# The Diagnosis of Diabetes and the Role of Glycated Proteins

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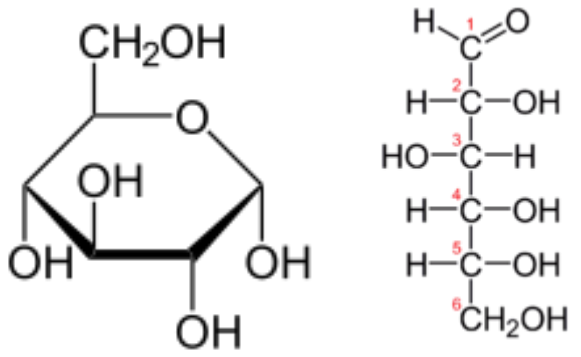
# Overview:


- What is Diabetes & How is it Classified?
- 2020 Diabetes Statistics
- 2021 ADA Guidelines for the Diagnosis of Diabetes
- Advantages & Disadvantages of Current Diagnostic Tests for Diabetes
- Closer Look at other Diagnostic Tests for Diabetes – Glycated Proteins?
- What we know about Glycated Proteins
- Review of 2 recent studies involving Glycated Albumin
- Summary

# What is Diabetes?

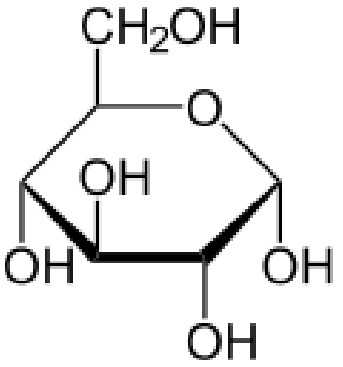

*Picture from Diabetes.co.uk*

- 300 BC – “Sweet urine”
  - Ancient Greek, Indian and Egyptian populations
  - Diabetes – “siphon, passing through, or large discharge of urine”
  - Mellitus – “pleasant tasting, like honey”
- Symptoms:
  - Polyuria
  - Polydipsia
  - Weight Loss
  - Polyphagia
  - Blurred Vision
- **Hyperglycemia**




https://en.wikipedia.org/wiki/Glucose

# What is Diabetes?




- Hyperglycemia:
  - Defects in Insulin Secretion
  - Defects in Insulin Action
  - Both

- End Result: Insulin can't act on target tissues, which then impacts carbohydrate, protein and fat metabolism

- Acute Hyperglycemia
  - Diabetic Ketoacidosis (DKA)
  - Hyperosmolar Syndrome

- Chronic Hyperglycemia
  - Long term damage, dysfunction and/or failure of many organs, like the eyes, kidneys, nerves, heart and/or blood vessels

# How is Diabetes Classified?

- Type 1 Diabetes – autoimmune β-cell destruction
  - Usually leads to absolute insulin deficiency
- Type 2 Diabetes – progressive loss of β-cell insulin secretion
  - Usually at same time as insulin resistance
- Gestational Diabetes Mellitus (GDM) – diabetes diagnosed in the 2$^{nd}$ or 3$^{rd}$ trimester
  - Not clearly overt prior to pregnancy
- Other Causes
  - Genetic (Mature Onset Diabetes of the Young)
  - Diseases of the Pancreas (Cystic Fibrosis or Pancreatitis)
  - Drug or Chemical Induced (HIV drugs or glucocorticoids)

# How is Diabetes Classified?

- Type 1 & Type 2 are Heterogeneous
  - Presentation and/or progression can vary a lot
  - Can’t always classify patients at initial diagnosis
  - Past – Type 1 occurs in youth, Type 2 in adults
    - No longer accurate
- Type 1 diagnosed in children, they generally present with polyuria and polydipsia; 1/3 with DKA
  - Type 1 diagnosed in adults - more variable presentation
- Type 2 – Can sometimes present with DKA
- Misdiagnosis at initial presentation is common

# National Diabetes Statistics Report 2020

**Table 1b. Estimated number of adults aged 18 years or older with diagnosed diabetes, undiagnosed diabetes, and total diabetes, United States, 2018**

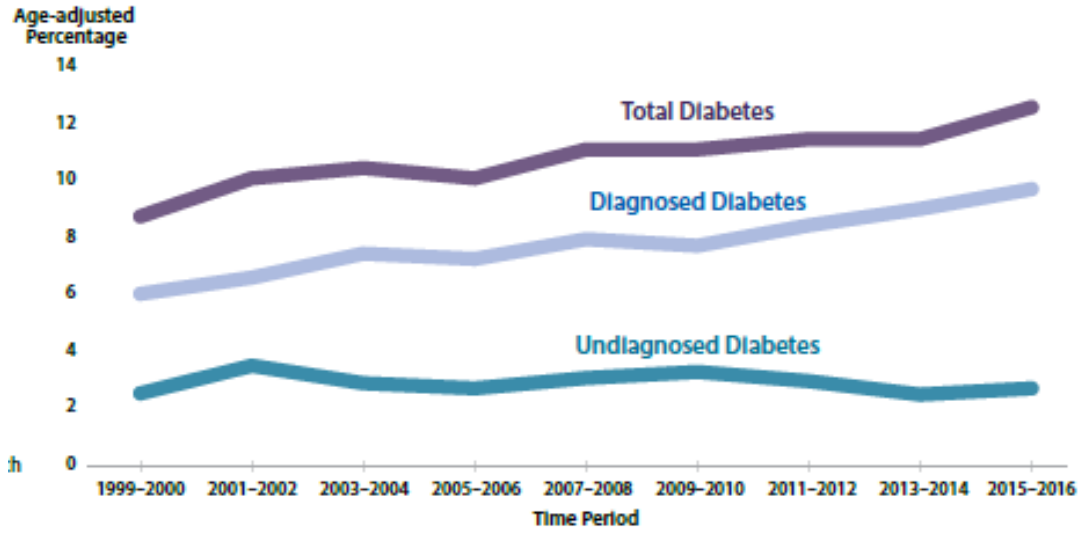
| Characteristic | Diagnosed diabetes Number in Millions (95% CI) | Undiagnosed diabetes Number in Millions (95% CI) | Total diabetes Number in Millions (95% CI) |
|---|---|---|---|
| Total | 26.8 (24.4–29.1) | 7.3 (6.3–8.4) | 34.1 (31.6–36.6) |
| Age in years | | | |
| 18–44 | 3.6 (3.0–4.1) | 1.4 (0.8–1.9) | 4.9 (4.0–5.8) |
| 45–64 | 11.7 (10.3–13.1) | 3.1 (2.3–3.9) | 14.8 (13.4–16.3) |
| ≥65 | 11.5 (10.1–12.8) | 2.9 (2.1–3.6) | 14.3 (12.7–15.9) |
| Sex | | | |
| Men | 14.0 (12.4–15.6) | 3.9 (2.8–5.0) | 17.9 (16.2–19.6) |
| Women | 12.8 (11.4–14.1) | 3.4 (2.7–4.1) | 16.2 (14.8–17.6) |
| Race/ethnicity | | | |
| White, non-Hispanic | 15.4 (13.8–17.0) | 4.1 (3.1–5.2) | 19.5 (17.9–21.2) |
| Black, non-Hispanic | 4.2 (3.8–4.7) | 0.9 (0.6–1.3) | 5.2 (4.7–5.7) |
| Asian, non-Hispanic | 1.6 (1.3–2.0) | 0.7 (0.4–1.0) | 2.3 (1.9–2.8) |
| Hispanic | 4.9 (4.1–5.6) | 1.5 (1.0–1.9) | 6.4 (5.4–7.3) |

Notes: CI = confidence interval. Estimated numbers for 2018 were derived from percentages for 2013–2016 applied to July 1, 2018 US resident population estimates from the US Census Bureau (See Detailed Methods). Diagnosed diabetes was based on self-report. Undiagnosed diabetes was based on fasting plasma glucose and A1C levels among people self-reporting no diabetes. Numbers for subgroups may not add up to the total because of rounding.

Data sources: 2013–2016 National Health and Nutrition Examination Survey; 2018 US Census Bureau data.

- 34.1 million adults (13%) aged 18 years or older
- 7.3 million adults aged 18 years or older who met laboratory criteria for diabetes were not aware - undiagnosed diabetes
- 14.3 million were 65 years or older

# National Diabetes Statistics Report 2020



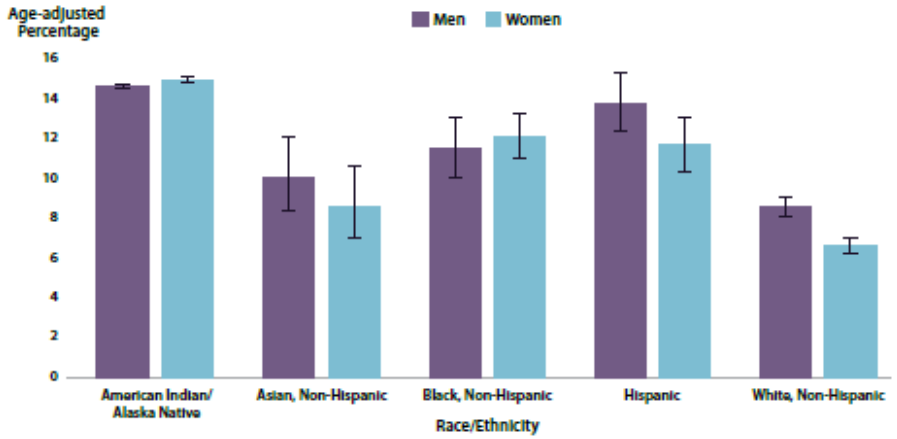

- Total diabetes, with age adjustments, increased from 1999-2016 in adults over 18 years old
- 9.5% in 1999-2002
- 12.0% in 2013-2016
- Significant increase in diagnosed diabetes from 1999-2016
- No significant change in undiagnosed diabetes

# National Diabetes Statistics Report 2020

**Figure 2. Age-adjusted estimated prevalence of diagnosed diabetes by race/ethnicity group and sex for adults aged 18 years or older, United States, 2017–2018**

Note: Error bars represent upper and lower bounds of the 95% confidence interval.

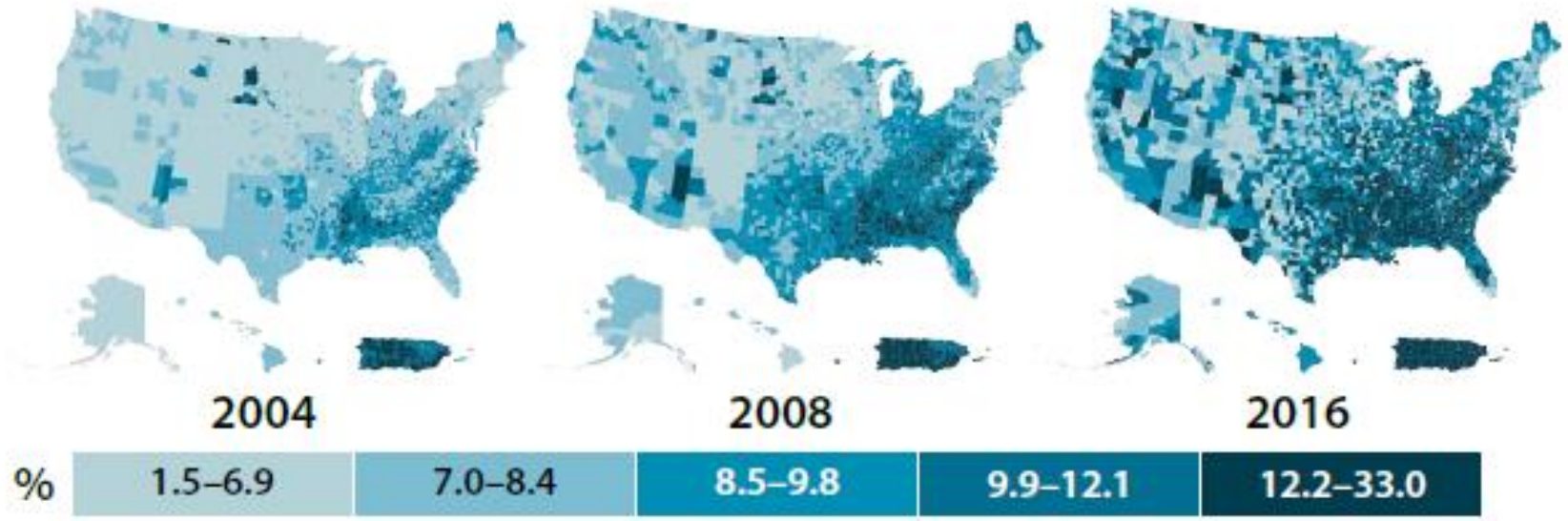
Data sources: 2017–2018 National Health Interview Survey; 2017 Indian Health Service National Data Warehouse (for American Indian/ Alaska Native group only).




- Age Adjusted Prevalence of diagnosed diabetes by ethnicity and gender:
- Highest for American Indians/Alaska Natives (14.7%), followed by Hispanics (12.5%), non-Hispanic Asians (9.2%), non-Hispanic Whites (7.5%)
- Further broken down by Hispanics - Mexicans (14.4%), Puerto Ricans (12.4%), Central/South Americans (8.3%), and Cubans (6.5%)
- Non-Hispanic Asians – Asian Indians (12.6%), Filipinos (10.4%), and Chinese (5.6%)
- Education level – 13.3% less than high school, 9.7% with high school, and 7.5% with college degrees

# National Diabetes Statistics Report 2020

**Figure 3. Age-adjusted, county-level prevalence of diagnosed diabetes among adults aged 20 years or older, United States, 2004, 2008, and 2016**




- In 2016, variable prevalence of diagnosed diabetes in US counties, from 1.5% to 33.0%
- Median prevalence of diagnosed diabetes increased from 7.8% in 2004 to 13.1% in 2016

# Criteria for the Diagnosis of Diabetes – American Diabetes Association (ADA) 2021 Guidelines

- **Must have at least 2 abnormal test results** (same or different samples):

  - Fasting Plasma Glucose (FPG) ≥ 126 mg/dL
    - Fasting = no caloric intake for 8 hours or more

    OR
  - 2 hour Plasma Glucose ≥ 200 mg/dL during an Oral Glucose Tolerance Test (OGTT)
    - WHO criteria – 75 g anhydrous glucose dissolved in water

    OR
  - A1C ≥ 6.5%
    - Using an NGSP certified assay, standardized to the DCCT assay

    OR
  - Random Plasma Glucose ≥ 200 mg/dL
    - Classic signs of hyperglycemia or hyperglycemic crisis

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- Prediabetes – individuals who have glucose levels above normal, but not high enough to meet criteria for diabetes
  - Not a separate disease
  - Increased risk for diabetes and cardiovascular disease (CVD)
  - Associated with obesity (abdominal or visceral), dyslipidemia (high triglycerides and/or low HDL) and hypertension

- Diagnosed with either:
  - FPG 100 to 125 mg/dL (Impaired Fasting Glucose or IFG)

    OR
  - 2 hour plasma glucose 140 to 199 mg/dL (Impaired Glucose Tolerance or IGT)

    OR
  - A1C 5.7 to 6.4%

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- Type 1 Diabetes
  - Immune Mediated
    - 5-10% of all diabetes cases
    - Cellular Mediated autoimmune destruction of pancreatic β–cells
    - Variable rate of β–cell destruction (fast in children, slower in adults)
  - Idiopathic
    - No known etiology - rare
    - Permanently low insulin levels, prone to DKA, no evidence of autoimmunity
    - Genetic
- Type 1 Diabetes Staging
  - Stage 1 – autoimmunity, normoglycemia and presymptomatic
    - Multiple Autoantibodies, No IGT or IFG
  - Stage 2 – autoimmunity, dysglycemia and presymptomatic
    - Multiple Autoantibodies, IFG or IGT, A1C between 5.7-6.4%
  - Stage 3 – New-onset hyperglycemia, symptomatic
    - Clinical symptoms, diabetes with ADA criteria

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- Type 2 Diabetes
  - Previously called “Noninsulin dependent diabetes” or “adult onset diabetes”
  - 90-95% of all diabetes
  - Relative (not absolute) insulin deficiency & peripheral insulin resistance
  - Most patients are overweight or obese – causes insulin resistance
  - DKA rarely occurs, but can occur with another stress like drugs, infection, or myocardial infection
  - Often undiagnosed for years; hyperglycemia happens gradually, not severe at first
  - Insulin levels normal or high, glucose levels high – insulin resistance by cells
  - Insulin resistance can improve with weight loss, exercise, drugs

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- Criteria for screening for diabetes or prediabetes in asymptomatic adults
  - Overweight or obese adults and one of the following risk factors: first degree relative with diabetes, ethnicity, history of CVD, Hypertension, Low HDL (<35 mg/dL)/High Triglycerides (>250 mg/dL), PCOS, low exercise
  - Prediabetes – test yearly
  - Women diagnosed with GDM – lifelong testing, every 3 years
  - Everyone else – lifelong testing every 3 years after age 45 (depending on other risk factors/results)

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- Gestational Diabetes Mellitus (GDM)
  - Any degree of glucose intolerance recognized during pregnancy – older definition
  - Initial screening at first prenatal visit, using classic criteria
    - "Diabetes complicating pregnancy" – usually Type 2 – most cases – pre-existing hyperglycemia
  - Rescreen others between 24-28 weeks for GDM
  - Increased obesity – more undiagnosed Type 2 Diabetes in women of reproductive age
  - Risk of adverse maternal, fetal and neonatal outcomes:
    - Hypertension of pregnancy, large for gestational age, macrosomia, birth trauma to mom or newborn, increased C-sections, perinatal mortality, cardiomyopathy of fetus/newborn, neonatal respiratory and/or metabolic issues, increased risk of type 2 diabetes and CVD for mom, increased risk of obesity, hypertension, metabolic issues for offspring
    - Risk increases as hyperglycemia increases – Hyperglycemia and Adverse Pregnancy Outcomes, or HAPO study

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- GDM Diagnosis – Either One-Step (ADA & International Association of Diabetes Pregnancy Study Groups or IADPSG) OR Two-Step (American College of Obstetricians & Gynecologists or ACOG)

One-Step:

- 75 gram OGTT with fasting patient, 24-28 weeks gestation, not previously diagnosed
  - Diagnosis – 1 or more values are met or exceeded:
    - Fasting ≥ 92 mg/dL
    - 1 hour ≥ 180 mg/dL
    - 2 hour ≥ 153 mg/dL

Two-Step:

- 50 gram Glucose Load Test (GLT) with non-fasting patient, 24-28 weeks gestation, not previously diagnosed, with 1 hour glucose measurement
  - If glucose is ≥ 130, 135 or 140 mg/dL, proceed to 100 gram OGTT
- 100 gram OGTT with fasting patient
  - Diagnosis – 2 or more values are met or exceeded:
    - Fasting ≥ 95 mg/dL
    - 1 hour ≥ 180 mg/dL
    - 2 hour ≥ 155 mg/dL
    - 3 hour ≥ 140 mg/dL

# Criteria for the Diagnosis of Diabetes – ADA 2021 Guidelines

- One-Step GDM Strategy:
  - Increase the incidence of GDM from 5-6% to 15-20%
  - ADA supports the IADPSG criteria because of the focus on pregnancy outcomes, rather than prediction of maternal diabetes later
  - Advantages: reduced rates of large for gestational age births and preeclampsia, catches women at risk of developing prediabetes, Type 2 diabetes, children who might have a higher risk of obesity
  - Disadvantages: substantial impact on costs, medical infrastructure needs, “medicalizes” normal pregnancies
- Two-Step GDM Strategy:
  - NIH and ACOG support due to lack of evidence of benefits of One-Step strategy and negatives of identifying so many with GDM
  - Advantages: Fasting not required, cost effective
  - Disadvantages: Could miss diagnosing mild GDM (use 1 abnormal result in 100 gram OGTT or use lower GLT cutoff?)
- “Long term studies underway to help find consensus and uniform approach”…

# Closer look: Diagnostic Tests for Diabetes

- Imperfect – variable concordance between tests
- All 3 tests have limitations…
- FPG and 2-hour OGTT (2h OGTT)
  - Inconvenience
    - Fasting – can't opportunistically screen with FPG and 2h OGTT
    - 2h OGTT – unpleasant for many
  - Acute illness impacts FPG and 2h OGTT
  - Preanalytical instability
    - Stringent sample handling – best to use sodium fluoride tubes, collect on ice, centrifuge right away, etc.
  - Intra-individual variability
    - 2h OGTT

# Closer look: Diagnostic Tests for Diabetes

- What about A1c?
  - Recommended by ADA for routine monitoring in 1988
  - Added to ADA diagnostic tests in 2010
  - Can be measured using immunoassays or HPLC (both are most common), chromatography, electrophoresis, enzymatic assays, etc.
    - Standardization of methods & harmonization of results – National Glycohemoglobin Standardization Program (NGSP) and International Federation of Clinical Chemistry (IFCC)
  - A1c Advantages:
    - Doesn’t require fasting
    - Not affected by acute illness, stress or diet
    - Not as much specialized sample handling
    - Lower intra-individual variability
    - Long term measure of glycemic control

# Closer look: Diagnostic Tests for Diabetes

- A1c Disadvantages & Factors that influence interpretation:
  - Age
    - 0.1% A1c decrease by decade
  - Race – maybe?
    - Black people with prediabetes have higher A1c levels than non-Hispanic white people for the same FPG and 2h OGTT concentrations
    - Black people with Type 1 Diabetes have higher average A1c for the same average glucose measured by continuous glucose monitoring
    - No racial differences in the association of A1c with diabetes complications
      - Thresholds for A1c by race probably not needed
  - Renal Failure
    - Chronic renal failure (CRF) is a common complication of diabetes
    - Diabetes is the leading cause of End-Stage Renal Disease
    - RBC survival is less in CRF, which then decreases A1c levels
    - Treatment with erythropoetic agents causes increase in young RBC's, and lowers A1c levels
    - A1c in patients with CRF ≠ glycemic control

# Closer look: Diagnostic Tests for Diabetes

- A1c Disadvantages & Factors that influence interpretation, continued:
  - Iron-Deficiency Anemia (IDA)
    - Opposing findings in several studies; either no difference between IDA and A1c, or IDA increases A1c levels
    - Caution should be used in patients with IDA that have A1c levels near the diagnostic thresholds
  - RBC Life Span & Turnover
    - RBC's approximately 120 days
      - A1c = 7%; 10 days shorter, A1c = 6.4% & 10 days longer, A1c = 7.4%
    - A1c not accurate if RBC life span is altered
      - Hemolytic anemia, severe β-thalassemia
    - Difficult to measure RBC life span, so can't apply a correction factor
    - Significant blood loss or transfusion
  - Conditions that change the A1c to glucose relationship:
    - Pregnancy, Cystic Fibrosis, GPDH deficiency, HIV, etc.
  - Variable glycation rates

# Closer look: Diagnostic Tests for Diabetes

- A1c Disadvantages & Factors that interfere with interpretation
  - Uremia
    - Isocyanic acid, derived from urea, can attach to hemoglobin (carbamylation)
    - Doesn’t interfere with most contemporary methods
  - Hemoglobin Variants
    - >1200 variants
    - β gene is responsible for 70% of these variants
    - Can’t measure A1c in anyone that is homozygous for these variants

- We have 3 tests to screen for Diabetes, do we need more???
  - What about glycated proteins?
    - Could they minimize the limitations of glucose testing?
    - Could they be independent of RBC’s?

# Closer look: Diagnostic Tests for Diabetes

**Clinical Chemistry** 68:3
379–381 (2022)

**Editorial**

## Glycated Albumin: Added Value or Redundancy in Diabetes Care?

M. Sue Kirkman[a,*] and David B. Sacks [b]

**Clinical Chemistry** 68:3
413–421 (2022)

**Endocrinology and Metabolism**

## Glycated Albumin for the Diagnosis of Diabetes in US Adults

Michael Fang,[a] Natalie Daya ,[a] Josef Coresh,[a] Robert H. Christenson,[b] and Elizabeth Selvin [a,*]

**Clinical Chemistry** 68:3
422–430 (2022)

**Epidemiological Studies**

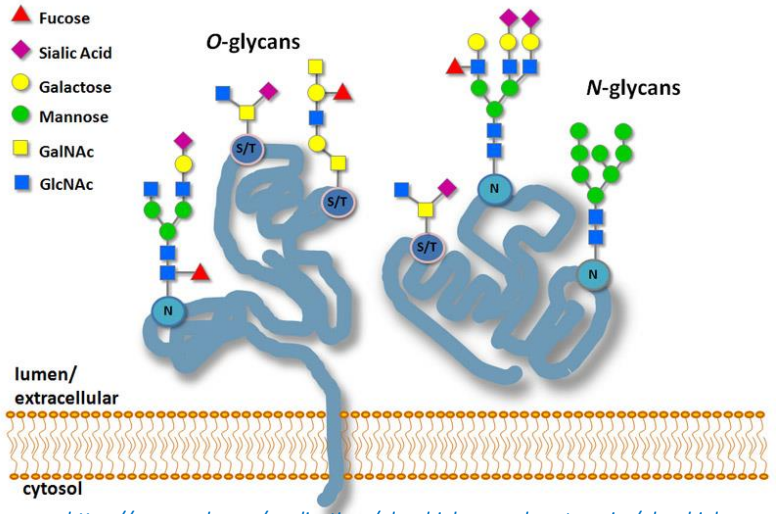
## Glycated Albumin and Risk of Mortality in the US Adult Population

Mary R. Rooney ,[a] Natalie Daya ,[a] Olive Tang,[a] John William McEvoy,[b] Josef Coresh,[a] Robert H. Christenson,[c] and Elizabeth Selvin [a,*]

# Glycosylation versus Glycation…

- Glycosylation – post-translational process that attaches oligosaccharides to proteins with the help of an enzyme
  - Alters protein function, protein life span, interactions with other proteins
  - Requires ATP
  - Regulated process, occurs in the ER or Golgi apparatus
  - Occurs with immature/unmodified proteins, increases stability of the protein

- Glycation – process that attaches monosaccharides (usually glucose) to a protein without enzymes
  - Glycated hemoglobin – condensation of Glucose with Lysine to form an unstable Schiff base (or pre-HbA1c), which then dissociates or undergoes an Amadori rearrangement to form a more stable ketoamine (HbA1c)
  - Not a regulated process
  - Occurs with mature proteins, makes them non-functional, decreases stability




https://www.neb.com/applications/glycobiology-and-proteomics/glycobiology

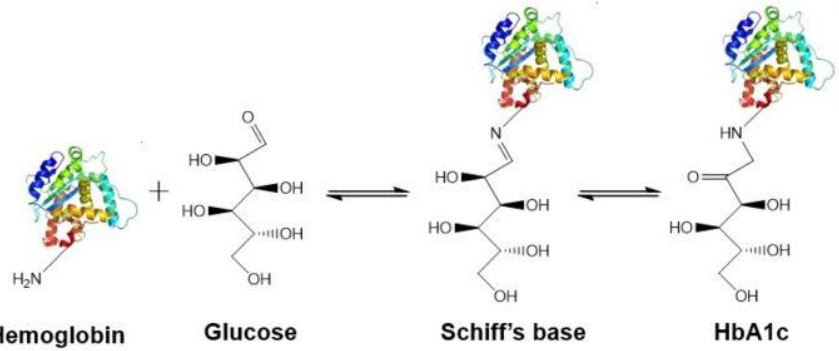



Figure 1. Glycation process between Hemoglobin and glucose

https://www.sciencedirect.com/science/article/pii/S0956566318304500

# Glycated Serum Proteins

- Advantages:
  - Turn over more quickly than RBC's
    - Albumin has a half-life of 14-20 days
    - Intermediate glycemic control
  - Not influenced by RBC life span or hemoglobin variants
  - Not influenced by hemolysis or blood transfusion

- Fructosamine, Glycated Albumin or Advanced Glycation End-products (AGE's)

# Fructosamine

- Common name → 1-amino-1-deoxy fructose
- Generic name → plasma protein ketoamines
- All glycated serum proteins are fructosamines
- Since albumin is the most abundant serum protein, most fructosamine is likely glycated albumin
  - Globulins and lipoproteins
- Readily automated (vendors with FDA-approved fructosamine assays)
  - Enzymatic/Colorimetric – lower pH, fructosamine acts a reducing agent
- Cheaper (?) than A1c assays
- Disagreement/unknown:
  - Independent of serum protein concentrations?
  - Does a correction factor need to be applied when serum proteins are low?
- Not valid when Albumin is <3 g/dL

# Fructosamine

- Earliest generations of Fructosamine assays were not very specific
  - Picked up other reducing substances in serum, like urates
  - Low clinical utility
- Starting in 1991 – Fructosamine assays modified
  - Specificity and precision improved
  - Strong correlation with HbA1c
  - Shown to have prognostic value for development of diabetes and microvascular complications
  - Automated assays

# Fructosamine

- Special populations where A1c doesn’t work well
  - GDM
    - Hyperglycemia develops rapidly, RBC turnover altered, A1c not useful
  - ESRD, Anemia, Transfusion
- Combine A1c with Fructosamine?
  - Could this better identify patients with prediabetes?
  - Studies have been neutral
- Lack of studies linking fructosamine to long term complications of diabetes
  - No treatment targets for fructosamine

# Glycated Albumin

- Albumin
  - 2/3 of total serum protein, >70% of total glycated serum proteins
  - 35 glycation sites (HPLC)
- Glycated Albumin
  - Expressed as a percentage to total albumin
  - FDA-approved open channel assays – Enzymatic/Colorimetric
  - Assays lack standardization
    - Varying reference intervals
  - Glycated albumin values are higher in Blacks than Whites
  - Altered albumin metabolism may alter glycated albumin levels, independent of glycemia
    - Nephrotic syndrome, cirrhosis, thyroid disease, hyperuricemia, hypertriglyceridemia, smoking
    - Liver, Renal and Thyroid diseases

# Glycated Albumin

- Limited clinical utility due to lack of studies relating it to clinical outcomes
  - Chronic complications of diabetes?
  - 2015-2021 studies looking at link between glycated albumin and microvascular and cardiovascular disease
- Potential role in diagnosis of prediabetes?
  - Used in combination with A1c in African immigrants, detected 78% of prediabetes cases
    - Compared to 50% of A1c alone, or 72% with A1c and Fructosamine
  - Japan – similar study, found Glycated Albumin comparable to A1c
    - Fructosamine – unsuitable
    - Japan - Glycated albumin is being used as a diabetes screening test for blood donors
- Non-obese patients – is Glycated Albumin better than A1c?
  - Japanese studies & the study with African immigrants - Lower BMI, Glycated Albumin better than A1c for detecting prediabetes
  - Opposite true?
    - Higher BMI, does Glycated Albumin underestimate glycemic index?

# Advanced Glycation End-products (AGE's)

- Glycation of tissue proteins - hyperglycemia and chronic complications of diabetes
  - Non-enzymatic attachment of glucose to proteins, lipids, nucleic acids can produce stable Amadori products
    - Further modification into AGE's
- Irreversible – cross-linking, altered structure/function
- More than 20 AGE's identified (glucosepane most common)
- Hyperglycemia corrected, AGE's remain
- AGE's accumulate with age, hyperglycemia also increases formation
  - Patients with diabetes have more AGE's than age-matched non-diabetics
- AGE's in the diet can also increase AGE levels in tissues
- AGE's may contribute to microvascular and cardiovascular complications of diabetes, along with nephropathy, retinopathy, neuropathy

# Advanced Glycation End-products (AGE's)

- AGE's might be better than A1c to predict retinopathy and nephropathy
- AGE's provide additional info to predict microvascular complications
  - Some patients with good glycemic control still develop complications (& vice versa)
- Patients taking diabetes medications have lower AGE levels
  - Can drugs inhibiting AGE formation reduce diabetic complications?
- Measurement methods:
  - Fluorescence – skin
    - Not all AGE's fluoresce
    - Not specific, other skin protein fluoresce in the same spectra as AGE's
  - Assays for specific AGE's
    - Immunoassays
      - Heterogenous – complex
      - Lack of assay standardization
    - Isotope dilution mass spectrometry
      - Works great
      - Expensive, specialized equipment, highly trained lab personnel, lack of commercial reagents, etc.

# Advanced Glycation End-products (AGE's)

- AGE receptor or RAGE
  - Receptor on endothelial and renal cells that AGE's can bind to producing inflammatory cytokines and oxidative stress
  - Can be broken down into a soluble form, or sRAGE
    - sRAGE can be measured (commercially available ELISA)
  - Important in causing diabetes complications?
  - Disagreement on clinical value
    - Do all AGE's activate RAGE?
    - Do levels of RAGE correlate with diabetes complications?
    - Genetics play a role in how much RAGE is expressed?

# Glycated Albumin for Diagnosis of Diabetes?

- Two 2022 studies from Johns Hopkins and the University of Maryland School of Medicine
  - Used samples collected by the CDC's National Health and Nutrition Examination Survey (NHANES) from 1999-2004
  - Each volunteer answered a series of questions regarding current health and lifestyle habits, had a physical exam & fasting blood was drawn
    - Used 3 survey cycles (1999-2000, 2001-2002 and 2003-2004)
  - Measured glucose, A1c and glycated albumin in 12,915 individuals (after excluding >2,500 participants for pregnancy, missing information on diabetic status, sample issues, etc.)
- What cut points should be used for glycated albumin?
- Can glycated albumin be used for the diagnosis of diabetes?
- Can glycated albumin predict all-cause and/or cardiovascular mortality?

# Glycated Albumin for Diagnosis of Diabetes?

- 1999-2004 data:
  - No diabetes: 45.5 years old, 51.3% female, 73.6% non-Hispanic whites
  - Glycated albumin levels ranged from 10.5% to 17.8%
- What percentile glycated albumin corresponds to A1c of 6.5% or a FPG of 126 mg/dL?
  - Weighted Pearson correlations and scatterplots
  - Sensitivity, specificity, receiver operating curve, positive and negative predictive values

**Table 1.** Equipercentile values of Hb $A_{1c}$, fasting plasma glucose, and glycine albumin in US adults without diagnosed diabetes, NHANES 1999-2004 (n = 4785).

| Hb $A_{1c}$, % | FPG, mg/dL[b] | Glycated albumin, % | Percentile |
|---|---|---|---|
| 4.7 | 79 | 10.5 | 2nd |
| 5.2 | 89 | 12.2 | 25th |
| 5.4 | 95 | 13.0 | 50th |
| 5.5 | 100 | 13.8 | 69th |
| 5.6 | 102 | 14.0 | 75th |
| 5.7 | 105 | 14.4 | 82nd |
| 6.2 | 126[a] | 16.5 | 97th |
| 6.5[a] | 138 | 17.8 | 98th |

[a]Hb $A_{1c}$ of 6.5% and FPG of 126 mg/dL (6.99 mmol/L) are the clinical cut points for diabetes diagnosis.
[b]To convert glucose concentrations from mg/dL to mmol/L, multiply by 0.05551.

# Glycated Albumin for Diagnosis of Diabetes?

- Comparison of diagnostic accuracy
  - Compared glycated albumin to FPG ≥ 126 mg/dL, A1c ≥ 6.5%, FPG ≥ 126 mg/dL OR A1c ≥ 6.5%, FPG ≥ 126 mg/dL AND A1c ≥ 6.5%
    - Glycated albumin performed well in all 4 categories, but better with more specific definitions of diabetes
  - Glycated albumin was just as good (but not better) than A1c and FPG in subgroups like anemia, iron deficiency, chronic kidney disease
- Looked at specificity and sensitivity using both the 16.5% and 17.8% glycated albumin cutoffs compared to FPG and A1c
  - Excellent specificity, low/moderate sensitivity
  - May miss patients with milder hyperglycemia

# Glycated Albumin for Diagnosis of Diabetes?

- Glycated albumin might be a suitable alternative or complementary test for the diagnosis of diabetes
  - Smaller studies have shown the same
  - Differences in disease severity (like chronic kidney disease using the NHANES samples which likely had mild/early stage kidney disease) might explain differences between studies
- Older age is a risk factor for increased glycated albumin, A1c and FPG
- Obesity not consistently associated with increased glycated albumin, but associated with increased A1c and FPG
  - Previous studies have shown the same
  - Might be related to inflammation (higher in obese individuals), and increased albumin turnover
- Weaker correlation between Glycated Albumin, A1c and FPG at normal levels, but strong at higher (or diabetic) levels

# Glycated Albumin and Long Term Outcomes?

- Hypothesized that all-cause and cardiovascular mortality predicted by glycated albumin would be similar to A1c
- Using the same NHANES group:
  - Measured A1c and Glycated Albumin
  - Set up 4 groups of percentile cutoffs for Glycated Albumin similar to A1c cutoffs in individuals without diabetes
    - <5% A1c for <12$^{th}$ %ile (<11.6%) Glycated Albumin – “low”
    - 5.0-5.6% A1c for 13$^{th}$ – 82$^{nd}$ %ile (11.6%-14.3%) Glycated Albumin – “normoglycemia”
    - 5.7-6.4% A1c for 83$^{rd}$ – 97$^{th}$ %ile (14.4%-17.7%) Glycated Albumin – “prediabetes”
    - ≥6.5% A1c for ≥98$^{th}$ %ile (>17.8%) Glycated Albumin – “undiagnosed diabetes”
  - Statistical Analysis

# Glycated Albumin and Long Term Outcomes?

- Adults without diagnosed diabetes had a median Glycated Albumin of 13% and an A1c of 5.4%
  - Adults without diagnosed diabetes and a Glycated Albumin ≥17.8% had more cardiometabolic risk factors
- Adults with diabetes had a median Glycated Albumin of 17.8% and an A1c of 7.0%
  - Adults with diagnosed diabetes, glycated albumin ≥24.5% were younger, less likely to be obese, more likely to have chronic kidney disease
- Inverse relationship with obesity and Glycated Albumin

**Table 1.** Baseline characteristics by glycated albumin categories among US adults aged 18 or older with and without diagnosed diabetes, NHANES (1999-2004).[a,b]

| | No diagnosed diabetes | | | | Diagnosed diabetes | | |
|---|---|---|---|---|---|---|---|
| Glycated albumin Categories Percentile | <11.6% <12th | 11.6%-14.3% 13th-82nd | 14.4%-17.7% 83rd-97th | ≥17.8% ≥98th | <17.9% <50th | 17.9%-24.4% 50th-83rd | ≥24.5% ≥84th |
| Unweighted n | 1300 | 7991 | 2153 | 269 | 582 | 407 | 213 |
| Mean HbA1c, % | 5.3 (0.01) | 5.4 (0.01) | 5.5 (0.02) | 7.4 (0.29) | 6.3 (0.03) | 7.8 (0.07) | 10.2 (0.18) |
| Mean age, years | 38.8 (0.6) | 43.8 (0.4) | 52.3 (0.7) | 55.4 (1.2) | 59.6 (0.7) | 60.4 (1.1) | 54.1 (1.4) |
| Female, % | 46.4 (1.3) | 50.0 (0.7) | 60.6 (1.4) | 39.3 (4.5) | 51.0 (1.9) | 49.4 (2.8) | 41.8 (5.3) |
| Race/ethnicity, % | | | | | | | |
| Non-Hispanic White | 75.0 (3.1) | 74.0 (1.6) | 68.7 (2.2) | 60.9 (6.0) | 69.6 (3.7) | 64.2 (3.4) | 56.8 (4.8) |
| Non-Hispanic Black | 3.9 (0.5) | 9.1 (0.9) | 15.4 (1.8) | 16.3 (2.7) | 11.3 (1.8) | 14.0 (2.2) | 19.3 (3.4) |
| Mexican American | 9.9 (1.5) | 7.4 (0.9) | 4.2 (0.7) | 7.5 (2.0) | 6.4 (1.3) | 7.5 (1.5) | 9.5 (2.8) |
| Other Hispanic | 11.1 (2.2) | 9.5 (1.0) | 11.8 (2.1) | 15.4 (4.2) | 12.7 (2.5) | 14.3 (3.0) | 14.4 (4.5) |
| Education, % | | | | | | | |
| Less than high school | 20.1 (1.4) | 19.3 (0.7) | 21.3 (1.6) | 42.5 (3.5) | 29.0 (2.3) | 37.6 (3.1) | 36.2 (4.4) |
| High school diploma | 31.7 (1.8) | 26.7 (0.8) | 24.5 (1.2) | 17.4 (3.1) | 28.3 (2.6) | 21.0 (2.1) | 23.1 (3.7) |
| More than high school | 48.1 (1.7) | 54.0 (1.2) | 54.1 (2.2) | 40.2 (3.3) | 42.7 (2.7) | 41.4 (3.2) | 40.7 (4.1) |
| Body mass index categories, % | | | | | | | |
| Normal weight, <25 kg/m$^2$ | 19.2 (1.4) | 37.1 (0.8) | 48.9 (1.6) | 22.1 (3.7) | 13.4 (1.8) | 15.9 (2.5) | 27.6 (5.3) |
| Overweight, 25 to <30 kg/m$^2$ | 30.7 (2.1) | 36.2 (0.7) | 31.9 (1.5) | 36.3 (3.4) | 28.2 (2.6) | 28.0 (2.7) | 37.1 (5.1) |
| Obese, ≥30 kg/m$^2$ | 50.2 (2.1) | 26.7 (0.8) | 19.2 (1.0) | 41.6 (4.5) | 58.5 (2.7) | 56.1 (3.1) | 35.4 (5.2) |
| Smoking status, % | | | | | | | |
| Current | 36.8 (2.0) | 25.1 (0.8) | 15.8 (1.5) | 18.2 (3.1) | 23.1 (2.1) | 15.3 (2.0) | 26.1 (4.3) |
| Former | 22.0 (1.8) | 24.7 (0.8) | 27.1 (1.0) | 36.6 (4.7) | 34.6 (2.3) | 34.0 (3.6) | 30.6 (4.9) |
| Never | 41.2 (2.4) | 50.2 (0.9) | 57.1 (1.8) | 45.2 (3.8) | 42.4 (2.0) | 50.8 (3.5) | 43.3 (5.0) |
| Moderate/vigorous physical activity, % | 67.2 (1.7) | 67.9 (0.9) | 64.3 (1.8) | 53.6 (6.1) | 56.8 (3.1) | 54.5 (3.3) | 51.7 (4.5) |
| Hypertension, % | 26.4 (1.9) | 26.6 (0.8) | 38.1 (1.9) | 43.5 (4.4) | 69.8 (2.5) | 67.8 (3.3) | 61.9 (5.3) |
| Hypercholesterolemia, % | 24.3 (1.9) | 23.2 (0.7) | 22.9 (1.1) | 29.7 (3.9) | 45.1 (2.5) | 48.1 (3.0) | 45.2 (5.8) |
| Chronic kidney disease, % | 9.8 (1.1) | 10.2 (0.5) | 19.2 (1.2) | 33.5 (4.5) | 36.8 (2.4) | 45.1 (2.9) | 49.1 (3.1) |
| Cardiovascular disease, % | 5.9 (0.8) | 7.0 (0.4) | 11.0 (1.0) | 17.1 (2.7) | 29.8 (2.7) | 31.2 (3.3) | 30.5 (3.7) |
| Anemia, % | 2.2 (0.4) | 4.5 (0.4) | 10.4 (0.7) | 9.3 (1.9) | 11.9 (1.5) | 17.5 (2.5) | 14.3 (3.4) |
| Iron deficiency, % | 7.3 (0.8) | 6.7 (0.3) | 8.7 (0.8) | 7.0 (1.7) | 8.5 (1.3) | 6.2 (1.4) | 5.6 (2.8) |

[a]Unweighted n;
Weighted percentages (SE) or mean (SE).
[b]Missing values for education n = 20, body mass index n = 344, smoking status n = 1351, moderate/vigorous physical activity n = 513, hypertension n = 390, hypercholesterolemia n = 1, chronic kidney disease n = 241, cardiovascular disease n = 1390, anemia n = 4, iron deficiency n = 1061.

# Glycated Albumin and Long Term Outcomes?

- Median follow-up of 16.8 years
- 2,818 deaths, 652 with cardiovascular disease as the primary cause
  - Increased A1c or Glycated Albumin had higher risks of all-cause mortality and cardiovascular mortality
  - Strongest associations in people with diagnosed diabetes
  - Looked at race/ethnicity and Glycated Albumin levels – similar higher risks of all-cause mortality
  - Looked at adults with chronic kidney disease, iron deficiency, anemia and diagnosed diabetes - Increased A1c and Glycated Albumin levels & higher risks of mortality

# Glycated Albumin and Long Term Outcomes?

- Higher levels of Glycated Albumin were associated with higher levels of mortality (all-cause and cardiovascular), in adults with and without diagnosed diabetes
  - Similar to what is seen with A1c – strong correlation
  - Glycated Albumin has similar prognostic value for mortality compared to A1c
- Use Glycated Albumin when A1c is not reliable
  - Conditions that alter hemoglobin levels, RBC levels or turnover, on treatments that affect either
- High correlation between Glycated Albumin, A1c and FPG
  - Differences due to half-life, obesity, inflammation, genetics
  - Obesity and inverse relationship to Glycated Albumin unclear but match previous studies

# Glycated Albumin for Diagnosis & Prognosis of Diabetes?

- Strengths and Limitations of the 2022 studies (Diagnosis and Outcomes/Prognosis Studies)
  - Strengths:
    - NHANES samples - nationally representative sample of adults
    - Very large sample size
    - Measured FPG, A1c and Glycated Albumin using contemporary and/or standardized methods
  - Limitations:
    - OGTT's were not administered to the NHANES study group
    - Glycated albumin was measured in samples that were stored for >10 years
    - Can't connect glycated albumin to future diabetes or complications (no additional testing in later years)
    - Self-reported information – couldn't distinguish between Type I and Type 2 Diabetes

# Take Home Messages on the Diagnosis of Diabetes & the Role of Glycated Proteins…

- 4 major classes of Diabetes Mellitus; Type 1, Type 2, Gestational and Other Causes (genetic, drugs, diseases)
- Prevalence of diagnosed diabetes has been increasing over the years
- Diagnosed with 2 abnormal lab tests (casual plasma glucose ≥ 200 mg/dL, FPG ≥126 mg/dL, 2 hour OGTT ≥200 mg/dL, or an A1c ≥6.5%)
- Current lab tests have limitations; ongoing research into clinical utility of glycated proteins
- Glycated Albumin shows most promise as an intermediate measure of glycemia…
  - Could be used as an alternative diagnostic test for diabetes (similar accuracy, specificity, not as sensitive as A1c and/or FPG)
  - Could be used prognostically, and to help monitor/predict future diabetes complications (cardiovascular and metabolic)

- Questions???