Laboratory Diagnosis & Monitoring of Diabetes Mellitus

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Objectives

- Explain the rationale for using HbA_{1c} to diagnose diabetes mellitus
- List the components of a HbA_{1c} result report based on international consensus
- Be able to describe how the estimated average glucose is calculated and the limitations of its use

Outline

- Overview of diabetes mellitus
- Clinical significance of HbA_{1c}
- Laboratory methods for HbA_{1c}
- HbA_{1c} and mean plasma glucose
- Effects of variant hemoglobins on HbA_{1c}
- Appropriate laboratory testing
- Conclusions

Statistics

- Afflicts 24 million Americans
- Approximately 6-7 million are undiagnosed
- 5-10% Type 1 (juvenile)
  - Insulin deficiency
- 90-95% Type 2 (adult)
  - Insulin resistance
  - Relative insulin deficiency

Diabetic Complications

- Leading cause of:
  - Kidney failure
  - Adult blindness
  - Non-traumatic amputations
- People with diabetes are 2-4 times more likely to have an MI or CVA
- Single most costly chronic disease
- Sixth leading cause of death in US

Non-enzymatic Glycation of Hb

- HbA_1: glycated Hb A (80% is HbA_{1c})
- HbA_{1c}: glucose at N-terminus of β chain
- HbA_{1a}: fructose-1,6-diphosphate (HbA_{1a1}) or glucose-6-phosphate (HbA_{1a2}) at N-terminus of β chain
- HbA_{1b}: pyruvate at N-terminus of β chain
- HbA can be glycated on lysine residues

Glycation of Hb

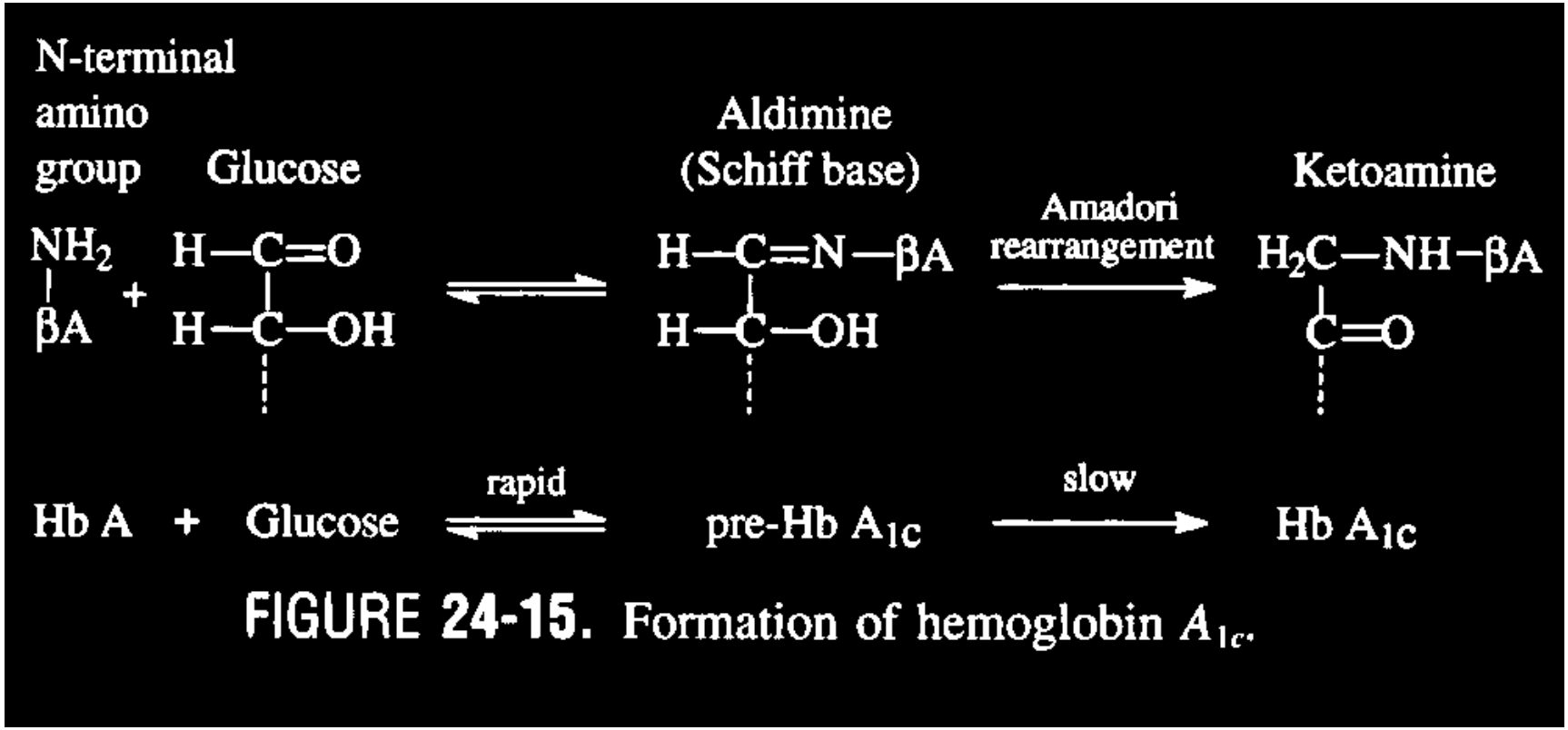


FIGURE 24-15. Formation of hemoglobin A_{1c}.

Tietz Textbook of Clinical Chemistry 3rd edition, 1999

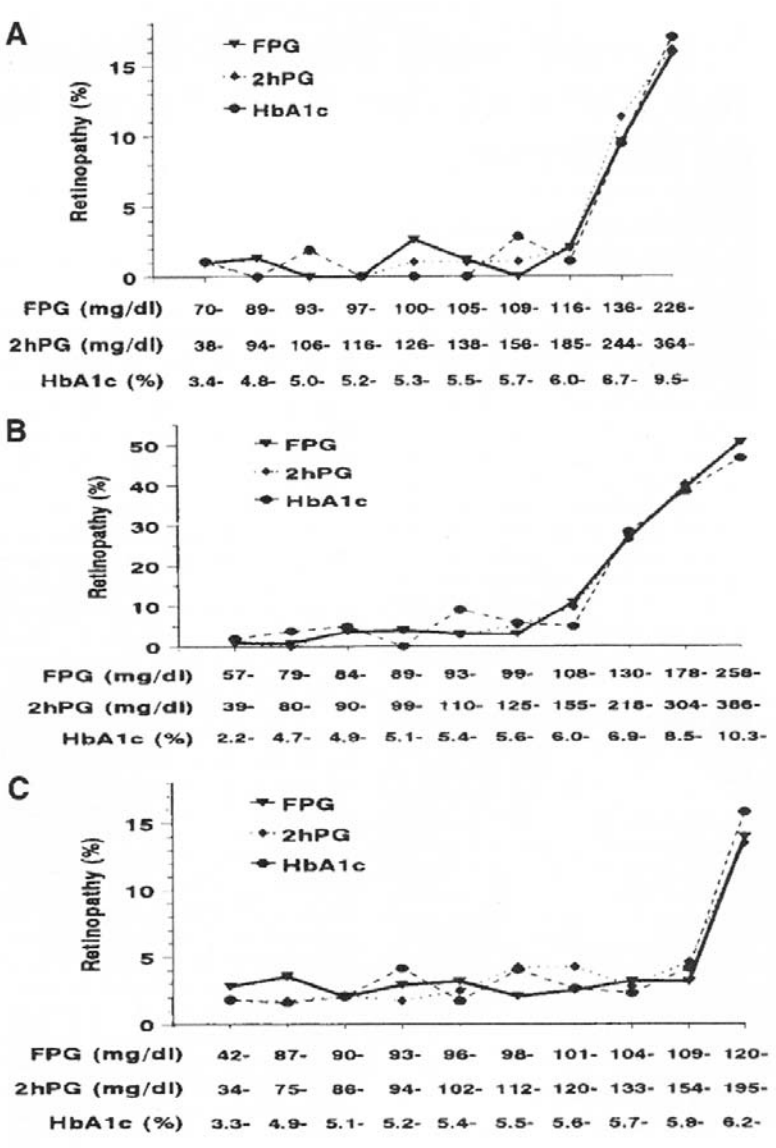
Clinical Utility of HbA_{1c}

- It reflects mean plasma glucose over the preceding 6-12 weeks
- Mean plasma glucose of preceding 1 month accounts for about 50% of HbA_{1c}
- It is the only marker that correlates well with long term complications
- Glycation of serum proteins reflects mean plasma glucose over the preceding 2-3 weeks

ADA Guidelines for Diagnosis

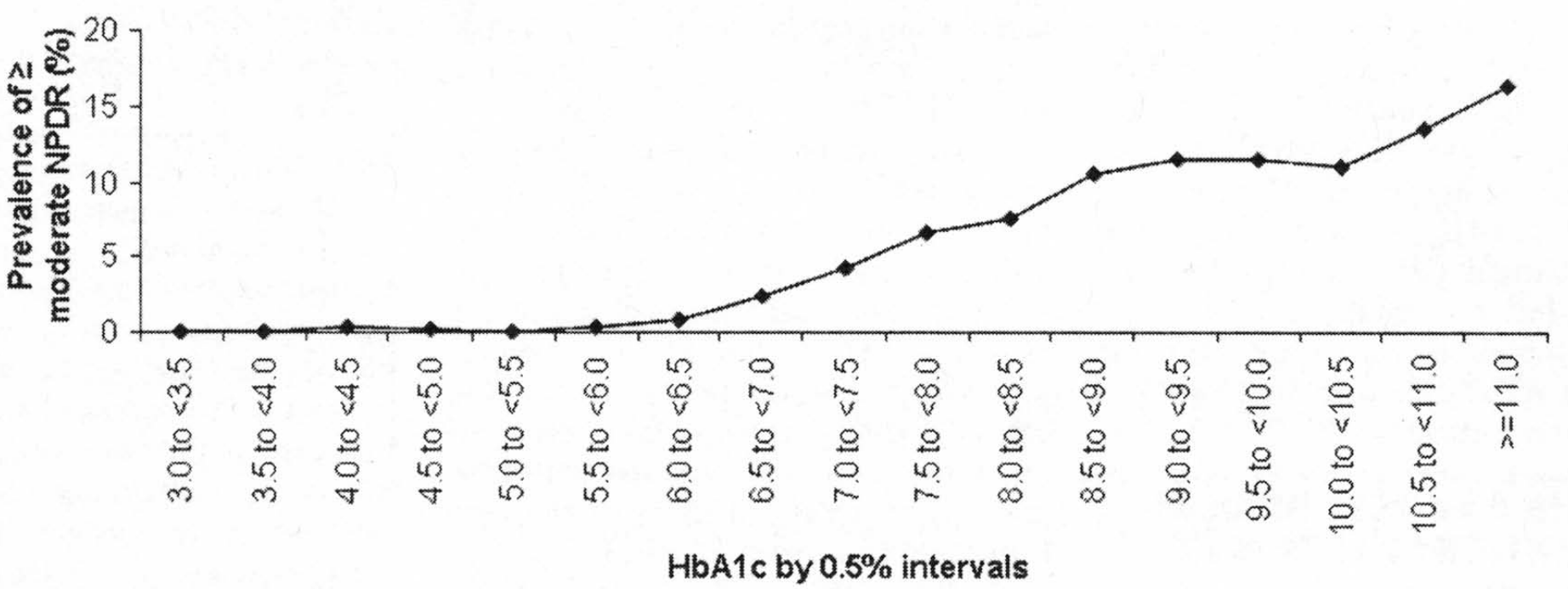
- Test people over 45 y.o. every 3 years
- Overweight or family history test earlier
- HbA_{1c} >6.4% (new in 2009)
- Fasting plasma glucose >125 mg/dL
- Symptoms plus random glucose >199 mg/dL
- 2 hr post glucose challenge >199 mg/dL (75g glucose load)

Retinopathy and FPG, 2hPG, HbA_{1c}



Diabetes Care 2009;32:1-8

Retinopathy and HbA_{1c}



The International Expert Committee, Diabetes Care 2009;32:1-8

Advantages of HbA_{1c} for Diagnosis

- Standardized and aligned to DCCT and UKPDS
- Better index of overall glycemic exposure and risk for long-term complications
- Less biologic variability than glucose
- Less preanalytic variability than glucose
- No need for fasting or timed samples
- Relatively unaffected by acute perturbations of glucose concentrations (e.g. stress or illness)
- Currently used to guide management and adjust therapy

Abnormal Glucose Homeostasis

- Metabolic stage intermediated between normal glucose homeostasis and diabetes (nearly normal HbA_{1c})
- Impaired fasting glucose (IFG)
  - Fasting plasma glucose 100-125 mg/dL
- Impaired glucose tolerance (IGT)
  - 2 hr post glucose challenge 140-199 mg/dL (75g glucose load)
- HbA_{1c} 6.1-6.4%

General Limitations of HbA_{1c}

- False increases
  - Iron deficiency
    - Non-diabetics subjects with severe iron deficiency had HbA_{1c} results up to 7.5%
  - Asplenia leads to increased RBC survival and increased HbA_{1c}
  - Analytical—Hb variants can comigrate with HbA_{1c}
- False decreases
  - Shortened RBC survival
    - Unstable hemoglobins
    - Autoimmune hemolytic anemias
  - Analytical
    - Hemoglobinopathies

DCCT

- Diabetes Control and Complications Trial
- Type 1 diabetic patients
- Two groups
  - Conventional management
  - Intensive management
    - Goal normalization of blood glucose
    - Goal not achieved
- Seven year average study period

DCCT Results

- Conventional group
  - Mean plasma glucose ~210 mg/dL
  - HbA_{1c} 9%
- Intensive treatment group
  - Mean plasma glucose ~160 mg/dL
  - HbA_{1c} 7.2%
- Non-diabetic
  - Mean plasma glucose ~100 mg/dL
  - HbA_{1c} <6%

Intensive Treatment Group

- Showed a 60% reduction in retinopathy, nephropathy, and neuropathy
- Threefold greater risk of hypoglycemia
- Increased costs of intensive control offset by decreased complications and more productive lives

UKPDS

- United Kingdom Prospective Diabetes Study
- Type 2 diabetic patients
- Followed an average of 10 years
- Conventional group HbA_{1c} 7.9%
- Intensive group HbA_{1c} 7.0%
- Microvascular complications reduced 25%
- Hyperglycemia causes these complications
- Intensive therapy may reduce CAD

Benefits of Nearly Normal HbA_{1c}

- Five extra years of life
- Delays progression of eye, kidney, and nerve disease
  - Eight additional years of sight
  - Six extra years free from kidney disease
- Increased work productivity
- Lower health care use

ADA Guidelines for Monitoring

- HbA_{1c} <5.7% is normal
- HbA_{1c} <7% meets goal
- HbA_{1c} 7-8%
- HbA_{1c} >8% additional action suggested

- AACE and ACE goal for HbA_{1c} is <6.5%

HbA_{1c} Methods

- Cation exchange chromatography
  - Measures HbA_{1c} as percent of total Hb A
- Boronate affinity chromatography
  - Measures glycated Hb as percent of total Hb
- Immunoassay
  - Measures glycated N-terminus of the β-chain as percent of total Hb
- Enzymatic
  - Measuresglycated N-terminus of the β-chain as percent of total Hb
- POCT vs. central laboratory

CAP PT Surveys

- Fresh whole blood PT samples
  - NGSP assigned values
  - Minimal matrix effects
  - Permit assessment of method performance in the field
  - Provide imprecision information across reagent lots and analyzers
- Performance has improved since introduction
- Accuracy based grading of +/- 8% of NGSP value

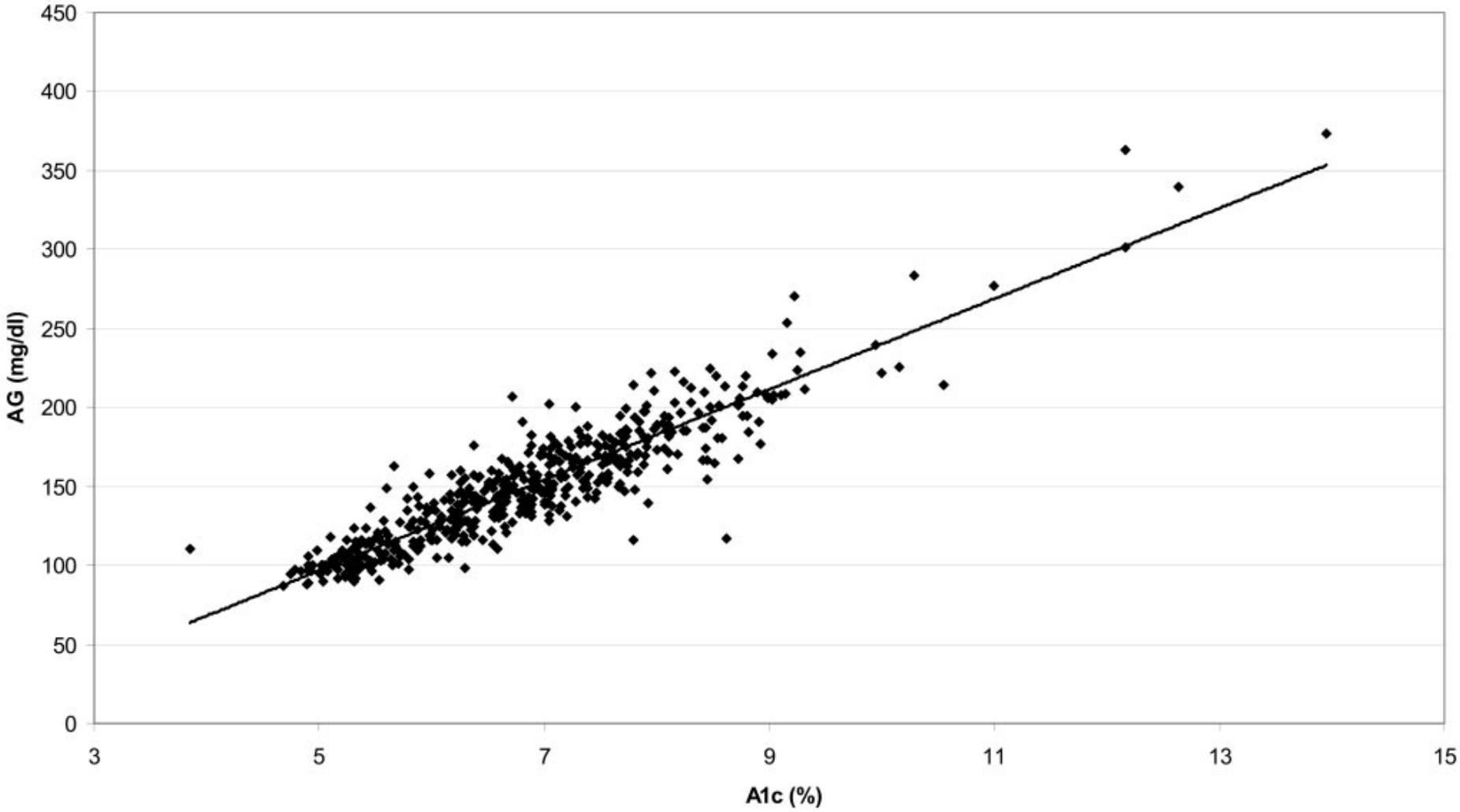
POCT for HbA_{1c}

- Atlanta study
  - Favorable impact of POCT on F/U HbA_{1c}
  - Enhanced patient and/or provider motivation
  - Thaler LM et al. Diabetes Care 1999;22:1415-21.
- Boston study
  - Decrease in HbA1c in POC group but not central lab group at 6 and 12 months
  - Cagliero E et al. Diabetes Care 1999;22:1785-9.
- UK study
  - No difference in proportion of patients with HbA_{1c} <7%
  - No difference in total cost
  - Khunti K et al. Br J Gen Pract 2006;56:511-7.
- Australian study sponsored by Australian DoH
  - POCT provided slightly better clinical effectiveness
  - POCT considerably more expensive than central lab alternative
  - Bubner TK et al. Med J Aust 2009;190:624-6.

Reporting Results

- ADA, EASD, IFCC, IDF
- Results for HbA_{1c} are to be reported worldwide
  - Mmol/mol (IFCC reference method equivalent)
  - % (NGSP equivalent, current reporting unit)
  - Estimated average glucose (eAG) which corresponds to average plasma glucose
- Worldwide study provided an equation to transform HbA_{1c} into eAG
  - eAG (mg/dL) = 28.7 x HbA_{1c} (NGSP %) – 46.7

Consensus Committee, Diabetes Care, 2007;30:2399-2400.
Nathan DM et al. Diabetes Care 2008;31:1473-8.



eAG (mg/dL) = 28.7 x HbA_{1c} (NGSP %) – 46.7 r = 0.92

Nathan DM et al. Diabetes Care 2008;31:1473-8.

Limitations of eAG

- Racial/ethnic limitations
  - Study was primarily conducted in Caucasian subjects
  - Different relationships between eAG and HbA_{1c} in Caucasian and African subjects, not statistically significant
  - Certain ethnic groups were not included
    - Subcontinent of India (specimens delayed in shipment)
    - Pacific Islanders
- Some groups of subjects were not included
  - Children
  - Pregnant women

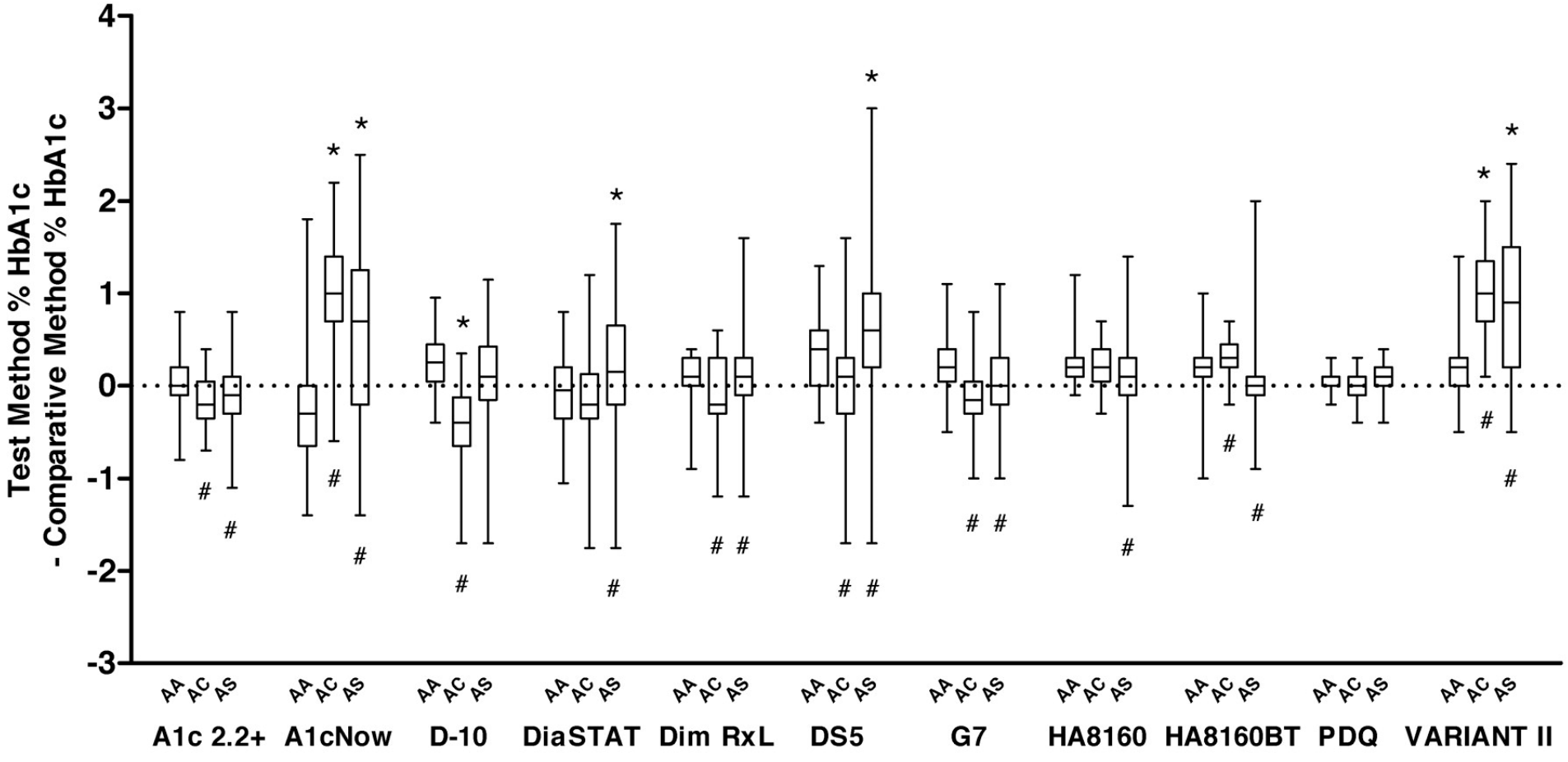
HbA_{1c} and Race

- African Americans have higher HbA_{1c} results than non-Hispanic whites
  - ~0.65% in diabetic patients
  - Kirk JK et al. Diabetes Care 2006;29:2130-6.
- African Americans have a higher HbA_{1c} for a given glucose concentration that whites
  - Difference is 4% at HbA_{1c} of 7% (~0.3% absolute)
  - Bleyer AJ et al. Diabet Med 2009;26:128–33
- American Indians and blacks with IFG have higher HbA_{1c} results than whites
  - Whites 5.78%, Hispanics 5.93%, Asians 6.00%, American Indians 6.12%, Blacks 6.18%
  - Herman WH et al. Diabetes Care 2007;30:2453-7.

Hemoglobin Variants

- Common variants in some populations
  - African and Mediterranean populations
    - Hb S
    - Hb C
  - Asian populations
    - Hb E
    - Hb D

HbC & S Trait



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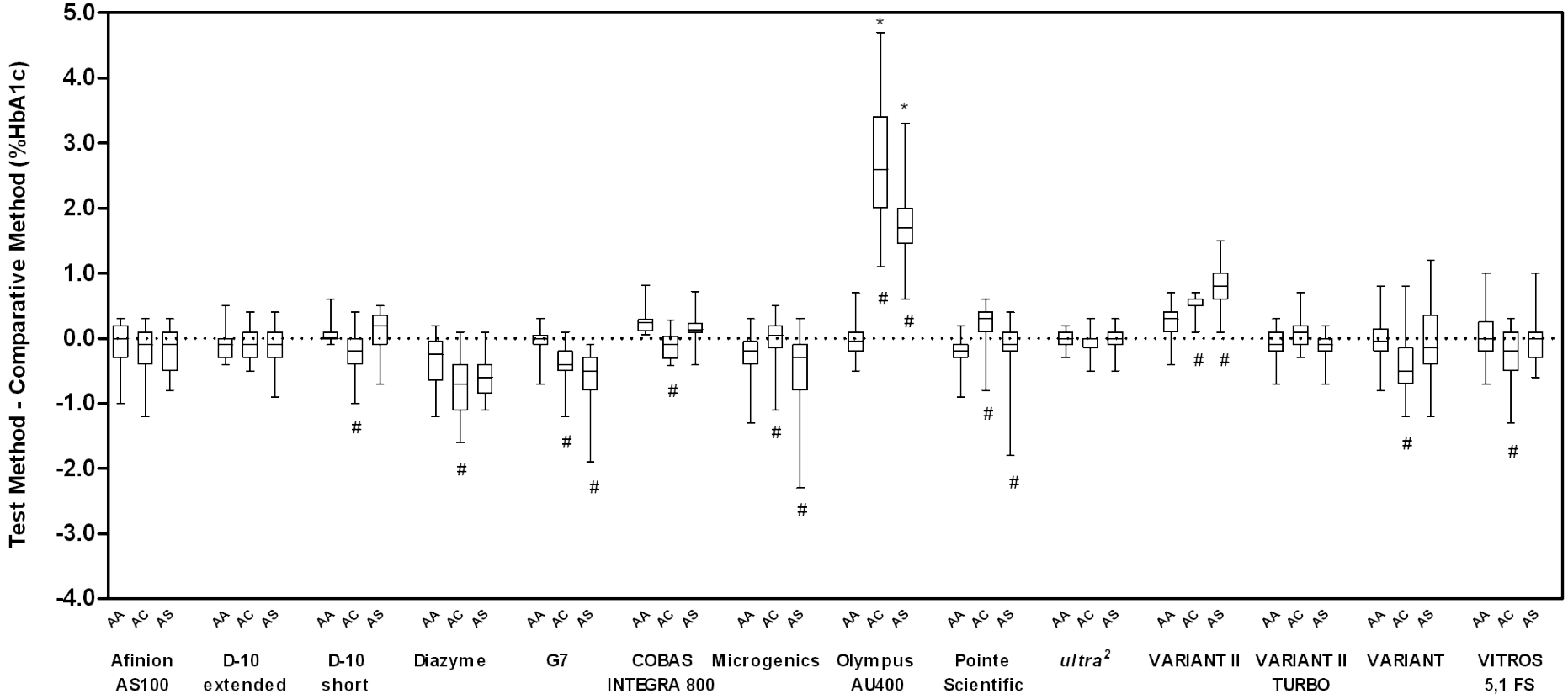
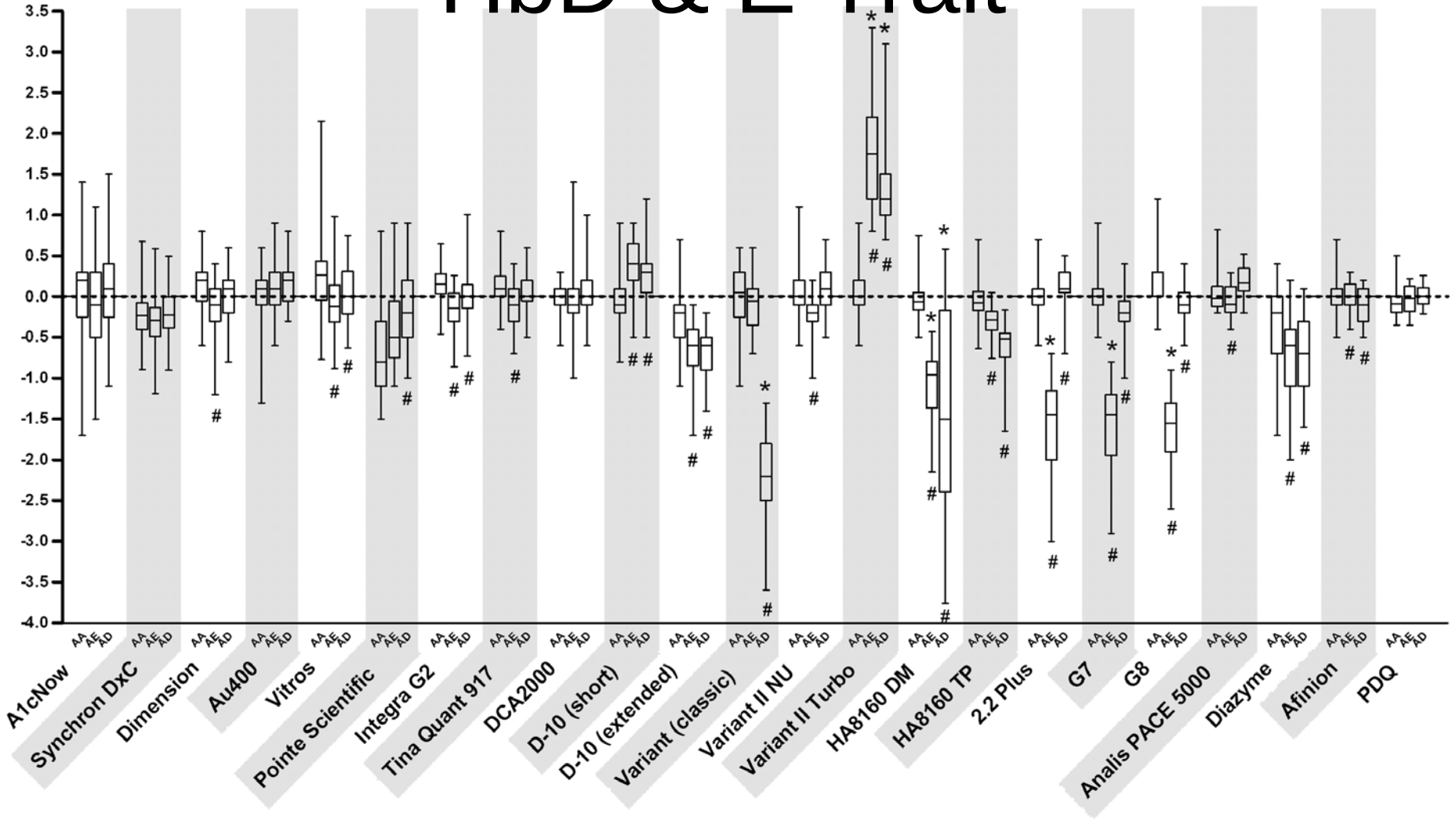
HbC & S Trait



Mongia SK, et al. Am J Clin Path 2008;130:136-40.

HbD & E Trait



Little RR et al. Clin Chem, 2008;54:1277-82.

Table 1: Interference of Heterozygous Variants S, C, D, E and elevated HbF with Specific HbA1c Methods

Manufacturer	Method	Interference from				
		HbAS	HbAC	HbAE	HbAD	↑HbF
Immunoassay						
Abbott	Architect/Aeroset	Yes	Yes	-	-	-
Bayer (Metrika)	A1cNOW	Yes	Yes	No	No	-
Beckman	Synchron System	No	No	No	No	-
Dade	Dimension	No	No	No	No	-
Olympus	AU system	Yes	Yes	No	No	-
Ortho-Clinical	Vitros	No	No	No	No	-
Point Scientific	HbA1c on Modular P	No	No	No	No	-
Roche	Cobas Integra	Yes	Yes	-	-	-
Roche	Cobas Integra Gen.2 (Tina Quant)	No	No	No	No	-
Roche/Hitachi	Hitachi (Tina Quant)	No	No	No	No	-
Siemens (Bayer)	Advia	Yes	Yes	-	-	-
Siemens (Bayer)	DCA 2000	No	No	No	No	Yes*
Ion-Exchange HPLC						
Bio-Rad	D-10 (short)	No	No	No	No	-
Bio-Rad	D-10 (extended)	No	No	No	No	-
Bio-Rad	Variant A1c	No	No	No	Yes	-
Bio-Rad	Variant II A1c	No	No	No	No	No
Bio-Rad	Variant II Turbo A1c	No	No	Yes	Yes	-
Menarini	HA8140 (diabetes mode)	Yes	No	-	-	-
Menarini	HA8160 (diabetes mode)	No	No	Yes	Yes	-
Menarini	HA8160 (TP mode)	No	No	No	Not Quantified	-
Tosoh	A1c 2.2 Plus	No	No	Yes	No	Yes*
Tosoh	G7	No	No	Yes	No	No[+]
Tosoh	G8	-	-	Yes	No	-
Boronate Affinity						
Axis-Shield	Afinion	No	No	No	No	-
Primus	Boronate affinity HPLC	No	No	No	No	Yes*
Other						
Diazyme	Direct Enzymatic A1c	No	No	No	No	-

* HbF levels above 15% cause clinically significant low bias

[+] Offline manual recalculation must be performed if the HbF peak is mislabeled as labile HbA1c (LA1C)

Routine Laboratory Tests

- HbA_{1c}
  - 2 times per year if stable and meeting treatment goals
  - 4 times per year if therapy changed or not meeting treatment goals
- Fasting lipids
  - Annually, LDL-C target <100 mg/dL, <70 mg/dL optional
- Liver function tests with further evaluation for fatty liver or hepatitis if abnormal
- Urine microalbumin
  - Yearly, after 5 years in type 1 and in all type 2 patients
- Serum creatinine and estimated GFR
- Urinalysis for ketones, protein, sediment
- TSH in all type 1 and if clinically indicated in type 2 patients

Health Care Delivery Challenges

- HEDIS data for 2008 (median for commercial, Medicare, Medicaid)
- Yearly HbA_{1c} 77-88%
- HbA_{1c} >9% 29-48%
- Yearly lipid profile 71-86%
- LDL <100 mg/dL 31-47%
- Yearly microalbumin 74-86%

Summary Points

- There are many different HbA_{1c} methods
- Certification of HbA_{1c} methodologies by the NGSP improves accuracy
- Laboratories need to be aware of potential interferences
- The greatest challenges are in appropriate test utilization and patient compliance

References

- DCCT Summary
  - Diabetes Care 1999;22(suppl 1):S24-S26.
- UKPDS Summary
  - Diabetes Care 1999;22(suppl 1):S27-S31.
- Lab Guidelines & Recommendations
  - http://www.aacc.org/members/nacb/LMPG/OnlineGuide/DraftGuidelines/diabetes_update/Pages/default.aspx
- NGSP web site
  - http://web.missouri.edu/~diabetes/ngsp.html