Small increments in blood glucose substantially increase the risk of developing diabetes mellitus; but preanalytical and analytical variables, such as the absence of harmonization for glucose assays, make it difficult to correctly apply these epidemiological insights to individual patients. Harmonization can be improved if 3 variables are addressed: changing proficiency test grading from consensus based to accuracy based, effectively controlling glycolysis, and taking into account the time of day blood was collected.

The continuous and graded quantitative relationship of fasting glucose measurements to the risk of developing diabetes was well documented recently by Tirosh et al. (1). They found an increased risk of type 2 diabetes across quintiles of fasting plasma glucose (FPG) concentrations within the newly defined reference range, <5.55 mmol/L (<100 mg/dL). For example, a person with an FPG between 4.83 and 5.00 mmol/L (87 and 90 mg/dL) has an age-adjusted risk of developing diabetes that is 1.81 that of a person with an FPG <4.55 mmol/L (82 mg/dL; 95% CI 1.16–2.83). Thus, a difference as small as 0.28 mmol/L (5 mg/dL) nearly doubles the risk. Higher concentrations of FPG were correlated with higher risk ratios, going as high as 3.05 times higher when FPG was between 5.27 and 5.49 mmol/L (95 and 99 mg/dL; CI 1.78–5.18). Unfortunately, until glucose measurements are harmonized these epidemiologically correct cut points cannot be applied with confidence to individual patients.

Three major variables must be addressed to achieve harmonization. First, proficiency test programs for glucose in the US should be accuracy based rather than consensus based. Second, glycolysis in the specimen must be effectively limited. Third, the time of day blood is collected must be taken into account.

The absence of an accuracy-based proficiency test program leads to unacceptably large differences among methods. For example, in the most recent College of American Pathologists survey C-B 2007, the mean glucose result for sample CHM-08—for different methods and instruments—ranged from a low of 7.92 mmol/L (144 mg/dL) to a high of 8.68 mmol/L (157.8 mg/dL) (2). The potential difference is even greater when you include in the analysis the mean imprecision (expressed as CV) of 1.9% for the method with the lowest result, and 2.5% for the method with the highest result. Thus, one-third of the time the result for an individual measurement could range between 7.75 mmol/L (141 mg/dL) and 8.91 mmol/L (162 mg/dL). This variation of 6.9% above or below the mean indicates that one-third of the time the difference between 2 different glucose results for an individual patient—assayed in 2 different laboratories—could differ by more than 14% even if the true difference was zero, an unacceptable situation.

Much of the disharmony is attributable to differences in calibrator accuracy. One cannot assume that a calibrator’s set point is accurate. For example, in 2003 and part of 2004 the NIST SRM for glucose in frozen human serum was not available (3). This situation forced manufacturers to use older retained calibrators to establish set points for new calibrators. Some of these secondary calibrator set points were too low by as much as 4.5%. Other calibrator problems include the variable instability of glucose in lyophilized serum samples and frozen serum samples (4, 5). In 1996, SRM 909a, which was a lyophilized product, manifested variable decreases in glucose content, a problem that was traced to a too-high moisture content in approximately 10% of the vials (6). I recommend the parallel use of stabilized aqueous glucose standards together with protein-based calibrators—both traceable to NIST. The aqueous standards are more stable, and their parallel use will ensure the accuracy and stability of the protein-containing calibrators.

In addition, accuracy and precision must be separately graded. Neither analytical bias nor imprecision should be permitted to consume the entire allowable total error of a proficiency test sample. In the absence of separately defined limits for bias and imprecision it is possible for a laboratory operating with very high precision to pass a proficiency test challenge in spite of clinically unacceptable analytical bias (7).

The most practical way to control glycolysis is to measure glucose immediately in whole blood or to separate serum or plasma from cells within 30 min of collection (8), true even if the specimen is collected in a tube that contains sodium fluoride. It is well documented, but not widely recognized, that the rates of decrease of glucose in the 1st hour after sample collection in tubes with and without fluoride are virtually identical (9). The decrease in glucose after 2 h in a fluoride tube can sometimes exceed 0.50 mmol/L (9 mg/dL). A change of this magnitude straddles 2 of the 4 top quintiles described by Tirosh et al. (1, 10).

Glucose concentrations change in relation to the time of day fasting blood is collected. Researchers at the CDC measured FPG in individuals who were randomly assigned to have blood collected either in the morning or afternoon (11). The mean fasting glucose was 5.41 mmol/L (97.4 mg/dL) in the morning and 5.13 mmol/L (92.4 mg/dL) in the afternoon—a 5% difference. Most of the decrease took place by 10:00 AM.

Even if we control all of these variables, fasting and 2-h postprandial glucose concentrations inherently have more biological day-to-day variability than does hemoglobin A1c (HbA1c) (12). In addition, HbA1c has been harmonized (13). Therefore, consideration should be given to including HbA1c in any screening program for diabetes. Inclusion of HbA1c, however, would not eliminate the need for rigorous harmonization of glucose testing.

Grant/funding support: None declared.
Financial disclosures: None declared.
References

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DOI: 10.1373/clinchem.2007.094466