Diabetes

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Learning Objectives

After this presentation, you should be able to:
1. Describe the regulation of glucose homeostasis.
2. Compare and contrast the various ways to diagnose diabetes.
3. Interpret the correlation between Hb A1c and average glucose.
4. Summarize the various methodologies used for glucose and Hb A1c measurement.
5. Discern the advantages and challenges when using methods for Hb A1c measurement.
Diabetes

• ADA: “A group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.”

• The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.
Age-adjusted Percentage of U.S. Adults Who Were Obese or Who Had Diagnosed Diabetes

**Obesity (BMI ≥30 kg/m²)**

- **1994**
- **2000**
- **2009**

**Diabetes**

- **1994**
- **2000**
- **2009**

Economic Cost* of Diabetes in the U.S.

* Includes both direct and indirect costs.

http://www.diabetesarchive.net/advocacy-and-legalresources/cost-of-diabetes.jsp
Regulation

Liver
- Glycogen
- Glucose

Glycogen
- Stimulates breakdown of glycogen
- Promotes insulin release

Insulin
- Stimulates glucose uptake from blood
- Promotes glucagon release

Glucagon
- Stimulates formation of glycogen
- Raises Blood Sugar

Pancreas
- Lowers Blood Sugar

Tissue Cells (muscle, kidney, fat)

High Blood Sugar
- Promotes insulin release

Low Blood Sugar
- Promotes glucagon release
Insulin Biochemistry

• Proinsulin produced by β-cells (only) in pancreatic islets in response to increased cell glucose

• When needed, proinsulin (86 aa) is cleaved to insulin and inactive connecting (C)-peptide

• Insulin’s half-life is ~4 minutes

• C-peptide’s half-life is 20-30 minutes; longer with renal insufficiency (increasing levels)
Endocrine Antagonists of Insulin Action

Counter-regulatory hormones:
- Glucagon
- Catecholamines
- Growth Hormone

The plasma concentrations of these hormones are elevated in untreated Type 2 & Type 1 DM.

Insulin treatment reverses the elevations.
Circulating Antagonists

- **Glucagon** regulates hepatic glucose production & insulin secretion.

- **Catecholamines** reduce the binding of insulin to its receptor by 30 - 40%.

  *Pheochromocytoma patients often present with DM.*

- **Growth Hormone** $\Rightarrow$ **Transferrin:**
  
  Transferrin is produced (by liver) 2 hours after Growth Hormone treatment. Human transferrin may:
  
  - Bind insulin directly
  - Compete with insulin for insulin receptor
  - Interact with a different receptor altering cell responsiveness to insulin.
Circulating Antagonists

Free Fatty Acids (FFA)

• The plasma concentration of FFA is elevated in Type 2 DM.

• Insulin fails to suppress plasma FFA concentration in Type 2 DM.

• Elevated plasma FFA levels inhibit insulin-stimulated glucose uptake and increase glucose production by the liver.
Diabetes

Classification:

1. Type 1
2. Type 2
3. Other types – (endocrinopathies, pharmacologically induced, genetic conditions, infections)
4. Gestational diabetes mellitus (GDM)
5. Impaired glucose tolerance (IGT)  
6. Impaired fasting glucose (IFG)  

Increased risk for diabetes
Type 1 DM (Insulin-dependent)

- β-cell destruction, usually leading to absolute insulin deficiency
- Commonly presents in childhood or adolescence, but can occur at any age.
- Patients are rarely obese (obesity does not rule out diagnosis).
- Late stage disease characterized by little or no insulin secretion as manifested by low or undetectable plasma C-peptide levels.
- Prone to other autoimmune diseases (Graves’ disease, Hashimoto’s thyroiditis, Addison’s disease, myasthenia gravis, pernicious anemia, etc.).
Type 1 DM

§ 5-10% of DM
§ ~ 70-80% of new onset diabetics have autoantibodies to:
  § Glutamic acid Decarboxylase autoAbs (GADA)
  § Tyrosine Phosphatase IA-2 and IA-2 β
  § Insulin autoantibodies
§ HLA association with linkage to DQA and DQB genes
Type 2 DM

- Non-insulin-dependent DM
- Insulin levels may appear normal or elevated, but high blood glucose levels indicate inadequate insulin (ie, insufficient insulin to compensate for insulin resistance).
- Risk increases with age, obesity, and decreased physical activity.

90 percent to 95 percent have type 2 diabetes
ADA’s criteria for diagnosis of diabetes (in non-pregnant adults)

Table 3—Criteria for the diagnosis of diabetes

| 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 |
| FPG ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.* | OR |
| 2-h 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 |

*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.

Diabetes Care. 2012
Increased Risk for Diabetes*

• FPG ≥ 100 mg/dL (5.6 mmol/L) to 125 mg/dL (6.9 mmol/L) = Impaired Fasting Glucose, or

• 2hr plasma glucose of 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L) in the 75 gm load OGTT = Impaired Glucose Tolerance, or

• A1C of 5.7 to 6.4%

Note: For all 3 tests risk is continuous extending below the lower limit of the range and becoming greater at higher ends of the range.
Diagnosis of Cystic Fibrosis Related Diabetes (CFRD)

• Cystic fibrosis–related diabetes (CFRD) is the most common co-morbidity in people with cystic fibrosis
• Occurs in 20% of adolescents and 40–50% of adults with CF
• The additional diagnosis of CFRD has a negative impact on pulmonary function and survival in CF
• Use of Hb A1c as a screening test for CFRD is not recommended
• Screening for CFRD should be performed using the 2-h 75-g OGTT
Precautions Expressed in the ADA Position Statement

• “Point-of-care A1C assays are not sufficiently accurate at this time to use for diagnostic purposes”
• “…greater cost, limited availability, …incomplete correlation…in certain individuals.”
• “rapidly evolving diabetes”
The Role of the Clinical Laboratory in Diabetes

What can we measure?

- Type 1 markers
- Glucose, A1c
- insulin, C-peptide
- ketones, lactate
- glycated proteins
- urine albumin
- creatinine, lipids, cardiac markers

\{ preclinical screening \\
\{ clinical diagnosis, acute management \\
\{ chronic monitoring \\
\} evaluate complications
Case 1

- Clinician from an outside clinic that just started seeing patients calls you and says something is wrong with the lab’s glucose assay. Every sample result has indicated hypoglycemia for the majority of the clinic’s patients that day.

What could be going on?
Why Sodium Fluoride (Gray Top) Tubes for Glucose?

Fluoride (F\(-\)) inhibits glycolysis (enolase), preventing metabolism of glucose.

Plasma more stable in NaF than serum, ADA recommends plasma
Stability of Glucose
Fluoride ◊ vs. Heparin

Volunteer A

Volunteer B
Glucose Measurement: Storage

• glycolysis causes 5-7% decrease per hr. in uncentrifuged blood
• in separated, non-hemolyzed serum, glucose is stable for 8 hr. at room T, 3+ days at 4 °C
Hemoglobin A1c (A1C)

- Used to estimate average blood glucose in diabetic patients
- Glucose attaches to many proteins via a non-enzymatic post-translational process

1st reaction reverses if glucose concentration returns to normal; formation of ketoamine is permanent
Sequence of first 16 amino acids
N-terminal \(\beta\) chain of Hb A
Sequence of first 16 amino acids

N-terminal  β chain of Hb A
Ketoamine (stable HbA1c)

Sequence of first 16 amino acids

MVHLTPEEKSAVTLW

N-terminal β chain of Hb A
Mean Blood Glucose and Hb A1c

The average amount of A1C changes in a dynamic way and indicates the mean blood glucose concentration over the life span of the red cell (8-12 wks):

Hb A1c: Measure of Average Glycemia

Blood glucose, mg/dL

Blood glucose, mmol/L

Not Glucose Control
DCCT & UKPDS Data

Mean A1C = 11%

Retinopathy: 11%
Nephropathy: 10%
Neuropathy: 9%
Microalbumin: 8%

Rate of Retinopathy Progression

Time During Study (yrs)
National Standardization Efforts

• The American Association for Clinical Chemistry (AACC) Standards Committee established a National Glycated Hb Standardization Subcommittee in April 1993.

• **Goal:** To standardize A1C test results so that clinical laboratory results are comparable to those reported in the DCCT where relationships to risk for diabetic complications have been established.

[Image: CAP Survey Results]

[Website: www.ngsp.org]
International Standardization Efforts

- Global approach to the standardization of A1C initiated by the International Federation of Clinical Chemistry (IFCC) in 1995.

- **Goal:**
  - Achieve uniform international A1C standardization
  - Develop an established reference method to specifically measure glycated Hb for the NGSP to use as a new target standard.

**Comparison of Two Standards**

- IFCC results showed a linear relationship with NGSP but were almost 2% lower.

\[
\text{NGSP} = 0.915 \times \text{IFCC} + 2.15
\]

- IFCC also recommends that results be reported in units of mmol/mol and eventually only as eAG.
Harmonizing Hb A\textsubscript{1C} reporting

• The ADA/EASD/IDF Working Group was established in 2004 to harmonize Hb A\textsubscript{1C} reporting.
• 2010 Consensus Statement:

**Patient: Smith, John**

<table>
<thead>
<tr>
<th></th>
<th>NGSP</th>
<th>IFCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A\textsubscript{1C}</td>
<td>6.1%</td>
<td>45 mmol/mol</td>
</tr>
</tbody>
</table>

Recommended by ADA/EASD/IDF/IFCC
Hb A₁c precision improvements and acceptable targets

- NGSP and IFCC groups have worked with method manufacturers to decrease the imprecision of their methods.

- The College of American Pathologists (CAP) began using accuracy grading based on target values set by the NGSP starting with the 2007 survey.

- Peer group means are no longer used.

### Hb A₁c Acceptable Proficiency Goals for CAP surveys*

<table>
<thead>
<tr>
<th>Survey Year</th>
<th>Acceptable (% Target)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>±15</td>
</tr>
<tr>
<td>2008</td>
<td>±12</td>
</tr>
<tr>
<td>2009</td>
<td>±10</td>
</tr>
<tr>
<td>2010</td>
<td>±8</td>
</tr>
<tr>
<td>2011</td>
<td>±7</td>
</tr>
<tr>
<td>2012</td>
<td>±7</td>
</tr>
<tr>
<td>2013</td>
<td>±6?</td>
</tr>
</tbody>
</table>

*within person biological variation ~ 2%
Is Hb A$_{1c}$ Measurement Good Enough?

- Calculating estimated average glucose
- Monitoring glycemic trends
- Diagnosing diabetes
Estimation of Average Glucose (eAG) from Hb A₁C

\[ eAG_{\text{mg/dL}} = 28.7(\text{Hb A}\,_1\,\text{C}) - 46.7 \]

\[ R^2 = 0.84 \]

eAG over 3 months vs. Hb A₁C at end of 3rd month

(n=507)
Hb A\textsubscript{1C} and estimated average glucose

<table>
<thead>
<tr>
<th>A1C (%)</th>
<th>eAG (estimated Average Glucose) (mmol/L)</th>
<th>(mg/dL)</th>
<th>Data in parenthesis are 95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.4 (4.2–6.7)</td>
<td>97 (76–120)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.0 (5.5–8.5)</td>
<td>126 (100–152)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8.6 (6.8–10.3)</td>
<td>154 (123–185)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10.2 (8.1–12.1)</td>
<td>183 (147–217)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>11.8 (9.4–13.9)</td>
<td>212 (170–249)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13.4 (10.7–15.7)</td>
<td>240 (193–282)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>14.9 (12.0–17.5)</td>
<td>269 (217–314)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>16.5 (13.3–19.3)</td>
<td>298 (240–347)</td>
<td></td>
</tr>
</tbody>
</table>

Calculated Estimated Average Glucose (mg/dL) = 28.7 × Hb A\textsubscript{1C} − 46.7

\textit{Diabetes Care.} 2008
How Good is Good Enough?

- In general, 0.5% Hb A\textsubscript{1c} is considered a clinically significant change (e.g. treatment guidelines from ADA/EASD and NICE)

Case 2
Is my patient really doing worse?

- Diabetic patient visited his endocrinologist for 6-month checkup. Hb A$_{1c}$ results for this visit and previous visit are below. Does this represent a statistically significant change?

<table>
<thead>
<tr>
<th>Date</th>
<th>Hb A$_{1c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/12/11</td>
<td>6.8 %</td>
</tr>
<tr>
<td>01/17/12</td>
<td>7.2 %</td>
</tr>
</tbody>
</table>

\[ \Delta + 0.4\% \text{ Hb A$_{1c}$} \]

Note: ADA goal is Hb A$_{1c}$ <7.0% for diabetics
Justification for precise methods? Monitoring Trends with Hb A₁c

<table>
<thead>
<tr>
<th>Analytical CV (%)</th>
<th>Critical Differences (%)</th>
<th>Difference in % Hb A₁c</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>7.2</td>
<td>0.5</td>
</tr>
<tr>
<td>3.0</td>
<td>9.5</td>
<td>0.7</td>
</tr>
<tr>
<td>4.0</td>
<td>11.9</td>
<td>0.8</td>
</tr>
<tr>
<td>5.0</td>
<td>14.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The value by which 2 serial results must **differ** to be considered statistically significant

\[
\text{Critical Differences (RCV) (\%) = } \sqrt{2} \times 1.96 \times \sqrt{\text{CV}_A^2 + \text{CV}_I^2}
\]

http://www.westgard.com/biodatabase1.htm
2011 Guidelines and Recommendations for Laboratory Analysis in the Diagnosis and Management of Diabetes Mellitus

• Within laboratory CV <2%
• Between-laboratory CV <3.5%
Small biases will cause the misdiagnosis of large numbers of people.
Insulin vs C-peptide

- evaluation of patients with hyperinsulinemic or nonhyperinsulinemic hypoglycemia
- insulinoma
- C-peptide
  - No hepatic metabolism, longer ½-life
  - Insulinomas
  - Factitious hypoglycemia
  - Must be low before some insurances will cover insulin pumps
Glycosuria

• Not used in the diagnosis of diabetes

• Average renal threshold for glucose: 
  ~ 150-170 mg/dL

• Renal threshold may be much higher 
  (250 to 300 mg/dL) in diabetes.
Limitations
Diabetes Diagnostic Methods

• All methods in modern times have relied on measures of glucose (blood, plasma, serum)
  - Fasting, casual, stress tests (OGTT)

Problems Using Plasma Glucose

1. Patient must fast ≥ 8 h

2. Repetition FPG on different days reveals large biological variability:
   - Within subject (intraindividual): CVs 4.6-8.3%
   - Between subject (interindivdual): CVs 7.5-12.5%

3. Lack of sample stability – *in vitro* glycolysis, even in the presence of fluoride
Variant Hb and diabetes

• 1,152 Hb variants identified
  – http://globin.cse.psu.edu/cgi-bin/hbvar/counter

• Hb S and Hb C represent 2 of the most commonly encountered Hb variants in the U.S.
  – ~10% of Hb A\textsubscript{1C} samples in Grady Hospital (Atlanta, GA) harbor Hb S

• Estimates suggest that >150,000 diabetic patients in the U.S. carry one of these genetic variants (Little, R.R. and W.L. Roberts. J Diabetes Sci Technol, 2009. 3(3): p. 446-51.)

• Variant Hb may increase, decrease or not alter Hb A\textsubscript{1C} (not enough evidence in most cases)
Analytical limitations

Ion Exchange Chromatography
Separates Hb species based on charge differences

\[
\text{% Hb } A_1C = 100 \times \frac{\text{Hb } A_1C}{\text{Total HbA}}
\]

\[\text{Clin Chem. 2001}\]
Analytical limitations
Immunoturbidimetry

• Closed tube principle
• 2nd generation assays use Ab’s that recognize the first 4 AA’s
• These assays still fail to recognize some variants
• They also report SS, SC, CC variants as “A”_{1c}

\[
\begin{align*}
\text{HbA} & \quad \text{MVHLTPEEKS}^{\text{AA}}\text{AVTALW} \\
\text{HbS} & \quad \text{MVHLTPVEK}^{\text{AA}}\text{AVTALW} \\
\text{HbC} & \quad \text{MVHLTPKEK}^{\text{AA}}\text{AVTALW}
\end{align*}
\]

Second-Generation Assay Epitope

Figure 1. Schematic showing the epitope locations for the antibody used in the second-generation immunoassays to determine glycated hemoglobin (Hb)A_{1c} values. The mutated amino acid for HbS and HbC is shown in gray.

## Potential hidden dangers

<table>
<thead>
<tr>
<th>Hb Variant</th>
<th>Variant II Turbo 1.0&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Synchron System&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Primus&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Integra&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>24.7</td>
<td>4.7</td>
<td>4.9</td>
<td>6.3</td>
</tr>
<tr>
<td>SC</td>
<td>22.0</td>
<td>4.3</td>
<td>4.7</td>
<td>6.1</td>
</tr>
<tr>
<td>SC</td>
<td>24.2</td>
<td>4.3</td>
<td>4.1</td>
<td>5.6</td>
</tr>
<tr>
<td>SC</td>
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</tr>
<tr>
<td>SC</td>
<td>25.9</td>
<td>4.6</td>
<td>4.3</td>
<td>5.5</td>
</tr>
<tr>
<td>SS</td>
<td>14.4</td>
<td>4.6</td>
<td>3.3</td>
<td>4.0</td>
</tr>
<tr>
<td>SS</td>
<td>13.8</td>
<td>4.4</td>
<td>3.1</td>
<td>3.9</td>
</tr>
<tr>
<td>SS</td>
<td>15.8</td>
<td>5.1</td>
<td>4.0</td>
<td>4.5</td>
</tr>
<tr>
<td>SS</td>
<td>17.7</td>
<td>4.4</td>
<td>3.1</td>
<td>4.0</td>
</tr>
<tr>
<td>SS</td>
<td>23.2</td>
<td>4.2</td>
<td>3.9</td>
<td>4.7</td>
</tr>
<tr>
<td>S-β-thalassemia</td>
<td>13.3</td>
<td>4.6</td>
<td>4.9</td>
<td>4.2</td>
</tr>
<tr>
<td>S-β-thalassemia</td>
<td>33.0</td>
<td>5.3</td>
<td>5.3</td>
<td>5.8</td>
</tr>
<tr>
<td>S-β-thalassemia</td>
<td>10.8</td>
<td>5.4</td>
<td>6.7</td>
<td>7.3</td>
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<tr>
<td>S-β-thalassemia</td>
<td>8.9</td>
<td>5.1</td>
<td>5.4</td>
<td>6.3</td>
</tr>
<tr>
<td>S-β-thalassemia</td>
<td>36.3</td>
<td>4.5</td>
<td>4.7</td>
<td>5.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Analytic interference noted. Result not reported in medical record.

<sup>b</sup> Bio-Rad, Inc, Hercules, California.

<sup>c</sup> Beckman, Brea, California.

<sup>d</sup> Trinity Biotech, Bray, Ireland.

<sup>e</sup> Roche, Indianapolis, Indiana.
Do common Hb variants affect my Hb $A_{1c}$ assay?
What do clinicians want to know?

• Factors that affect their clinical interpretation
  – iron deficiency and sickle cell disease, polycythemia, altered red blood cell lifespan, and pregnancy

Blood. 2008
Hb A1C values are influenced by RBC survival

Analytically accurate, but not clinically useful?

<table>
<thead>
<tr>
<th>High Hb A1C</th>
<th>Low Hb A1C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Hemolysis</td>
</tr>
<tr>
<td>B12 deficiency</td>
<td>Treatment</td>
</tr>
<tr>
<td>Folate deficiency</td>
<td>Hb variants</td>
</tr>
</tbody>
</table>

For conditions with abnormal red cell turnover, such as anemias from hemolysis and iron deficiency, the diagnosis of diabetes must employ glucose criteria exclusively.

*Diabetes Care. 2012*
Fructosamine

Characteristics & Advantages:
Estimates average glycemia over the preceding 1-3 weeks.

\[ \text{Hb A1c: preceding } \sim 2-3 \text{ months} \]

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Avoids problems of hemoglobin variants</td>
<td>- Uremia, icterus and lipemia (increase)</td>
</tr>
<tr>
<td></td>
<td>- Hemolysis (decreases)</td>
</tr>
<tr>
<td></td>
<td>- Dependent on total [protein]</td>
</tr>
<tr>
<td></td>
<td>- Assays not standardized</td>
</tr>
<tr>
<td></td>
<td>- No correlation to risk for diabetic complications</td>
</tr>
</tbody>
</table>
Glycated Albumin

Glycated Albumin:
An alternative to fructosamine with better analytical specificity.

Estimates average glycemia over the preceding 1 to 2 week period.

Standardization is still a problem.
Self Assessment Questions

1. Which of the following is **NOT** a criterion for the diagnosis of diabetes?
   a) Hb A1c ≥ 6.5%
   b) Fasting Plasma Glucose ≥ 126 mg/dL (7.0 mmol/L)
   c) 3-h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an OGTT
   d) 2-h plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an OGTT
2. In a normal healthy individual, glucose measurement in whole blood is approximately how much lower when compared to plasma glucose?

a) 2-5%

b) 10-12%

c) 18-20%

d) None of the above
3. Hb A1c provides an assessment of the average glucose concentration within an individual for the preceding:

a) 1-2 weeks
b) 2-3 months
c) 6-8 months
d) None of the above
4. Which of the following tests is an alternative to Hb A1c for the assessment of average glycemia for the preceding 1-2 week period?

a) Hyperglycosylated HCG
b) Glycated Albumin
c) 1,5-Anhydroglucitol
d) Galectin 3
Thank you!