Hemoglobin A1c in the Diagnosis and Management of Diabetes

- A1c Assay
- Standardization
- Diabetes Monitoring
- Non-enzymatic Biochemistry
- Separation Science

DCCT       UKPDS

Ignoring Biological Variation in A1c
Objectives

1. Describe what clinical laboratories actually measure as HbA1c

2. Show that HbA1c and mean blood glucose are not interchangeable estimates of glycemic control

3. Explain how biological variation in HbA1c complicates new guidelines for the diagnosis and management of diabetes
Main Take-home Message

The quantitative relationship between blood glucose concentration and hemoglobin A1c is not the same in all people.

\[ \text{MBG} \neq \text{A1c} \]

\[ \text{MBG} = \text{A1c} \]
Hemoglobin Structure

- Four subunits
  - two $\alpha$ globins
  - two $\beta$
- Iron
- Heme $(A_{415} \text{ nm})$
- Binds 4 $O_2$
Globin Association is Dynamic

MW 15258  15867  31125  62249

α + β → αβ + αβ ↔ αβαβ

Monomers  Dimers  Tetramer

Higher Hemoglobin Concentration
Higher pH
Oxygenation
Known Glucose Binding Sites

Order of affinity
1. $\beta$Val1
2. $\beta$Lys66
3. $\alpha$Lys61
4. $\beta$Lys17
5. $\alpha$Val1
6. $\alpha$Lys40
7. $\beta$Lys8
Non-enzymatic Hb glycation

Maillard Reaction

Labile A1c (aldimine/Schiff base)

Stable A1c (ketoamine/Amadori)

β-globin

Glucose
Role of lysine and ε-amino groups

\[ \text{β globin} + \text{Glucose} \rightarrow \text{Schiff base} \rightarrow \text{Amadori} \]

1. Rapid: \[ \text{K-NH}_2 + \text{V-NH}_2 \rightarrow \text{N=CH} + \text{H}_2\text{O} \]
2. Slow: \[ \text{K} \rightarrow \text{HCOH} \rightarrow \text{CH}_2\text{OH} \]
Discovery of Hemoglobin Heterogeneity

Hemoglobin Heterogeneity in Normal Blood

Fig. 1. Chromatogram of normal adult oxyhemoglobin.


Identification of Hemoglobin $A_{1c}$


Fast Hb
Sickle $A_{1c}$


Hemoglobin $A_I$ Heterogeneity
Peaks designated a, b, c in order of appearance

Identification of Hemoglobin $A_{1c}$

“The designations, $A_I$, $A_{II}$ and others, are given for convenience of reference in this discussion. It is not our purpose to propose a system of nomenclature”

Hemoglobin $A_I$ Heterogeneity
Peaks designated a, b, c in order of appearance

Identification of Hemoglobin $A_{1c}$


- Fast Hb
- Sickle $A_{1c}$

Nomenclature Problem

Additional heterogeneity of normal hemoglobin

Clegg MD, Schroeder WA.
Holmquist, W. R. & Schroeder, W. A. A new N-terminal blocking group involving a Schiff base in hemoglobin A\textsubscript{lc}. *Biochemistry* 5: 2489-2503, 1966

Fraction 5 of hemoglobin A\textsubscript{lc}, but not AII, contained chemically modified N-terminal beta globin peptides
The studies described in the present report indicate that a hexose is linked to the N-terminal of the βAlc chain - the possible attachment of other structures to the hexose has not been excluded...”
Hemoglobin A\textsubscript{1c} Elevated in Diabetes


Fast Hb Higher in People with Diabetes

Diabetic
Normal
Fetal
Hemoglobin A\textsubscript{1c} Elevated in Diabetes


Fast Hb Higher in People with Diabetes

Hemoglobin A\textsubscript{1c} Elevated in Diabetes


Blood glucose variation within individuals

AIc correlated with blood glucose
Hemoglobin $A_{1c}$ Elevated in Diabetes

Assessing Glycemia

Blood glucose variation within individuals


Labile fraction removed by RBC incubation in physiological saline >5 h

Bio-Rex 70 Cation Exchange


Clinical Monitoring of Blood Glucose

Commercial Glycated Hemoglobin Assays

- Fast Hb
- Sickle
- $A_{1c}$
- Diabetes
- DCCT
- Feasibility
- Complications

Cation Exchange HPLC
Boronate Affinity Chromatography
Immunoaffinity

**FIGURE 3.** Chromatographic separations of hemolysates from (a) diabetic and (b) nondiabetic individuals.
Commercial Glycated Hemoglobin Assays

Vicinal diol

Doesn’t measure labile A1c

Hemoglobin modified by vicinal diols bind (G, glycated)

Non-diol hemoglobins pass through (N, non-glycated)

Figure 1: GlycoGel B boronic acid affinity chromatography of a diabetic erythrocyte hemolysate containing 10 mg Hb CO. The first peak, eluted in the void volume, consists of nonglycosylated Hb. The addition of sorbitol to the column permits the elution of glycosylated hemoglobin.

Cation Exchange HPLC

Boronate Affinity Chromatography

Immunoaffinity

Antibodies made from synthetic N-terminal beta globin peptides or purified A1c chromatography fractions

Doesn't measure labile A1c

Commercial Glycated Hemoglobin Assays


Fast Hb Sickle $A_{lc}$ and Diabetes DCCT Feasibility Complications

1960 1970 1980

Sickle AIc and Diabetes

1980 1990

DCCT Feasibility

2000 2010 2020

Complications

1960 1970 1980

DCCT Feasibility

2000 2010 2020

Complications

2007/01/04
What do these assays measure?

**A1c**
Whatever is present in the A\textsubscript{1c} peak; modified \(\beta\text{Val1}\)

**Cation Exchange HPLC**

**Boronate Affinity Chromatography**
Whatever is present in the bound fraction; vicinal diols on any amino group

**Immunoaffinity**
Whatever the antibody recognizes
Fig. 1. Flowchart showing NGSP organization and processes.
Fig. 1. Flowchart showing NGSP organization and processes.
Hempe JM, Craver RD. Quantification of hemoglobin variants by capillary isoelectric focusing. Clin Chem 40:2288-95, 1994

vs DCCT Assay
Comparison of A1c and MBG in Children
Comparison of A1c and MBG in Children

A1c and

Children’s Hospital Study:
2.3 years
128 children
Type 1 diabetes
HbA1c levels are influenced by factors in addition to glucose
Hemoglobin Glycation Phenotypes

Hempe et al. J Diab Comp 16:313, 2002
Hemoglobin Glycation Index

\[ HGI = \text{Observed HbA1c} - \text{Predicted HbA1c} \]

\[ \text{Predicted HbA1c} = (\text{MBG} \times \text{slope}) + \text{intercept} \]

- \( HGI > 0 \)
- \( HGI = 0 \)
- \( HGI < 0 \)
Diabetes Control and Complications Trial (DCCT)

Mean Blood Glucose (mg/dl)

% HbA1c

0 100 200 300 400

AA

55

1010

1515

BB

High HGP

Moderate HGP

Low HGP

n=1441

Diabetes Control and Complications Trial (DCCT)

--2.2

--1.2

--0.2

0.8

1.8

2.8

HGI

Low HGP

Moderate HGP

High HGP

Frequency

150

100

50

0

Hypothesis: High HGP patients are more susceptible to microvascular complications

First 100 patients

Three individual patients
Diabetes Control and Complications Trial (DCCT)

Retinopathy

Nephropathy

Risk Probability

Years of Follow-up

0 1 2 3 4 5 6 7

0 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20 0.22 0.24 0.26 0.28 0.30 0.32 0.34 0.36

Frequency

-2.2 -1.2 -0.2 0.8 1.8 2.8

Frequency

0 50 100 150

HGI

Low HGP

Moderate HGP

High HGP

Conclusion: HGI reflects biological variation in A1c and complications risk due to factors other than mean blood glucose

McCarter et al. Diabetes Care 27: 1259, 2004
## HGI and Biological Variation in A1c

<table>
<thead>
<tr>
<th>Study</th>
<th>Reference</th>
<th>Patients</th>
<th>A1c</th>
<th>Blood glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children’s Hospital</td>
<td>JDC 2002</td>
<td>Type 1 children</td>
<td>CIEF</td>
<td>MBG - 30 d meter data</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Care 2004</td>
<td>Type 1 adults</td>
<td>BioRex HPLC</td>
<td>MBG - 7 pt profile set</td>
</tr>
<tr>
<td>UKPDS</td>
<td>ADA 2005</td>
<td>Type 2 adults</td>
<td>BioRad HPLC</td>
<td>Fasting glucose</td>
</tr>
<tr>
<td>DirecNet</td>
<td>Diabetes Care 2007</td>
<td>Type 1 children</td>
<td>NGSP Immunoassay</td>
<td>MBG - CGM</td>
</tr>
<tr>
<td>Children’s Hospital</td>
<td>Pediatric Diab 2010</td>
<td>Type 1 children</td>
<td>NGSP Immunoassay</td>
<td>MBG – Raw meter data</td>
</tr>
</tbody>
</table>
Consensus Statement on the Worldwide Standardization of the Hemoglobin A1c Measurement

_Diabetes Care_ 30(9):2399-2400, 2007

1. The NGSP cation exchange reference A1c method is non-specific
2. The IFCC developed a mass spectrometry based reference method that quantifies N-terminal hexapeptides of β globin modified by glucose
3. IFCC standardized results were 25% lower than NGSP standardized results
4. Recommended reporting A1c in mmol A1c/mol of Hb
5. And since MBG = A1c, consider reporting A1c as “estimated average glucose” - eAG
Translating the A1C Assay Into Estimated Average Glucose Values

AG (mg/dl) = 28.7 x HbA1c – 46.7

r² = 0.84, p < 0.0001

Underlying assumption: A1c is related to MBG the same way in all people

Caveats regarding the use of HbA\textsubscript{1c} for prediction of mean blood glucose

S. Chalew • J. M. Hempe

To achieve the most comprehensive and safest interpretation of HbA\textsubscript{1c} for patient care, clinicians must recognise that HbA\textsubscript{1c} may not be a fully reliable surrogate for MBG. In this case, both patients and clinicians will need to learn how to individualise treatment decisions by taking into account the patient’s HbA\textsubscript{1c} and preceding self-monitored MBG, as well as idiosyncratic between-patient differences that also contribute to the level of HbA\textsubscript{1c}. 
Now, there is a new way to discuss blood glucose control with your patients — estimated average glucose — or eAG.

The results of the A1C-Derived Average Glucose study (ADAG), recently published in Diabetes Care, have confirmed the existence of a linear relationship between A1C and average blood glucose levels.

In light of the study results, the American Diabetes Association is joining with EASD and IDF to promote a new term in diabetes management, estimated average glucose or eAG. Health care providers can now report A1C results to patients using the same units (mg/dl or mmol/L) that patients see routinely in blood glucose measurements.

When discussing the effectiveness of an overall treatment plan in diabetes management, the use of eAG can help simplify the discussion between a patient and provider, using terminology and measurements that are familiar to the patient. Using eAG may enhance the diabetes education process by focusing on a single set of values for both daily glucose checks and long-term control.

For more information about eAG including an on-line eAG calculator, education materials, and a link to the full article from the ADAG study group, visit the American Diabetes Association website at diabetes.org/professional/eAG.

Help your patients make the connection between daily and long-term glycemic control.

Introducing estimated Average Glucose (eAG), a new way to talk to patients about diabetes management.

For years, the A1C test has given patients and health care providers an invaluable tool for measuring diabetes control and guiding treatment decisions. However, A1C as an indicator of diabetes control is not always easy to explain to patients. The measurement — expressed as a percentage — is not something that intuitively relates to the glucose measurements that patients encounter through home glucose monitoring or their lab values. This may make A1C targets difficult for patients to translate into action.

1-800-DIABETES
diabetes.org/professional/eAG

1-800-DIABETES
diabetes.org/professional/eAG
Purpose

The purpose of this study was to evaluate the hypothesis that using estimated average glucose (eAG) while instructing patients yields better knowledge retention than using the term hemoglobin A1C (A1C).

Figure 1. Percentage of questions answered correctly for baseline and follow-up survey.

eAG does not improve patient understanding of diabetes management

**Estimated Average Glucose and Self-Monitored Mean Blood Glucose Are Discordant Estimates of Glycemic Control**

\[ eAG = 28.7 \times A1c - 46.7 \]

A1c 6% = eAG of 126

- **Low**
- **Moderate**
- **High**

- **eAG < MBG** underestimates
- **eAG = MBG**
- **eAG > MBG** overestimates

- Failure to reach target MBG
- Exceed target MBG/risk for hypoglycemia

Hempe, J.M., Soros, A.A. & Chalew, S.A. Diabetes Care 33:1449, 2010
Table 11 — Summary of glycemic recommendations for non-pregnant adults with diabetes

<table>
<thead>
<tr>
<th>A1C Preprandial capillary plasma glucose</th>
<th>Key concepts in setting glycemic goals:</th>
</tr>
</thead>
<tbody>
<tr>
<td>70–130 mg/dl (3.9–7.2 mmol/l)</td>
<td>• A1C is the primary target for glycemic control</td>
</tr>
<tr>
<td>&lt;180 mg/dl (&lt;10.0 mmol/l)</td>
<td>• Goals should be individualized based on:</td>
</tr>
<tr>
<td></td>
<td>- duration of diabetes</td>
</tr>
<tr>
<td></td>
<td>- age/life expectancy</td>
</tr>
<tr>
<td></td>
<td>- comorbid conditions</td>
</tr>
<tr>
<td></td>
<td>- known CVD or advanced microvascular complications</td>
</tr>
<tr>
<td></td>
<td>- hypoglycemia unawareness</td>
</tr>
<tr>
<td></td>
<td>- individual patient considerations</td>
</tr>
<tr>
<td></td>
<td>• More or less stringent glycemic goals may be appropriate for individual patients</td>
</tr>
<tr>
<td></td>
<td>Postprandial glucose may be targeted if A1C goals are not met despite reaching preprandial glucose goals</td>
</tr>
</tbody>
</table>

*Referenced to a nondiabetic range of 4.0–6.0% using a DCCT-baselines should be made 1–2 h after the beginning of the meal, diabetes.

Deaths Partially Halt Diabetes Study

(WASHINGTON) — An unexpected number of deaths among patients receiving intense therapy to lower their blood sugar forced the National Institutes of Health to abruptly cut short part of a major study on the effects of the therapy.

The therapy was aimed at reducing blood sugar levels among type 2 diabetics at especially high risk of heart attack and stroke. There were 2,114 patients enrolled in the 12-week intervention study, and 41 of them died, The New England Journal of Medicine said.

The study, which was designed to test the long-term effects of the treatment compared with standard treatment, was a collaboration between the National Heart, Lung, and Blood Institute and the National Institute of Diabetes and Digestive and Kidney Diseases.

The study used two measures of blood sugar: the glycosylated hemoglobin A1c and the mean blood glucose (MBG).

The graph shows the MBG and A1c values for the low, moderate, and high HGI groups. The red line represents the target MBG of 125 mg/dl. The graph indicates the failure to reach the target MBG and the exceedance of target MBG for hypoglycemia.
## Standards of Medical Care in Diabetes—2010

**Table 17—Plasma blood glucose and A1C goals for type 1 diabetes by age-group**

<table>
<thead>
<tr>
<th>Values by age (years)</th>
<th>Plasma blood glucose goal range (mg/dl)</th>
<th>A1C</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toddlers and preschoolers (0–6)</td>
<td>100–180 Before meals/110–200 Bedtime/overnight</td>
<td>&lt;8.5% (but &gt; 7.5%)</td>
<td>High risk and vulnerability to hypoglycemia</td>
</tr>
<tr>
<td>School age (6–12)</td>
<td>90–180 Before meals/100–180 Bedtime/overnight</td>
<td>&lt;8%</td>
<td>Risks of hypoglycemia and relatively low risk of complications prior to puberty</td>
</tr>
<tr>
<td>Adolescents and young adults (13–19)</td>
<td>90–130 Before meals/90–150 Bedtime/overnight</td>
<td>&lt;7.5%</td>
<td>Risk of severe hypoglycemia</td>
</tr>
</tbody>
</table>

Key concepts in setting glycemic goals:
- Goals should be individualized and lower goals may be reasonable based on benefit-risk assessment.
- Blood glucose goals should be higher than those listed above in children with frequent hypoglycemia or hypoglycemia unawareness.
- Postprandial blood glucose values should be measured when there is a discrepancy between pre-prandial blood glucose values and A1C levels and to help assess glycemia in those on basal/bolus regimens.
Table 2—Criteria for the diagnosis of diabetes

1. A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
   OR
2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*
   OR
3. Two-hour plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGGT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
   OR
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.
Non-proliferative retinopathy graded by fundus photography (n=28,000 from 9 countries)
CONCLUSIONS — In patients with known diabetes, correlations between glucose and A1C are strong; however, moderate correlations were found in the general population. In addition, based on the diagnostic properties of A1C defined by ROC curve analysis, the advantage of A1C compared with OGTT for the diagnosis of diabetes is limited.
Hypoglycemia

Hyperglycemia

Coma and Death

Microvascular and Macrovascular Disease
Survival as a function of HbA$_1c$ in people with type 2 diabetes: a retrospective cohort study

Craig J Currie, John R Peters, Aodán Tynan, Marc Evans, Robert J Heine, Oswaldo L Bracco, Tony Zagar, Chris D Poole

Summary

Background Results of intervention studies in patients with type 2 diabetes have led to concerns about the safety of aiming for normal blood glucose concentrations. We assessed survival as a function of HbA$_1c$ in people with type 2 diabetes.

Racial disparity in A1C in children with diabetes

Table 1—Results of ANOVA/ANCOVA (controlling for MBG, participant age, and diabetes duration) evaluating differences in A1C and HGI between African American and Caucasian participants

<table>
<thead>
<tr>
<th></th>
<th>African American</th>
<th>Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>78</td>
<td>198</td>
</tr>
<tr>
<td>Unadjusted (mean ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>9.4 ± 1.5</td>
<td>8.2 ± 1.0*</td>
</tr>
<tr>
<td>HGI (%)</td>
<td>0.65 ± 1.44</td>
<td>−0.15 ± 0.79*</td>
</tr>
<tr>
<td>Adjusted (mean ± SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>9.1 ± 0.1</td>
<td>8.3 ± 0.1*</td>
</tr>
<tr>
<td>HGI (%)</td>
<td>0.64 ± 0.11</td>
<td>−0.15 ± 0.07*</td>
</tr>
</tbody>
</table>

*P < 0.001; values within a row are significantly different.
Translational Research Program

Hemoglobin Analysis

Development of multidimensional hemoglobin separation techniques
Identification of chemical modifiers

Clinical Research

Prospective Diabetes Study

Best way to use HGI and hemoglobin assays to improve patient care

Analysis of Existing Clinical Trial Data

HGI association with clinical phenotype and outcome in large populations

Mechanisms of Non-enzymatic Hemoglobin Glycation

Quantitative in vitro bioassay of non-enzymatic hemoglobin glycation

Mouse Model of Hemoglobin Glycation

In vivo assessment of factors that influence non-enzymatic hemoglobin glycation
Objectives

1. Describe what clinical laboratories actually measure as HbA1c

2. Show that HbA1c and mean blood glucose are not interchangeable estimates of glycemic control

3. Explain how biological variation in HbA1c complicates new guidelines for the diagnosis and management of diabetes
Conclusions

1. MBG $\neq$ A1c
2. Consider both MBG and A1c for more personalized patient care
3. Even expert committee recommendations should be taken with a grain of salt
It Takes a Long Time to Change Course
Funding
Children’s Hospital RIC
ADA, JDRF,
P & C Carroll Foundation

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Thanks
Children’s Hospital Lab Endocrine Nurses
"An important scientific innovation rarely makes its way by gradually winning over and converting its opponents. What does happen is that its opponents gradually die out and that the growing generation is familiar with the idea from the beginning."

Max Planck
1858-1947