles per liter) that would obviate the IFCC objection to having laboratory results expressed as a percentage. This path forward was then ratified in an official consensus statement issued by all four organizations (37).

The rationale for another study examining the relationship between mean blood glucose and A1C stemmed from the belief that the previously published reports used a variety of measures of glucose concentration, recruited only small numbers of subjects (and mostly those with type 1 diabetes), performed measurements over a relatively short time period, and, most notably, performed relatively infrequent sampling of blood glucose (mostly during the daytime). For example, the often-cited conversion table in the American Diabetes Association Standards of Medical Care in Diabetes—2007 (38) was based on very limited capillary glucose sampling in the DCCT, and the study was actually not intended to establish the relationship between average glucose and A1C. Thus, greater confidence was needed that A1C truly represents an average glucose.

The results of the international study are now reported (1) and confirm and extend previous findings. The strengths of the study are that it examined the relationship between average glucose and A1C across a wide spectrum of A1C values—from ~5% to as high as 13%—and in more people than ever before studied. Also, both normal subjects and subjects with type 1 and type 2 diabetes were enrolled in numbers sufficient to conclude that the relationship between the two variables was consistent between these subgroups and also in relation to other important variables (i.e., age, ethnicity, smoking). Finally, the study obtained ~2,700 glucose measurements in each participant, which is far greater than the number obtained in nearly all previous studies. The results clearly support the hypothesis that there is a strong linear relationship between mean blood glucose

and A1C, with a coefficient of correlation (R^2) of 0.84.

The data from ADAG indicate that at any mean glucose or A1C level, there is some scatter (see Fig. 1 in the ADAG report), thereby conveying a less than perfect correlation. Is that primarily due to measurement error, or does it suggest that an A1C level reflects processes beyond a straightforward time and glucose concentration–dependent glycation of hemoglobin? Addressing this uncertainty would require an even larger study conducted ideally at only one site, with more diverse subjects, uninterrupted continuous glucose monitoring for months at a time, and, most important, a measurement error much lower than currently seen. The report by Nathan et al. (39) in which 24,000 glucose measurements were done on each participant resulted in an R^2 (0.81) and regression equation very similar to that reported in the ADAG study, suggesting that performing more measurements will not in itself improve the correlation.

Thus, we have ~16–19% of the variation unaccounted for, but given that there is a small measurement error in the determination of A1C (perhaps 2–5%), and a larger coefficient of variation in the measurement of glucose (10–20%), the constraints imposed by methodology can explain the residual variation.

We are unable, of course, to conclude from the study that the relationship holds for all populations. That is, many populations (e.g., Asians, Pacific Islanders, children) were not studied, and it is conceivable that the physiology of glycation differs in such groups, although there is no obvious reason why that would be so. A recent study (40) that showed a relatively poor correlation between average glucose and A1C in children should not raise doubts about the translation of the ADAG study to other populations. In that report (40), it is unclear whether the A1C values were stable throughout the study and how many glucose measurements were obtained in each participant, and there are doubts regarding the precision and accuracy of the continuous glucose-monitoring system device used and other issues (1).

In the ADAG study, the differences between various ethnic groups were not statistically significant. However, the study was not adequately powered to detect such differences and, in one group, the differences came close to being significant. Although other reports have shown

an association between ethnicity and A1C at similar levels of glycemia (41,42), in all the studies, glucose measurements were very infrequent, the populations studied were not controlled for hemoglobinopathy, and there were no measures of the rate of glycation as it relates to ethnicity. Clearly, this is an area that needs further investigation.

It is important to note that the ADAG investigators attempted to study patients with “stable” glycemia—predefined as a change in A1C of <1% during the study—and all but 4% were stable as so defined. It is not surprising that some change would occur, particularly since patients were doing considerably more self-monitoring than in real life. However, a 1% change with a baseline of 11% has implications different from those associated with a similar change at 7%. However, the investigators quite rightly chose the end-of-study A1C to relate to estimated average glucose (eAG); therefore, clinicians can have confidence that the average glucose reflects antecedent glycemia over a 3-month period.

So what does this mean for clinical practice? At the simplest and most basic level, when clinicians explain to patients what A1C “means,” they should have greater confidence that the common explanation that has essentially been in effect for decades—“it’s your average blood glucose over the last few months”—is true. In addition, knowing one’s average glucose level should be beneficial to clinicians and patients in that the measure of long-term glucose control (A1C) can reliably be conveyed in the same units as those provided to patients at the time of diagnosis and the values obtained from patient self-monitoring.

Finally, we have a new opportunity for (re)education on the importance of glycemic control and the seriousness of diabetes. Because the study results fulfilled the a priori criteria, the agreement forged in the consensus statement from the European Association for the Study of Diabetes, International Diabetes Federation, and IFCC (37) will take effect. Thus, we hope that clinicians who order an A1C test will receive a lab report containing the familiar A1C value, an eAG derived from that measurement, and a likely-to-be-ignored IFCC unit (in millimoles per mol). The American Diabetes Association and European Association for the Study of Diabetes are planning to begin a comprehensive educational effort on knowing one’s average glucose and are publishing a

new conversion table in guidelines that is based on the equation derived from the ADAG study.

Patients may still get confused that the “average” glucose on their own meters does not match the eAG. However, this is also an opportunity to educate patients about fluctuations in glucose that may occur at times different from their own testing schedules. In addition, the 95% CIs of eAG for any A1C value imply uncertainty of the “true” mean—and more so at very high A1C levels. But it should be remembered that every point estimate in medicine has uncertainty related to laboratory imprecision and inaccuracy and that this variation is almost always ignored. If necessary, however, these CIs give clinicians an opportunity to present patients a “range” in which their average glucose lies.

Another potential limitation of the study is the specific and careful exclusion of individuals with conditions likely to affect A1C, e.g., hemoglobinopathy. Inadequate recognition of the latter in clinical practice remains a limitation to the interpretation of A1C and will thereby limit the utility of discussing eAG in such patients.

Despite the limitations discussed above, the study by Nathan and colleagues (1) is likely to remain a key reference regarding the relationship between A1C and average glucose. To be sure, the term A1C, along with its current units and normal range, will not vanish or change. Also, whatever instrument and assay is used in a clinical laboratory will continue to remain the same, even though the reference method used for calibration will now be more precise. A provider wedded to conveying an A1C to his or her patients will certainly be able to continue doing so. But for those interested in adding another strategy to improve outcomes, we now have a new term that will likely be easier to explain to patients and to convey more meaning and importance to glucose control.

RICHARD KAHN, PHD¹
VIVIAN FONSECA, MD²

From the ¹American Diabetes Association, Alexandria, Virginia; and the ²Tulane University School of Medicine, New Orleans, Louisiana.

Corresponding author: Richard Kahn, rkahn@diabetes.org.

Received 8 May 2008 and accepted 11 May 2008. The opinions expressed in this article are those of the authors and do not necessarily represent the views of the American Diabetes Association.

DOI: 10.2337/dc08-0878

© 2008 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See <http://creativecommons.org/licenses/by-nc-nd/3.0/> for details.

References

1. Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ, the A1c-Derived Average Glucose (ADAG) Study Group: Translating the A1C assay into estimated average glucose values. *Diabetes Care* 31: 1473–1479, 2008
2. Allen DW, Schroeder WA, Balog J: Observations on the chromatographic heterogeneity of normal adult and fetal human hemoglobin. *J Am Chem Soc* 80:1628–1634, 1958
3. Holmquist WR, Schroeder WA: A new N-terminal blocking group involving a Schiff base in hemoglobin A1c. *Biochemistry* 5:2489–2503, 1966
4. Mortensen HB, Christophersen C: Glycosylation of human hemoglobin A: kinetics and mechanisms studied by isoelectric focusing. *Biochim Biophys Acta* 707:154–163, 1982
5. Huisman THJ, Dozy AM: Studies on the heterogeneity of hemoglobin. V. Binding of hemoglobin with oxidized glutathione. *J Lab Clin Med* 60:302–319, 1962
6. Rahbar S: An abnormal hemoglobin in red cells of diabetics. *Clin Chem Acta* 22:296–298, 1968
7. Rahbar S, Blumenfeld O, Ranney HM: Studies of a unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Comm* 36:838–843, 1969
8. Bunn HF, Haney DN, Kamin S, Gabbay KH, Gallop PM: The biosynthesis of human hemoglobin A1C. *J Clin Invest* 57: 1652–1659, 1976
9. Koenig RJ, Peterson CM, Kilo C, Cerami A, Williamson JR: Hemoglobin A1C as an indicator of the degree of glucose intolerance in diabetes. *Diabetes* 25:230–232, 1976
10. Gabbay KH, Hasty K, Breslow JL, Ellison RC, Bunn HF, Gallop PM: Glycosylated haemoglobins and long-term blood glucose control in diabetes mellitus. *J Clin Endocrinol Metab* 44:859–864, 1977
11. Gonen B, Rubenstein AH, Rochman H, Tanega SP, Horwitz DL: Hemoglobin A1: an indicator of the metabolic control of diabetic patients. *Lancet* 2:734–737, 1977
12. Bunn HF: Evaluation of glycosylated hemoglobin in diabetic patients. *Diabetes* 30:613–617, 1981
13. Jovanovic L, Peterson CM: The clinical utility of glycosylated hemoglobin. *Am J Med* 70:331–338, 1981
14. Mayer TK, Fredman ZR: Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their

- clinical utility. *Clin Chim Acta* 127:147–184, 1983
15. Nathan DM, Singer DE, Hurxthal K, Goodson JD: The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341–346, 1984
 16. Mortensen HB, Christophersen C: Glucosylation of human haemoglobin A in red blood cells studied in vivo: kinetics of the formation and dissociation of haemoglobin A1c. *Clin Chem Acta* 134:317–326, 1983
 17. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A: Correlation of glucose regulation and haemoglobin A1c in diabetes mellitus. *N Engl J Med* 295:417–420, 1976
 18. Peterson CM, Jones RL, Dupuis A, Bernstein R, O'Shea M: Feasibility of improved glucose control in patients with insulin dependent diabetes mellitus. *Diabetes Care* 2:329–335, 1979
 19. Paisley RB, MacFarlane DG, Sheriff RJ, Hartog M, Slade RR, White DA: The relationship between blood glycosylated haemoglobin and home capillary blood glucose levels in diabetics. *Diabetologia* 19:31–34, 1980
 20. Svendsen PA, Lauritzen T, Soegaard U, Nerup J: Glycosylated haemoglobin and steady-state mean blood glucose concentration in type 1 (insulin-dependent) diabetes. *Diabetologia* 23:403–405, 1982
 21. Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE: Defining the relationship between plasma glucose and HbA1c: an analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. *Diabetes Care* 25:275–278, 2002
 22. Murata GH, Hoffman RM, Duckworth WC, Wendel CS, Shah JH: Contributions of weekly mean blood glucose values to hemoglobin A1c in insulin-treated type 2 diabetes: the diabetes outcomes in veterans study. *Am J Med Sci* 327:319–323, 2004
 23. Tahara Y, Shima K: The response of GHb to stepwise plasma glucose over time in diabetic patients. *Diabetes Care* 9:1313–1314, 1993
 24. Makris K, Spanou L, Rambaouni-Antonelitis A, Koniari K, Drakopoulos I, Rizos D, Haliassos D: Relationship between mean blood glucose and glycosylated haemoglobin in type 2 diabetic patients. *Diabet Med* 25:174–178, 2008
 25. Little RR, Wiedmeyer HM, England JD, Wilke AL, Rohlfing CL, Wians FH: Inter-laboratory standardization of measurements of glycohemoglobins. *Clin Chem* 38:2472–2478, 1992
 26. Diabetes Control and Complications Trial Research Group: Feasibility of centralized measurements of glycosylated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. *Clin Chem* 33:2267–2271, 1987
 27. Little RR, Goldstein DE: Endocrine standardization of glycohemoglobin measurements. *Anal Chem* 67:393R–397R, 1995
 28. Goldstein DE, Little RR, Lorenz RA, Malone JJ, Nathan D, Peterson CM: Tests of glycemia in diabetes. *Diabetes Care* 27:1761–1773, 2004
 29. 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 the Inter-laboratory Standardization of HbA1c Determination, the Japan Diabetes Society. *J Jpn Diabetes Soc* 37:855–864, 1994
 30. Arnquist H, Wallensteen M, Jeppsson JO: Standardization of longterm glucose measurements established. *Lakartidningen* 50:4789–4790, 1997
 31. Muller MM: Implementation of reference systems in laboratory medicine. *Clin Chem* 46:1907–1909, 2000
 32. Hoelzel W, Miedema K: Development of a reference system for the international standardization of HbA1c/glycohemoglobin determinations. *J Int Fed Clin Chem* 9:62–76, 1996
 33. Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hosino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Theinpoint L, Umamoto M, Wiedmeyer HM, on behalf of the 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* 50:166–174, 2004
 34. Nordin G, Dykaer R: International Federation of Clinical Chemistry and Laboratory Medicine—Scientific Division: recommendation for term and measurement unit for “HbA1c.” *Clin Chem Lab Med* 45:1081–1082, 2007
 35. Hanas R: Psychological impact of changing the scale of reported HbA1c results affects metabolic control. *Diabetes Care* 25:2110–2111, 2002
 36. ADA/EASD/IDF: Report of the ADA/EASD/IDF working group of the HbA1c assay. *Diabetologia* 47:R53–R54, 2004
 37. ADA/EASD/IFCC/IDF: Consensus statement on the worldwide standardization of the hemoglobin A1c measurement. *Diabetes Care* 30:2399–2400, 2007
 38. American Diabetes Association: Standards of medical care. *Diabetes Care* 30 (Suppl. 1):S4–S41, 2007
 39. Nathan DM, Turgeon H, Regan S: Relationship between glycosylated haemoglobin levels and mean glucose levels over time. *Diabetologia* 50:2239–2244, 2007
 40. Diabetes Research in Children Network (DirecNet) Study Group: Relationship of A1c to glucose concentrations in children with type 1 diabetes. *Diabetes Care* 31:381–385, 2008
 41. Saaddine JB, Fagot-Campagna A, Rolka D, Narayan KMV, Geiss L, Eberhardt M, Flegal KM: Distribution of HbA1c levels for children and young adults in the U.S.: Third National Health and Nutrition Examination Survey. *Diabetes Care* 25:1326–1330, 2002
 42. Herman WH, Ma Y, Uwaifo G, Haffner S, Kahn SE, Horton ES, Lachin JM, Montez MG, Brenneman T, Barrett-Connor E, for the Diabetes Prevention Program Research Group: Differences in A1c by race and ethnicity among patients with impaired glucose in the diabetes prevention program. *Diabetes Care* 30:2453–2457, 2007