Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

University of East Anglia

Assuming glycaemic control and the impact of anaemia in DM CKD

Garry John
Norfolk and Norwich University Hospital
& The Norwich Medical School, UEA
UK

Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

University of East Anglia

History of Diabetes Investigations

A DOCTOR OF MEDICINE
MAGDALEN COLLEGE, 1508

1679: Willis

Urine of diabetics had a sweet taste

Associated with:

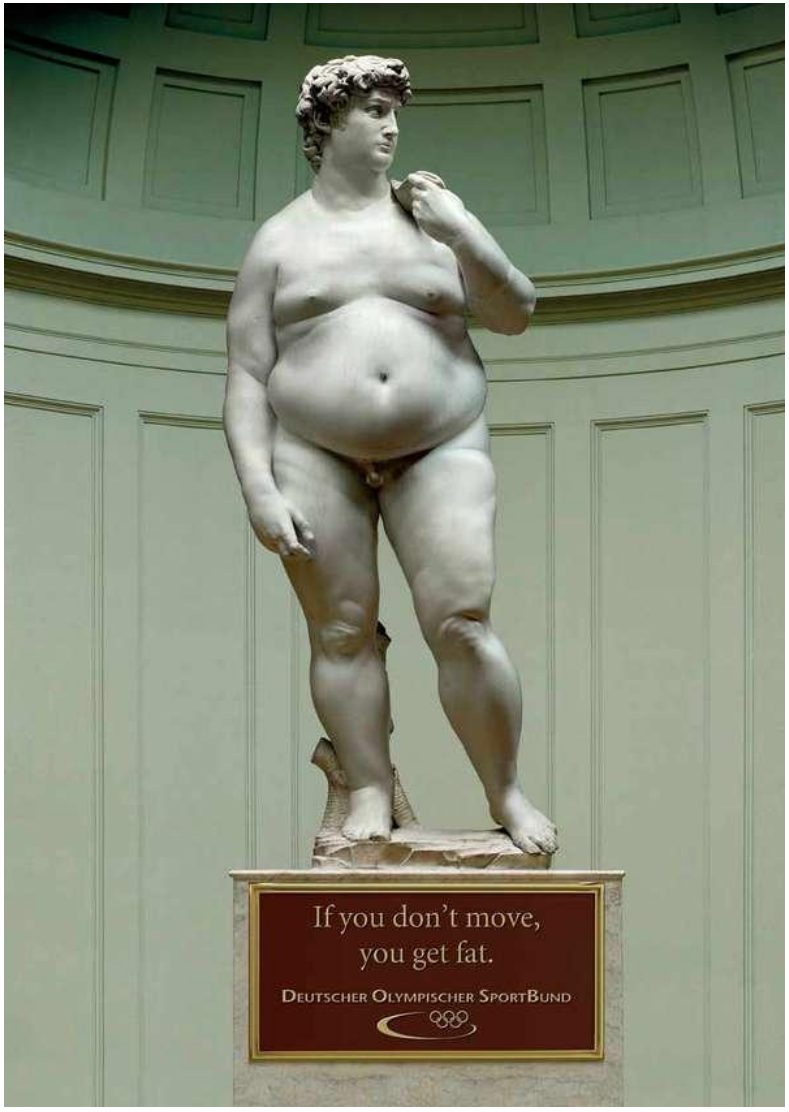
"good fellowship and guzzling down of ... wine."

1776: Dobson

Isolated a “dry residue” from urine of diabetics which looked and tasted like “brown sugar”

Michelangelo's ***David*** created between 1501 and 1504

Michelangelo's David 2014



Easily available, cheap, energy dense foods!

12 rashers of bacon
12 sausages
Six eggs
Four black pudding slices
Four slices of bread and butter
Four slices of toast
Four slices of fried bread
Two hash browns
Eight-egg cheese and potato omelette
Saute potatoes
Mushrooms
Beans
Tomatoes

Cost: £15 ($19)
Free if eaten within an hour!

An abnormal haemoglobin in red cells of diabetics

Rahbar S

Clin Chim Acta 1968; 22: 296-298

International Federation of Clinical Chemistry and Laboratory Medicine

An abnormal hemoglobin in red cells of diabetics

In a survey carried out on 1200 patients from Tehran University Hospitals, in addition to three rare hemoglobins which are under investigation both in our department here and at the University of Cambridge, two patients also showed an abnormal fast moving hemoglobin fraction : both were suffering from diabetes mellitus.

Studies were started to investigate the occurrence of this abnormal fraction in other diabetics, and in 47 cases examined in the last three months, including 11 children with severe diabetes mellitus, the additional fraction was detected. Routine hematological examination according to standard methods[2] gave normal results in the majority of cases.

Electrophoresis of hemoglobin was carried out on cellulose acetate according to Graham and Gruenbaum[3]; the abnormal fraction does not separate well by this method, but there is a broadening of the Hb A band. In starch gel electrophoresis with tris–EDTA–borate buffer pH 8.1 (ref. 1) the additional fraction moves a little faster than Hb A and slower than Hb J (Iran)[8] (Fig. 1).

Agar gel electrophoresis in citrate buffer pH 6.2 by the method of Robinson *et al.*[4] is the method of choice for the separation and demonstration of this fraction which moves in front of Hb A to the cathode in the same position as Hb F (Fig. 2).

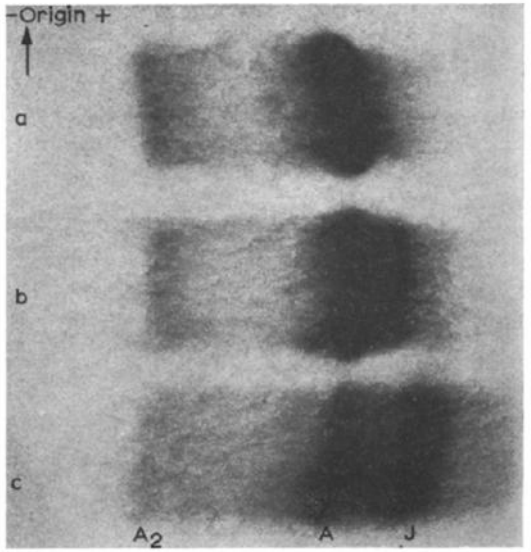
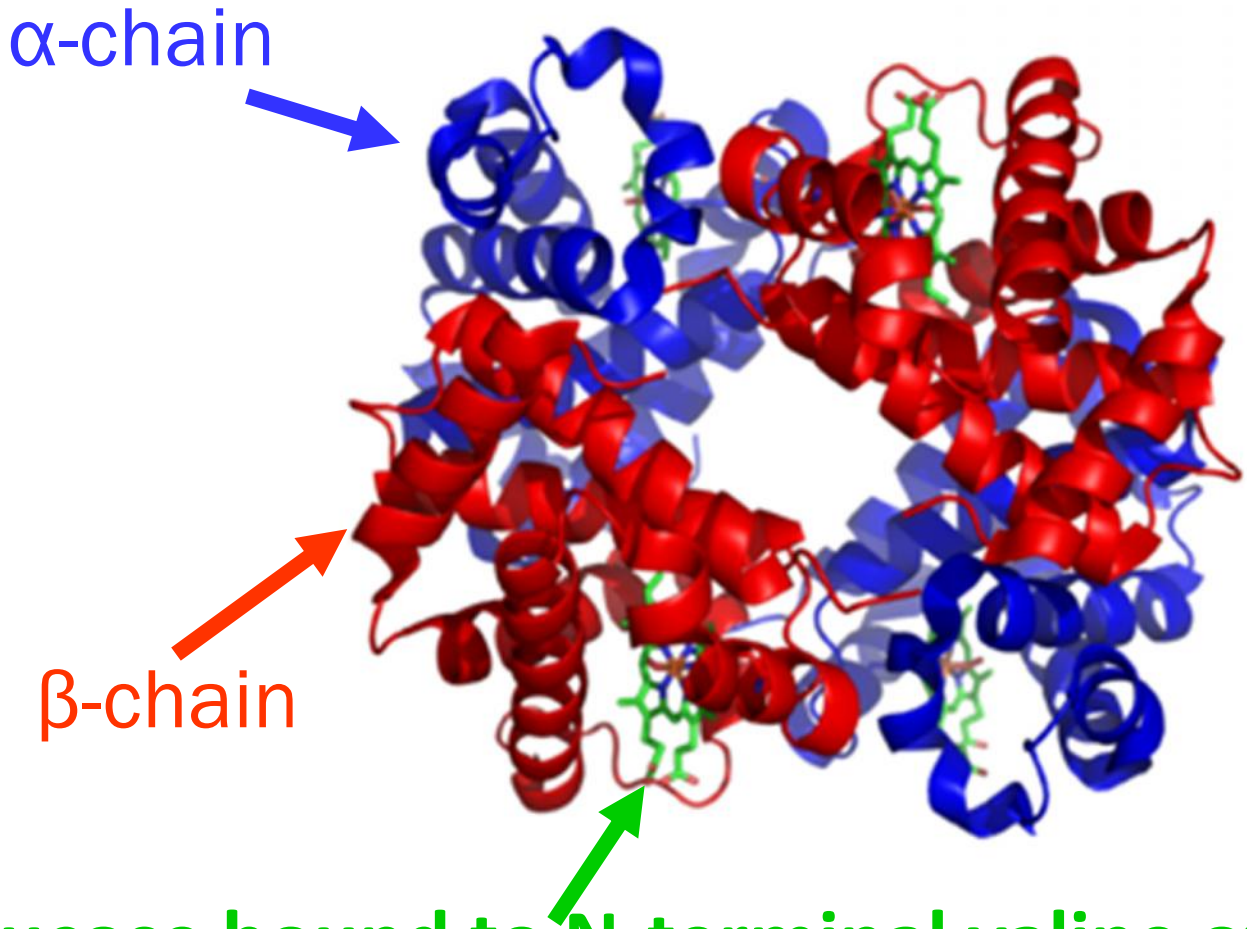


Fig. 1. Starch gel electrophoresis in tris–EDTA–borate buffer, pH 8.1. *o*-Dianizidine stain, ref. 7. a: normal; b: Hb A + Hb x; c: Hb A + Hb J (Iran).

Clin. Chim. Acta, 22 (1968) 296–298

HbA_{1c} – N terminal glycation

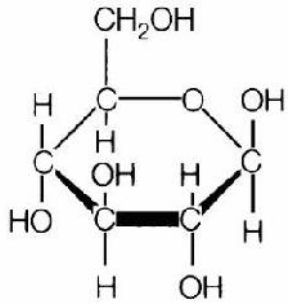


Glucose bound to N-terminal valine of β-chain

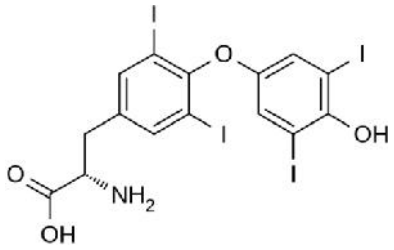
The use of Haemoglobin A_{1c} in Clinical Practice

HbA_{1c} is not like:

Glucose



Thyroxine



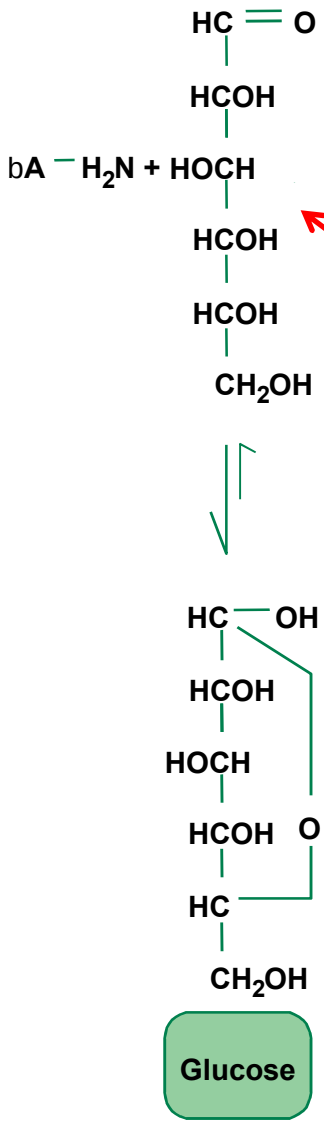
These are <u>single</u> molecular structures of known composition; their concentration in the body is tightly regulated and <u>concentrations controlled</u>.

The use of Haemoglobin A_{1c} in Clinical Practice

HbA_{1c} is:

A product of a non-enzymatic Glycation reaction; the reaction follows the Law of Mass action. The reaction is not controlled and the Glycated Haemoglobin formed is not a single molecular structure.

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

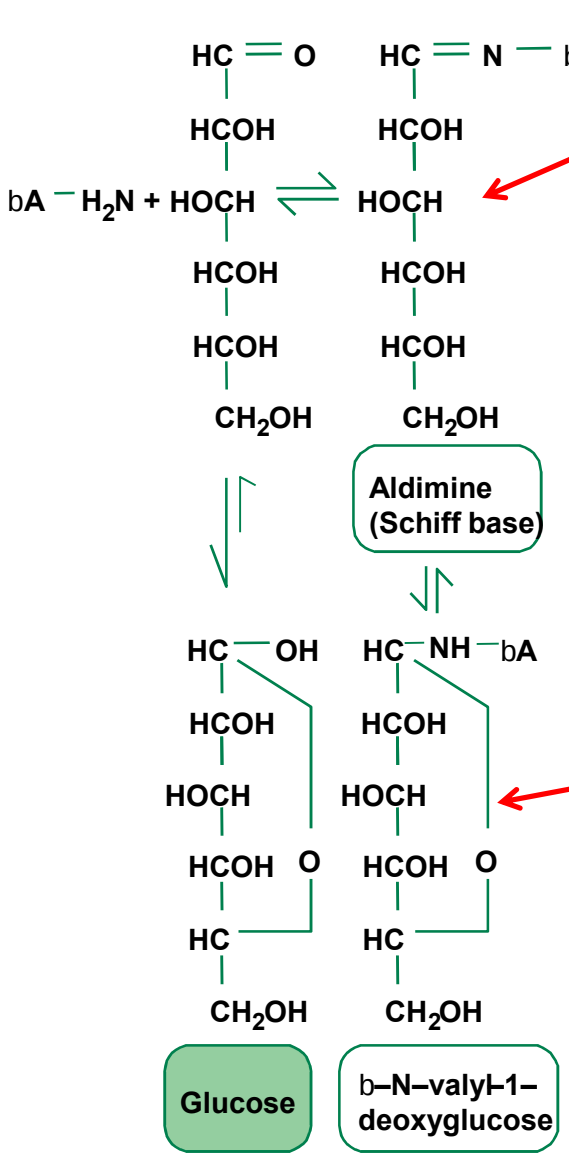


All proteins will react with glucose in the open chain format.

The amino acid Lys is the usual site of reaction: in Hb the N-terminal Val is favoured

Glucose is present in the body in two forms:
open chain format
a ring structure (majority of glucose)

Only glucose in the open chain format will react with protein.

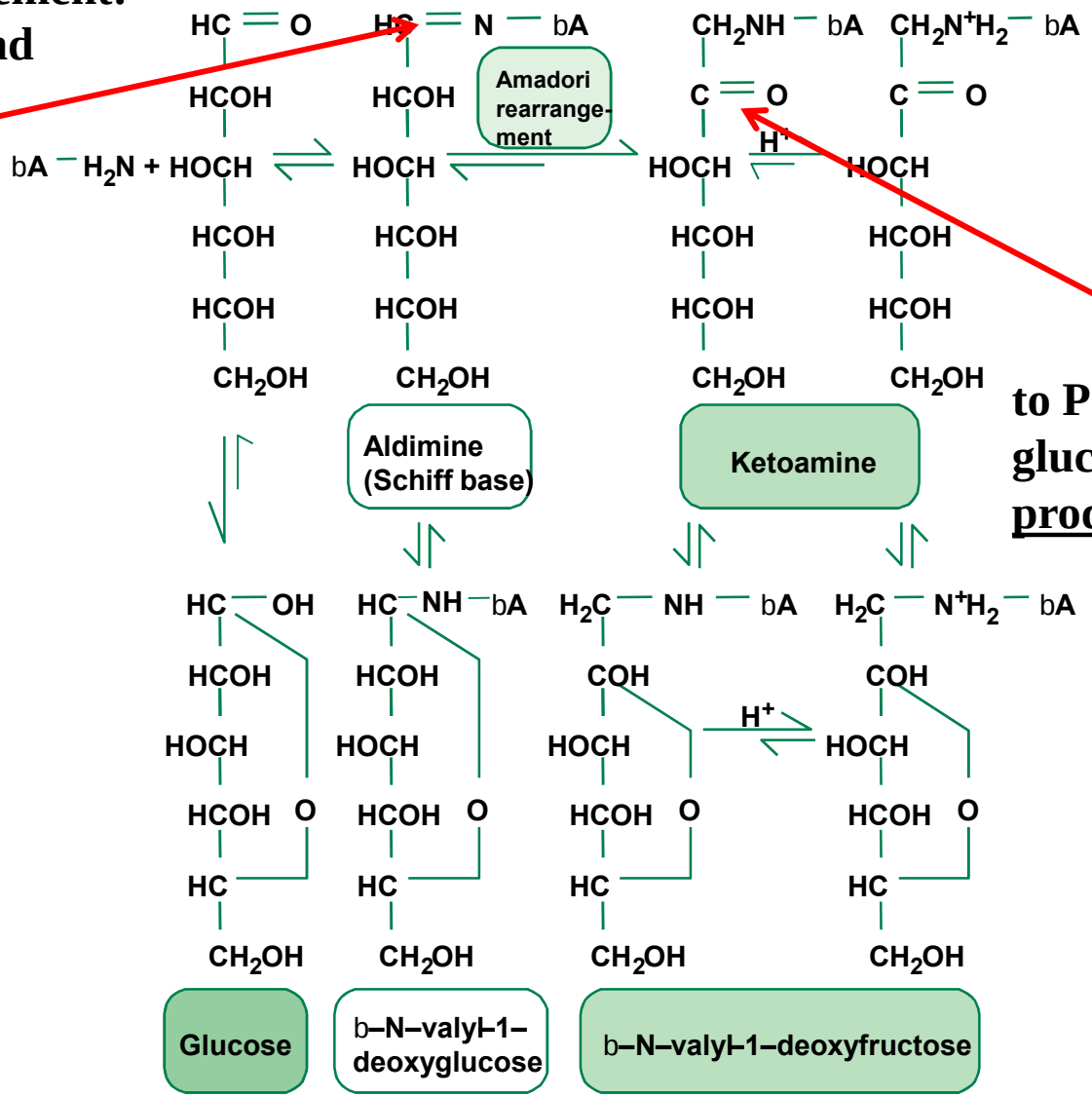


Protein reversibly reacts with the glucose forming a double bond at position 1 on the open glucose form.

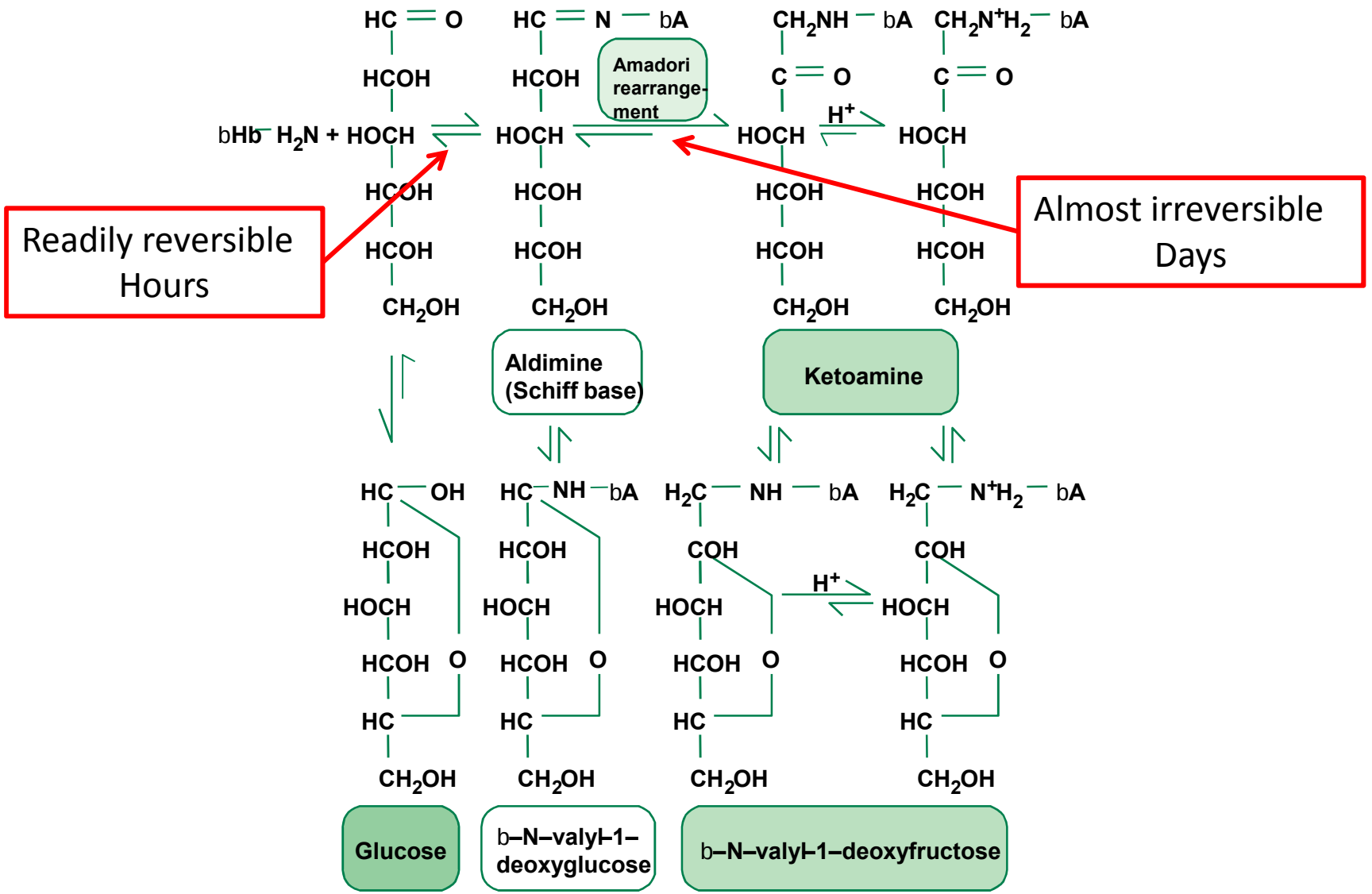
Termed a Shiff Base

The glucose may form a ring structure at this point. No further reaction will take place.

The almost irreversible Amadori rearrangement: Moving double bond from Position 1



to Position 2 on the glucose <u>stabilises the product.</u>

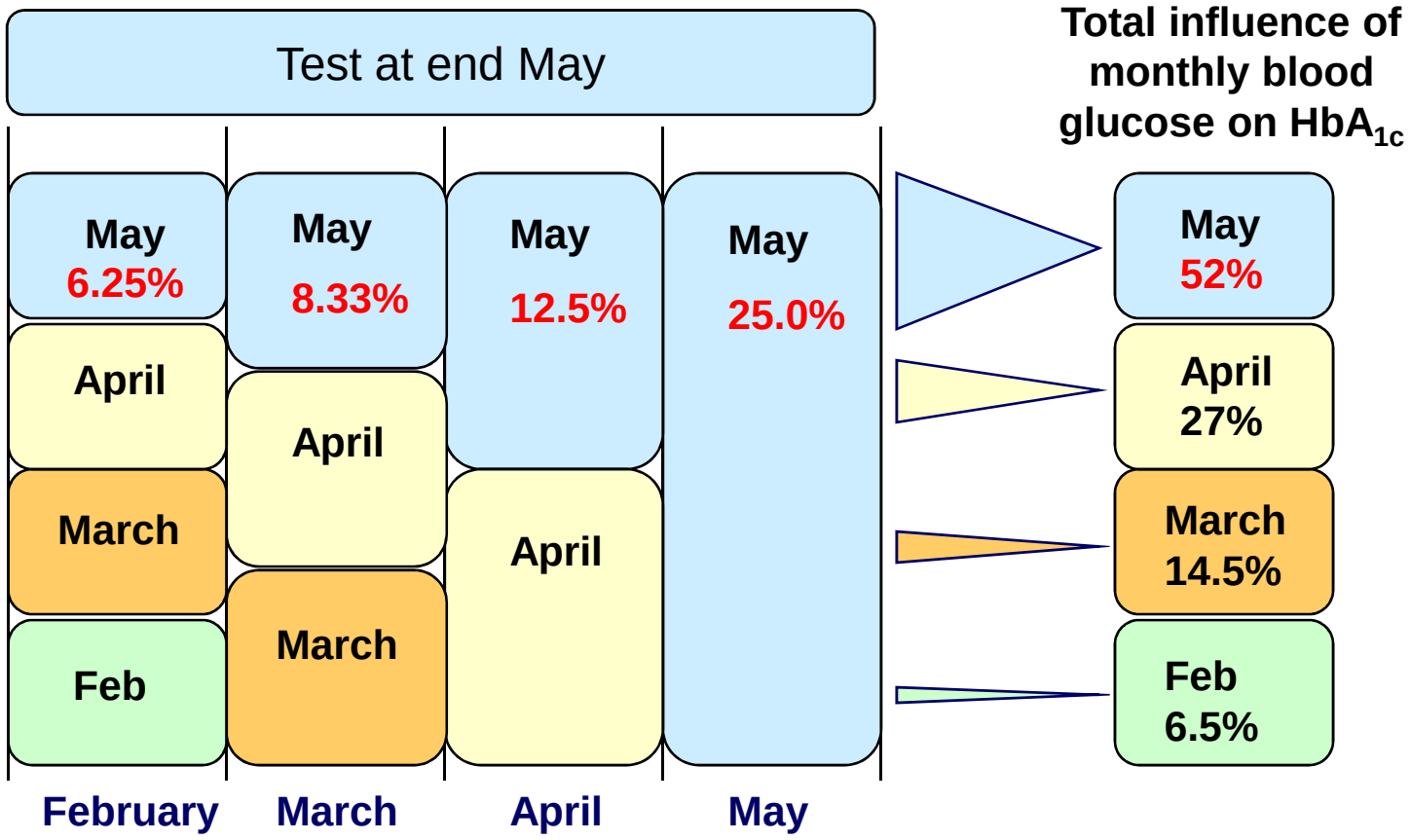


A Non-enymatic reaction following the Law of Mass Action.

GLUCOSE $\neq$ HbA_{1c}

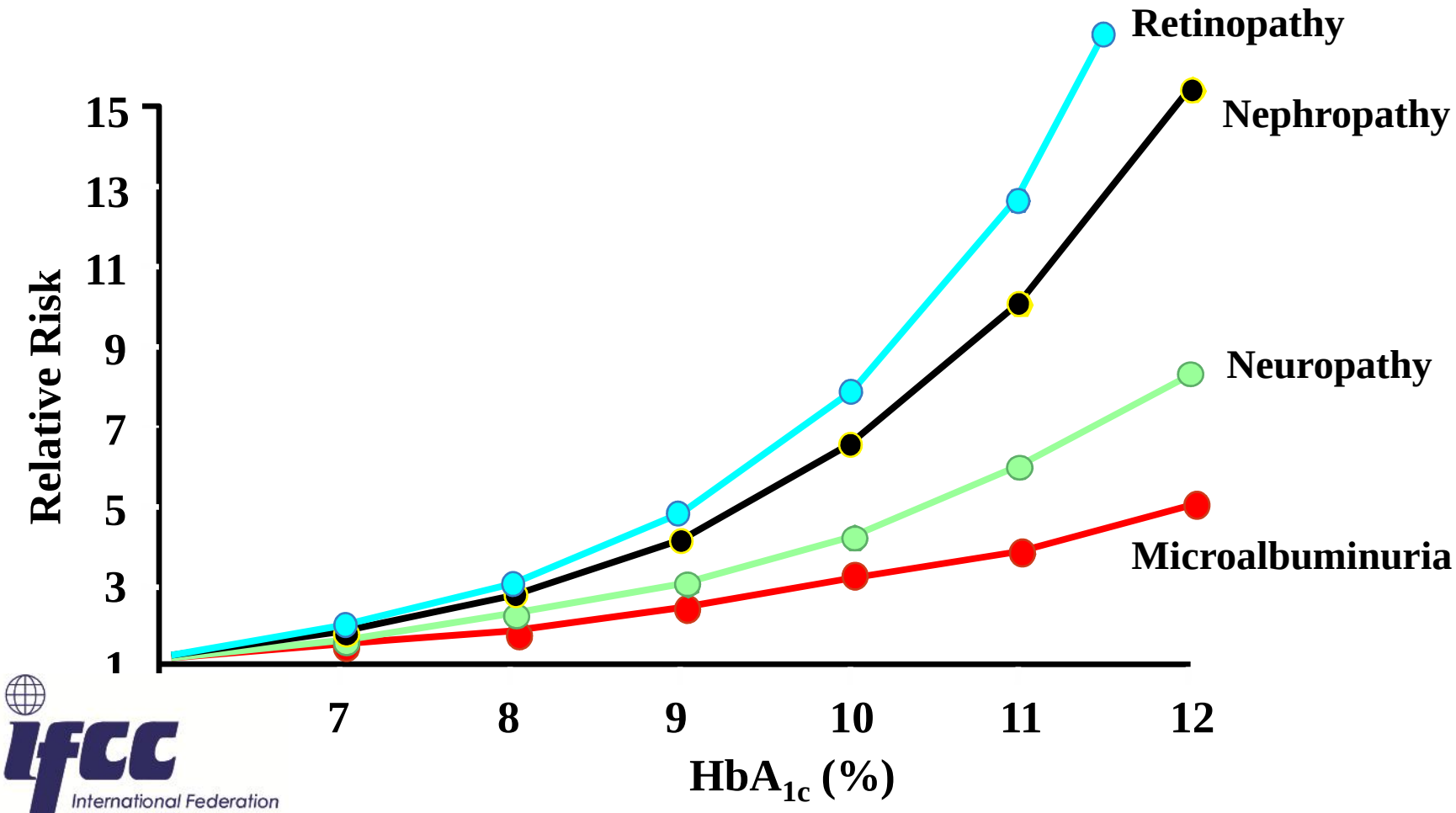
Diagnosis with GLUCOSE $\neq$
Diagnosis with HbA_{1c}

What Period is Measured?



IFCC International Federation of Clinical Chemistry and Laboratory Medicine

DCCT: HbA_{1c} and risk of microvascular complications

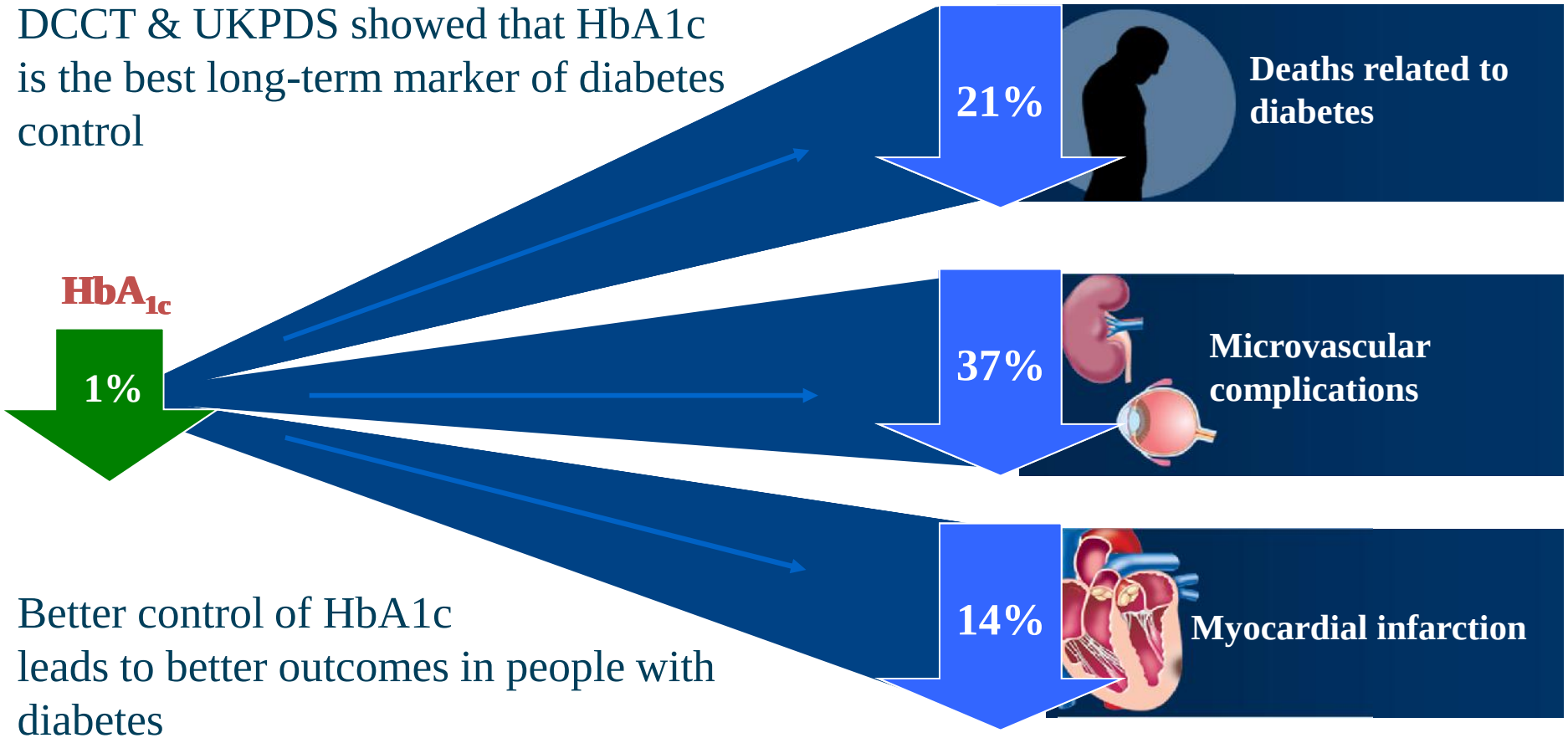


IFCC International Federation of Clinical Chemistry and Laboratory Medicine

Skyler. Endocrinol Metab Clin 1996;25:243–254

Why is HbA_{1c} so Important?

DCCT & UKPDS showed that HbA1c is the best long-term marker of diabetes control

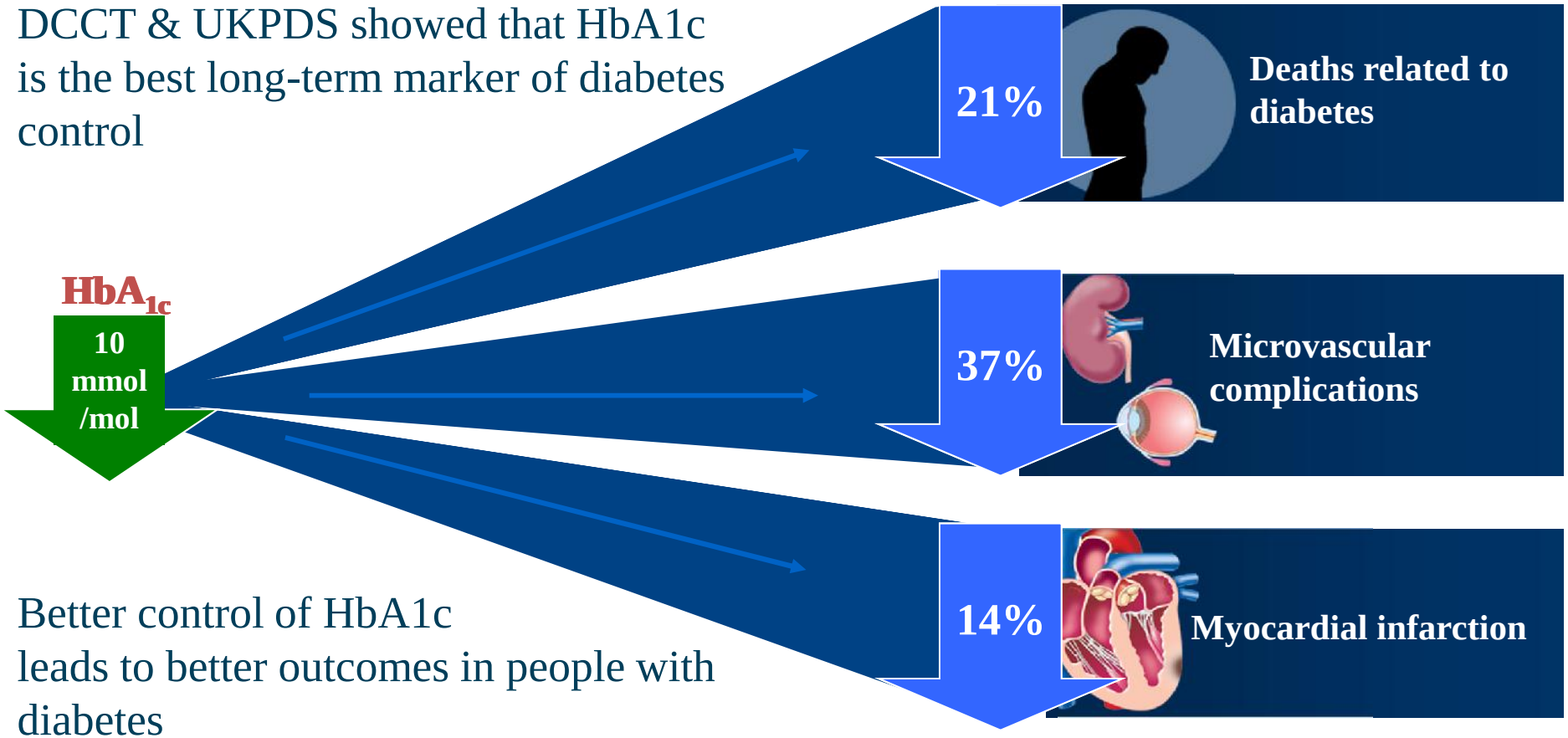


Better control of HbA1c leads to better outcomes in people with diabetes

Stratton IM, *et al*. *BMJ* 2000; 321:405–412.

Why is HbA_{1c} so Important?

DCCT & UKPDS showed that HbA1c is the best long-term marker of diabetes control



Better control of HbA1c leads to better outcomes in people with diabetes

Stratton IM, *et al*. *BMJ* 2000; 321:405–412.

Assumptions for use of HbA_{1c} as a monitor of glycaemia:

Haemoglobin is present at a cons… … "normal" concentration

- Within an individual thi… …y true (? Anaemia)

Life span of Red Bloo… …onstant and "normal"

- Within an i… …is is probably true; This may not be true betw… …viduals

ANAEMIA

Micro-environment is constant

- Within an individual this is probably true.

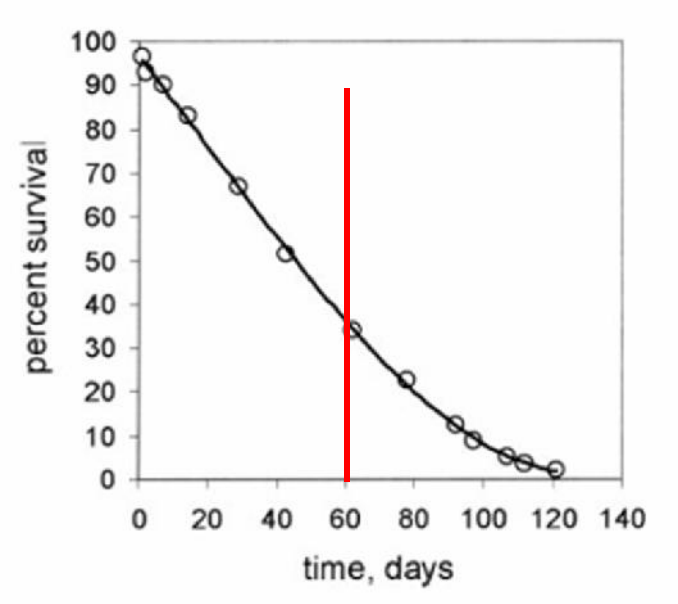
Hence: Glucose is the ONLY variable.

Consider: The HbA_{1c} result is for the **INDIVIDUAL patient**

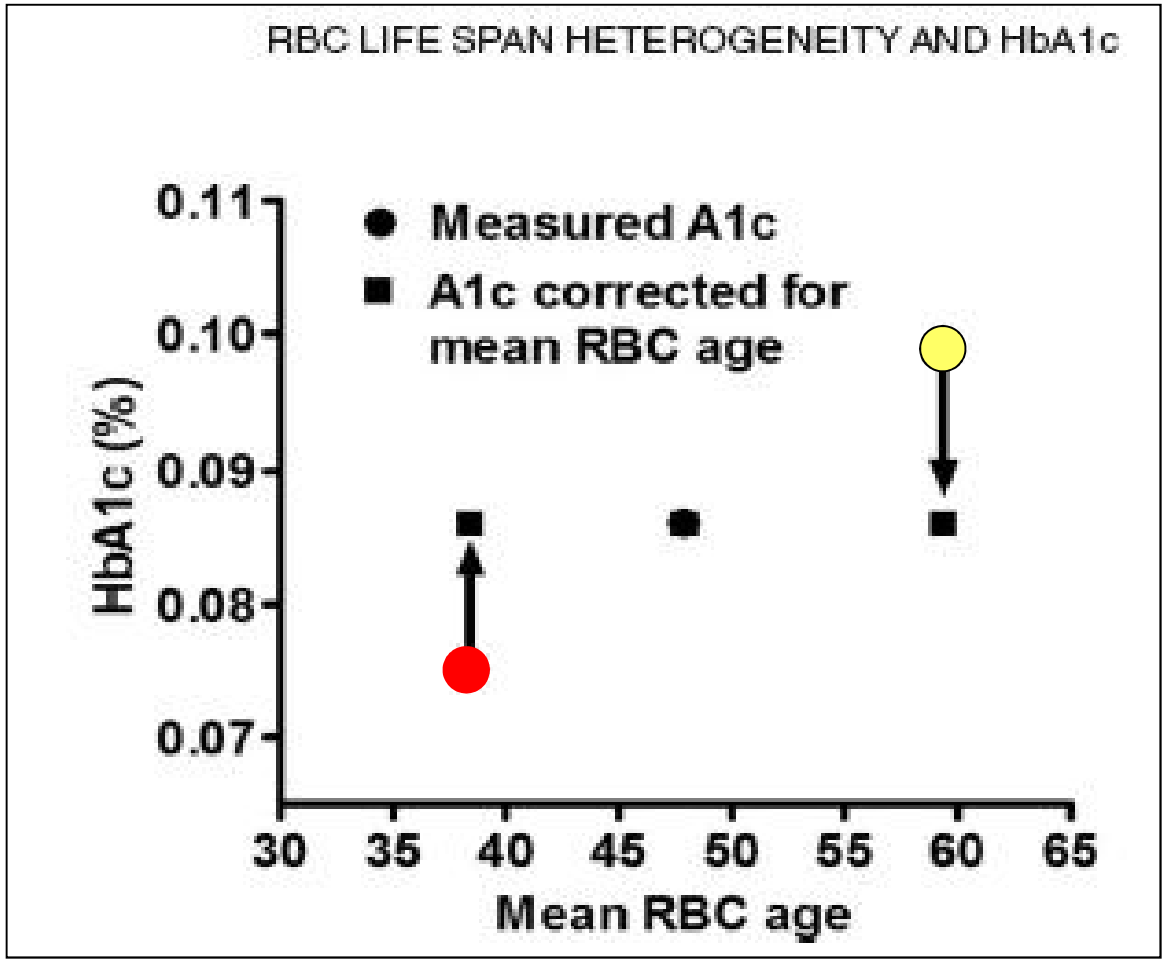

LEECHES
QUAIFE

Red cell life span heterogeneity in haematologically normal people is sufficient to alter HbA_{1c}

Red cell survival

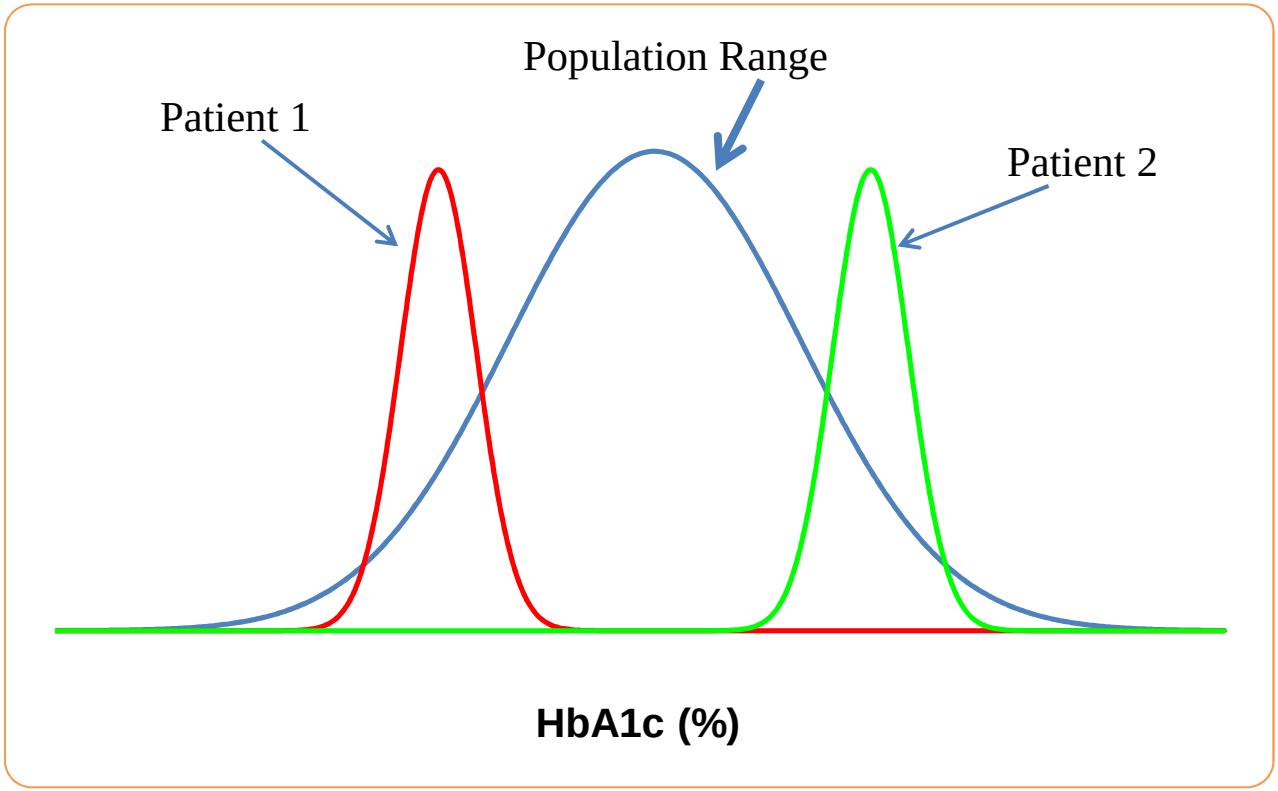


Franco RS. Am J Hematol 2009;84:109-114



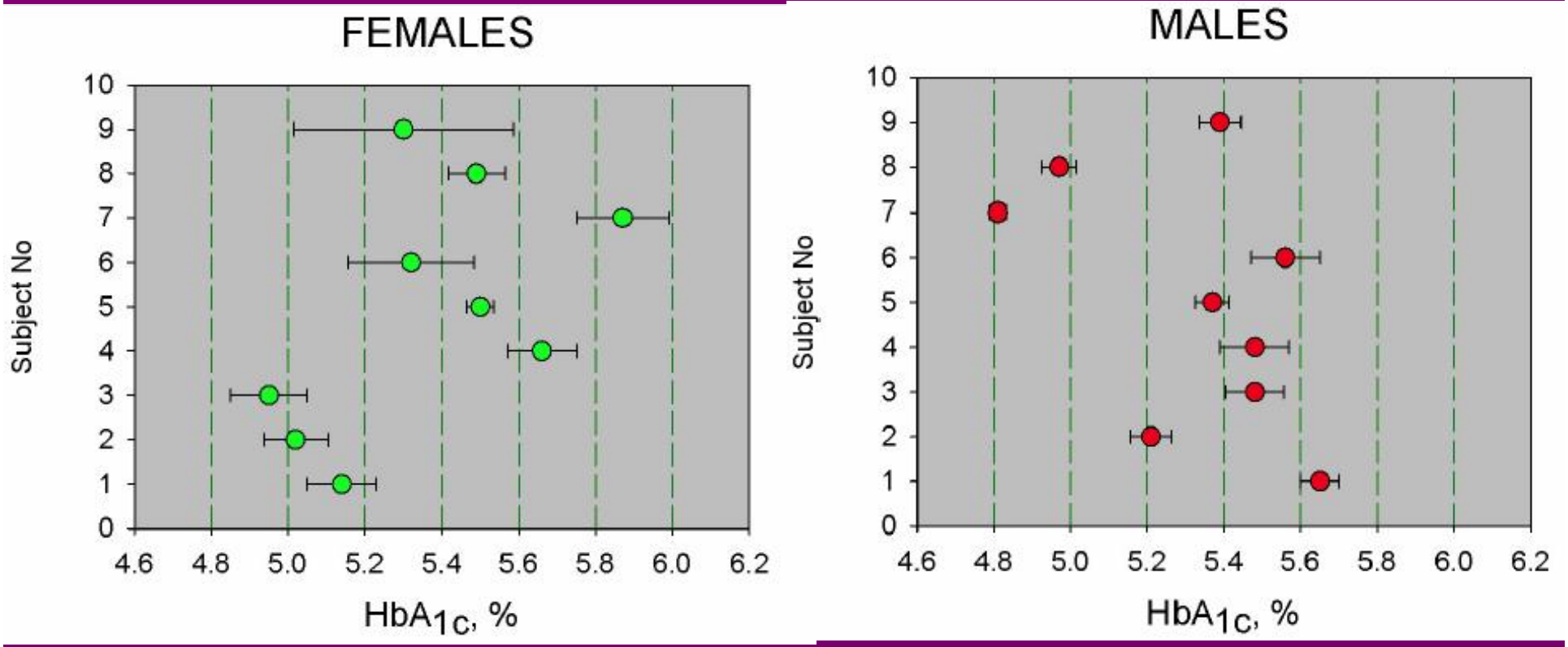
Cohen et al. Blood 2008;112:4284-4291

Biological Variation of HbA_{1c}



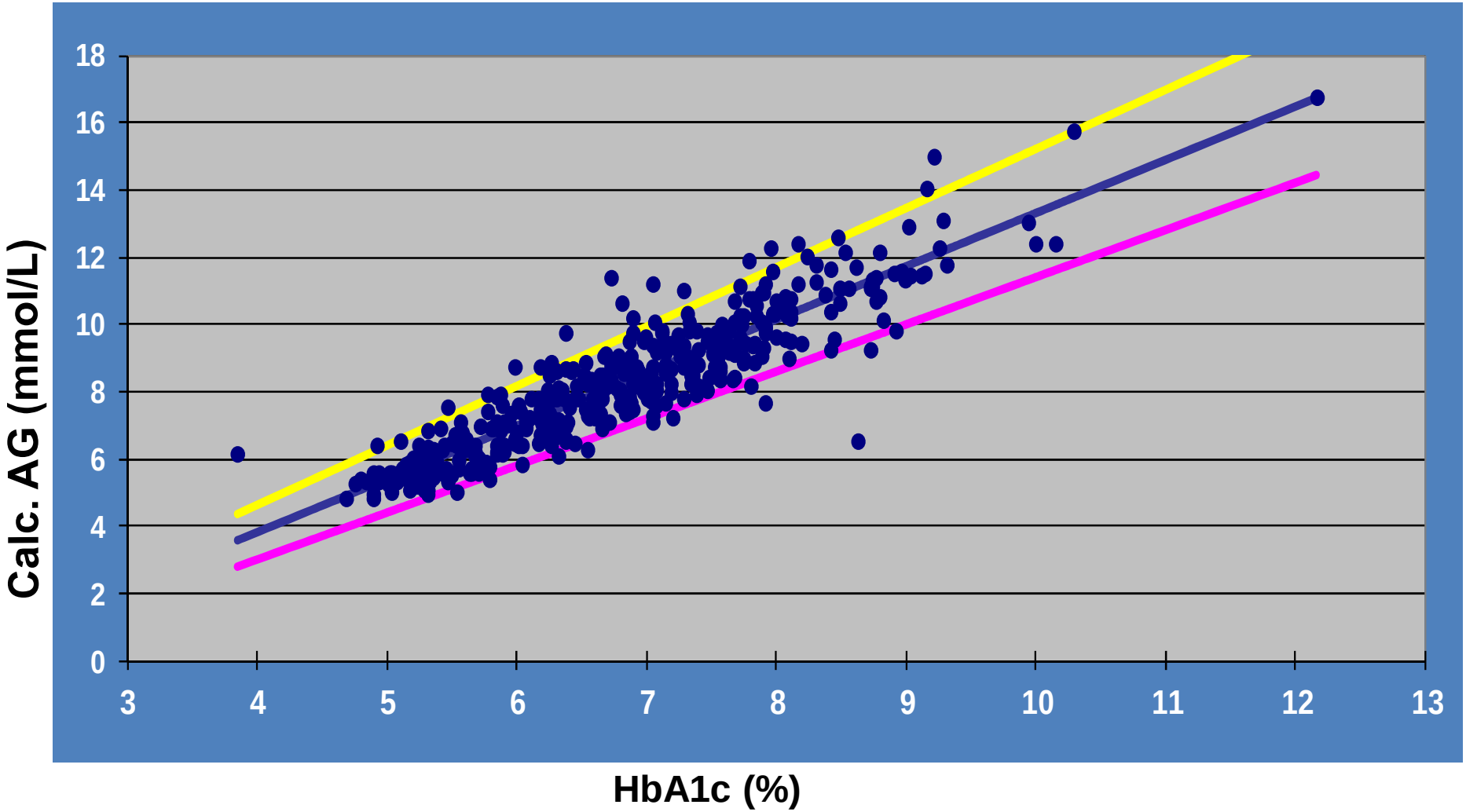
International Federation of Clinical Chemistry and Laboratory Medicine

Biological Variability HbA_{1c}



(Mosca et al.)

Acceptability of Data (ADAG)



Diabetes Care 2008;31:1–6

Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

Predicting mean glucose using CGMS

University of East Anglia

HbA_{1c} (%)	eAG (mmol/L)	95% Predictive Limits (mmol/L)
5	5.4	4.2 to 6.7
6	7.0	5.5 to 8.5
7	8.6	6.8 to 10.3
8	10.1	8.1 to 12.1
9	11.7	9.4 to 13.9
10	13.3	10.7 to 15.7
11	14.9	12.0 to 17.5
12	16.5	13.3 to 19.3

Diabetes Care 2008;31:1–6

HbA_{1c} and anaemia

Why?

We know that anaemia can give rise to an elevated HbA_{1c}; but is this true in all cases?

Norfolk and Norwich University Hospitals NHS
NHS Foundation Trust

Effect of Fe deficiency anaemia

University of East Anglia

50 patients (30 women, 20 men, mean age 35.7 ± 11.9 years) with IDA and 50 controls

HbA_{1c} in healthy group 5.9% ± 0.5%

HbA_{1c} in IDA 7.4% ± 0.8% ($p<0.001$)

Following 3 months iron HbA_{1c} 6.2% ± 0.6%

3.3million US females estimated to have IDA

Acta Haematol 2004;112:126-128

JAMA 1997;277:973-6

The Effect of Iron and Erythropoietin Treatment on the A1C of Patients With Diabetes and Chronic Kidney Disease

JEN M. NG, MRCP[1]
MICHELLE COOKE, BSC[2]
SUNIL BHANDARI, PHD, FRCP[2,3]
STEPHEN L. ATKIN, PHD, FRCP[1]
ERIC S. KILPATRICK, MD, FRCPATH[4]

Diabetes Care 2010; 33: 11

Effect of Intravenous Iron Therapy

Table 1—*Patients on iron therapy*

	Before iron mean (95% CI)	After iron mean (95% CI)	P*
A1C (%)	7.40 (6.60–8.19)	6.96 (6.27–7.25)	<0.001
Hb (g/dl)	9.71 (9.32–10.05)	10.46 (9.97–10.75)	0.001
Hct	0.302 (0.285–0.316)	0.334 (0.314–0.354)	0.007
Ferritin (μg/l)	122 (67–176)	307 (211–403)	<0.001
MBG (mmol/l)	9.55 (8.20–10.90)	9.71 (8.29–11.13)	0.071
Estimated glomerular filtration rate	34.0 (31.9–36.2)	32.8 (30.4–35.2)	0.137

*Paired *t* test.

Conclusion:

Despite a lack of change of glycaemic control HbA_{1c} concentrations fell significantly ($P < 0.001$). There was no linear relationship between the change in HbA_{1c} and haemoglobin concentration values.

Mean Blood Glucose did not change

Diabetes Care 2010; 33: 11

Effect of Erythropoietin-stimulating Agents

Table 2—*Patients on ESA*

	Before ESA mean (95% CI)	After ESA mean (95% CI)	P*
A1C (%)	7.31 (6.42–8.54)	6.63 (6.03–7.36)	0.013
Hb (g/dl)	9.52 (9.18–9.86)	11.51 (11.15–11.85)	<0.001
Hct	0.324 (0.296–0.350)	0.378 (0.341–0.398)	<0.001
Ferritin (μg/l)	344 (241–447)	332 (211–354)	0.37
MBG (mmol/l)	8.72 (7.31–10.12)	8.78 (7.47–9.99)	0.893
Estimated glomerular filtration rate	30.5 (28.6–33.4)	31.0 (27.3–33.8)	0.613

*Paired *t* test.

Conclusion:

Despite a lack of change of glycaemic control HbA_{1c} concentrations fell significantly (P 0.013), respectively, for groups A and B).

There appeared to be a nonsignificant trend toward ESA leading to a further decrease in HbA_{1c} following the initial fall due to iron.

In contrast, the group of patients receiving ESA therapy without iron had a significant fall in HbA1c from 7.3 to 6.5% (P = 0.02).

Mean Blood Glucose did not change

Diabetes Care 2010; 33: 11

The Effect of Iron and Erythropoietin Treatment on the A1C of Patients With Diabetes and Chronic Kidney Disease

JEN M. NG, MRCP[1]
MICHELLE COOKE, BSC[2]
SUNIL BHANDARI, PHD, FRCP[2,3]
STEPHEN L. ATKIN, PHD, FRCP[1]
ERIC S. KILPATRICK, MD, FRCPATH[4]

Diabetes Care 2010; 33: 11

Conclusions:
HbA_{1c} can be unreliable and can fall following treatment with both iron and ESA therapy.

Alternative methods for measuring glycaemic control such as capillary glucose testing and CGM should be used, and therapy should not be based on the HbA_{1c} value alone.

Glycated albumin has been suggested as an alternative marker to represent glycaemic control in patients with iron deficiency but, before this measurement is widely used further studies are required to better understand the correlation between glycated albumin and glycaemic control.

What affect does anaemia have on HbA_{1c}?

Diabetologia (2015) 58:1409–1421
DOI 10.1007/s00125-015-3599-3

SYSTEMATIC REVIEW

The effect of anaemia and abnormalities of erythrocyte indices on HbA_{1c} analysis: a systematic review

Emma English[1] · Iskandar Idris[1] · Georgina Smith[1] · Ketan Dhatariya[2] · Eric S. Kilpatrick[3] · W. Garry John[4]

DOI: 10.1007/s00125-015-3599-3
Pubmed ID: 25994072

HbA_{1c} and anaemia

IDA and ID clearly have an effect but studies are small and in limited ethnic groups commonly resulting in increased HbA_{1c} values

No clear cut point at which ‘anaemia’ becomes a problem.

Non-IDA show either no change in HbA_{1c} or decreased values in HbA_{1c} so knowing Hb levels alone will be insufficient to judge whether HbA_{1c} likely to be affected.

How to apply this information to everyday clinical practice

1. During monitoring of people with diabetes, when glucose and HbA_{1c} are discordant, consider abnormalities of erythrocyte indices.

2. When HbA_{1c} is normal/elevated but Hb is low, do not assume that HbA_{1c} is falsely elevated

- check erythrocyte indices, in particular MCV and MCH; if low, consider iron deficiency by TSAT or ferritin. If MCV and MCH are not low then consider other forms of anaemia—HbA_{1c} may be falsely decreased in these cases.

How to apply this information to everyday clinical practice

3. Iron deficiency, as well as IDA, may be sufficient to cause a change in HbA_{1c} values; this is highly relevant in women of childbearing age.

4. If abnormalities are identified, consider correction of the abnormality before using HbA_{1c} for diagnosis or monitoring.

5. It may take up to 6 months after treatment is initiated to normalise erythrocyte indices.

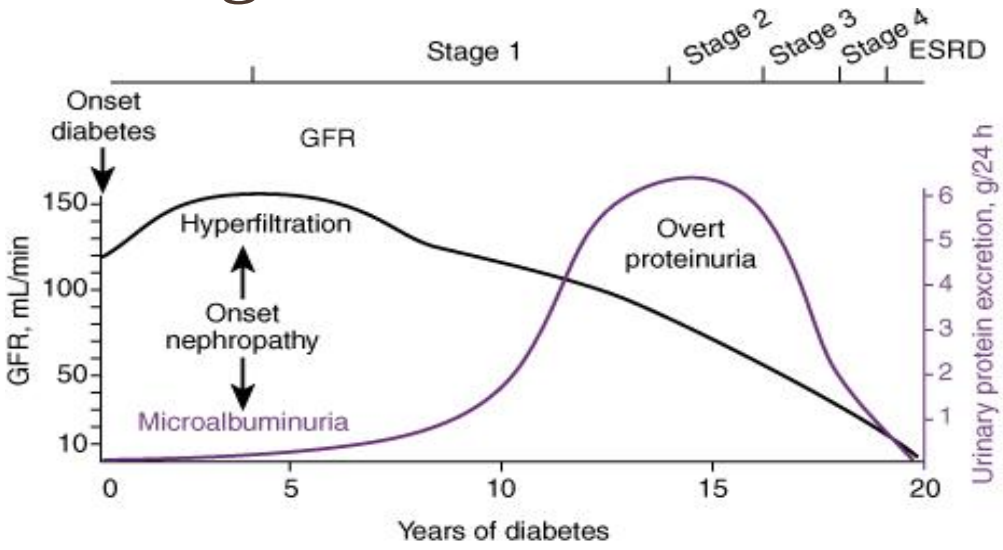
6. RDW may provide an additional indicator of normalisation of the erythrocyte population and erythrocyte lifespan.

HbA_{1c} in renal disease (systematic review)

- Does CKD affect HbA_{1c} values when compared to glucose markers?
  - No direct studies addressing whether or not CKD has an impact on HbA_{1c} analysis
  - Carbamlyated Hb no longer a major issue
  - Can HbA_{1c} be used in renal disease?

So what do we know?

- HbA_{1c} was reported to be positively associated with eGFR in both diabetics and non-diabetics at various stages of CKD, but particularly in early CKD
- May be attributable to glomerular hyperfiltration

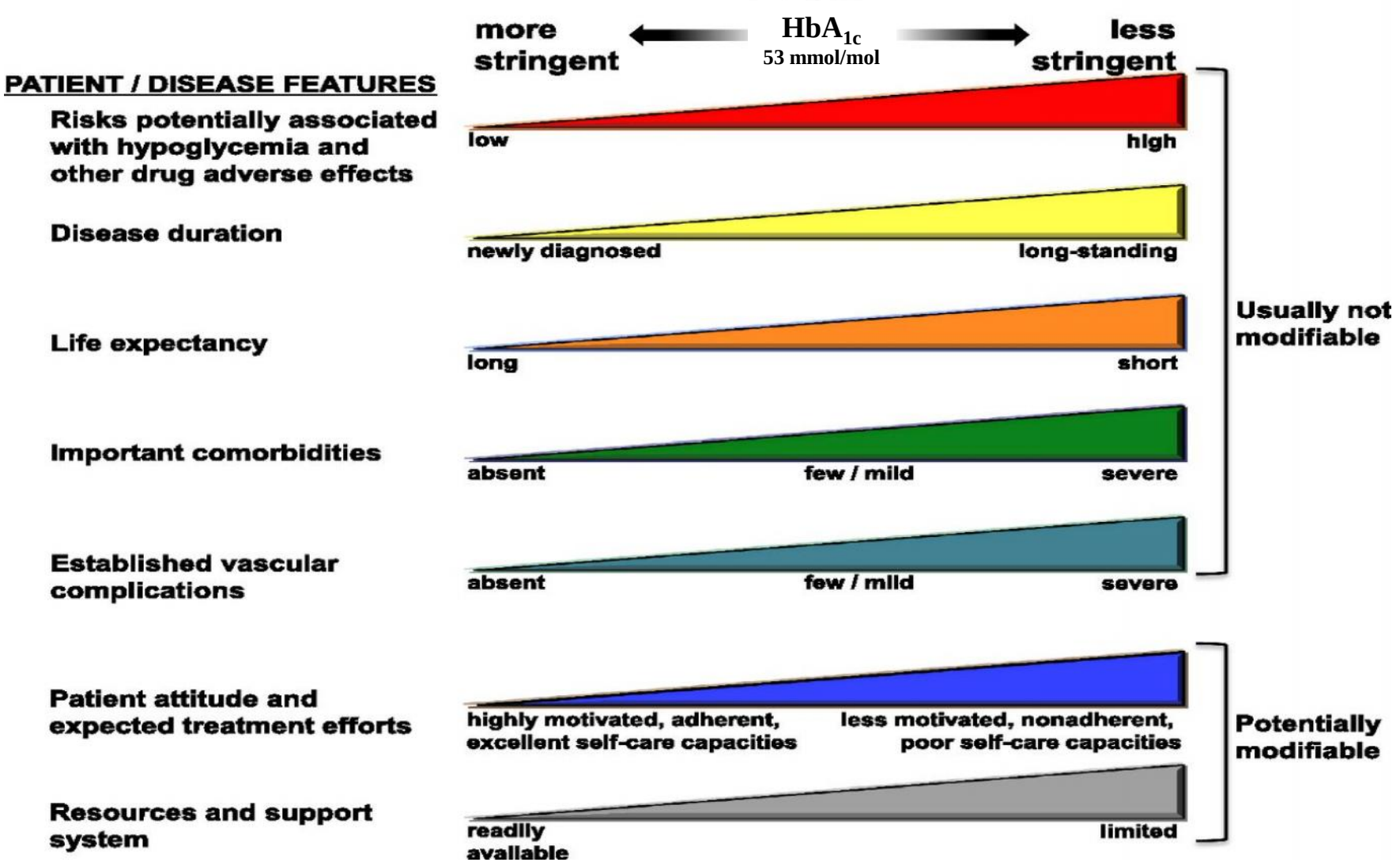


HbA_{1c} and glomerular hyperfiltration

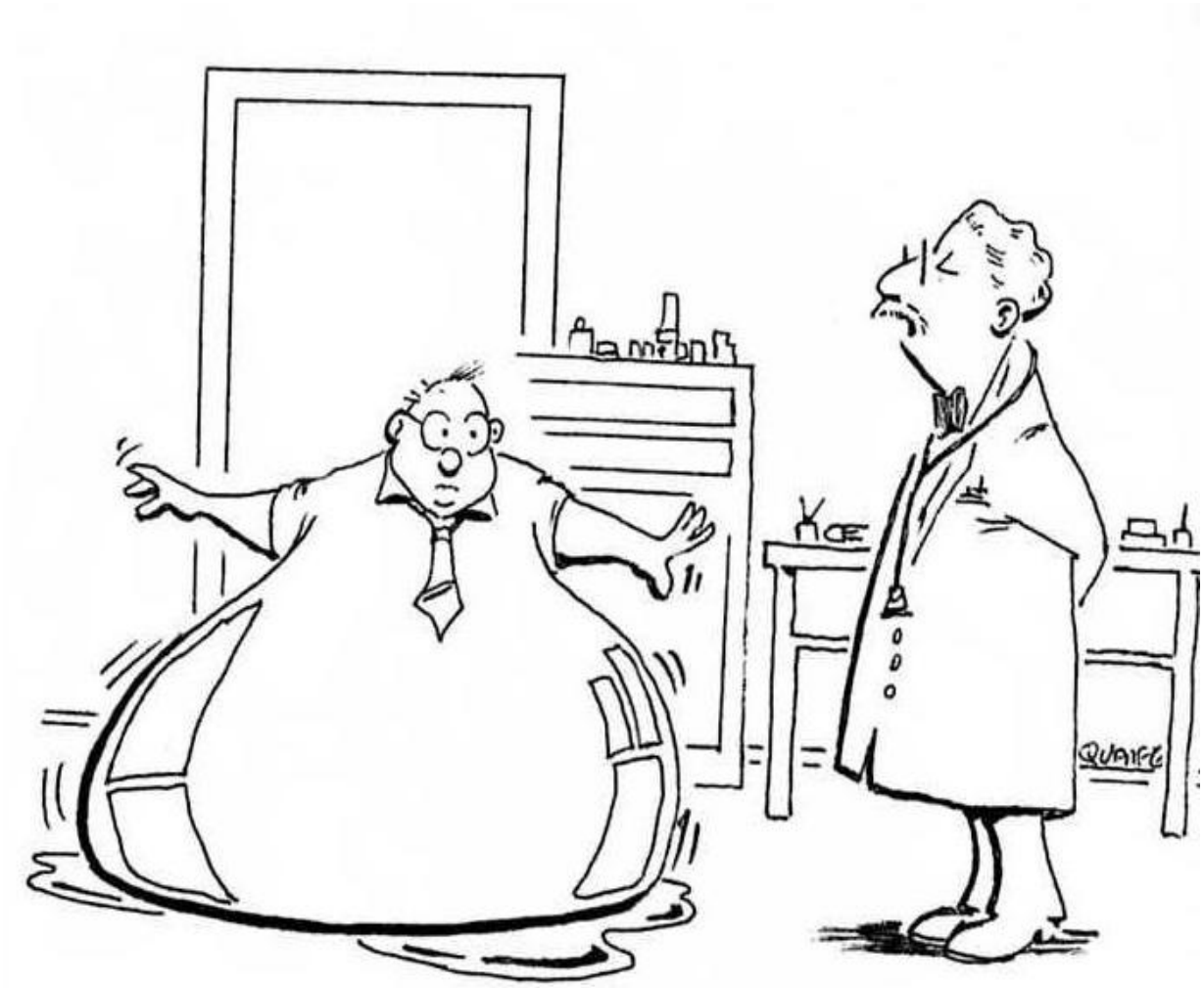
- An elevated baseline HbA_{1c}, results in a greater annual decline in eGFR, particularly in people with a baseline eGFR above 60 ml/min/1.73 m^2.

- Individuals in later stages of CKD, particularly stage 3-4, show eGFR to be negatively affected by preceding HbA_{1c}, suggesting that poor glycemic control occurred prior to the decline in eGFR in those with later stages of CKD, and that good glycemic control at these stages may be critical in delaying the progression to end-stage renal disease (ESRD)

Determination of optimal HbA_{1c} targets in patients with type 2 diabetes.



Inzucchi SE et al. Diabetes Care 2015;38:140–149



Renal Disease if not HbA_{1c} then what?

Fructosamine:

- Mixture of proteins
- No standardisation
- Affected by several interferences
- No evidence base

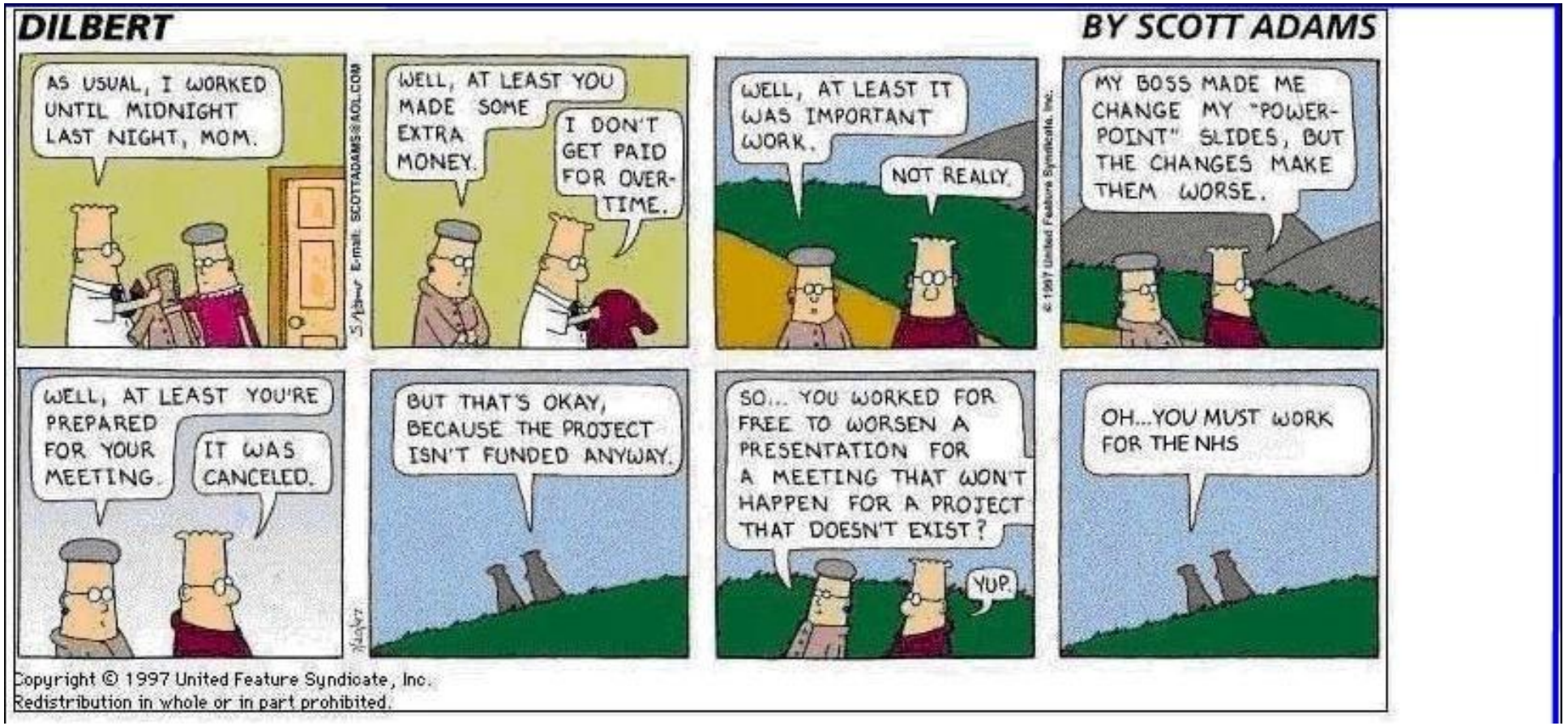
Glycated Albumin:

- Single protein with known half life
- ? Effect of Renal disease on clearance
- No evidence base

Glucose and CGM:

- Measures the substance required
- Currently the only reliable measurement

Thank You


DILBERT
BY SCOTT ADAMS
AS USUAL, I WORKED UNTIL MIDNIGHT LAST NIGHT, MOM.
WELL, AT LEAST YOU MADE SOME EXTRA MONEY.
I DON'T GET PAID FOR OVER-TIME.
WELL, AT LEAST IT WAS IMPORTANT WORK.
NOT REALLY.
MY BOSS MADE ME CHANGE MY "POWER-POINT" SLIDES, BUT THE CHANGES MAKE THEM WORSE.
WELL, AT LEAST YOU'RE PREPARED FOR YOUR MEETING.
IT WAS CANCELED.
BUT THAT'S OKAY, BECAUSE THE PROJECT ISN'T FUNDED ANYWAY.
SO... YOU WORKED FOR FREE TO WORSEN A PRESENTATION FOR A MEETING THAT WON'T HAPPEN FOR A PROJECT THAT DOESN'T EXIST?
YUP.
OH...YOU MUST WORK FOR THE NHS
Copyright © 1997 United Feature Syndicate, Inc.
Redistribution in whole or in part prohibited.