Effects of Different Hematocrit Levels on Glucose Measurements With Handheld Meters for Point-of-Care Testing

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• ***Objectives.*—To determine the effects of low, normal, and high hematocrit levels on glucose meter measurements and to assess the clinical risks of hematocrit errors.**

***Design.*—Changes in glucose measurements between low and high hematocrit levels were calculated to determine hematocrit effects. The differences between glucose measured with meters and with a plasma glucose method (YSI 2300) also were compared.**

***Setting.*—Six handheld glucose meters were assessed in vitro at low (19.1%), normal (38.5%), and high (58.3%) hematocrit levels, and at 6 glucose concentrations ranging from 2.06 mmol/L (37.1 mg/dL) to 30.24 mmol/L (544.7 mg/dL).**

***Results.*—Most systems, regardless of the reference to which they were calibrated, demonstrated positive bias at lower hematocrit levels and negative bias at higher hematocrit levels. Low, normal, and high hematocrit levels progressively lowered Precision G and Precision QID glucose measurements. Hematocrit effects on the other systems were more dependent on the glucose concentration. Overall, Accu-Chek Comfort Curve showed the least sensitivity to hematocrit changes, except at the lowest glucose concentration.**

***Conclusions.*—We strongly recommend that clinical professionals choose glucose systems carefully and interpret glucose measurements with extreme caution when the patient's hematocrit value changes, particularly if there is a simultaneous change in glucose level.**

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The use of glucose meters for point-of-care testing with critically ill patients is controversial.[1–4] Error sources are poorly understood. Nonetheless, handheld glucose meters are in widespread use in critical care settings. Few data are available for the latest generation of devices. Variations in P_{O_2}, P_{CO_2}, pH, and some drugs used in critical care can affect glucose measurements.[5,6] The objectives of this study were (1) to determine hematocrit effects on glucose measurements obtained with the latest generation of handheld glucose meters; (2) to quantitate changes in glucose measurements observed with low, normal, and high hematocrit levels; and (3) to discuss the clinical risks of hematocrit errors when using point-of-care glucose testing.

MATERIALS AND METHODS

Glucose Meters

Table 1 summarizes the characteristics of each of the following 6 glucose meter systems evaluated: (1) Accu-Chek Advantage H and (2) Accu-Chek Comfort Curve (Roche Diagnostics, Indianapolis, Ind), (3) Precision G and (4) Precision QID (Abbott Laboratories, Bedford, Mass), (5) SureStep (LifeScan, Milpitas, Calif), and (6) Glucometer Elite (Bayer Corporation, Elkhart, Ind). Systems 1, 2, 3, 4, and 6 use electrochemical biosensors. System 5 uses a reflectance photometric system. Systems 1 through 5 are appropriate for hospital use. System 6 was included as an example of a glucose meter intended for home use.

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Principles of Glucose Measurements

Quantitative measurement of glucose in whole blood with an electrochemical biosensor begins when a drop of blood is introduced on the top, tip, or side of the test strip. Plasma from the whole-blood sample diffuses into and solvates the reagent layer, which contains glucose oxidase or glucose dehydrogenase and electrodes. Glucose is catalyzed to form gluconic acid by the glucose dehydrogenase or glucose oxidase reagent. The electrons produced from the reaction form a current. Under the potential provided from the meter, a current is generated from the electrons produced during glucose oxidation. The current is calibrated to measure the glucose concentration in the whole-blood sample.[7]

Photometric test strips, such as the SureStep, have a porous membrane on top. The porous membrane separates the erythrocytes from the plasma in the sample. The plasma diffuses into the reagent layer, where impregnated glucose oxidase facilitates the oxidation of glucose. Gluconic acid and hydrogen peroxide are produced by the reaction. Peroxidase then catalyzes the hydrogen peroxide, which oxidizes the dye in the strip to produce a blue color. The intensity of color developed is proportional to the glucose concentration in blood and is transformed into glucose readings by the meter.[8] Other test strips (not evaluated here) use alternate enzymes, such as glucose dehydrogenase (Simplicity, Roche Diagnostics) and hexokinase (Encore, Bayer).

Comparison Methods

A biosensor-based whole-blood/plasma analyzer, the YSI 2300 (Yellow Springs Instrument Inc, Yellow Springs, Ohio), served as

Table 1. Glucose System Specifics*

System	Test Strip	Method/Reference	Enzyme	Blood Sample	Sample Dosing	Sample Volume, μL
1	Accu-Chek Advantage H	Electrochemical/Hitachi 717, plasma	GD	C, V, A, N	Top	9–14
2	Accu-Chek Comfort Curve	Electrochemical/Hitachi 717, plasma	GD	C, V, A, N	Side	4
3	Precision G	Electrochemical/YSI, plasma	GO	C, V, A, N	Top	3.5
4	Precision QID	Electrochemical/YSI, plasma	GO	C, V, A, N	Top	3.5
5	SureStep	Reflectance photometric/YSI, plasma	GO	C, V	Top	‡
6	Glucometer Elite	Electrochemical/Hitachi 704, plasma	GO	C, V	Tip	3.5

* GD indicates glucose dehydrogenase; GO, glucose oxidase; O, capillary; V, venous; A, arterial; and N, neonate.
† Hematocrit limits at the given glucose concentrations (mg/dL); mmol/L = 0.05551 × mg/dL.
‡ Sample volume 5 μL for pipette application; otherwise, 10–30 μL.

the plasma glucose comparison method for all glucose meters tested. The YSI analyzer uses glucose oxidase to measure the glucose concentration in duplicate with 2 glucose channels. The linearity is 0 to 1000 mg/dL. The YSI analyzer self-calibrates every 15-minute interval or after 5 measurements. The hematocrit of each blood sample was measured on a Micro-Capillary Centrifuge (Model MB, International Equipment Company, Needham Heights, Mass) by centrifuging the sample at 10 000 rpm for 5 minutes.

Protocol

The study followed the guidelines of the Human Subjects Committee. Two hundred milliliters of venous blood were collected in lithium heparin vacutainer tubes from a healthy volunteer. The blood was allowed to undergo glycolysis overnight at room temperature to a glucose concentration near zero. The blood was pooled and then spun down to separate the erythrocytes from plasma. Separated erythrocytes and plasma were reconstituted to achieve the desired target hematocrit levels of approximately 20%, 40%, and 60%. Each hematocrit level had 6 target glucose concentrations (40 mg/dL [2.22 mmol/L], 100 mg/dL [5.55 mmol/L], 130 mg/dL [7.22 mmol/L], 230 mg/dL [12.77 mmol/L], 380 mg/dL [21.09 mmol/L], and 480 mg/dL [26.64 mmol/L]), which were prepared by spiking with appropriate volumes of concentrated dextrose solution (20 000 mg/dL [1110.20 mmol/L]). The maximum dilution after dextrose spiking was 2.4%. There were 18 hematocrit/glucose samples prepared in total.

Two different lots of test strips for each glucose system were tested. Two glucose meters for each system were used. Each meter was tested 10 times with each lot of test strips. Twenty measurements per lot were obtained for each hematocrit/glucose sample. Before and after testing, the glucose meters and the reference analyzer were checked with aqueous quality control solutions, respectively. All test strips were dosed with 6 μL of sample using a pipette, except for Advantage H, which was dosed with 14 μL. These volumes were used to ensure that adequate sample was applied. The hematocrit/glucose sample test order was randomized before each sample preparation. After sample preparation, testing was performed immediately to minimize the effect of glycolysis or changes in P_{O_2}. All systems were tested simultaneously under identical conditions by trained personnel.

At the start and the end of each sample testing, an aliquot of the sample was centrifuged. Plasma glucose was measured with the YSI 2300 using 2 glucose channels. The measurements (n = 4) were averaged, and the mean served as the plasma comparison level.

Comparison of Glucose Differences

Comparisons between meter whole-blood glucose measurements and YSI 2300 plasma glucose measurements were done by subtracting as a percentage (1) the mean of the glucose differences between the meter and the YSI (meter minus YSI) at 60% hematocrit divided by the mean YSI measurement at 60% hematocrit, and subtracting this percentage from (2) the mean of the glucose differences between the meter and the YSI at 20% hematocrit divided by the mean YSI measurement at 20% hematocrit, all at the same target glucose level. The formula for the calculation is as follows: $[(\text{Meter Measurement}_{20\%\ \text{Hct}} - \text{YSI Measurement}_{20\%\ \text{Hct}})/\text{YSI Measurement}_{20\%\ \text{Hct}}] - [(\text{Meter Measurement}_{60\%\ \text{Hct}} - \text{YSI Measurement}_{60\%\ \text{Hct}})/\text{YSI Measurement}_{60\%\ \text{Hct}}]$.

Glucose meter measurements also were compared to YSI 2300 plasma glucose measurements to show the effects of changes in glucose concentrations on the glucose measurements for individual hematocrit levels. This calculation is [(Meter Measurement − YSI Measurement)/YSI Measurement] × 100%. Differences between the meter and YSI 2300 glucose measurements were plotted as a function of glucose concentrations at the 3 hematocrit levels.

Precision

Quality control solutions provided by the manufacturer were tested 20 consecutive times to assess within-day precision. The precision of the YSI analyzer was evaluated with NERL 1343-Standard Glucose Solutions (New England Reagent Laboratory, East Providence, RI). The precision was expressed as coefficient of variation (CV).

Statistics

Statistical calculations included the mean and standard deviation (SD) of paired differences with results reported as mean ± SD in percent. Glucose is expressed in mmol/L and mg/dL; mmol/L = 0.05551 × mg/dL. Paired differences were analyzed using the Student *t* test to determine statistically significant differences between 2 lots of glucose test strips. Analysis of variance was used to compare hematocrit-related effects at each glucose concentration. A *P* value less than .05 was considered to be statistically significant. Coefficient of variation is the standard deviation divided by the mean expressed as a percentage: [CV = (SD/mean) × 100%].

RESULTS

In total, 4320 measurements were made from 18 samples; each sample was tested 20 times on 6 glucose meter systems with 2 different test strip lots (4320 = 18 × 20 × 6 × 2). Mean (SD) plasma glucose concentrations measured in different samples (n = 3) with the YSI analyzer were 37.1 (2.0) mg/dL (2.06 [0.11] mmol/L), 104.4 (6.4) mg/dL (5.80 [0.36] mmol/L), 139.6 (8.8) mg/dL (7.75 [0.49] mmol/L), 259.5 (14.8) mg/dL (14.4 [0.82] mmol/L),

Table 1. Extended

Glucose Range, mg/dL	Hematocrit Range, %†
10–600	20–65 at <200 mg/dL; 20–55 at >200 mg/dL
10–600	20–65 at <200 mg/dL; 20–55 at >200 mg/dL
20–600	20–70
20–600	20–70
0–500	25–60
40–500	20–60 at <300 mg/dL; 55 at >300 mg/dL

422.1 (26.6) mg/dL (23.43 [1.48] mmol/L), and 544.7 (42.8) mg/dL (30.24 [2.38] mmol/L). Hematocrit means (SD) were 19.1% (0.7), 38.5% (0.8), and 58.3% (0.7) (n = 6 for each).

Effects of Hematocrit on Glucose Measurement

Figure 1 shows the hematocrit effect on glucose measurements at hematocrit levels of 19.1% versus 58.3% for glucose concentrations of 37.1 mg/dL (2.06 mmol/L) to 544.7 mg/dL (30.24 mmol/L). Sixty percent of the 2 test strip lots showed no statistically significant differences between test lot results. Differences in lots that were statistically significant were not necessarily clinically significant. The Accu-Chek Advantage H (system 1), SureStep (system 5), and the Elite (system 6) glucose meters showed little hematocrit dependency at a glucose concentration of 37.1 mg/dL (2.06 mmol/L) (Figure 1, A). Compared with other systems, the Accu-Chek Comfort Curve (system 2) showed less hematocrit dependency at the other glucose concentrations (Figure 1, B through F). The Precision G (system 3) and Precision QID (system 4) showed large hematocrit differences in glucose measurements at the 6 different glucose concentrations. At each glucose concentration, variations in results among the 6 systems were statistically significant ($P < .01$, analysis of variance). The SureStep (system 5) did not give readings at a glucose concentration of 544.7 mg/dL (30.24 mmol/L) because the limit for meter glucose measurement is 500 mg/dL (27.76 mmol/L). Hence, there are no SureStep measurements shown in Figure 1, F.

Figure 2 shows the effects of different glucose concentrations on the test strip measurements of each glucose system at low, normal, and high hematocrit levels. The intent of Figure 2 is to demonstrate the glucose dependency of hematocrit effects, not to assess the accuracy of the systems, since not all of the systems are calibrated to the YSI 2300 plasma glucose reference method (see Table 1). At low hematocrit levels (dashed line), most glucose systems yielded a higher glucose level relative to the YSI 2300 plasma glucose measurements and at high hematocrit levels (solid line), they yielded a lower glucose level, except the Precision G and the Precision QID, for which the glucose levels at the 3 hematocrit levels were lower than those determined by the YSI 2300 (Figure 2, C and D).

At normal hematocrit levels (38.5%), the glucose systems showed smaller differences (Figure 2), except Precision G and Precision QID, for which smaller differences were observed at the lowest hematocrit. Similar findings were observed on the second strip lot for each of the glucose systems. At glucose concentrations less than 100 mg/dL (5.55 mmol/L), the Accu-Chek Comfort Curve yielded a lower glucose level (Figure 2, B), but at the other glucose concentrations, this system matched the YSI 2300 plasma glucose value fairly closely. Results were most variable at the lowest glucose concentration, where in some cases scatter may be related to the small sample volume used.

Precision

Table 2 summarizes within-day precision for the 7 glucose devices when tested with aqueous controls. The CVs for within-day precision of the glucose meter systems ranged from 2.0% to 5.5%. Generally, the largest CVs resulted when testing the lowest glucose levels in quality control solutions.

COMMENT

This study shows that hematocrit differences can significantly affect glucose measurements determined using the latest test strip technologies, and that the degree of the hematocrit effect depends on the glucose concentration. Increases in hematocrit are known to decrease glucose meter measurements and, conversely, decreases in hematocrit can increase glucose measurements.[9–13] Recognition of these facts is important for clinical decision making. A wide range of hematocrit values was tested to observe the range of effects on glucose meter measurements. The Precision G and Precision QID systems were tested within their vendor-specified hematocrit ranges (Table 1). The Advantage H, Comfort Curve, Elite, and SureStep were tested outside their stated hematocrit ranges. The rationale for this approach was (1) all systems were compared equally, (2) meter systems cannot detect or exclude samples by hematocrit, and (3) often the hematocrit of an individual patient is not known at the time of glucose measurement, particularly in a critical care situation.

The Precision G and the Precision QID glucose meters have a third background compensation electrode that lacks glucose oxidase enzyme and measures the signal from potentially interfering substances. This nonspecific signal is subtracted to give the correct glucose readings. The third electrode in the Precision G and Precision QID did not show apparent advantages with different hematocrit levels. Changes in hematocrit levels decreased the Precision G and Precision QID glucose readings. Also, at a hematocrit of 58.3% and at a glucose concentration of 544.7 mg/dL (30.24 mmol/L), the Precision G and the Precision QID sometimes gave error (''Err'') messages, although both the hematocrit and glucose concentration were within the manufacturer's claims for measurement ranges. Other systems showed crossover of glucose differences relative to the comparison method for the 3 hematocrit levels as the glucose concentration increased. The glucose differences observed with the Accu-Chek Advantage H, Accu-Chek Comfort Curve, SureStep, and the Elite glucose meter systems varied inconsistently in relation to the glucose concentrations. This variation may make it more difficult for the clinician to predict hematocrit effects on these meter systems compared to the Precision G and the Precision QID. We cannot rule out that possible P_{O_2} changes[4] from sample preparation affected these results.

Several possible mechanisms may explain hematocrit effects on glucose meter measurements. For example, an in-

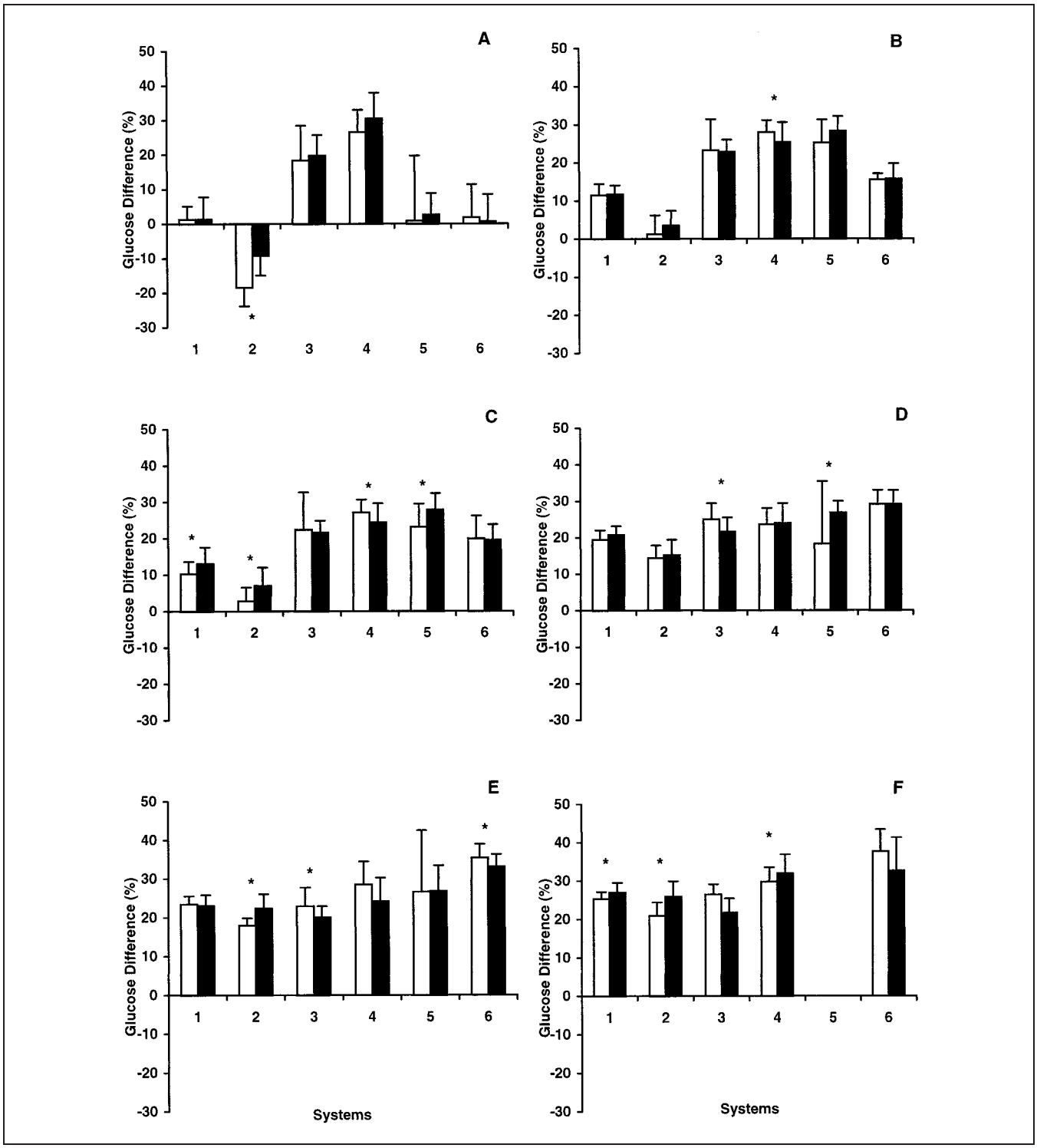

Figure 1. *The relative effects of different hematocrit levels on glucose measurements performed on each meter system (n = 20, mean ± SD shown). The vertical axis represents the glucose difference in percent between the glucose measurements at hematocrit levels of 19.1% and 58.3%. Glucose concentrations were as follows: A, 37.1 mg/dL (2.06 mmol/L); B, 105.3 mg/dL (5.85 mmol/L); C, 138.4 mg/dL (7.68 mmol/L); D, 259.1 mg/dL (14.38 mmol/L); E, 428.8 mg/dL (23.80 mmol/L), and F, 550.1 mg/dL (30.54 mmol/L). The 2 sets of bars (open and filled) represent 2 test strip lots. The asterisks indicate statistically significant differences between the 2 test strip lots at* $P < .05$. *System 1 was Accu-Chek Advantage H; 2, Accu-Chek Comfort Curve; 3, Precision G; 4, Precision QID; 5, SureStep; and 6, Glucometer Elite.*

creased number of erythrocytes in the whole-blood sample may mechanically impede diffusion of plasma into the reagent layer,[14] block the ''holes'' in the mesh membrane,[12] or decrease the volume of plasma available to diffuse. Also, hematocrit changes may alter blood viscosity, thereby decreasing fluid permeability into the reagent layer. The more viscous a solution, the slower the rate of diffusion of a solute within it.[15] Other factors, such as (1) mi-

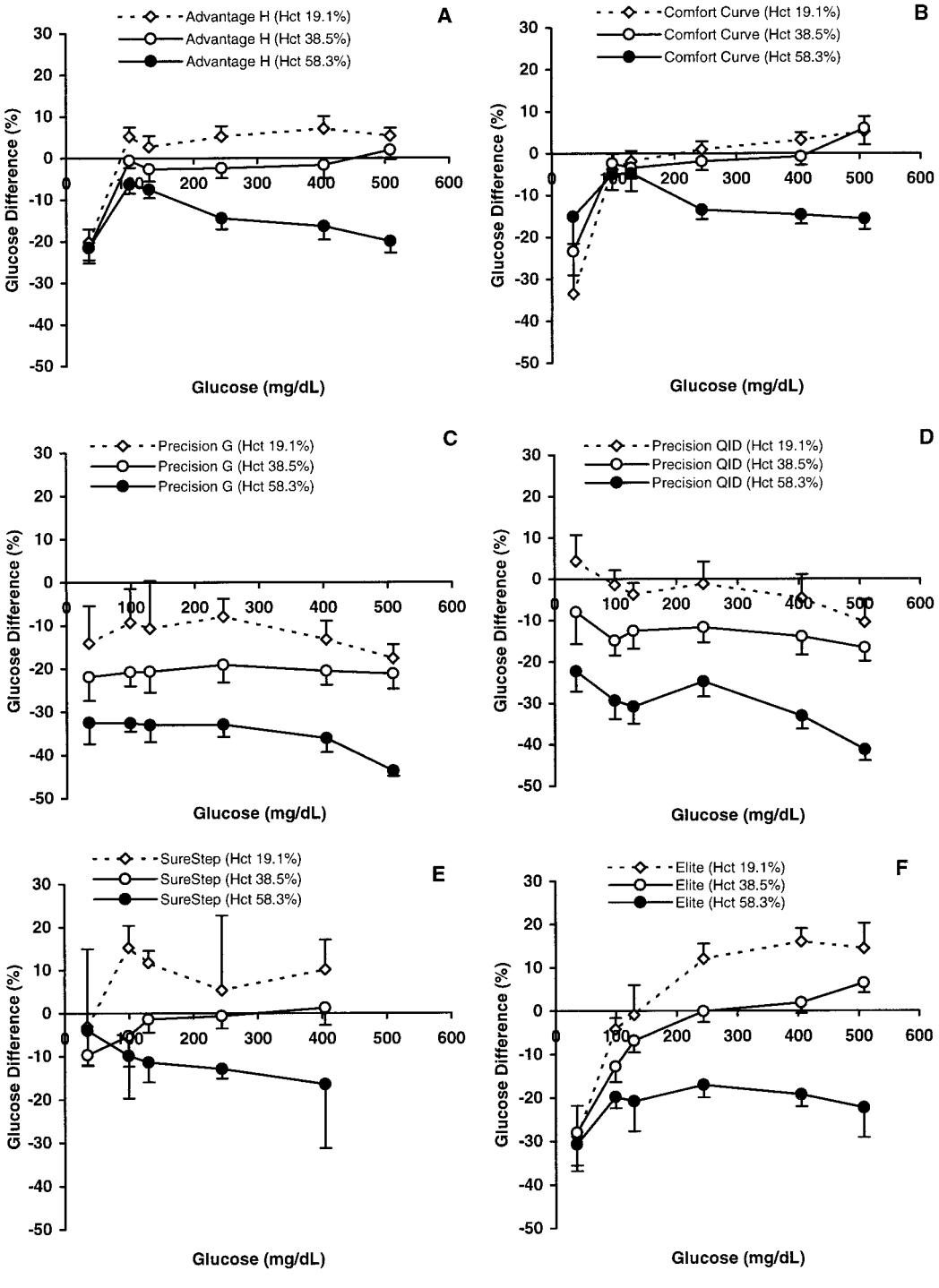

Figure 2. *Effects of glucose concentrations on glucose meter system measurements (n = 20, mean ± SD shown) at low (19.1%), normal (38.5%), and high (58.3%) hematocrit levels. The 6 glucose concentrations were 37.1 mg/dL (2.06 mmol/L); 104.4 mg/dL (5.80 mmol/L); 139.6 mg/dL (7.75 mmol/L); 259.5 mg/dL (14.40 mmol/L); 422.1 mg/dL (23.43 mmol/L), and 544.7 mg/dL (30.24 mmol/L). The largest differences compared to the plasma glucose measurements were observed with the Precision G and the Precision QID measurements (C and D) at a high hematocrit level (solid line). Results from the second test strip lot were comparable.*

croclot formation in the samples or on the test strips, (2) hemolysis,[16] (3) protein deposition, (4) fibrin aggregation, (5) the experimental model itself, and (6) platelet or other cellular phenomena triggered by the test strips, may add to hematocrit error or may produce other undetected errors in glucose measurements. In addition, changes occurring in vivo, such as blood-borne or hematologic disease, may introduce errors that we were unable to observe.

Since critically ill patients may have unpredictable changes in hematocrit, the effects of different hematocrit

Table 2. Within-Day Precision

Glucose Meters	N	Glucose Control Levels, mg/dL: Low Mean ± SD	Low CV	Middle Mean ± SD	Middle CV	High Mean ± SD	High CV
Accu-Chek Advantage H	20	44.1 ± 1.8	4.2	121.1 ± 6.5	5.4	255.7 ± 7.2	2.8
Accu-Chek Comfort Curve	20	60.7 ± 3.1	5.1	134.9 ± 4.5	3.4	333.1 ± 12.0	3.6
Precision G*	20	48.4 ± 2.6	5.5	. . .	. . .	279.9 ± 12.5	4.5
Precision QID*	20	51.2 ± 2.8	5.4	. . .	. . .	283.4 ± 7.9	2.8
SureStep	20	49.2 ± 1.0	2.0	116.0 ± 2.3	2.0	360.5 ± 16.4	4.6
Glucometer Elite	20	61.0 ± 2.8	4.5	94.6 ± 3.9	4.2	281.3 ± 15.5	5.5

Glucose Analyzer		Glucose Control Levels, mg/dL: Low (50) Mean ± SD	Low (50) CV	Middle (200) Mean ± SD	Middle (200) CV	High (400) Mean ± SD	High (400) CV
YSI 2300	20	48.2 ± 0.5	1.0	197.7 ± 5.6	2.8	401.4 ± 2.9	0.7

* Precision G and Precision QID were tested with 2 quality control levels.

levels on glucose measurements could mask hyperglycemia in patients with polycythemia or abnormally high hematocrit, or could mask hypoglycemia in patients with anemia or low hematocrit. Hematocrit levels in newborns may be as high as 62.9%.[17] Polycythemia occurs in 2% to 5% of all newborn infants.[18,19] These high hematocrit levels could falsely lower the glucose measurements. Low hematocrit levels are observed commonly in several conditions, including renal failure,[20] hemodialysis,[21] and cardiopulmonary bypass.[22] These low hematocrit levels could falsely increase the glucose measurements. Because anemia, polycythemia, and unexpected changes in hematocrit values are common, it is important to understand the effects of hematocrit levels on glucose meter performance for point-of-care testing and also to be aware of ''dual ranges'' for hematocrit claims, if more than 1 range is specified by the manufacturer.

Different hematocrit levels can affect glucose meter measurements significantly. The most pronounced hematocrit effects occur at low and at high hematocrit levels, which generally increase and decrease glucose measurements, respectively. Additionally, hematocrit effects are both system and glucose dependent. Meter systems respond uniquely to changes in glucose concentration at fixed hematocrit levels, that is, each has its own characteristic ''signature.''

Solutions to the hematocrit effect as well as its dependency on glucose concentration are needed badly. Improvements could include simultaneous measurement of the patient's hematocrit with algorithmic adjustment of glucose results, warning of potential errors, or results lock out, as well as fundamental improvements in the approach to glucose measurement that will eliminate hematocrit effects. Note that hematocrit effects in vitro and in vivo may differ.

Clinically, the hematocrit level may change profoundly and unexpectedly in critically ill patients. We strongly recommend that physicians and medical professionals carefully choose glucose meters for point-of-care glucose testing and interpret glucose readings with extreme caution when the glucose testing is performed under conditions such as acute blood loss, transfusion, and surgery, where hematocrit and glucose changes may be rapid and possibly encountered simultaneously.

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