

A1C Versus Glucose Testing: A Comparison

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Diabetes was originally identified by the presence of glucose in the urine. Almost 2,500 years ago it was noticed that ants were attracted to the urine of some individuals. In the 18th and 19th centuries the sweet taste of urine was used for diagnosis before chemical methods became available to detect sugars in the urine. Tests to measure glucose in the blood were developed over 100 years ago, and hyperglycemia subsequently became the sole criterion recommended for the diagnosis of diabetes. Initial diagnostic criteria relied on the response to an oral glucose challenge, while later measurement of blood glucose in an individual who was fasting also became acceptable. The most widely accepted glucose-based criteria for diagnosis are fasting plasma glucose (FPG) ≥ 126 mg/dL or a 2-h plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test (OGTT) on more than one occasion (1,2). In a patient with classic symptoms of diabetes, a single random plasma glucose ≥ 200 mg/dL is considered diagnostic (1). Before 2010 virtually all diabetes societies recommended blood glucose analysis as the exclusive method to diagnose diabetes. Notwithstanding these guidelines, over the last few years many physicians have been using hemoglobin A1C to screen for and diagnose diabetes (3). Although considered the “gold standard” for diagnosis, measurement of glucose in the blood is subject to several limitations, many of which are not widely appreciated. Measurement of A1C for diagnosis is appealing but has some inherent limitations. These issues have become the focus of considerable attention with the recent publication of the Report of the International Expert Committee that recommended

the use of A1C for diagnosis of diabetes (4), a position that has been endorsed (at the time of writing) by the American Diabetes Association (ADA) (1), the Endocrine Society, and in a more limited fashion by American Association of Clinical Endocrinologists/American College of Endocrinology (5). This review will provide an overview of the factors that influence glucose and A1C testing.

FACTORS CONTRIBUTING TO VARIATION IN RESULTS

—Before addressing glucose and A1C, it is important to consider the factors that impact the results of any blood test. While laboratory medicine journals have devoted some discussion to the sources of variability in results of blood tests, this topic has received little attention in the clinical literature. Factors that contribute to variation can conveniently be divided into three categories, namely biological, pre-analytical, and analytical. Biological variation comprises both differences within a single person (termed intraindividual) and between two or more people (termed interindividual). Preanalytical issues pertain to the specimen before it is measured. Analytical differences result from the measurement procedure itself. The influence of these factors on both glucose and A1C results will be addressed in more detail below.

GLUCOSE MEASUREMENT

FPG

Measurement of glucose in plasma of fasting subjects is widely accepted as a diagnostic criterion for diabetes (1,2). Advantages include inexpensive assays on automated instruments that are available

in most laboratories worldwide (Table 1). Nevertheless, FPG is subject to some limitations. One report that analyzed repeated measurements from 685 fasting participants without diagnosed diabetes from the Third National Health and Nutrition Examination Survey (NHANES III) revealed that only 70.4% of people with FPG ≥ 126 mg/dL on the first test had FPG ≥ 126 mg/dL when analysis was repeated ~ 2 weeks later (6). Numerous factors may contribute to this lack of reproducibility. These are elaborated below.

Biological variation. Fasting glucose concentrations vary considerably both in a single person from day to day and also between different subjects. Intraindividual variation in a healthy person is reported to be 5.7–8.3%, whereas interindividual variation of up to 12.5% has been observed (6,7). Based on a CV (coefficient of variation) of 5.7%, FPG can range from 112–140 mg/dL in an individual with an FPG of 126 mg/dL. (It is important to realize that these values encompass the 95% confidence interval, and 5% of values will be outside this range.)

Preanalytical variation. Numerous factors that occur before a sample is measured can influence results of blood tests. Examples include medications, venous stasis, posture, and sample handling. The concentration of glucose in the blood can be altered by food ingestion, prolonged fasting, or exercise (8). It is also important that measurements are performed in subjects in the absence of intercurrent illness, which frequently produces transient hyperglycemia (9). Similarly, acute stress (e.g., not being able to find parking or having to wait) can alter blood glucose concentrations.

Samples for fasting glucose analysis should be drawn after an overnight fast (no caloric ingestion for at least 8 h), during which time the subject may consume water ad lib (10). The requirement that the subject be fasting is a considerable practical problem as patients are usually not fasting when they visit the doctor, and it is often inconvenient to return for phlebotomy. For example, at an HMO affiliated with an academic medical center, 69% (5,752 of 8,286) of eligible participants were screened for diabetes (11).

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See accompanying editorial, p. 524.

Table 1—FPG for the diagnosis of diabetes**Advantages**

- Glucose assay easily automated
- Widely available
- Inexpensive
- Single sample

Disadvantages

- Patient must fast ≥ 8 h
- Large biological variability
- Diurnal variation
- Sample not stable
- Numerous factors alter glucose concentrations, e.g., stress, acute illness
- No harmonization of glucose testing
- Concentration varies with source of the sample (venous, capillary, or arterial blood)
- Concentration in whole blood is different from that in plasma
- Guidelines recommend plasma, but many laboratories measure serum glucose
- FPG less tightly linked to diabetes complications (than A1C)
- Reflects glucose homeostasis at a single point in time

However, FPG was performed on only 3% (152) of these individuals. Ninety-five percent (5,452) of participants were screened by random plasma glucose measurements, a technique not consistent with ADA recommendations. In addition, blood drawn in the morning as FPG has a diurnal variation. Analysis of 12,882 participants aged 20 years or older in NHANES III who had no previously diagnosed diabetes revealed that mean FPG in the morning was considerably higher than in the afternoon (12). Prevalence of diabetes (FPG ≥ 126 mg/dL) in afternoon-examined patients was half that of participants examined in the morning. Other patient-related factors that can influence the results include food ingestion when supposed to be fasting and hypocaloric diet for a week or more prior to testing.

Glucose concentrations decrease in the test tube by 5–7% per hour due to glycolysis (13). Therefore, a sample with a true blood glucose value of 126 mg/dL would have a glucose concentration of ~ 110 mg/dL after 2 h at room temperature. Samples with increased concentrations of erythrocytes, white blood cells, or platelets have even greater rates of glycolysis. A common misconception is that sodium fluoride, an inhibitor of glycolysis, prevents glucose consumption. While fluoride does attenuate in vitro glycolysis, it has no effect on the

rate of decline in glucose concentrations in the first 1 to 2 h after blood is collected, and glycolysis continues for up to 4 h in samples containing fluoride (14). The delay in the glucose stabilizing effect of fluoride is most likely the result of glucose metabolism proximal to the fluoride target enolase (15). After 4 h, fluoride maintains a stable glucose concentration for 72 h at room temperature (14). A recent publication showed that acidification of the blood sample inhibits glycolysis in the first 2 h after phlebotomy (16), but the collection tubes used in that study are not commercially available. Placing tubes in ice water immediately after collection may be the best method to stabilize glucose initially (2,16), but this is not a practical solution in most clinical situations. Separating cells from plasma within minutes is also effective, but impractical.

The nature of the specimen analyzed can have a large influence on the glucose concentration. Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended by both the ADA and World Health Organization (WHO) for diagnosis (1,2). However, many laboratories measure glucose in serum, and these values may differ from those in plasma. There is a lack of consensus in the published literature, with glucose concentrations in plasma reported to be lower than (17), higher than (16,18,19), or the same as (20) those in serum. Importantly, glucose concentrations in whole blood are 11% lower than those in plasma because erythrocytes have a lower water content than plasma (13). The magnitude of the difference in glucose between whole blood and plasma changes with hematocrit. Most devices (usually handheld meters) that measure glucose in capillary blood use whole blood. While the majority of these report a plasma equivalent glucose value (21), this result is not accurate in patients with anemia (22) (unless the meter measures hematocrit).

The source of the blood is another variable. Although not a substantial problem in the fasting state, capillary glucose concentrations can be 20–25% higher (mean of 30 mg/dL) than venous glucose during an OGTT (23). This finding has practical implications for the OGTT, particularly because the WHO deems capillary blood samples acceptable for the diagnosis of diabetes (2).

Analytical variation. Glucose is measured in central laboratories almost exclusively using enzymatic methods, predominantly with glucose oxidase or

hexokinase (24). The following terms are important for understanding measurement: *accuracy* indicates how close a single measurement is to the “true value” and *precision* (or repeatability) refers to the closeness of agreement of repeated measurements under the same conditions. Precision is usually expressed as CV; methods with low CV have high precision. Numerous improvements in glucose measurement have produced low within-laboratory imprecision (CV $< 2.5\%$). Thus, the analytical variability is considerably less than the biological variability, which is up to 8.3%. Nevertheless, accuracy of measurement remains a problem. There is no program to standardize results among different instruments and different laboratories. Bias (deviation of the result from the true value) and variation among different lots of calibrators can reduce the accuracy of glucose results. (A calibrator is a material of known concentration that is used to adjust a measurement procedure.) A comparison of serum glucose measurements (target value 98.5 mg/dL) was performed among $\sim 6,000$ laboratories using 32 different instruments (25). Analysis revealed statistically significant differences in bias among clinical laboratory instruments, with biases ranging from -6 to $+7$ mg/dL (-6 to $+7\%$) at a glucose concentration of 100 mg/dL. These considerable differences among laboratories can result in the potential misclassification of $> 12\%$ of patients (4). Similarly, inspection of a College of American Pathologists (CAP) survey comprising $> 5,000$ laboratories revealed that one-third of the time the results among instruments for an individual measurement could range between 141 and 162 mg/dL (26). This variation of 6.9% above or below the mean reveals that one-third of the time the glucose results on a single patient sample measured in two different laboratories could differ by 14%.

OGTT

The OGTT evaluates the efficiency of the body to metabolize glucose and for many years has been used as the “gold standard” for diagnosis of diabetes. An increase in postprandial glucose concentration usually occurs before fasting glucose increases. Therefore, postprandial glucose is a sensitive indicator of the risk for developing diabetes and an early marker of impaired glucose homeostasis (Table 2). Published evidence suggests that an increased 2-h plasma glucose during an OGTT is a better predictor of both all-cause

Table 2—OGTT for the diagnosis of diabetes

Advantages
<ul style="list-style-type: none"> • Sensitive indicator of risk of developing diabetes • Early marker of impaired glucose homeostasis
Disadvantages
<ul style="list-style-type: none"> • Lacks reproducibility • Extensive patient preparation • Time-consuming and inconvenient for patients • Unpalatable • Expensive • Influenced by numerous medications • Subject to the same limitations as FPG, namely, sample not stable, needs to be performed in the morning, etc.

mortality and cardiovascular mortality or morbidity than the FPG (27,28). The OGTT is accepted as a diagnostic modality by the ADA, WHO/International Diabetes Federation (IDF) (1,2), and other organizations. However, extensive patient preparation is necessary to perform an OGTT. Important conditions include, among others, ingestion of at least 150 g of dietary carbohydrate per day for 3 days prior to the test, a 10- to 16-h fast, and commencement of the test between 7:00 A.M. and 9:00 A.M. (24). In addition, numerous conditions other than diabetes can influence the OGTT (24). Consistent with this, published evidence reveals a high degree of intraindividual variability in the OGTT, with a CV of 16.7%, which is considerably greater than the variability for FPG (6). These factors result in poor reproducibility of the OGTT, which has been documented in multiple studies (29,30). The lack of reproducibility, inconvenience, and cost of the OGTT led the ADA to recommend that FPG should be the preferred glucose-based diagnostic test (1). Note that glucose measurement in the OGTT is also subject to all the limitations described for FPG (Table 1).

A1C MEASUREMENT—A1C is formed by the nonenzymatic attachment of glucose to the N-terminal valine of the β -chain of hemoglobin (24). The life span of erythrocytes is ~120 days, and consequently A1C reflects long-term glycemic exposure, representing the average glucose concentration over the preceding 8–12 weeks (31,32). Both observational studies (33) and controlled clinical trials (34,35) demonstrate strong correlation between A1C and retinopathy, as well as

other microvascular complications of diabetes. More importantly, the A1C value predicts the risk of microvascular complications and lowering A1C concentrations (by tight glycemic control) significantly reduces the rate of progression of microvascular complications (34,35).

Biological variation. Intraindividual variation of A1C in nondiabetic people is minimal (36) (Table 3), with CV <1% (37). Variability between individuals is greater. Data derived from several investigators imply that A1C values may not be constant among all individuals despite the presence of similar blood glucose or fructosamine concentrations (38). Some investigators have termed this a “glycation gap” and proposed that there are differences in the rate of glycation of hemoglobin (“low and high glycaters”) (39). Studies of twins with type 1 diabetes support a genetic contribution to A1C values (40), and heritability of the glycation gap was observed in healthy female twins (41). However, the glycation gap is essentially a measure of A1C adjusted for fructosamine. Importantly, measurement of fructosamine, which is glycated albumin and protein, suffers from several limitations (42). In addition, some authors have questioned the statistical analysis (which is not standard) used in determining the glycation gap and noted the statistical tautology that the outcome is correlated with the residual from a regression (43). Importantly, the postulate of a glycation gap remains unsubstantiated by data because glycation rates cannot be measured accurately in vivo. In addition, the hemoglobin glycation index (difference between observed A1C and that predicted from blood glucose) is not an independent predictor of the risk of microvascular complications (43), and the possible clinical significance of the glycation gap is unclear.

Accumulating evidence supports the hypothesis that race influences A1C. Initial studies in patients with diabetes reported statistically significant differences in A1C concentrations among races (44). While adjusted for factors that may influence glycemia, it remains possible that these differences may be due to variations in glycemic control. More compelling support was provided in NHANES III where Mexican Americans and blacks had higher average A1C values than whites (45,46). Similar findings were observed in adults with impaired glucose tolerance in the Diabetes Prevention Program (47) and validated in a cross-sectional analysis

Table 3—A1C for the diagnosis of diabetes

Advantages
<ul style="list-style-type: none"> • Subject need not be fasting • Samples may be obtained any time of the day • Very little biological variability • Sample stable • Not altered by acute factors, e.g., stress, exercise • Reflects long-term blood glucose concentration • Assay standardized across instruments • Accuracy of the test is monitored • Single sample, namely whole blood • Concentration predicts the development of microvascular complications of diabetes • Used to guide treatment
Disadvantages
<ul style="list-style-type: none"> • May be altered by factors other than glucose, e.g., change in erythrocyte life span, ethnicity • Some conditions interfere with measurement, e.g., selected hemoglobinopathies • May not be available in some laboratories/areas of the world • Cost

of two studies (48). Collectively these data suggest that there are differences in A1C concentrations among racial groups. However, it is not clear that these changes have clinical significance. A1C was measured in the Atherosclerosis Risk in Communities (ARIC) study in 11,092 adults who did not have a history of diabetes or cardiovascular disease (49). Consistent with prior publications, blacks had mean A1C values 0.4% higher than whites. Nevertheless, race did not modify the association between the A1C value and adverse cardiovascular outcomes and death (49). Because follow-up revealed that blacks with biochemically defined incident diabetes were significantly less likely than whites to report having received a diagnosis of diabetes by a physician, the authors speculate that delays in diagnosis may explain the higher A1C values in blacks.

The molecular mechanism underlying the racial and ethnic differences remains to be established. Possibilities include differences in rates of glucose uptake into erythrocytes, rates of intracellular glucose metabolism, rates of glucose attachment to or release from hemoglobin or erythrocyte life span (50,51). Regardless of the mechanism, the variations in A1C concentrations are

relatively small ($\leq 0.4\%$), and no consensus has been reached on whether different cutoffs should be used for different races.

Preanalytical variation. Most factors that alter FPG do not significantly affect A1C concentrations. Acute illness, short-term lifestyle changes (e.g., exercise), recent food ingestion, and sample handling do not significantly alter A1C values (Table 3). Importantly, whole blood samples are stable for 1 week at 4°C and for at least 1 year at -70°C or colder (13,52).

The interpretation of A1C depends on the erythrocytes having a normal life span. Patients with hemolytic disease or other conditions with shortened erythrocyte survival have a substantial reduction in A1C (53). Similarly, individuals with acute blood loss have spuriously low A1C values because of an increased fraction of young erythrocytes. False increases in A1C have been reported with some methods in patients with hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, or chronic ingestion of salicylates (13). Because most interferences are method specific, in many cases they can be overcome by selecting an appropriate method that is not subject to the interference.

Individuals with iron deficiency anemia have increased A1C and fructosamine concentrations (54), both of which are reduced by therapy with iron (54,55). A mechanism for the higher A1C was recently identified by the demonstration that malondialdehyde, which is increased in subjects with iron deficiency anemia (54), augments glycation of hemoglobin (56). However, the magnitude of the increase in A1C is probably small. Examination of 10,535 adults without self-reported diabetes in NHANES III revealed that while 13.7% of women had iron deficiency, only 4.74% and 0.48% had A1C $\geq 5.5\%$ or $\geq 6.5\%$, respectively (57). Iron deficiency in women was associated with a small (odds ratio 1.39) yet significant greater odds of A1C $\geq 5.5\%$ but not with greater odds of A1C $\geq 6.5\%$. Iron deficiency was rare in men ($< 0.5\%$) (57). Nevertheless, it would seem prudent to correct the iron deficiency before measuring A1C in individuals with severe iron deficiency anemia.

Analytical variation. There are ~ 100 different methods used to measure A1C. The most widely used commercial methods use either antibodies (immunoassays) or cation-exchange chromatography (most commonly high-performance liquid chromatography) to separate the glycated

(A1C) from the nonglycated hemoglobin (24). The National Glycohemoglobin Standardization Program (NGSP) has been instrumental in standardizing A1C testing among laboratories (58,59), particularly (but not exclusively) in the U.S. The NGSP has markedly improved the performance of A1C testing (58). At the time of writing, the vast majority (93%) of clinical laboratories that participate in CAP surveys use methods with between-laboratory CVs $< 5\%$ (www.ngsp.org). Within laboratory CVs for some methods are as low as $< 0.5\%$. In addition, the International Federation for Clinical Chemistry (IFCC) developed a reference method using mass spectrometry (or capillary electrophoresis) for A1C measurement, which should result in international harmonization as it facilitates traceability to a metrologically sound accuracy base. It is important to emphasize that the IFCC method is technically complex, time consuming, and expensive and is not designed for routine analysis of patient samples.

Hemoglobin variants affect some A1C measurements. The most common variants are HbS, HbE, HbC, and HbD. A1C measurement is not appropriate in subjects homozygous for HbS or HbC, with HbSC or with any other variant that alters erythrocyte survival. However, A1C can be measured accurately in individuals heterozygous for HbS, HbE, HbC, or HbD and in those with increased HbF, provided an appropriate assay is used (53,60). Only $\sim 4\%$ of the 3,378 clinical laboratories that participated in the 2010 GH2 College of American Pathologists survey (which measures A1C) use methods in which HbAS or HbAC has clinically significant interference. In addition, if the sample is analyzed by high-performance liquid chromatography method, careful inspection of the chromatogram usually reveals the aberrant peaks produced by the variant hemoglobin. The presence of a hemoglobin variant should be considered if A1C is $> 15\%$ or if a large change in A1C coincides with a change in laboratory A1C method (53). In these situations, hemoglobin electrophoresis should be performed. It is important to emphasize that, like any other test, A1C results that are inconsistent with the clinical presentation should be investigated.

PERSPECTIVE—Notwithstanding the use of glucose (FPG and/or the OGTT) as the “gold standard” for the diagnosis of diabetes for many years, glucose testing

suffers from several deficiencies. The requirement that the subject be fasting at the time the blood is drawn is a considerable inconvenience. While our ability to measure glucose has improved, inherent biological variability can produce very large differences within and among individuals. In conjunction with lack of sample stability, which is difficult to overcome in clinical practice, these factors result in lack of reproducibility of glucose testing.

A1C, which reflects chronic blood glucose values, is routinely used in monitoring glycemic control and guiding therapy. The significant reduction in microvascular complications with lower A1C and the absence of sample lability, combined with several other advantages (Table 3), have led to the recommendation by some organizations that A1C be used for screening and diagnosis of diabetes (1). Accumulating evidence suggests that racial differences in A1C values may be present, and the possible clinical significance of this needs to be determined. Importantly, A1C cannot be measured in certain conditions. Despite these caveats, A1C can be measured accurately in the vast majority of people. A comprehension of the factors that influence A1C values and the conditions where it should not be used will produce accurate and clinically meaningful results. The convenience of sampling at any time without regard to food ingestion makes it likely that measurement of A1C will result in the detection of many of the millions of people with diabetes who are currently undiagnosed.

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