Unravelling the Biochemistry in Diabetes Mellitus

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Overview

• Epidemiology update

• Biochemistry
  - HbA1c in screening and limitations
  - HbA1c in new IFCC units
  - C-peptide, Insulin and Diabetes Autoantibodies
Diabetes Tsunami

- Worldwide there are 150 million people with diabetes…and rising
  - Will rise to 300 million by 2025

  - Every day 275 people develop diabetes (50% undiagnosed)

- Prevalence of T2D increases progressively with age

  - >20% of the population age >60 have T2D
Obesity Epidemic

Australia is today ranked as one of the fattest nations in the developed world. The prevalence of obesity in Australia has more than doubled in the past 20 years.

- 14 millions are overweight or obese
- If weight gain continues at current rate, 80% Aussie adults and 1/3 children will be overweight or obese.
- Obesity has overtaken smoking as the leading cause of premature death and illness in Australia
Prevalence of Overweight 2008, ages 20+, both sexes
Prevalence of Obesity 2008, ages 20+, both sexes
Lack of Physical Activity

Figure 10. Age-standardized prevalence of insufficient physical activity in adults aged 15+ years, by WHO Region and World Bank income group, comparable estimates, 2008.
Disease Progression with Deteriorating Islet Cell Function

Will HbA1c help?
Current Australian Guideline for Screening

MJA 2003 Vol79: 379-383

HbA1c
>6.5% (48 mmol/mol)

Are there limitations?
56 y.o. man
Health Screen

• Fasting glucose 7.1 mmol/L

• Repeat fasting glucose to confirm
56 y.o. man

Health Screen

• Fasting glucose 7.1 mmol/L

• OGGT:
  Time (hr)  Glucose (mmol/L)
  0        6.8 (<6.0)
  1        16.9
  2        13.5 (<11.1)

• HbA1c 6.4%

Labelled Type 2 DM - Health Insurance implications

Will not alter clinical management eg: treatment
HbA1c in MBS 2013

- Only for monitoring in patients with established diabetes mellitus

- Not for use in diagnosis and screening
What is the Best Screening Test?

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accurate</td>
<td>✓</td>
<td>?</td>
</tr>
<tr>
<td>Sen &amp; Spe</td>
<td>&gt;7 mmol/L</td>
<td>&gt;6.0 or 6.5%</td>
</tr>
<tr>
<td>Standardised</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Readily available</td>
<td>✓</td>
<td>x</td>
</tr>
<tr>
<td>Patient’s preparation</td>
<td>x</td>
<td>✓</td>
</tr>
<tr>
<td>Affordability</td>
<td>✓</td>
<td>x</td>
</tr>
</tbody>
</table>
Practicality of HbA1c

- Non-fasting, random sample
- Patient’s preparation not required
- Not affected by short-term lifestyle changes
- Abnormal fasting glucose or OGTT not F/U
- Correlation with microvascular complications
- Point of care, whole blood specimen
- Physicians familiar with HbA1c in diabetes monitoring

Saudeck et al. *JCEM* 2008;93:2447
Proposed Use of HbA1c in Diagnosis

• International Expert Committee
• American Diabetes Association

• WHO Executive Summary 2011:

- Diagnosis: HbA1c $\geq 6.5\%$

- Insufficient evidence to make any formal recommendation on interpretation of HbA1c <6.5%
HbA1c: Areas of Uncertainty

• Surrogate marker of hyperglycaemia – discrepancies between HbA1c and glucose in certain individuals
  - Sensitivity & Specificity
HbA1c vs Plasma Fasting Glucose (NHAMES III)

• Comparing sensitivity and specificity for the diagnosis of diabetes based on FPG/OGTT:

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6%</td>
<td>83%</td>
<td>84%</td>
</tr>
<tr>
<td>6.1%</td>
<td>63%</td>
<td>97%</td>
</tr>
<tr>
<td>6.3%</td>
<td>67%</td>
<td><strong>95%</strong> (Shanghai)*</td>
</tr>
<tr>
<td>6.5%</td>
<td>43%</td>
<td>99.6%</td>
</tr>
<tr>
<td>7.0%</td>
<td>28%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

Carson et al. Diabetes Care 2010;33:95
HbA1c: Areas of Uncertainty

- **Ethnicity**
  - Blacks: 0.2-0.4% higher than whites

- **Age**  – children and adolescents, elderly

- **Seasonal Variation**

  ![Graph showing seasonal variation in HbA1c levels](image)

HbA1c: Areas of Uncertainty

• Some Clinical Settings

  - Acute presentation of Type 1 DM

  - Gestational DM
    New ADIPS Guidelines:
    Abolish Glucose Challenge
    2-hr OGTT for all women with new cut-offs
Disadvantages of HbA1c

- Larger differences in results between labs than glucose
  - Accuracy
  - Standardisation
  - Variability
Accuracy & Standardisation of HbA1c

- National Glycohaemoglobin Standardization Program (NGSP)
  - Responsible for calibration
- To DCCT “Reference Standard Method”
  - BioRex 70 HPLC
- 99% lab methods are standardised to IFCC transferable to DCCT/NGSP
Impact of Reporting in mmol/mol

- **IFCC Units**

<table>
<thead>
<tr>
<th></th>
<th>Current (%)</th>
<th>IFCC traceable methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference interval (non-diabetics)</td>
<td>4–6%</td>
<td>20–42 mmol/mol</td>
</tr>
<tr>
<td>Target for treatment in diabetics</td>
<td>&lt;7%</td>
<td>&lt;53 mmol/mol</td>
</tr>
<tr>
<td>Change of therapy in diabetics</td>
<td>&gt;8%</td>
<td>&gt;64 mmol/mol</td>
</tr>
</tbody>
</table>

*refer to methods aligned to the U.S. National Glycohemoglobin Standardization Program.

* as recommended by American Diabetes Association.

- Derived NGSP units (%) using IFCC-NGSP master equation:
HPLC – Biorad Variant II

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Calibrated Area %</th>
<th>Area %</th>
<th>Retention Time (min)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1a</td>
<td>—</td>
<td>0.4</td>
<td>0.104</td>
<td>9055</td>
</tr>
<tr>
<td>A1b</td>
<td>—</td>
<td>1.7</td>
<td>0.191</td>
<td>37575</td>
</tr>
<tr>
<td>F</td>
<td>—</td>
<td>1.3</td>
<td>0.242</td>
<td>28267</td>
</tr>
<tr>
<td>LA1c</td>
<td>—</td>
<td>0.4</td>
<td>0.342</td>
<td>8661</td>
</tr>
<tr>
<td>CHb</td>
<td>—</td>
<td>2.7</td>
<td>0.390</td>
<td>60182</td>
</tr>
<tr>
<td>A1c</td>
<td>3.2*</td>
<td>—</td>
<td>0.537</td>
<td>15579</td>
</tr>
<tr>
<td>P3</td>
<td>3.5</td>
<td>—</td>
<td>0.745</td>
<td>79664</td>
</tr>
<tr>
<td>A0</td>
<td>—</td>
<td>48.7</td>
<td>0.818</td>
<td>1092831</td>
</tr>
<tr>
<td>Variant Window</td>
<td>—</td>
<td>40.7</td>
<td>0.867</td>
<td>912471</td>
</tr>
</tbody>
</table>

*Values outside of expected range

Total Area: 2244284

A1c Concentration: 3.296*

Analysis Comments:
HbA1c Report

Glycated Hb 8.0 %  (<6.0)
Glycated Hb(IFCC) 64 mmol/mol  (<42)

6.0 – 7.0%  42-53 mmol/mol Good glycaemic control
7.1 - 8.0%  54-64 mmol/mol May be sub-optimal. Suggest clinical review
>8.0%  >64 mmol/mol Poor glycaemic control

Prior to conception, glycated Hb< 6% (42 mmol/mol) is advisable to reduce the incidence of birth defects

From 11/01/2012 HbA1C will be reported in dual units in keeping with current Australian recommendations.
Ref: Change of HbA1c reporting to the new SI units, MJA Position Statement, 2011
## Variability

<table>
<thead>
<tr>
<th></th>
<th>HbA1c</th>
<th>PFG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biological</strong></td>
<td>1.9%</td>
<td>6-14%</td>
</tr>
<tr>
<td>Eg: 6.5%: 6.3-6.7</td>
<td>Eg: 7 mmol/L: 5.7-8.3</td>
<td></td>
</tr>
<tr>
<td><strong>Imprecision</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal CV</td>
<td>2.15%</td>
<td>1.3%</td>
</tr>
<tr>
<td>(HbA1c 5.5%)</td>
<td>(PFG 7 mmol/L)</td>
<td></td>
</tr>
</tbody>
</table>
RCPA-AACB EQAP HbA1c

2012

2009
RCPA-AACB QAP 2012

Glucose

Regression Lines (634)

- Median Low 2.3
- Median High 25.0

Coefficient of Variation

- No. of Laboratories
- Best 5.0
- 50%
- >8.8

HbA1c

Regression Lines (288)

- Median Low 5.6
- Median High 10.5

Coefficient of Variation

- No. of Laboratories
- Best 5.0
- 50%
- >7.4
How we currently perform in WA

2011 AACB QCSC HbA1c survey
Patient pools

* Clinipath uses the Biorad Variant 2 Turbo
Cost Restriction: HbA1c

- Unavailable in certain countries
- Limited resources
- Limited accredited laboratories in India

Mahajan B and Mishra B 2011
Cost per test

Glucose  $ 0.05
HbA1c    $ 2.00

(POCT  $10.00)
Medicare Cost 2012

85% Bulk Bill Rate

- HbA1c (not for diagnosis or screening) $14.40
- Glucose $8.30
- U&Es & LFTs & Lipids & Glu $15.15
- HDL $9.45
- Urine Alb/Creat $17.25
- OGTT $16.25
Medicare Benefits Claimed
July 2011 – June 2012

Medicare Item 66551
— HbA1c in patients with established diabetes mellitus

<table>
<thead>
<tr>
<th>State</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSW</td>
<td>367,602</td>
</tr>
<tr>
<td>VIC</td>
<td>285,681</td>
</tr>
<tr>
<td>QLD</td>
<td>195,422</td>
</tr>
<tr>
<td>WA</td>
<td>85,513</td>
</tr>
<tr>
<td>TAS</td>
<td>26,255</td>
</tr>
<tr>
<td>ACT</td>
<td>16,793</td>
</tr>
<tr>
<td>NT</td>
<td>9,599</td>
</tr>
</tbody>
</table>

Total 1,084,502 tests @ $14.40

$15.6 millions

OGTT: 305,685 @ $16.25
~$5 millions
Is the HbA1c correct?

68 y.o. woman

Type 2 DM and CRF on haemodialysis

Plasma Creatinine  386 µmol/L (50-95)
Plasma Glucose  (F)  11.3 mmol/L

HbA1c  6.0%
Haemodialysis and HbA1c

• Shortening of erythrocyte lifespan

• Erythropoetin use → changing proportion of new and old RBCs

• Carbamylated Hb – on certain assay
  - Urea derived isocynate modifies Hb at the amino terminal valine (interferes with ion-exchange HPLC)
68 y.o. woman

Type 2 DM and CRF on haemodialysis

Plasma Creatinine  \(386 \, \mu\text{mol/L} \,(50-95)\)

Plasma Glucose (F)  \(11.3 \, \text{mmol/L}\)

HbA1c  \(6.0\%\)

Fructosamine  \(416 \, \mu\text{mol/L} \,(<285)\)

(HbA1c \(~10\\%)\)
HbA1c Measurement: Analytical Issues

Erythropoiesis
- increased new RBC production

Erythrocyte destruction
- increased red cell turnover

Altered Hb
- eg: Hb variant (Hb S, C, D and E) or haemoglobinopathies

Glycation

Assays
- method based on molecular charge or structure

WHO 2011
## Factors that can affect HbA1c Measurement

<table>
<thead>
<tr>
<th>Factors</th>
<th>Decreased HbA1c</th>
<th>Increased HbA1c</th>
<th>Variable change in HbA1c</th>
</tr>
</thead>
</table>
| Erythropoiesis | Treated Fe deficiency anaemia  
B12 therapy  
EPO therapy  
Reticulocytosis | Fe deficiency anaemia  
Vit B12 and folate deficiency  
Decreased erythropoiesis |                                                                                                   |                                          |

---

### HbA1c Assay Interferences

<table>
<thead>
<tr>
<th>Altered Haemoglobin</th>
<th>Glycation</th>
<th>Assays</th>
</tr>
</thead>
</table>
| Foetal Hb  
Haemoglobinopathies  
Methaemoglobin | Aspirin  
Vit C and E  
Haemoglobinopathies | Hypertriglyceridaemia  
Hyperbilirubinaemia  
Carbamylated Hb  
Large doses of aspirin  
Chronic opiate use |
# Artefactually Lower HbA1c

<table>
<thead>
<tr>
<th>Factors</th>
<th>Decreased HbA1c</th>
</tr>
</thead>
</table>
| Erythropoiesis   | Treated Fe deficiency anaemia  
B12 therapy  
EPO therapy  
Reticulocytosis  
Recurrent Phlebotomy  
Haemolytic anaemia  
  eg: sickle cell, thalassaemia, G6PD deficiency  
RBC transfusion (haemodilution) |
| Erythrocyte Destruction | Reduced RBC lifespan  
Splenomegaly  
Drugs eg: dapsone, antiretrovirals  
ribavirin  
Rheumatoid arthritis  
Chronic malaria |
| Altered Haemoglobin |  |
| Glycation         | Aspirin  
Vit C and E  
Haemoglobinopathies |
| Assays            | Hypertriglyceridaemia |
## Artefactually Higher HbA1c

<table>
<thead>
<tr>
<th>Factors</th>
<th>Increased HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoiesis</td>
<td>Fe deficiency anaemia</td>
</tr>
<tr>
<td></td>
<td>Vit B12 and folate deficiency</td>
</tr>
<tr>
<td></td>
<td>Decreased erythropoiesis</td>
</tr>
<tr>
<td>Erythrocyte Destruction</td>
<td>Increased RBC lifespan</td>
</tr>
<tr>
<td></td>
<td>Splenectomy</td>
</tr>
<tr>
<td>Altered Haemoglobin</td>
<td></td>
</tr>
<tr>
<td>Glycation</td>
<td>Alcoholism</td>
</tr>
<tr>
<td></td>
<td>Chronic Renal Failure</td>
</tr>
<tr>
<td>Assays</td>
<td>Hyperbilirubinaemia</td>
</tr>
<tr>
<td></td>
<td>Carbamylated Hb</td>
</tr>
<tr>
<td></td>
<td>Large doses of aspirin</td>
</tr>
<tr>
<td></td>
<td>Chronic opiate use</td>
</tr>
</tbody>
</table>
WA Data
PathWest QEII Data - WA

- HbA1c requests Jan 2007-Sept 2012
  - 242,093 records (150 daily)

- Non-diabetic patients 26%

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>5.9 %</td>
</tr>
<tr>
<td>Median</td>
<td>5.8 %</td>
</tr>
<tr>
<td>Mode</td>
<td>5.7 %</td>
</tr>
<tr>
<td>SD</td>
<td>0.71</td>
</tr>
<tr>
<td>2.5&lt;sup&gt;th&lt;/sup&gt; centile</td>
<td>5.0 %</td>
</tr>
<tr>
<td>97.5&lt;sup&gt;th&lt;/sup&gt; centile</td>
<td>7.2 %</td>
</tr>
</tbody>
</table>

Courtesy of Rob Wardrop
PathWest QEII Data – WA
Making the distribution Gaussian

N 50,696
Mean 5.7 %
Median 5.7 %
Mode 5.7 %
2.5th centile 5.0 %
97.5th centile 6.4 %

ADA Category of Increased Risk of Diabetes
HbA1c 5.7-6.4%
(indeterminate range)

Median 6.4%
2.5th to 97.5th: 5.8-8.8%

Courtesy of Rob Wardrop
Evolving Recommendations:

HbA1c
Evolving Recommendations:

- **2008 Consensus Statement**
  - \( \text{HbA1c} \geq 6.5\% \) would be diagnostic if confirmed by another test (fasting, random, “OGTT”)
  - \( \text{HbA1c} \geq 6.0\% \) as screening as well as plasma glucose of IFG – further diagnostic evaluation and closer follow-up

  *JCEM 2008;93:2447*

- **International Expert Committee**
  - Diagnosis of diabetes if \( \text{HbA1c} \geq 6.5\% \). Diagnosis should be confirmed with a repeat HbA1c unless clinical symptoms and plasma glucose >11.1 mmol/L

  *Diabetes Care 2009;32*

- **ADA Criteria**
  - \( \text{HbA1c} >6.5\% \) or fasting or random plasma glucose or OGTT
  - repeated in asymptomatic patients
  - categories for increased risk of diabetes

  *Diabetes Care 2010;33:S11*
Recommendation

HbA1c can be used as a diagnostic test for diabetes providing that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement.

An HbA1c of 6.5% is recommended as the cut point for diagnosing diabetes. A value of less than 6.5% does not exclude diabetes diagnosed using glucose tests.

Quality of evidence assessed by GRADE: moderate

Strength of recommendation based on GRADE criteria: conditional

Summary: Limitations

• Relationship between plasma glucose and HbA1c is not perfect
  - Is 6.5% appropriate cut-off? Low sensitivity!
  - Use glucose or HbA1c but not both
• Accredited laboratories and International reference values
  - Accuracy and precision between labs
  - POCT not currently recommended
• Not appropriate for Gestational diabetes
• Impact of reporting HbA1c in mmol/mol
• Interferences - “No conditions present which preclude accurate measurement”
• Cost
HbA1c – Is It Better?

- For the rich?
- For the hungry?
- For the lazy?

Personal views for Diagnosis with HbA1c:

- Fasting glucose 5.6-6.9 mmol/L
- OGTT can be avoided
- Cost-effectiveness
HbA1c Control and Beyond

Diabetes... Think!

“What fits your busy schedule better, exercising one hour a day or being dead 24 hours a day?”
**HbA₁c goals:**

What needs to be considered

---

**Approach to management of hypoglycaemia:**

<table>
<thead>
<tr>
<th>Category</th>
<th>More stringent</th>
<th>Less stringent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient attitude and expected treatment efforts</td>
<td>Highly motivated, adherent, excellent self-care capacities</td>
<td>Less motivated, non-adherent, poor self-care capacities</td>
</tr>
<tr>
<td>Risks potentially associated with hypoglycaemia, other adverse events</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Disease duration</td>
<td>Newly diagnosed</td>
<td>Long-standing</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>Important comorbidities</td>
<td>Absent</td>
<td>Few/mild</td>
</tr>
<tr>
<td>Established vascular complications</td>
<td>Absent</td>
<td>Few/mild</td>
</tr>
<tr>
<td>Resources, support system</td>
<td>Readily available</td>
<td>Limited</td>
</tr>
</tbody>
</table>

Complexity of glycaemic goals recognised in ADS/EASD position

“It is important to individualise treatment targets”

<table>
<thead>
<tr>
<th>Suggested clinical situations</th>
<th>Recommended HbA&lt;sub&gt;1c&lt;/sub&gt; goals</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td>&lt;7%*</td>
</tr>
<tr>
<td>Diabetes of short duration</td>
<td></td>
</tr>
<tr>
<td>No clinically significant CVD</td>
<td>6.0–6.5%*</td>
</tr>
<tr>
<td>Long life expectancy</td>
<td></td>
</tr>
<tr>
<td>History of severe hypoglycaemia</td>
<td>7.5–8.0%</td>
</tr>
<tr>
<td>Limited life expectancy</td>
<td>(or slightly higher)</td>
</tr>
<tr>
<td>Advanced complications</td>
<td></td>
</tr>
<tr>
<td>Extensive comorbid conditions</td>
<td></td>
</tr>
<tr>
<td>When glycaemic goals difficult to attain despite intensive self-management education, repeated counselling, and effective doses of multiple glucose-lowering agents, including insulin</td>
<td></td>
</tr>
</tbody>
</table>

*If can be achieved without significant hypoglycaemia or other adverse effects of treatment

Biochemistry of Diabetes Mellitus

- **Type 1** vs **Type 2**
  - Diabetes
  - Autoantibodies

**Fasting plasma glucose and C-peptide**

(Random plasma c-peptide +/- insulin with GLP-1 agonist and/or DPP4 inhibitors?)
Diabetes Autoantibodies

• **Islet cell autoantibodies (ICA)**
  - Abs to islet cell-cytoplasm
  - seen in 1-2% of health individuals
  - no longer available

• **Anti-GAD**
  - Abs to 65 kDa isoform of glutamic acid decarboxylase

• **IAA or IA-2 or IA-2A antibodies**
  - Abs to two tyrosine phosphatase-like islet antigens

• **Insulin autoantibodies**
  - previous exposure to insulin therapy
Diabetes Autoantibodies

• Type 1 DM
  - Abs positive in 85-90%
  - Negative Abs seen in African or Asian
  - Positive multiple Abs associated with >95% risk of Type 1 DM

• Type 2 DM
  - 5-10% Caucasian adults with DM2
  - Usually positive Anti-GAD65
Case 1
52 year old housewife

- Gestational Diabetes Mellitus 1990 (diet controlled)
- Type 2 diabetes mellitus diagnosed 2001
- Diet controlled

<table>
<thead>
<tr>
<th>Year</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>6.4%</td>
</tr>
<tr>
<td>2007</td>
<td>8.3%</td>
</tr>
<tr>
<td>04/08</td>
<td>10.9%</td>
</tr>
</tbody>
</table>
April 2008

- Metformin 500mg TDS introduced but not tolerated with diarrhoea and nausea.
- Could not tolerate gliclazide
- Restricted carbohydrate intake with improvement in HbA1c 6.8% July 08
- Lost 20kg weight in 12 weeks (58kg)
  - lethargic, given up aerobics exercise
Investigations

• OGTT:

  Plasma Glucose (mmol/L)

  Fasting        8.3       (<7.0)
  1 hour         19.5      
  2 hour         25.5      (<7.8)

• Anti-GAD Abs  94 U/ml   (<10)
• IA-2 Abs      <10 U/ml   (<10)
Diagnosis

- LADA (Latent Autoimmune Diabetes of Adults)
  or
- Slow to Evolve Type 1 DM
- Commenced Insulin therapy
Follow-up

- On Lantus 8 units nocte
- Stopped NovoRapid because of hypoglycaemia
- Fingerprick BSLs: 5-8 mmol/L

- Last follow-up in 2012
  - Gradual weight gain over the years (72 kg)
  - HbA1c 2012 6.1% (infrequent hypos)
  - Novorapid 0-7 units with meals and Lantus 10 units
Thank You

We ran blood tests, did M.R.I. scans, took stool samples and performed a colonoscopy... and we’ve determined that the “bloating sensation” you’re experiencing is “fat.”