Accurate measurements of plasma glucose are required for diagnosis of diabetes, particularly of gestational diabetes, for which hemoglobin A\textsubscript{1c} cannot substitute for glucose measurements [1]. While laboratories have improved the measurement procedures for plasma glucose, loss of glucose from blood samples before analysis remains a threat to accuracy of results. The traditional use of sodium fluoride (NaF) alone is unsatisfactory as it does not begin to inhibit glycolysis until 90–120 min have elapsed after blood is collected [2, 3]. The failure of NaF to properly control glycolysis leads to falsely low plasma glucose concentrations and, potentially, failure to detect diabetes. By contrast, the use of blood collection tubes with a low-pH citrate buffer stops glycolysis immediately [4].

An article by Garcia del Pino et al. [5] in this issue of Clinical Chemistry and Laboratory Medicine confirms the simplicity and effectiveness of citric acid as an immediate inhibitor of glycolysis. The authors document significantly lower measured glucose concentrations in NaF samples than in paired citrate tubes (which also had a much lower rate of hemolysis). Importantly, their paper is the first to examine the effect of using the citrate tubes on the rate of diagnosis of diabetes. The effect on diagnosis is a critical issue if we are to move beyond the current state of affairs in which uncontrolled glycolysis introduces error into measurement of glucose and diagnosis of diabetes.

The average error in measured glucose when NaF is used as the glycolytic inhibitor ranges between 0.28 and 0.39 mmol/L (5 and 7 mg/dL) in published studies, but the actual error for an individual patient can be as great as 1.1 mmol/L (20 mg/dL) if more than 3 h elapse after blood is drawn before plasma is separated from cells or if the patient has increased white blood cell and platelet counts [4]. Such potential errors are widely unrecognized as a significant source of error in the diagnosis and management of diabetes mellitus and in assessing risk of developing diabetes.

Some published reports fail to detect the NaF error. It is essential that any study of glycolysis inhibitors include zero-time reference plasma samples that are placed in an ice slurry immediately after collection and centrifuged within 30 min of collection. If, for example, glucose concentrations in NaF plasma separated within 2 h of collection are compared with serum glucose concentrations in serum separated from cells at the same time as the plasma samples, no difference will be seen. In such studies serum and NaF plasma are undergoing uncontrolled glycolysis at the same rate.

Measurements of glucose are used to assess risk of adverse events, such as the risk of developing complications of diabetes or developing diabetes. The cut points for diagnosis of diabetes are based on studies of the risk of developing diabetic retinopathy and other adverse outcomes over the subsequent follow-up period. Errors in glucose measurement obscure the relationship between glucose concentration and outcomes and affect patient management.

In 2005 Tirosh et al. [6] reported that higher fasting plasma glucose concentrations within the reference interval were an independent risk factor for type 2 diabetes among young men. A multivariate model revealed a progressively increased risk of eventual type 2 diabetes with progressively higher fasting glucose concentrations within the reference interval, measured at the time of an Israel Defense Forces Staff Periodic Examination. They divided the normal fasting glucose concentrations of 13,163 apparently healthy men into quintiles within the reference interval. The first quintile ranged from 2.78 to 4.5 mmol/L (50–81 mg/dL) and the fifth quintile ranged from 5.28 to 5.5 mmol/L (95–99 mg/dL). The hazard ratio of developing type 2 diabetes was 2.84 among those in the fifth quintile when compared with those in the first quintile. Even those in the second quintile (4.56–4.7 g mmol/L; 82–86 mg/dL) were 1.43 times more likely to develop type 2 diabetes than those in the first quintile. A 0.2 mmol/L (3–4 mg/dL) increase in glucose concentration moved subjects into progressively higher quintiles and a higher risk of developing type 2 diabetes; and a similar decrease moved subjects into progressively lower risk quintiles. Therefore, a measurement error of 1.1 mmol/L (20 mg/dL) secondary to improperly controlled glycolysis would totally eliminate the ability to correctly define the true risk
of developing type 2 diabetes in subjects whose glucose concentrations were within the reference interval.

The landmark HAPO study of 25,505 pregnant women [7] documented the relationships of hyperglycemia and adverse pregnancy outcomes. In this study, in which glycolysis in blood samples was controlled by use of ice baths, participants were divided into seven categories according to glucose concentrations. For categories 2 through 6 of fasting glucose, the plasma glucose increments were 5 mg/dL or 0.28 mmol/L (e.g., category 2 included patients with fasting glucose of 75–79 mg/dL [4.17–4.39 mmol/L], category 3 included all patients with fasting glucose of 80–84 mg/dL [4.44–4.67 mmol/L], etc.; categories 1 and 7 included women with fasting glucose concentrations <75 and ≥100 mg/dL [4.17 and 5.56 mmol/L, respectively]. The risk of adverse events increased progressively with increasing categories of fasting glucose and of plasma glucose concentrations measured at 1 and 2 h after ingestion of a solution that contained 75 g of glucose. For fasting glucose, between category 2 [75–79 mg/dL (4.17–4.39 mmol/L)] and category 6 [95–99 mg/dL (5.28–5.5 mmol/L)] the risk of a birth weight above the 90th percentile doubled and the risk of increased cord-blood C-peptide tripled. A loss of 20 mg/dL (1.1 mmol/L) of glucose from glycolysis would move a patient from the midpoint of category 6 [97.5 mg/dL (5.42 mmol/L)] to the midpoint of category 2 [77.5 mg/dL (4.31 mmol/L)], completely obscuring the enormous difference in risk of adverse events between these categories. Even an increase in the fasting plasma glucose level of 1 SD of the population, or 6.9 mg per deciliter (0.4 mmol/L), carried a significant odds ratio for birth weight above the 90th percentile (1.38, 95% confidence interval 1.32–1.44). One SD in the HAPO population is similar to the loss of glucose due to hemolysis in 1 h.

Clearly, the use of risk categories based on the HAPO study, in management of patients, requires the use of properly stabilized samples for measurements of glucose.

This instability of glucose in blood, with or without NaF, not only leads to errors if the classification of individual patients but also introduces noise into epidemiological and research studies [8]. The prevalence of diabetes will be underestimated in studies in which sample handling is delayed. The magnitude of the underestimation cannot be known. Collection of blood samples in citrate buffered blood tubes eliminates this pitfall. Similarly, the poor reproducibility of the oral glucose tolerance test almost certainly reflects, in part, variable loss of glucose from samples on different days as influenced by vagaries of sample handling, room temperature, white blood cell counts and other variables that alter the rates of glycolysis.

In our view, there is a need for additional studies of the effect of prompt inhibition of glycolysis on the diagnosis of diabetes. The epidemiological studies that defined the cut points used for diagnosis of diabetes (and of impaired glucose tolerance and other categories of risk) used NaF tubes for sample collection. In most cases (the HAPO study, as mentioned above, being an exception) these studies did not document the mean length of time from venipuncture until plasma was separated from cells to stop glycolysis, nor did they document the variability of that delay in stopping glycolysis. Thus it is not a straight-forward exercise to adjust the current diagnostic cut points for use with sample-handling techniques, such as the citrate-buffered tubes, that control glycolysis effectively. There is no simple factor that can be used to adjust the current diagnostic cut points to remove the noise contributed by glycolysis in those studies. If this cannot be resolved, it appears necessary to perform new studies in which glycolysis is completely inhibited. However, clearly, it is time to move ahead and use properly collected blood samples for patient care and research [9].

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*Corresponding author: David E. Bruns, MD, Department of Pathology, University of Virginia School of Medicine, PO Box 800168, Charlottesville, VA 22908, USA, E-mail: DEB6J@hscmail.mcc.virginia.edu
Raymond Gambino: Quest Diagnostics, Miramar, FL, USA