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Assessment of Trueness of a Glucose Monitor Using Interstitial Fluid and Whole Blood as Specimen Matrix

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ABSTRACT

Background: Interstitial fluid (ISF) is a specimen of increasing interest for glucose measurements because it can be obtained in a minimally invasive manner. Our previous study showed that sufficient ISF can be obtained using microneedles to measure glucose with a conventional electrochemical glucose monitor. The aim of this study was to assess the trueness of this glucose monitor using split-sample comparison with whole blood. We used ISF as specimen and our gas chromatography/mass spectrometry (GC/MS) method as reference.

Methods: We obtained 50 ISF samples and 40 whole blood samples from hairless Sprague-Dawley rats and analyzed for glucose by both methods.

Results: For whole blood, a non-significant bias of 5.7% (±2 SD: −54.9% to 66.3%) was determined. ISF glucose measurements showed a significant constant bias of 29.5% (±2 SD: −85.0% to 144%), which seems to be caused in part by the lack of red blood cells in ISF. The correlation coefficients were 0.782 and 0.679 for whole blood and ISF, respectively.

Conclusions: The assessed electrochemical glucose monitor shows a close agreement with our GC/MS reference method for whole blood, for which this monitor was optimized. When glucose measurements are performed with ISF as matrix, the observed bias needs to be taken into consideration. Further studies are necessary to elucidate the reasons for the wide dispersion of data for ISF.

INTRODUCTION

MONITORING OF GLYCEMIC STATUS is a cornerstone of diabetes care. Frequent measurement of blood glucose levels and tight metabolic control in patients with type 1 and 2 diabetes have been shown to reduce microvascular and other complications.[1,2] The American Diabetes Association recommends that all diabetes treatment programs should encourage daily self-monitoring of blood glucose, with three or more measurements per day in people with type 1 diabetes.[3] Most glucose monitoring methods rely on blood collection by finger stick. This procedure can be uncomfortable and painful for the patient, which results in poor compliance with currently recommended glucose monitoring regimens.

To overcome this problem, minimally invasive technologies have been developed to mea-

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sure glucose in interstitial fluid (ISF), which is obtained from the intradermal and subcutaneous regions of the skin.[4,5] Because this approach is bloodless, it lends itself to methods that are less invasive, and thereby less painful. ISF extraction is suitable for conventional glucose measurements at discrete time points, as well as for continuous ISF extraction for frequent, automated glucose measurements. Previous studies have shown that ISF glucose levels correlate with both static and changing blood glucose levels,[6] which indicates that ISF is a suitable specimen to assess glycemia in patients with diabetes.

ISF glucose concentrations can be measured by a variety of methods, including subcutaneous implemented sensors,[7] laser-ablated micropores,[8] iontophoresis,[9] ultrasound,[10] and microdialysis.[11] Subcutaneous implanted sensors and microdialysis tubes measure glucose directly in ISF below the skin; micropores are used as conduits to extract ISF out of the skin for ex vivo measurement. The other two methods render the skin permeable through different mechanisms to obtain ISF.

As a novel approach to ISF glucose measurement, arrays of microscopic needles can be used to increase skin permeability and thereby extract ISF in a minimally invasive manner.[12,13] Related studies[14,15] have shown that microneedles can be used to deliver drugs, including insulin,[16–20] across the skin and can be used in a painless manner.[21]

Our previous work using hairless rats demonstrated that microneedles can extract sufficient ISF through skin to perform glucose measurements with a conventional electrochemical glucose monitor. ISF glucose concentration correlated well with blood levels based on 140 measurements on 15 rats and six measurements on six human subjects, where 95% of rat data and 100% of human data fell within the clinically acceptable A+B region in Clarke Error Grid analysis,[13] which is in agreement with recent findings obtained with ISF from patients with diabetes.[22] However, conventional glucose monitors are optimized to measure glucose in whole blood. Trueness of such measurement systems can vary with the specimen used and needs to be assessed for each specimen separately. The trueness of such instruments for ISF as the specimen matrix is not known. Therefore, this study assesses the trueness of this electrochemical glucose monitor against a gas chromatography/mass spectrometry (GC/MS) method using whole blood and ISF as specimen matrices. We accomplished this through split-sample comparison using a previously described capillary-based specimen collection procedure.[23]

MATERIALS AND METHODS

We obtained ISF from hairless Sprague-Dawley rats (300–450 g, male, Charles River Laboratories, Wilmington, MA) according to a procedure described earlier.[13] In brief, glass microneedles with a tip radius of 15–40 μm and a cone-angle of 20–30° were fabricated using a micropipette puller (P97, Sutter Instrument, Novato, CA). We gently inserted the microneedles into the skin of isoflurane-anesthetized rats at the back to a depth of 700–1,500 μm within a 1-cm^2 area and then removed them to create seven to 10 microholes within a 0.5-mm^2 area. We obtained ISF by applying vacuum at 200–500 mm Hg below atmospheric pressure for 2–10 min using a small, flanged bell chamber adhered to the skin. ISF drops (2–3 μL) were collected from the skin surface by wiping the edge of the glucose strip along the skin surface, thereby sequentially contacting the individual ISF droplets. Measurements were performed with an electrochemical glucose monitor (Freestyle™ blood glucose monitoring system, Abbott Diabetes Care, Alameda, CA). We also filled a 1-μL glass capillary with ISF. The ISF was then immediately transferred into a stable isotope-labeled internal standard solution as described below and frozen until further processing for GC/MS analysis.[23] Immediately after ISF extraction, we collected whole blood by lateral tail vein laceration and analyzed the blood for glucose using the glucose monitor and the GC/MS method with the 1-μL capillary blood collection procedure described below. The Georgia Institute of Technology Institutional Animal Care and Use Committee has approved these experiments.

For GC/MS analysis, we transferred the specimen collected in 1-μL glass capillaries into

a microcentrifuge tube containing 500 μL of internal standard solution, an aqueous solution of [$^{13}C_6$]glucose (Cambridge Isotope Labs, Andover, MA) at a concentration of 15 mmol/L (270 mg/dL). After the tube was closed and shaken vigorously, we removed the specimen from the capillary and stored it at −70°C until further processing. This specimen collection procedure allows the collection and analysis of glucose in whole blood by minimizing any loss of analyte. We then measured the glucose by GC/MS as described previously.[23] Calibration was performed using Standard Reference Material (SRM) 917b [D-glucose (dextrose), National Institute of Standards and Technology (NIST), Gaithersburg, MD]. The GC/MS method shows the same results as the YSI 2300 STAT plus analyzer (YSI Corp., Yellow Springs, OH) for serum and aqueous glucose solutions.[24] We assessed the within- and among-day variability of this method using frozen serum pools NIST SRM 965 and EDTA-whole blood. This method has a mean recovery of 100.3%, as determined by addition of calibrators to SRM 965. The among-day imprecision (over 20 days) was 0.88% for whole blood and 1.55%, 1.15%, and 0.93% for NIST SRM 965 at level 1, level 2, and level 3, respectively.[23] NIST SRM 965 was used as quality control materials during analysis of the ISF and blood samples.

The Freestyle blood glucose monitoring system has been designed for capillary whole blood and requires a specimen of less than 1 μL. The instrument has a built-in self-calibration routine and displays results as plasma glucose concentration using a mathematical formula to convert whole blood glucose values to plasma glucose values. The instrument performance of this monitor was assessed by testing the glucose monitor using the manufacturer's control solutions and comparing the obtained results against the manufacturer's specifications. All measurements were found to be within the manufacturer's specifications (data not shown).

We assessed the correlation between methods using Passing-Bablok regression and determined the bias between the glucose monitor and the GC/MS reference method using a bias plot according to the guidelines described by the Clinical Laboratory Standards Institute (Document EP-9A2).[25]

RESULTS

The first goal of this study was to assess the trueness of glucose measurement in whole blood using an electrochemical glucose monitor against a GC/MS reference method. We measured whole blood glucose concentration in 40 samples using both of the measurement procedures. We analyzed data to determine the bias of the glucose monitor to the GC/MS reference method (Fig. 1a). The glucose concentrations determined by GC/MS ranged from 33.0 mg/dL to 285 mg/dL. Analysis of the data shows that the glucose monitor had a non-significant bias of 3.6 mg/dL or 5.7% relative to GC/MS reference measurements. The 95% confidence interval (CI) of this bias ranged from −6.9 mg/dL to 14.0

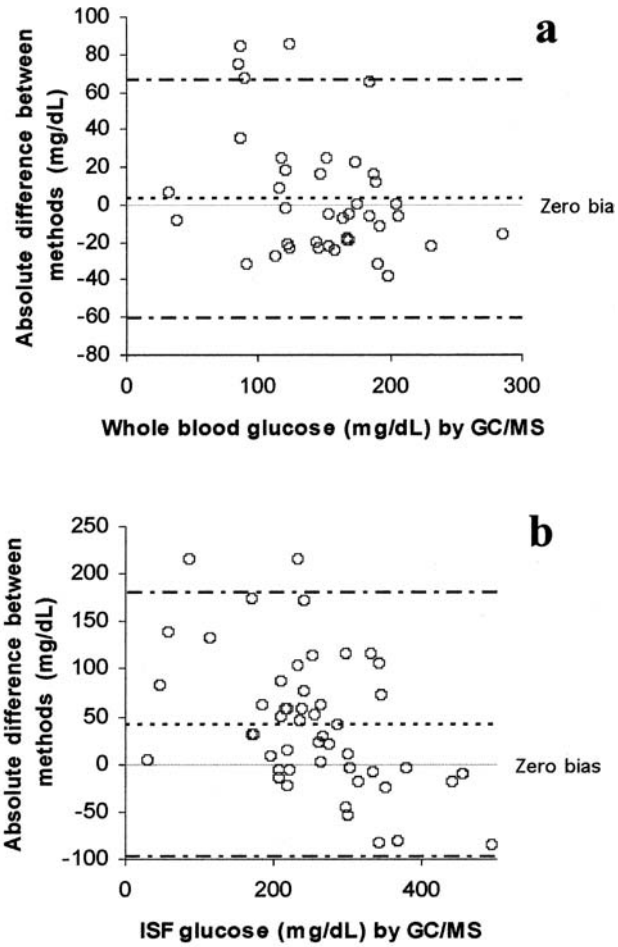

FIG. 1. Bias plot of glucose values determined by the GC/MS reference method and electrochemical glucose monitor for whole blood (**a**) and ISF (**b**). The dashed lines indicate the bias level, and the dot-dash lines indicate the upper and lower limits of agreement (± 2SD).

mg/dL. The dispersion of data points described by the limits of agreement (±2 SD) ranged from −60.4 mg/dL to 67.5 mg/dL (−54.9% to 66.3%). Regression analysis gave a slope of 1.151 (CI 0.935 to 1.447) and an intercept of −34.1 mg/dL (CI −80.9 to 4.5 mg/dL) with a correlation coefficient of 0.782.

The second goal of the study was to assess the trueness of glucose measurement in ISF for this glucose monitor against a GC/MS reference method. The glucose concentrations of 50 ISF samples obtained from the same set of rats used for the whole blood study were used for this comparison (Fig. 1b). The ISF glucose concentrations determined by the GC/MS reference method ranged from 31.0 mg/dL to 494 mg/dL. Analysis of the ISF data shows that the glucose monitor had a significant constant bias to the reference method of 41.4 mg/dL or 29.5% with a CI ranging from 21.1 mg/dL to 61.6 mg/dL. The dispersion of data points described by the limits of agreement ranged from −98.2 mg/dL to 181 mg/dL (CI −85.0% to 144%). Regression analysis gave a slope of 0.978 (CI 0.703 to 1.435) and an intercept of 33.3 mg/dL (CI −80.2 to 109.0 mg/dL) with a correlation coefficient of 0.679.

DISCUSSION

Accurate measurement of blood glucose by glucose monitors is crucial for optimal patient care. The assessed glucose monitor shows close agreement with the GC/MS reference method for whole blood measurements. No statistically significant difference from the reference method was observed. However, a wide dispersion of data points for whole blood was observed, which is within the range found with other glucose meters and clinical analyzers.[24]

In contrast to the whole blood glucose measurements, the monitor showed a significant mean bias of 29.5% and a wide dispersion of data around this bias for ISF glucose. Because of the close agreement of this glucose monitor observed for whole blood, the bias and dispersion of data determined for ISF seem to be caused by the specimen matrix. One apparent difference between ISF and whole blood is the lack of red blood cells in ISF. The glucose monitor used in this study is designated for use at hematocrit levels of 15–65%.[26] Because ISF does not contain red blood cells, its characteristics such as viscosity and cell-free volume are significantly different from those of whole blood. This may cause alterations in the measurement of the glucose in the monitor and may lead to the observed bias and dispersion of data. The observed increase in data dispersion and bias is in agreement with findings obtained with other cell-free matrices such as plasma (Dr. Ben Feldman, Abbott Diabetes Care, personal communication). Also, the reduced protein content of the ISF as compared with plasma or whole blood may be a source of variability and bias as indicated by Collison et al.[27] While the observed bias may easily be corrected by appropriate calibration of the monitor, further investigations are necessary to properly assess the reasons for the wide dispersion of data observed with ISF as the specimen matrix.

For whole blood, the glucose monitor met the analytical performance goal of 20% total error set by the Clinical Laboratory Improvement Act of 1988. Measurements performed in ISF did not meet this goal. However, the bias for glucose measured in ISF is similar in magnitude to the biases reported for other Food and Drug Administration-approved glucose monitors using other specimen matrices such as capillary whole blood or venous EDTA-whole blood.[24,28,29]

In conclusion, assessment of trueness of this electrochemical glucose monitor using split-sample comparison shows close agreement to our GC/MS reference method for measurements in whole blood. When ISF is used as the specimen matrix, the observed bias needs to be taken into consideration when reporting results.

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