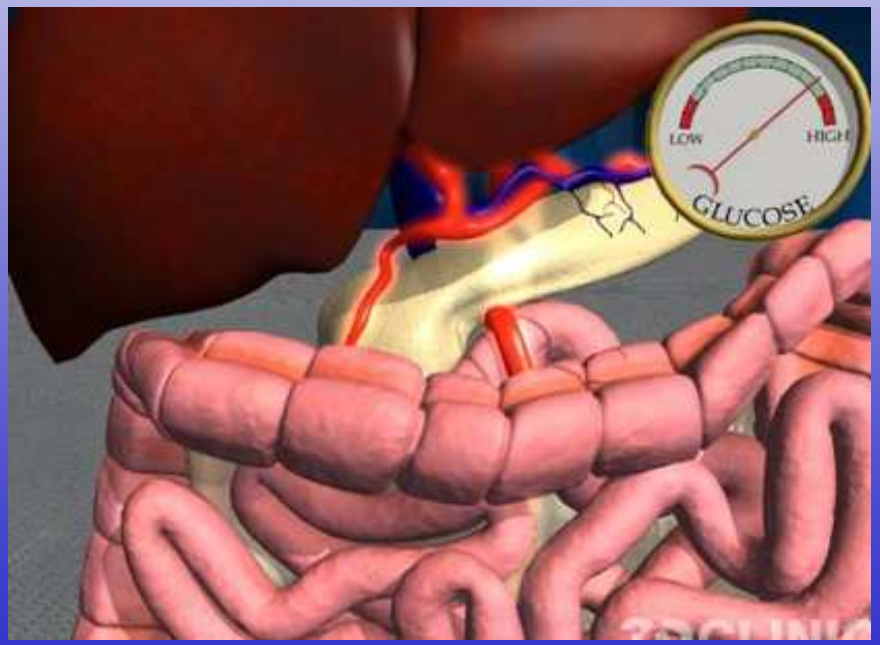
Department of Health

GOVERNMENT OF WESTERN AUSTRALIA

PathWest
LABORATORY MEDICINE WA

Unravelling the Biochemistry in Diabetes Mellitus



Ee Mun LIM
Chemical Pathology
PathWest QEII
Endocrinologist,
Sir Charles Gairdner Hospital
Western Australia

Overview

- Epidemiology update

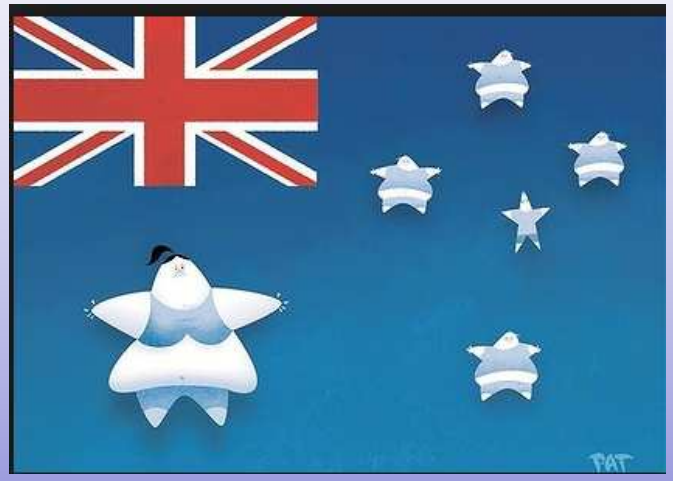
- Biochemistry
  - HbA1c in screening and limitations
  - HbA1c in new IFCC units
  - C-peptide, Insulin and Diabetes Autoantibodies

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

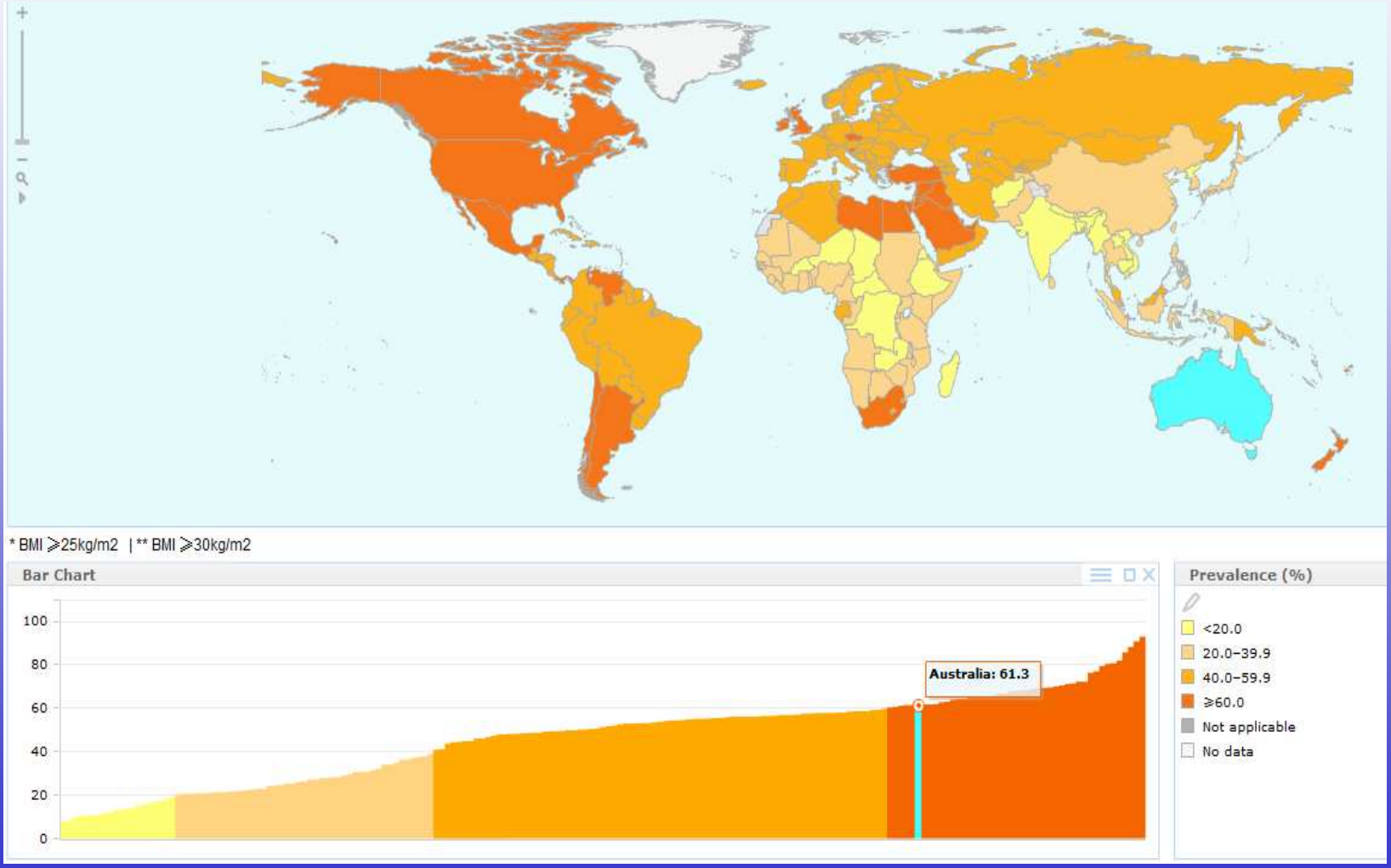
Obesity Epidemic

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.



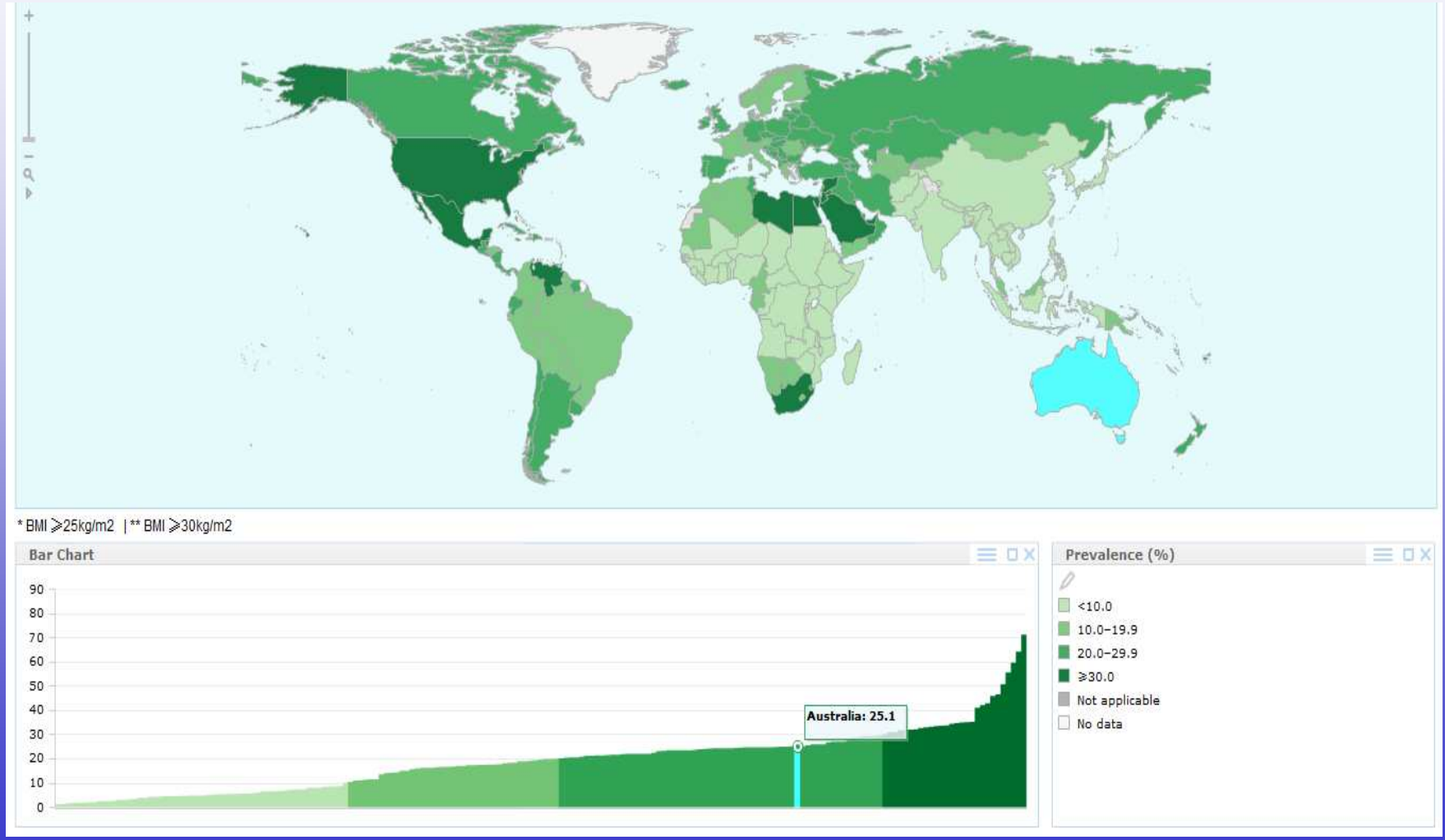
- 14 millions are overweight or obese
- If weight gain continues at current rate, 80% Aussie adults and 1/3 children will be overweight or obese.
- Obesity has overtaken smoking as the leading cause of premature death and illness in Australia

Prevalence of Overweight 2008, ages 20+, both sexes



World Health Organization

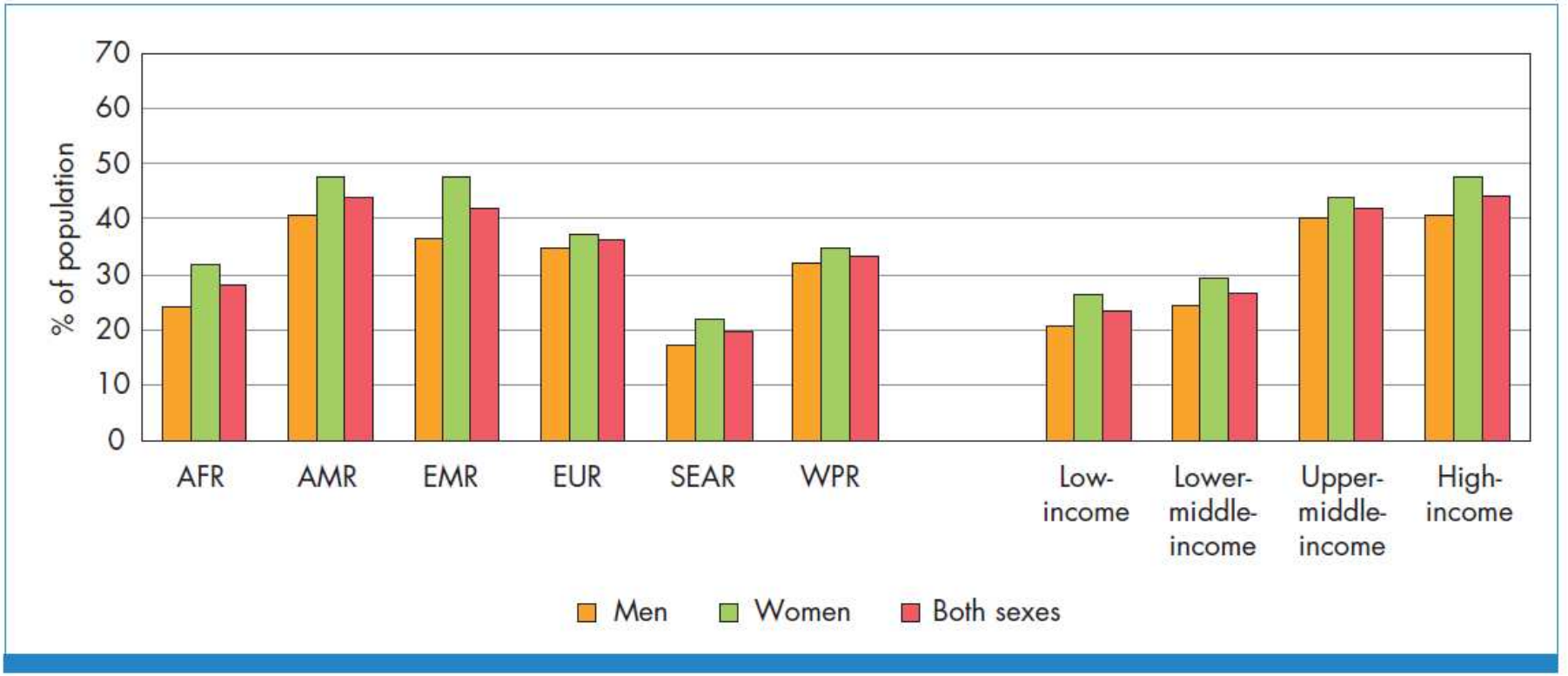
Prevalence of Obesity 2008, ages 20+, both sexes



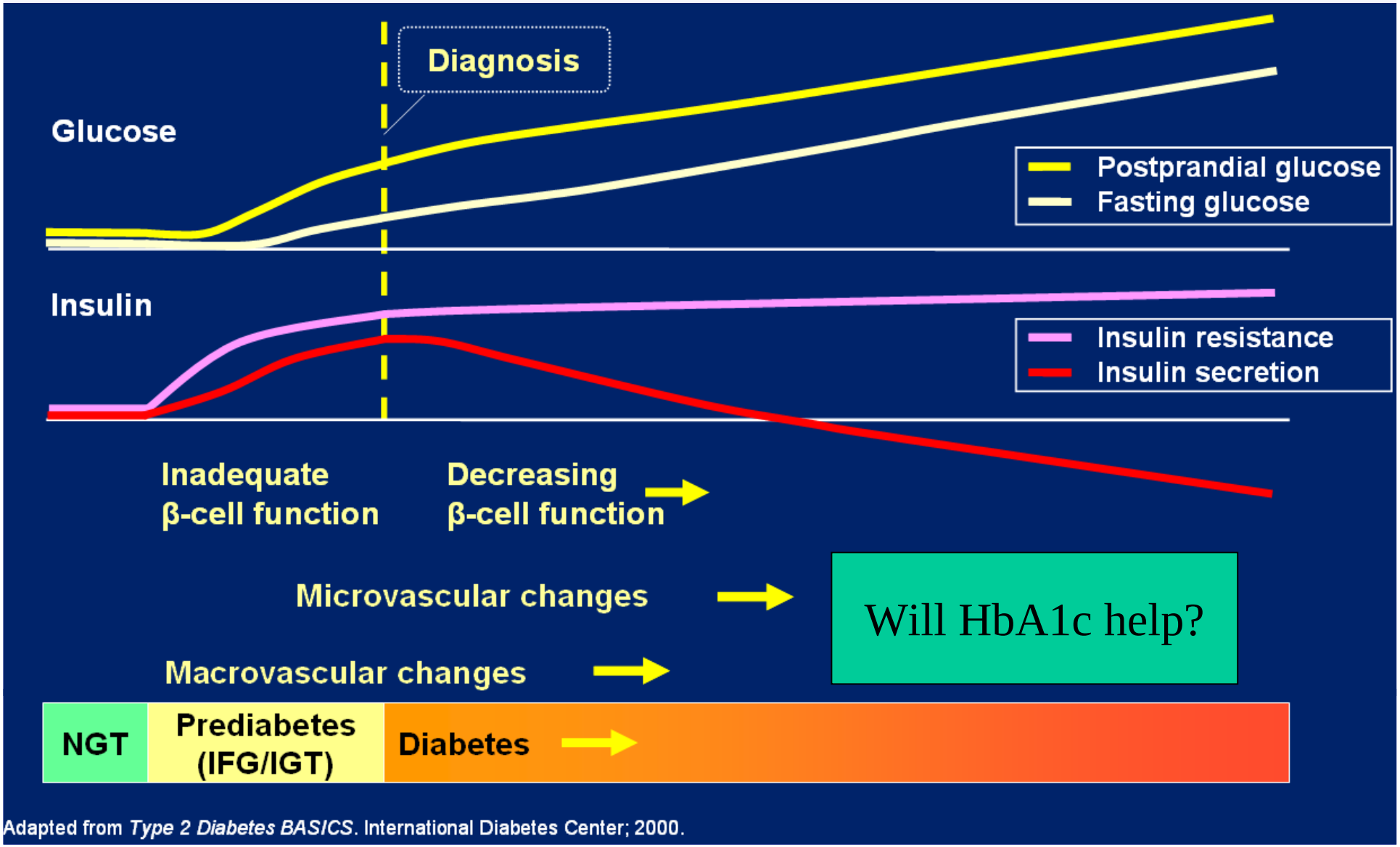
World Health Organization

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

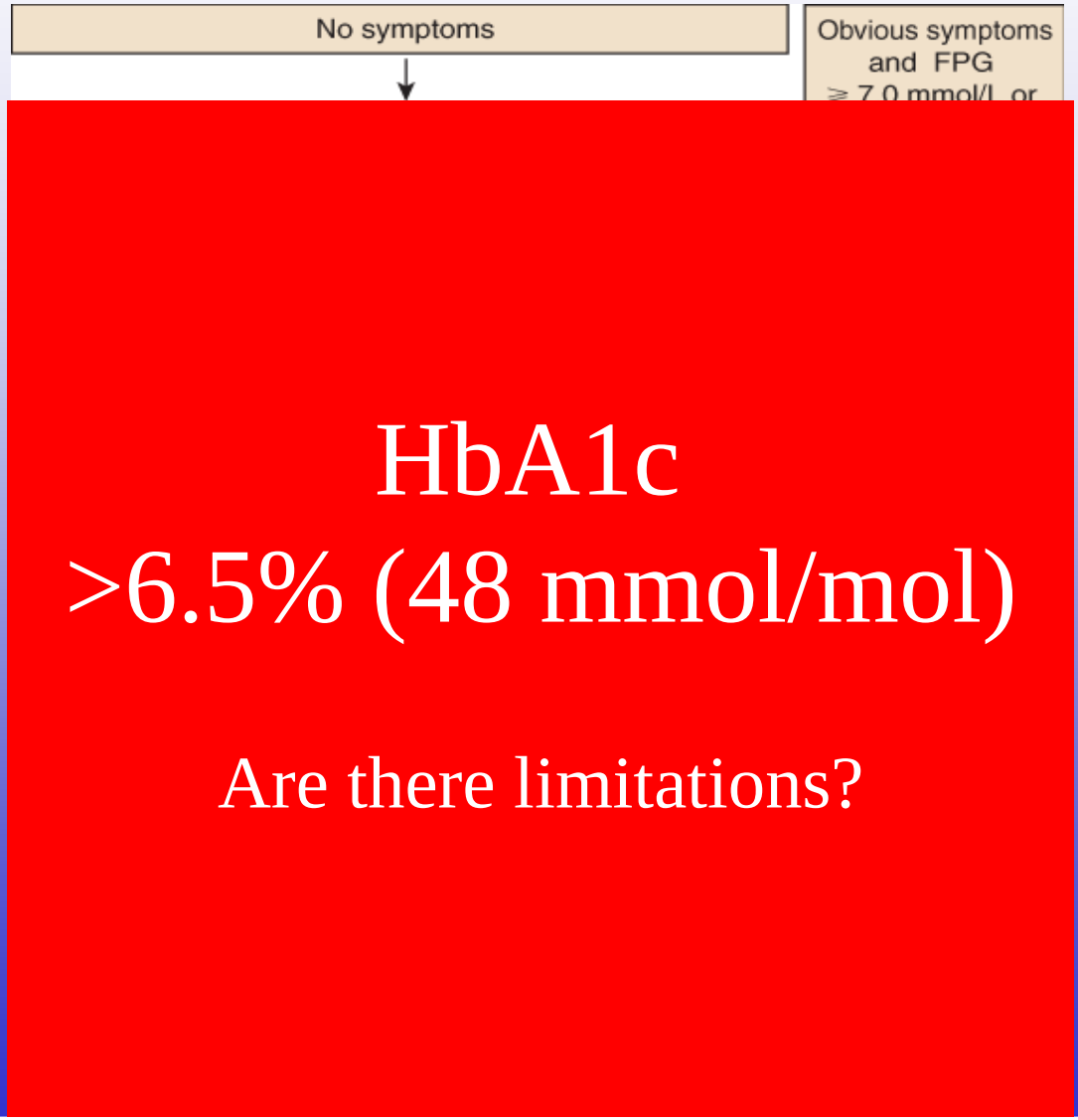


Disease Progression with Deteriorating Islet Cell Function



Adapted from *Type 2 Diabetes BASICS*. International Diabetes Center; 2000.

Current Australian Guideline for Screening MJA 2003 Vol79: 379-383



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

- OGTT:

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

Labelled Type 2 DM
- Health Insurance implications

- HbA1c 6.4%

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?

	Glucose	HbA1c
• Accurate	√	?
• Sen & Spe	>7 mmol/L	>6.0 or 6.5%
• Standardised	√	√
• Readily available	√	x
• Patient's preparation	x	√
• Affordability	√	x

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

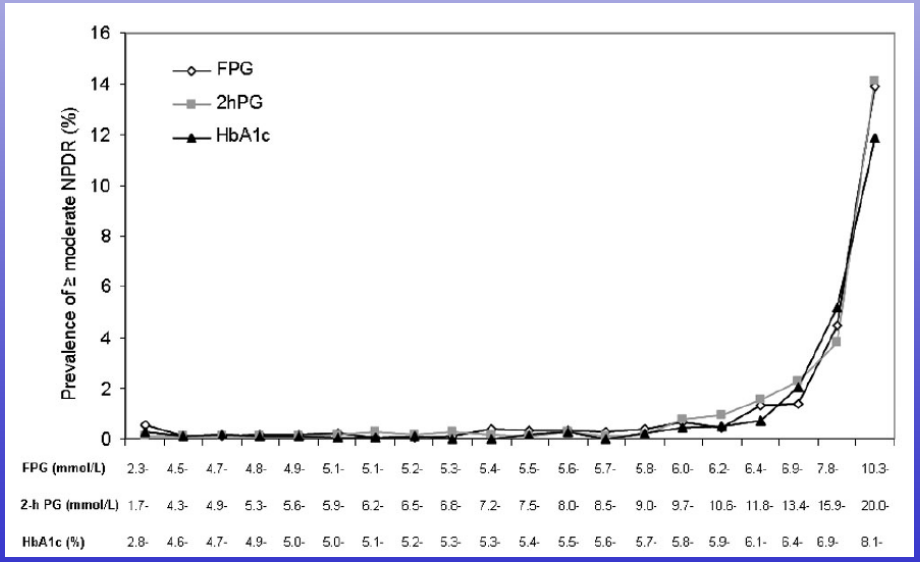
Proposed Use of HbA1c in Diagnosis

- International Expert Committee
- American Diabetes Association

- WHO Executive Summary 2011:

  - Diagnosis: **HbA1c ≥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)

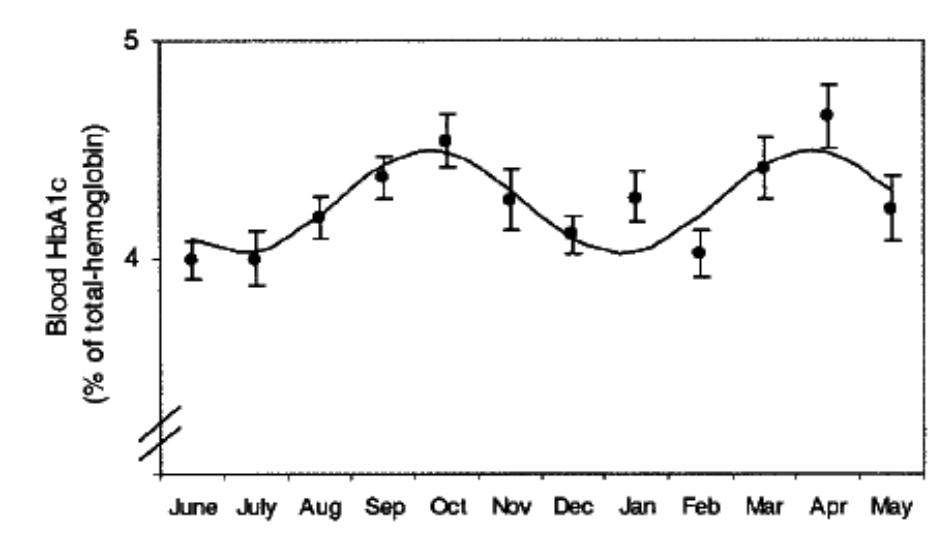
- Comparing sensitivity and specificity for the diagnosis of diabetes based on FPG/OGTT:

HbA1c	Sensitivity	Specificity
5.6%	83%	84%
6.1%	63%	97%
6.3%	**67%**	**95% (Shanghai)***
6.5%	43%	99.6%
7.0%	28%	99.9%

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



Garde AH et al. *Clin Chem* 2000

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

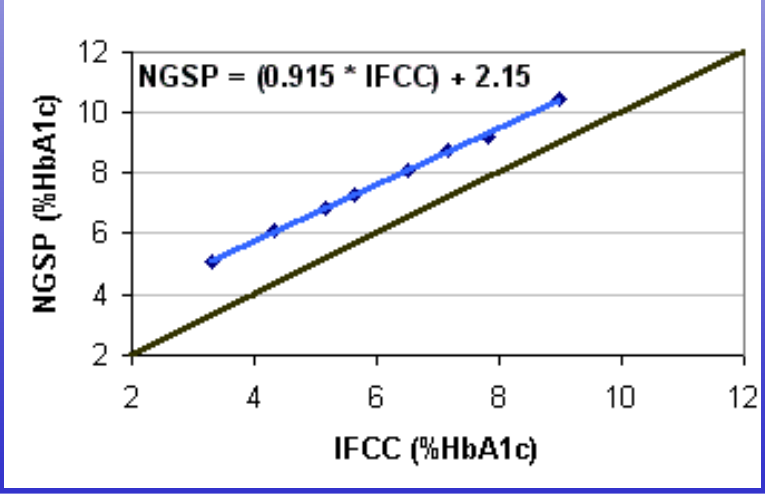
- IFCC Units

	Current[a]	IFCC traceable methods
Reference interval (non-diabetics)	4–6%	20–42 mmol/mol
Target for treatment in diabetics[b]	<7%	<53 mmol/mol
Change of therapy in diabetics[b]	>8%	>64 mmol/mol

[a] refer to methods aligned to the U.S. National Glycohemoglobin Standardization Program.
[b] as recommended by American Diabetes Association.

&

- Derived NGSP units (%) using IFCC-NGSP master equation:



HPLC – Biorad Variant II

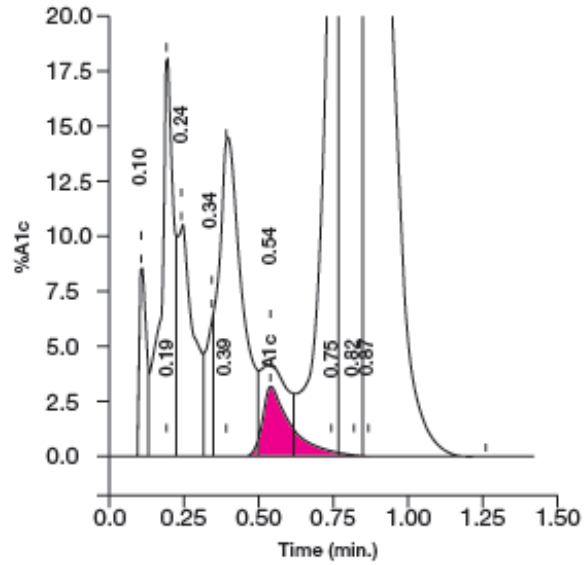
Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
A1a	—	0.4	0.104	9055
A1b	—	1.7	0.191	37575
F	—	1.3	0.242	28267
LA1c	—	0.4	0.342	8661
CHb	—	2.7	0.390	60182
A1c	3.2*	—	0.537	15579
P3	—	3.5	0.745	79664
A0	—	48.7	0.818	1092831
Variant Window	—	40.7	0.867	912471

*Values outside of expected range

Total Area: 2244284

A1c Concentration: 3.2%*

Analysis Comments:



IFCC HbA1c

NGSP 5.1%

HbA1c Report

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

6.0 – 7.0%	42-53	glycaemic control
7.1 - 8.0%	54-64	e sub-optimal. st clinical review
>8.0%	>64 m	lycaemic control

Prior to conception, glycate /mol) is advisable to reduce the incidence of birth defects

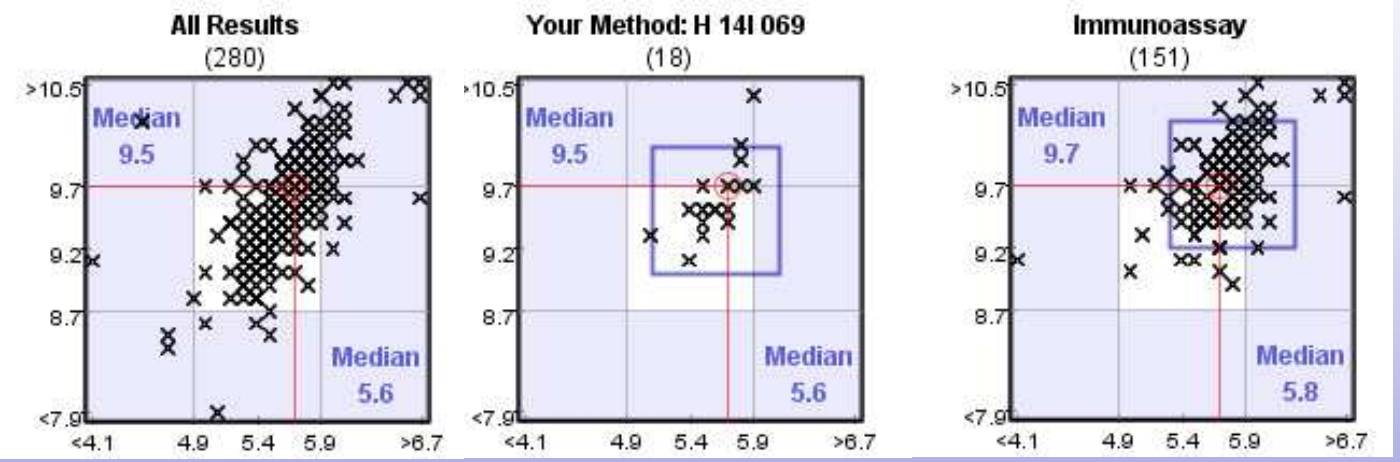
From 11/01/2012 HbA1C will be r rrent Australian recommendations.
Ref: Change of HbA1c reporting to nent, 2011

Variability

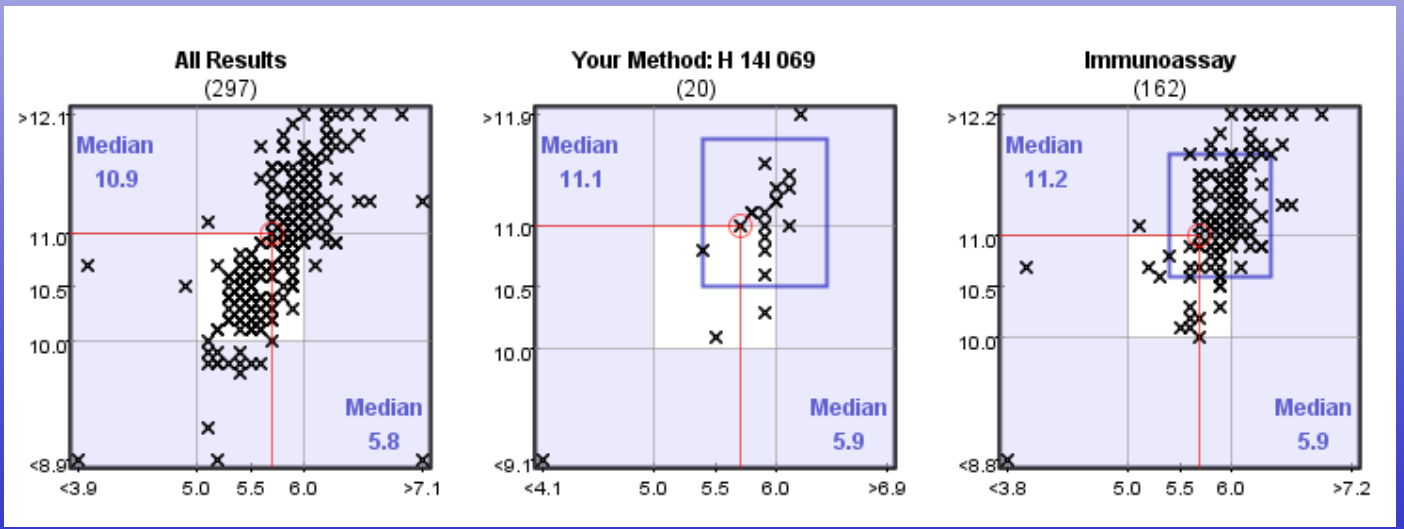
	HbA1c	PFG
Biological	1.9%	6-14%
	Eg: 6.5%: 6.3-6.7	Eg: 7 mmol/L : 5.7-8.3
Imprecision		
Internal CV	2.15%	1.3%
	(HbA1c 5.5%)	(PFG 7 mmol/L)

RCPA-AACB EQAP HbA1c

2012

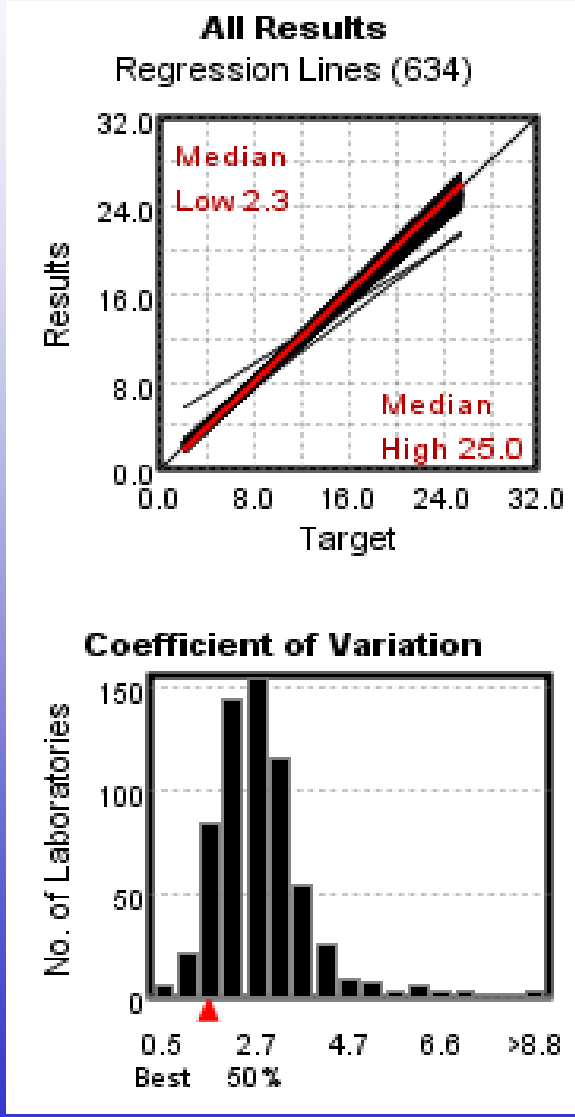


2009

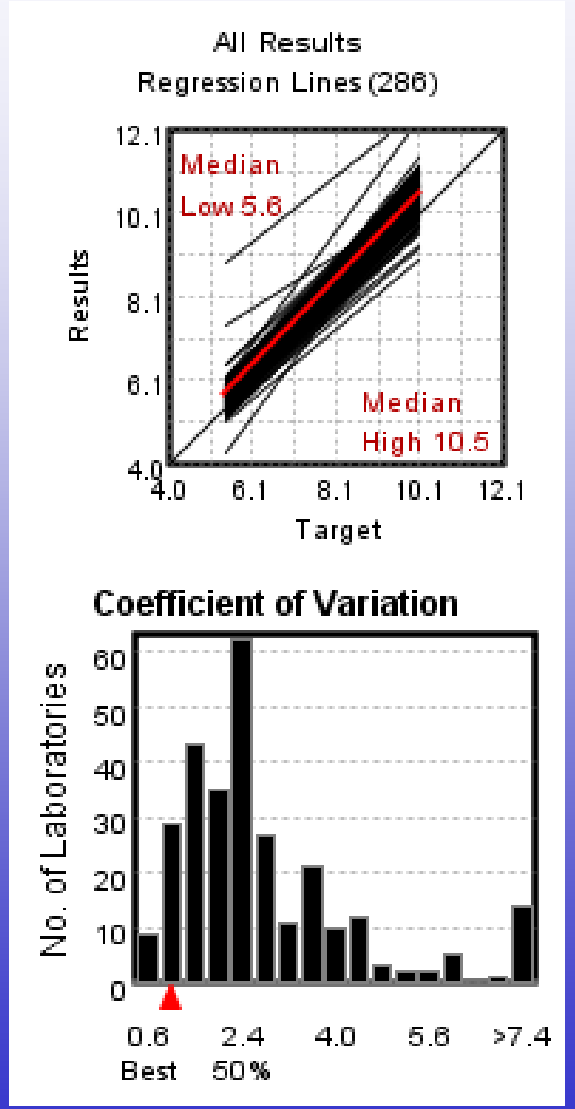


RCPA-AACB QAP 2012

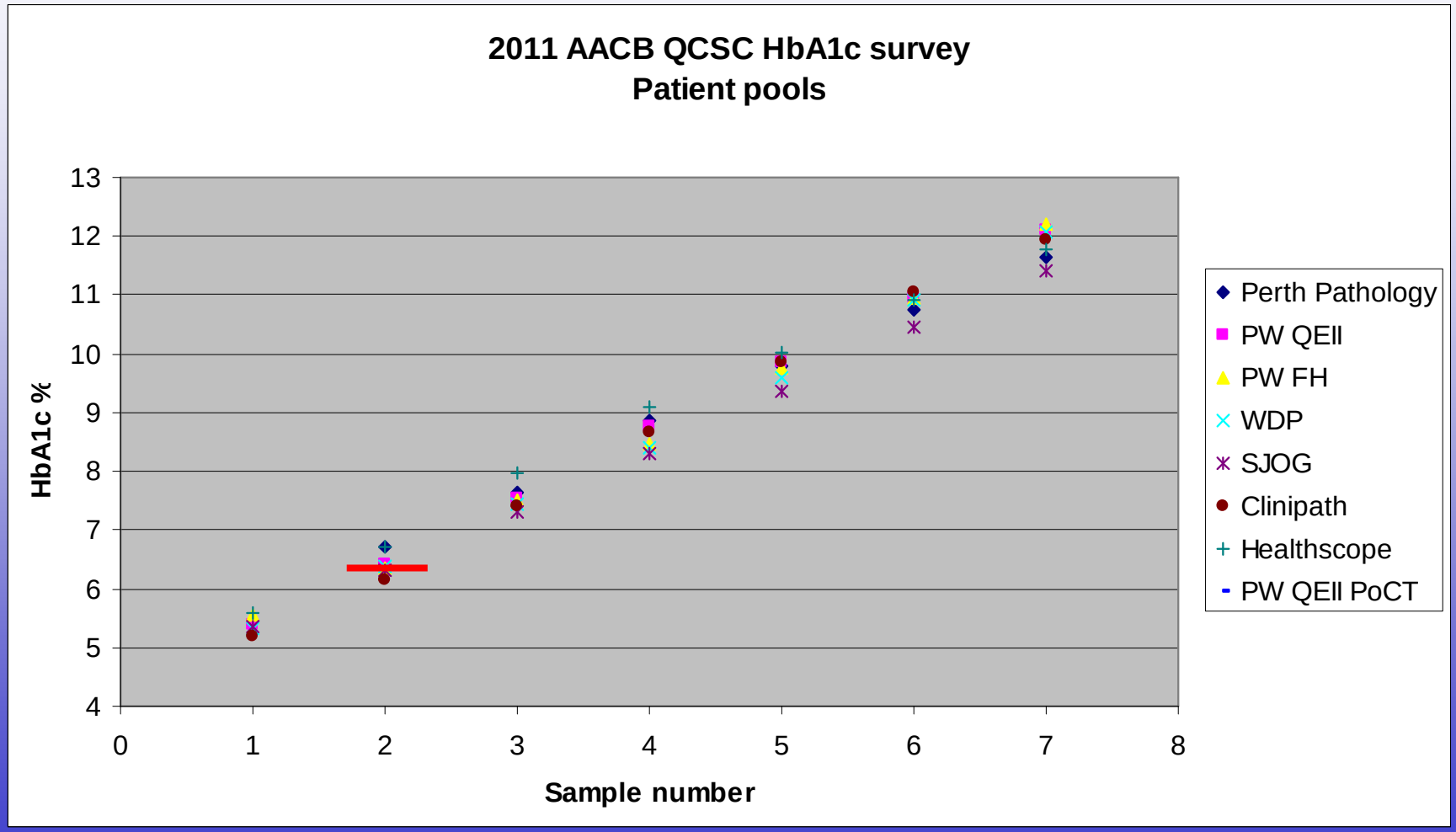
Glucose



HbA1c



How we currently perform in WA



* 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

Australian Government
Department of Human Services
medicare

Medicare Item 66551

– HbA1c in patients with established diabetes mellitus

NSW	367,602
VIC	285,681
QLD	195,422
WA	85,513
TAS	26,255
ACT	16,793
NT	9,599

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** μmol/L (50-95)
Plasma Glucose (F)	**11.3** mmol/L
HbA1c	6.0%
Fructosamine	**416** μmol/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

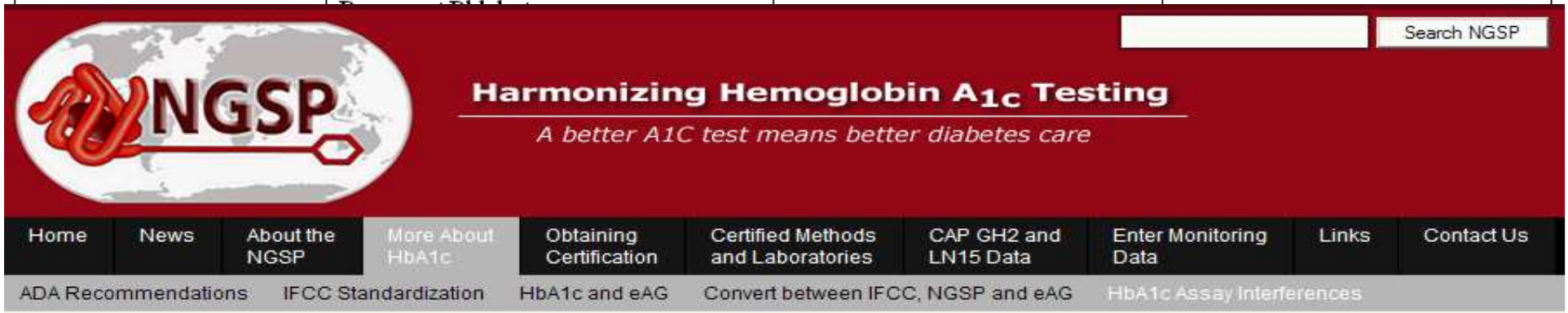
Assays

- method based on molecular charge or structure

WHO 2011

Factors that can affect HbA1c Measurement

Factors	*Decreased HbA1c*	*Increased HbA1c*	*Variable change in HbA1c*
Erythropoiesis	Treated Fe deficiency anaemia B12 therapy EPO therapy Reticulocytosis	Fe deficiency anaemia Vit B12 and folate deficiency Decreased erythropoiesis	



Altered Haemoglobin			Foetal Hb Haemoglobinopathies Methaemoglobin
Glycation	Aspirin Vit C and E Haemoglobinopathies	Alcoholism Chronic Renal Failure	
Assays	Hypertriglyceridaemia	Hyperbilirubinaemia Carbamylated Hb Large doses of aspirin Chronic opiate use	

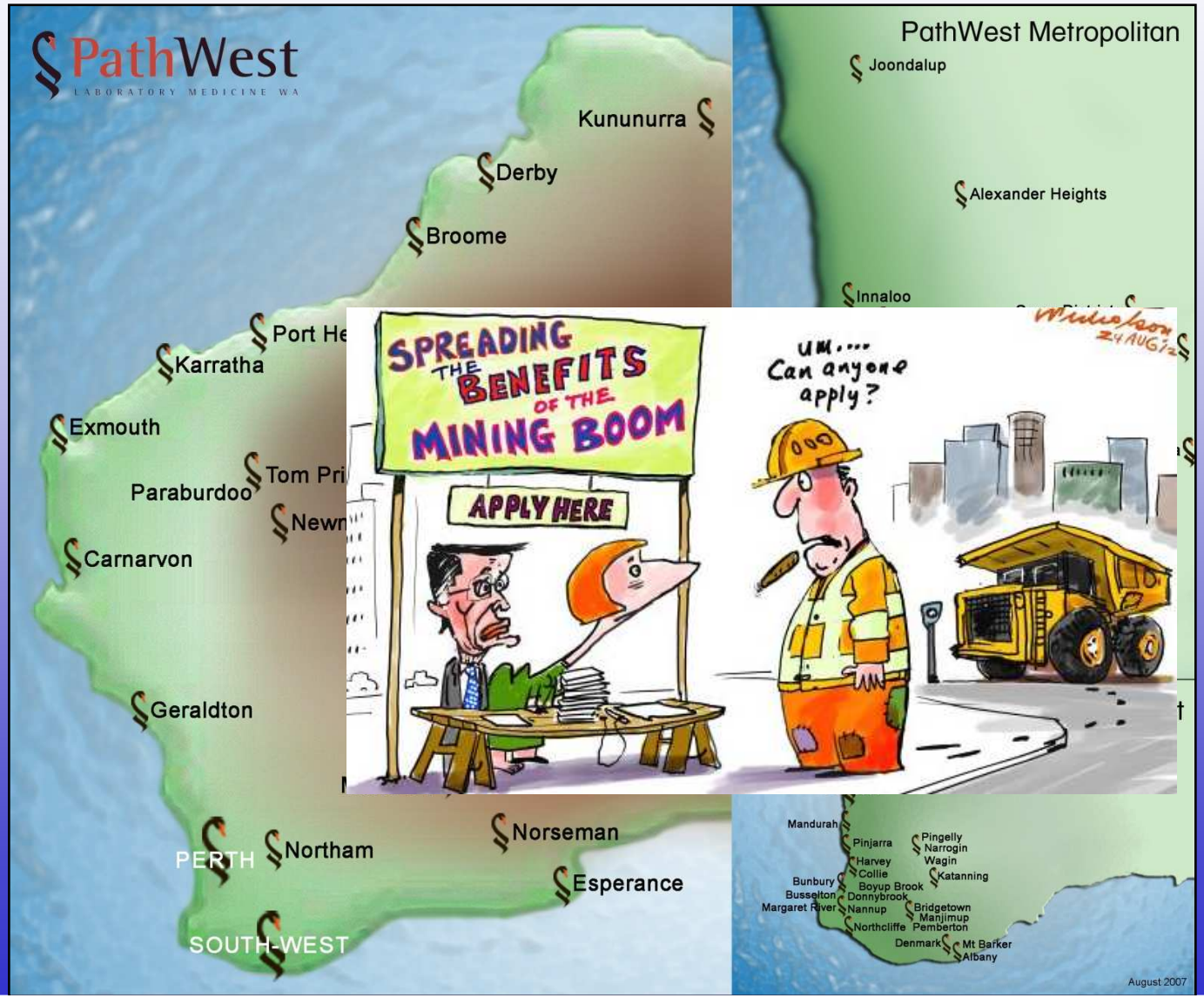
Artefactually Lower HbA1c

Factors	*Decreased Hb41c*
Erythropoiesis	Treated Fe deficiency anaemia B12 therapy EPO therapy Reticulocytosis Recurrent Phlebotomy Haemolytic anaemias eg: sickle cell, thalassaemia, G6PD deficiency RBC transfusion (haemodilution)
Erythrocyte Destruction	Reduced RBC lifespan Splenomegaly Drugs eg: dapsone, antiretrovirals ribavarin Rheumatoid arthritis Chronic malaria
Altered Haemoglobin	
Glycation	Aspirin Vit C and E Haemoglobinopathies
Assays	Hypertriglyceridaemia

Artefactually Higher HbA1c

Factors	*Increased HbA1c*
Erythropoiesis	Fe deficiency anaemia Vit B12 and folate deficiency Decreased erythropoiesis
Erythrocyte Destruction	Increased RBC lifespan Splenectomy
Altered Haemoglobin	
Glycation	Alcoholism Chronic Renal Failure
Assays	Hyperbilirubinaemia Carbamylated Hb Large doses of aspirin Chronic opiate use

WA Data


PathWest
LABORATORY MEDICINE WA
PathWest Metropolitan
Joondalup
Kununurra
Derby
Broome
Alexander Heights
Innaloo
Port He
Karratha
Exmouth
Tom Pri
Paraburdoo
Newn
Carnarvon
Geraldton
PERTH
Northam
Norseman
Esperance
SOUTH-WEST
SPREADING THE BENEFITS OF THE MINING BOOM
APPLY HERE
UM.... Can anyone apply?
Mandurah
Pinjarra
Pingelly
Narrogin
Wagin
Harvey
Collie
Katanning
Bunbury
Boyup Brook
Busselton
Donnybrook
Margaret River
Nannup
Bridgetown
Manjimup
Northcliffe
Pemberton
Denmark
Mt Barker
Albany
August 2007

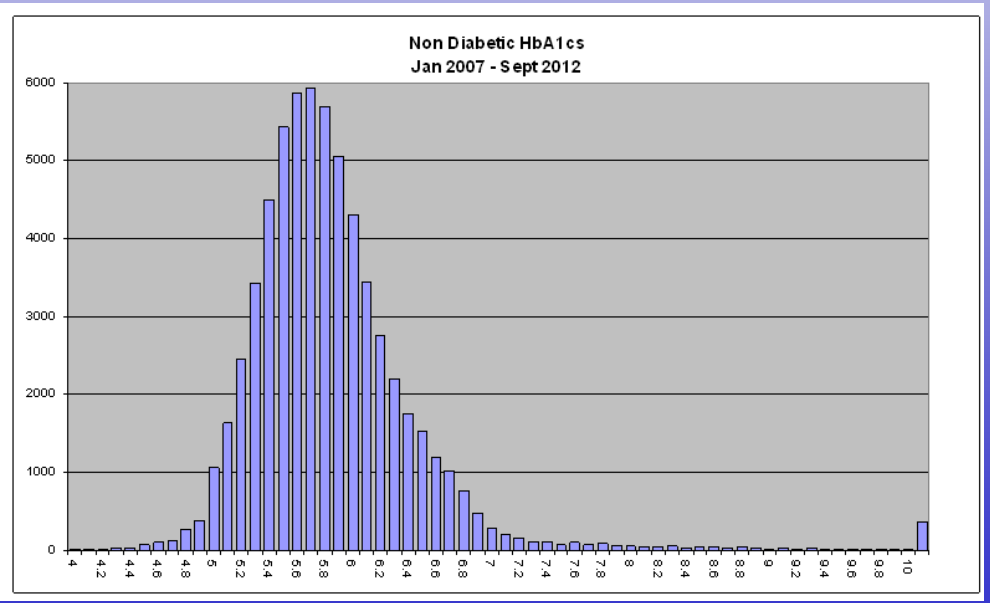
PathWest QEII Data - WA

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

- Non-diabetic patients 26%

Mean	5.9 %
Median	5.8 %
Mode	5.7 %
SD	0.71
2.5th centile	5.0 %
97.5th centile	7.2 %

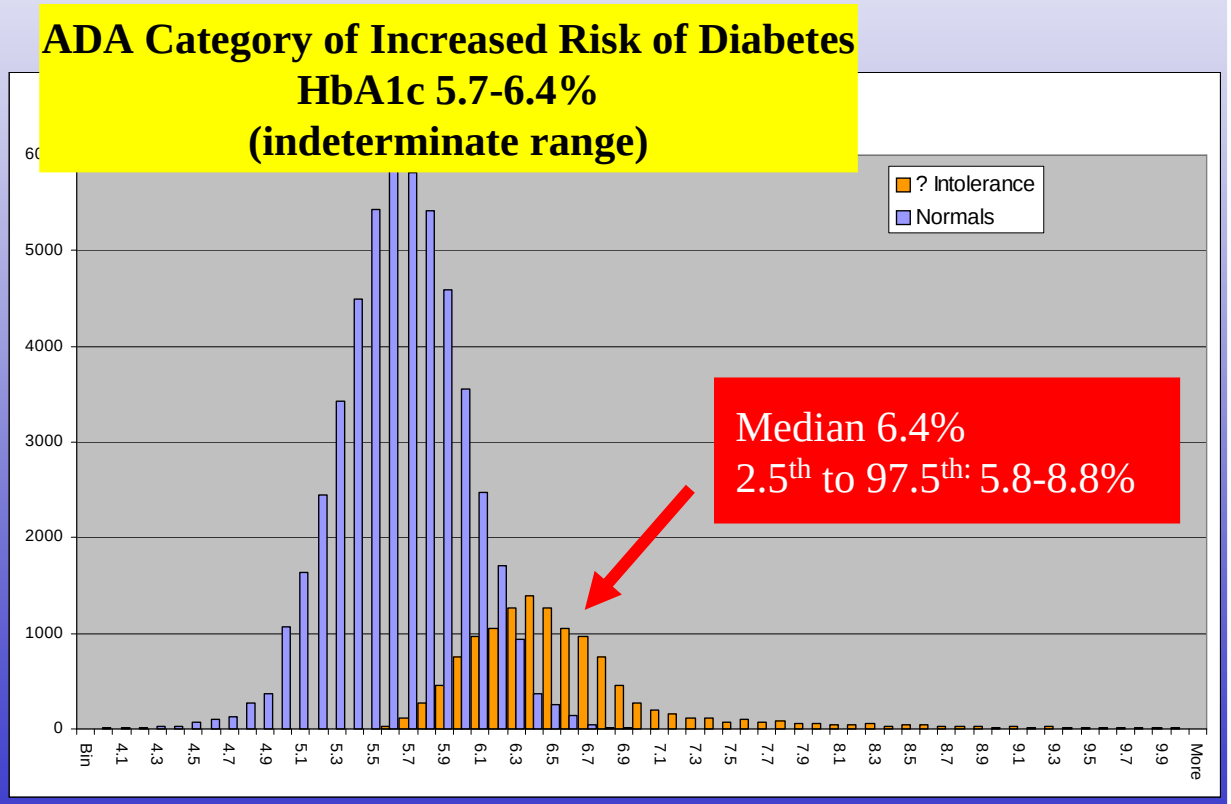
Courtesy of Rob Wardrop



PathWest QEII Data – WA

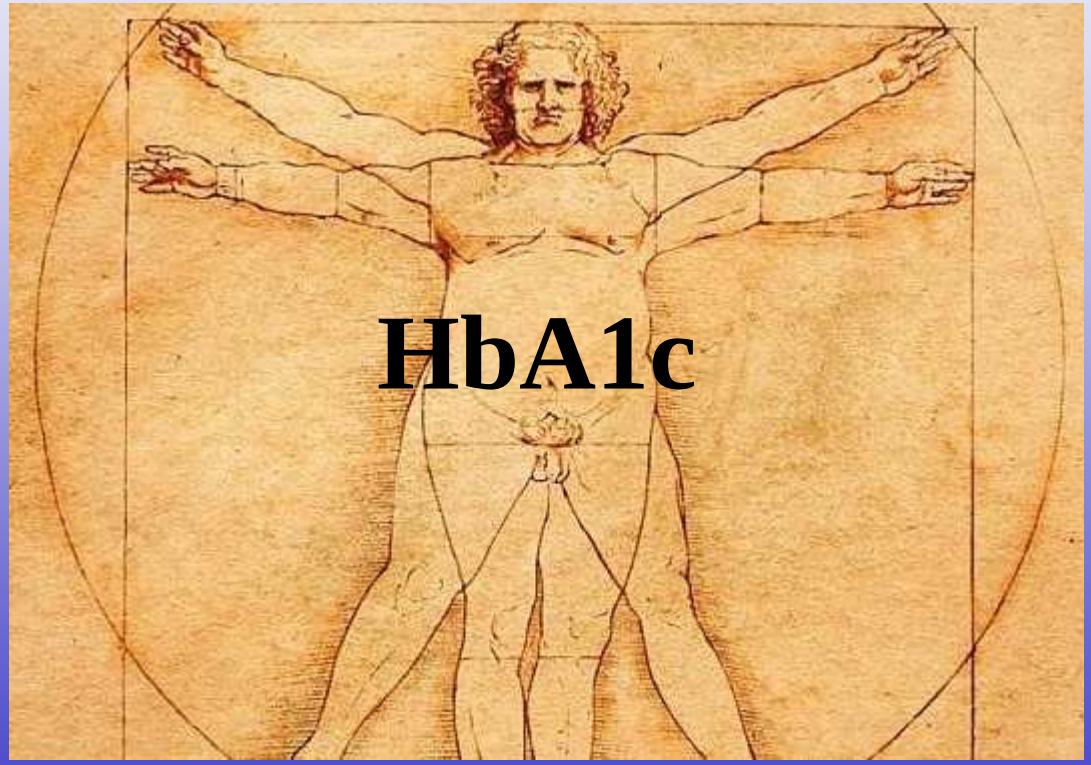
Making the distribution Guassian

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



Courtesy of Rob Wardrop

Evolving Recommendations:



Evolving Recommendations:

- **2008 Consensus Statement**

  - HbA1c ≥ 6.5% would be diagnostic if confirmed by another test (fasting, random, "OGTT")

  - HbA1c ≥ 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 HbA1c ≥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**

  - 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

WHO Executive Summary 2011

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

http://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf

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

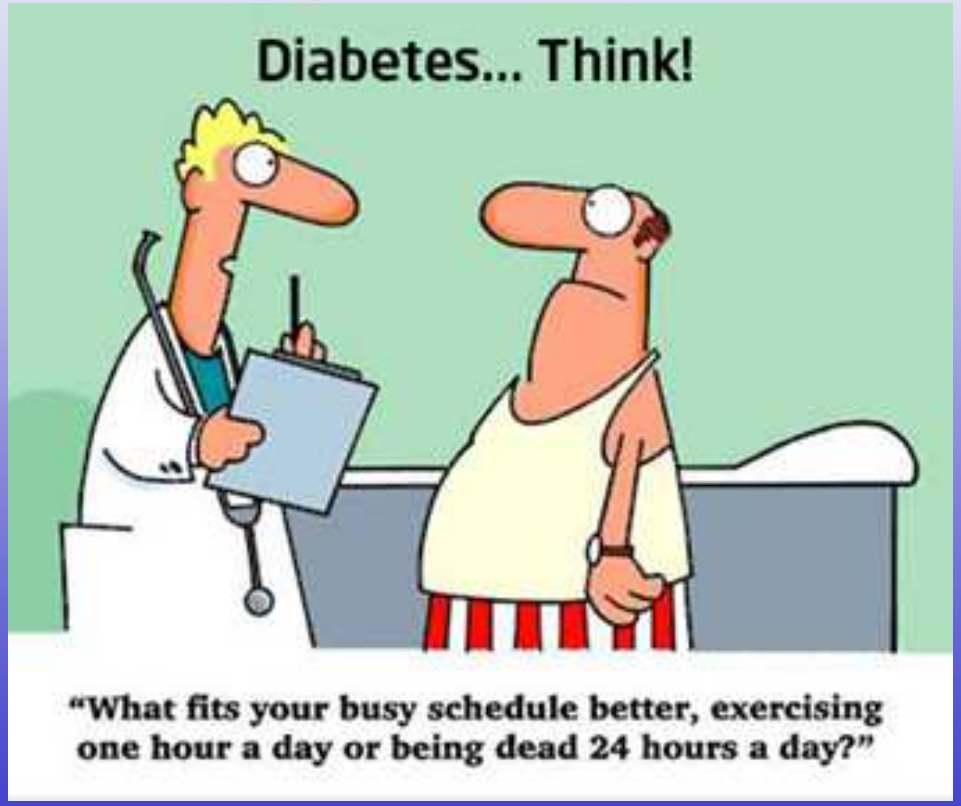
HbA1c – Is It Better?

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

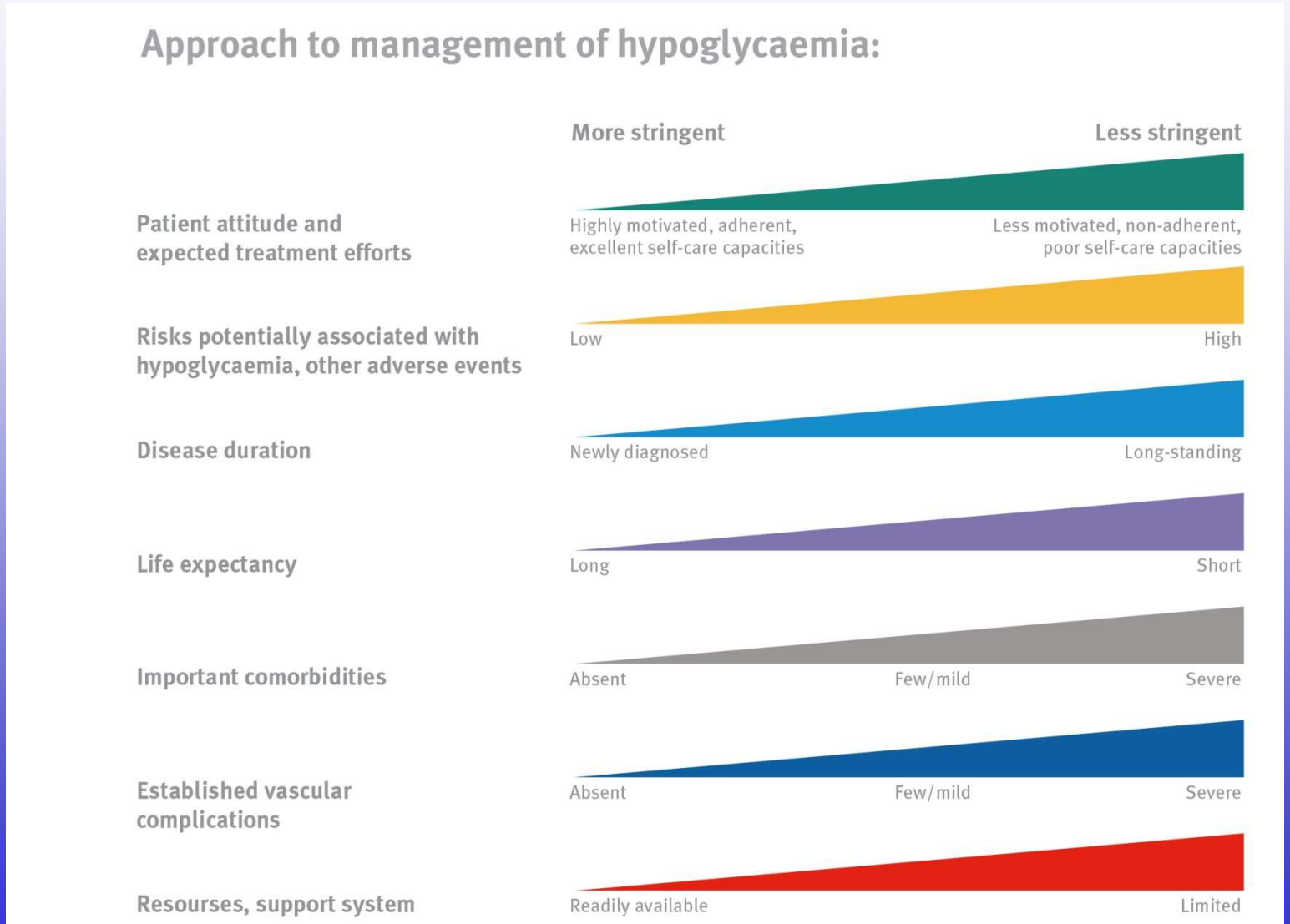
Personal views for Diagnosis with HbA1c :

- Fasting glucose 5.6-6.9 mmol/L
- OGTT can be avoided
- Cost-effectiveness

HbA1c Control and Beyond



HbA_{1c} goals: What needs to be considered



Inzucchi SE, et al. Diabetes Care 2012;35:1364–79.

Complexity of glycaemic goals recognised in ADS/EASD position

"It is important to individualise treatment targets"

Suggested clinical situations	Recommended HbA_{1c} goals
General	<7%*
Diabetes of short duration No clinically significant CVD Long life expectancy	6.0–6.5%*
History of severe hypoglycaemia Limited life expectancy Advanced complications Extensive comorbid conditions When glycaemic goals difficult to attain despite intensive self-management education, repeated counselling, and effective doses of multiple glucose-lowering agents, including insulin	7.5–8.0% (or slightly higher)

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

Inzucchi SE, et al. Diabetes Care 2012;35:1364–79.

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

	HbA1c
2006	6.4%
2007	8.3%
04/08	10.9%

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

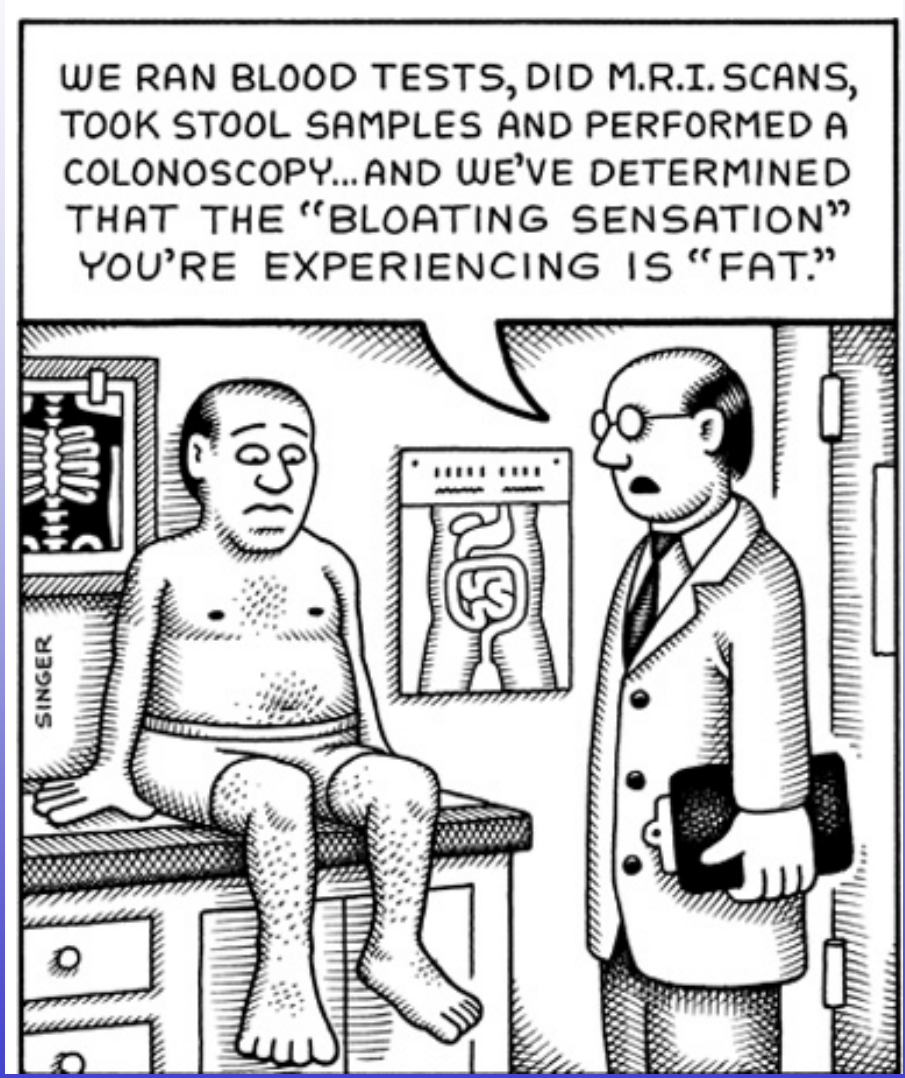
- Commenced Insulin therapy

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."
SINGER