Do Sensor Glucose Levels Accurately Predict Plasma Glucose Concentrations During Hypoglycemia and Hyperinsulinemia?

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OBJECTIVE — The MiniMed Continuous Glucose Monitoring System (CGMS) measures subcutaneous interstitial glucose levels that are calibrated against three or more fingerstick glucose levels daily. The objective of the present study was to examine whether the relationship between plasma and interstitial fluid glucose is altered by changes in plasma glucose and insulin levels and how such alterations might influence CGMS performance.

RESEARCH DESIGN AND METHODS — Arterialized plasma glucose, sensor glucose, and interstitial fluid glucose were measured by microdialysis in 11 healthy subjects during a 1.0 $mU \cdot kg^{-1} \cdot min^{-1}$ stepped euglycemic-hypoglycemic-hyperglycemic (plasma glucose ~5, 3.1, and 8.6 mmol/l, respectively) insulin clamp that raised plasma insulin to ~360–390 pmol/l.

RESULTS — When the CGMS was calibrated versus plasma glucose levels before insulin infusion, basal sensor and plasma glucose were similar (5.0 ± 0.3 vs. 5.2 ± 0.3 mmol/l, respectively); dialysate glucose was 3.3 ± 0.9 mmol/l. During the hyperinsulinemic-euglycemia study (plasma glucose 4.9 ± 0.3 mmol/l), dialysate glucose fell by 30–35%, accompanied by a significant reduction in sensor glucose (to 3.7 ± 0.6 mmol/l; $P < 0.001$ vs. plasma). Subsequently, sensor levels remained lower than plasma values during mild hypoglycemia (2.5 ± 0.6 vs. 3.1 ± 0.3 mmol/l; $P < 0.01$) and during recovery from hypoglycemia (7.3 ± 1.2 vs. 8.6 ± 0.6; $P < 0.01$). However, when the CGMS was calibrated against plasma glucose levels before and during each step of the clamp, sensor glucose levels increased throughout the study and did not differ from plasma glucose values during hypoglycemia.

CONCLUSIONS — Although hyperinsulinemia may contribute to modest discrepancies between plasma and sensor glucose levels, the CGMS is able to accurately track acute changes in plasma glucose when calibrated across a range of plasma glucose and insulin levels.

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Abbreviations: ECF, extracellular fluid; CGMS, Continuous Glucose Monitoring System.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

The development of continuous glucose-sensing systems is potentially the most important advance in the management of type 1 diabetes since the introduction of meters for self-monitoring of blood glucose more than 20 years ago. The MiniMed Continuous Glucose Monitoring System (CGMS) was the first device approved by the U.S. Food and Drug Administration for 3-day periods of "Holter-style" rather than real-time continuous glucose monitoring. Even such limited use of the glucose sensor system has provided a wealth of data on glycemic excursions in diabetes that were previously unavailable. With this system, we (1) and others (2–4) have demonstrated that there are often marked increases in plasma glucose levels after meals, even in patients with low HbA_{1c} levels who are using premeal bolus doses of rapid-acting insulin analogs. The CGMS has also revealed frequent and prolonged periods of unsuspected hypoglycemia at night during sleep.

Although the extent of problems with asymptomatic nocturnal hypoglycemia in type 1 diabetes is especially worrisome, a note of caution needs to be raised regarding interpretation of CGMS results. The relative error of all methods of glucose monitoring increases to some extent at the lower limit of the detection range. With the CGMS, the current generated by the oxidation of interstitial glucose is calibrated against three to four finger-stick blood glucose levels daily. The tacit assumption is that the relationship between plasma and interstitial fluid glucose is fairly constant across the range of plasma glucose values. However, results from recent studies that have used microdialysis to measure glucose levels directly in interstitial fluid of muscle and adipose tissue during insulin-induced hypoglycemia (5) suggest that this assumption may not be correct. In comparison to values at euglycemia, the concentration gradient between plasma and interstitial glucose

increased when plasma glucose was lowered (5). If the same were true in subcutaneous tissue, the net result might be to artificially lower sensor estimates of blood glucose levels.

The present study was undertaken to examine this issue. Direct measurements of interstitial fluid glucose levels were obtained at different levels of plasma glucose and insulin using microdialysis and compared to sensor and plasma glucose readings. The hyperinsulinemic clamp technique was used to produce an increase in plasma insulin levels and to vary plasma glucose concentrations.

RESEARCH DESIGN AND METHODS

Study subjects

The study participants were 11 healthy young adult volunteers (9 women, 2 men). The age of the subjects was 24 ± 4 years (range 18–31) and the BMI was 22.1 ± 2.8 kg/m^2 (range 18.4–26.6). Volunteers were instructed to maintain their normal activity and diet for the 3 days before the study. The study was approved by the Yale Human Investigation Committee, and all subjects gave written consent after the aims, methods, and risks of the study were described to them.

The CGMS system

The MiniMed (Northridge, CA) CGMS system consists of a subcutaneous sensor connected by a cable to a pager-sized glucose monitor. The sensor is a glucose oxidase–based platinum electrode that is inserted through an insertion needle into the subcutaneous tissue of the anterior abdominal wall or other appropriate site using a spring-loaded device (the Senserter). Enzyme-mediated oxidation of glucose in the interstitial fluid generates an electrical current that is carried by a cable to a monitoring device. Sensor readings are acquired by the monitor every 10 s, and average values are reported every 5 min. The sensor outputs are stored in the monitor's memory, downloaded after the study, and analyzed by CGMS software. The program used (MiniMed Download Program Model 7310, version 1.6B) is a retrospective calibration approach using a modified form of linear regression to determine calibration factors based on three to four plasma glucose levels and simultaneous sensor outputs.

In this study, sensor outputs were downloaded into a Microsoft Excel file that allowed us to perform two separate calibrations. In the first analysis of sensor data, the sensor outputs were calibrated against four plasma glucose readings obtained during the baseline period before the start of the insulin clamp procedure (i.e., at −60, −40, −20, and 0 min). In the second analysis, the sensor outputs were calibrated against four plasma glucose levels distributed throughout the study—one during the baseline period (0 min) and one at the end of each step of the clamp at 60, 120, and 180 min.

In clinical use, glucose levels outside the 2.2- to 22.2-mmol/l range are reported as ≤2.2 or ≥ 22.2 mmol/l. For readings ≤ 2.2 mmol/l in this study, the actual glucose level was calculated from sensor outputs using the calibration factors.

Microdialysis technique

The microdialysis technique has been described in detail elsewhere (6). In this study, 30 × 0.62 mm microdialysis probes with a 20-kDa molecular weight cutoff (CMA/Microdialysis, Acton, MA) were used. Each probe was connected to a portable microinfusion pump and continuously perfused with artificial extracellular fluid (ECF) (NaCl 135 mmol/l, KCl 35 mmol/l, $MgCl_2$ 1 mmol/l, $CaCl_2$ 1.2 mmol/l, ascorbate 300 umol/l, and 2 mmol/l Na phosphate buffer, adjusted to pH 7.4) at a rate of 0.3 μl/min. The artificial ECF flows through the outer tubing into the space between the concentric cylinders to the distal end of the probe. The exchange of molecules between the extracellular fluid and the artificial ECF occurs across the semi-permeable dialysis membrane, after which the fluid enters the inner cannula in a retrograde direction. Dialysates were collected in timed fractions for later analysis. Two separate probes were used to measure interstitial glucose concentrations in each subject.

Experimental protocol and insulin clamp procedure

All subjects were studied in the supine position after an overnight fast. The sensor was inserted into the subcutaneous tissue of the anterior abdominal wall by the same investigator (T.P.M.) in all subjects. The microdialysis catheters were also inserted percutaneously in the subcutaneous adipose tissue of the anterior abdominal wall at a distance of at least 30 mm apart. Before insertion, intradermal local anesthesia was administered. A retrograde cannula was inserted into a vein in the dorsum of the left hand, which was positioned in a heated box (60–65°C) for sampling of arterialized venous blood. A second intravenous catheter was inserted into an antecubital vein for infusion of glucose and insulin.

After insertion of the sensor, microdialysis probes, and intravenous catheters, there was a 60-min rest period during which the glucose sensor was initialized. This was followed by a 60-min baseline period (−60 to 0 min) when the sensor was calibrated against at least four plasma glucose measurements from arterialized venous blood, and baseline plasma insulin and interstitial fluid dialysates were collected. Thereafter (at 0 min), an intravenous infusion of insulin (1.0 mU · kg^{-1} · min^{-1}) was initiated while adjusting a variable glucose (dextrose 20% wt/vol)) infusion to maintain euglycemia (~5 mmol/l). During the second step of the clamp, plasma glucose was allowed to fall rapidly to a target of ~3.1 mmol/l, and during the third step, plasma glucose was acutely raised to a target of ~8.6 mmol/l (Fig. 1). The 1.0 mU · kg^{-1} · min^{-1} insulin infusion was maintained throughout the clamp procedure. Each step of the clamp was maintained for 60–75 min, including the 10–15 min interval between each step that was needed to achieve the target level of glycemia. Plasma samples for insulin were collected every 30 min. Collections of interstitial fluid dialysates were obtained every 15 min. The timing of dialysate collections was adjusted for the transit time between the efferent arm of the probe to the collection tube.

Analytical procedures

Plasma glucose levels were measured by the glucose oxidase method with a Beckman glucose analyzer (Beckman Instruments, Brea, CA). Plasma insulin was measured by a double-antibody radioimmunoassay (Linco Research, St Louis, MO). Glucose in dialysates was measured by an enzymatic fluorometric method using an automated multianalyzer (CMA/600; CMA/Microdialysis, Stockholm, Sweden).

Statistics

Data in text and figures are presented as means ± SD. Because the interstitial glucose concentrations did not differ significantly between the two microdialysis

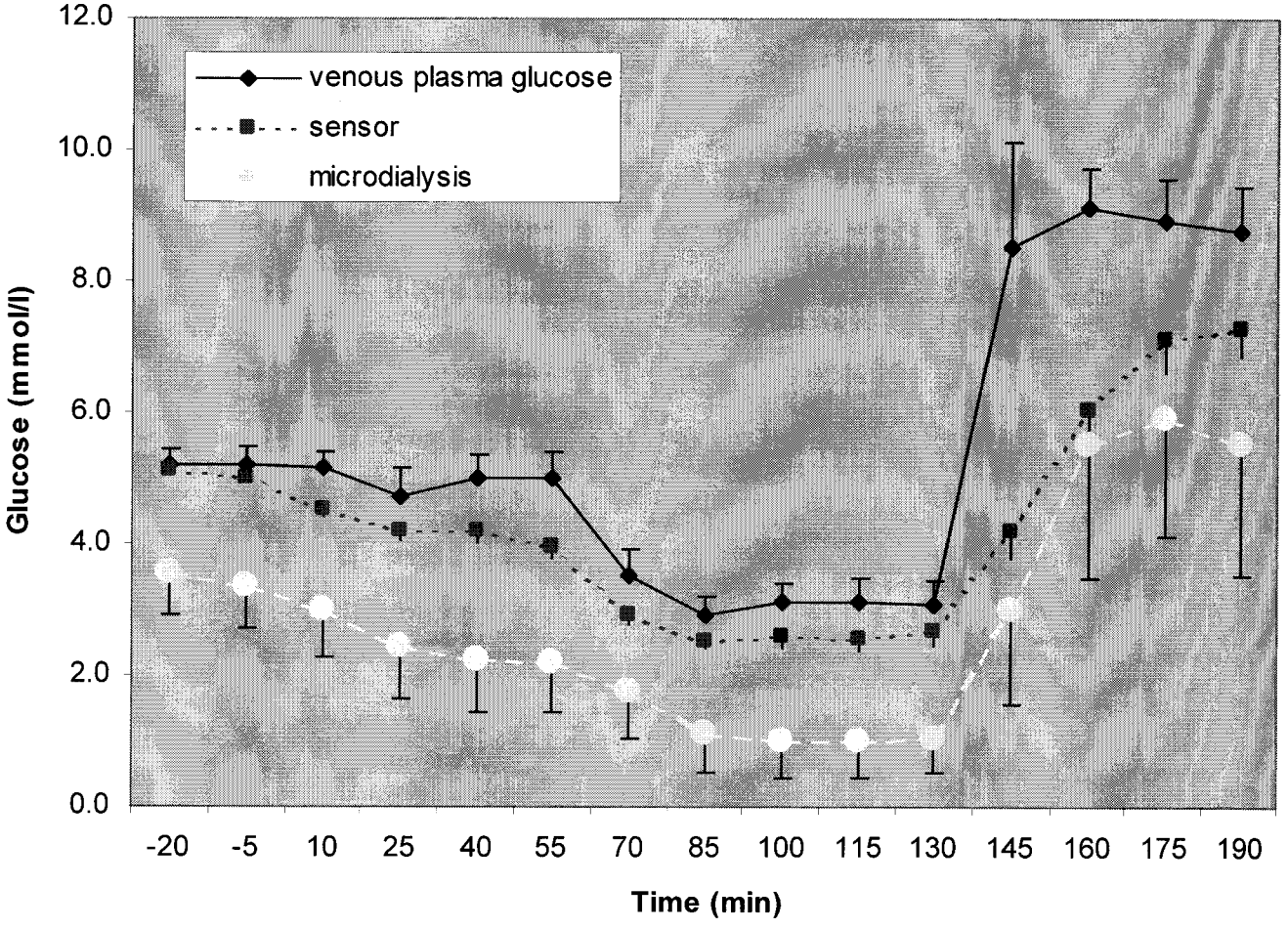

Figure 1—*Mean plasma and dialysate glucose levels over time and the effect of these changes on sensor readings when the CGMS was calibrated against plasma glucose levels during the baseline period.*

probes at any time point, the mean of the two dialysate samples in each subject at each time point was used for analysis. Comparison of glucose values at different time points was performed by repeated measures ANOVA. When there was a significant difference between the methods of measuring glucose, paired *t* tests were used to localize the effects. All analyses were performed using SPSS version 10.0 software and Microsoft Excel 97.

RESULTS — As shown in Table 1, plasma insulin concentrations (57 ± 12 pmol/l at baseline) increased to 395 ± 78 pmol/l during the euglycemic phase of the insulin clamp and remained at this level during the remainder of the study. In addition, target levels of glucose were achieved during each of the three steps of the clamp procedure. The relationship between plasma and interstitial (by microdialysis) glucose levels at baseline and during the three steps of the clamp is also

Table 1—*Mean plasma insulin, plasma glucose, dialysate glucose, and sensor glucose levels at baseline and during the three stages of the glucose clamp*

	Baseline	Euglycemia	Hypoglycemia	Hyperglycemia
Plasma insulin (pmol/l)	57 ± 12	395 ± 78	341 ± 51	396 ± 129
Plasma glucose (mmol/l)	5.2 ± 0.3	4.9 ± 0.3	3.1 ± 0.3	8.6 ± 0.6
Dialysate glucose (mmol/l)	3.3 ± 0.9†	2.3 ± 0.6†	1 ± 0.6†	5.7 ± 0.6†
Sensor glucose (mmol/l)				
Basal calibration	5 ± 0.3	3.7 ± 0.6†	2.5 ± 0.6†	7.3 ± 1.2†
Study calibration	5.4 ± 0.6*	3.9 ± 0.6†	2.8 ± 0.6	7.8 ± 0.9*

*Data are means ± SD, $n = 11$. Basal calibration, sensor calibrated against four plasma glucose levels during baseline period; study calibration, sensor calibrated against four plasma glucose levels obtained throughout the study. *$P < 0.05$ vs. plasma glucose at the same time point; †$P < 0.001$ vs. plasma glucose at the same time point.

shown in Table 1. At basal plasma glucose and insulin levels, the mean dialysate glucose concentration was 3.3 ± 0.9 mmol/l, or 65 ± 5% of plasma values. In contrast, during the first step of the insulin clamp, dialysate concentrations fell to 2.3 ± 0.6 mmol/l ($P < 0.001$ vs. baseline), even though plasma glucose levels were maintained at euglycemic levels by exogenous glucose infusion. Moreover, when plasma glucose was allowed to fall to ~3 mmol/l, dialysate glucose fell to 1.0 ± 0.6 mmol/l. The relatively greater reduction in interstitial compared to plasma glucose was statistically significant ($P < 0.001$ for group by time interaction). When plasma glucose was acutely raised to ~8.6 mmol/l, dialysate glucose concentrations also sharply increased to 66 ± 9% of plasma values (NS vs. baseline).

The time course of changes in plasma and dialysate glucose levels and the effects of these changes on sensor readings when the CGMS was calibrated against plasma glucose levels before the start of the clamp are shown in Fig. 1. Raw sensor outputs did not significantly change from the beginning (25.7 ± 11.7 nA) to the end of the basal period (25.0 ± 11.9 nA).

Because the CGMS was calibrated exclusively against plasma glucose levels before the start of the insulin infusion, basal sensor and plasma glucose values were similar (5 ± 0.3 vs. 5.2 ± 0.3 mmol/l) (Table 1). In contrast, during euglycemic hyperinsulinemia, sensor glucose levels fell to 3.7 ± 0.6 mmol/l ($P < 0.001$ vs. plasma), a 20% reduction in glucose levels that paralleled the fall in dialysis concentrations during this step of the clamp. Subsequently, sensor readings remained 20% lower than plasma glucose concentrations during the hypoglycemia step (2.5 ± 0.6 vs. 3.1 ± 0.3 mmol/l; $P < 0.05$). During recovery from hypoglycemia, sensor readings lagged behind increases in plasma glucose and remained ~15% lower than plasma glucose at the end of the study (7.3 ± 1.2 vs. 8.6 ± 0.6 mmol/l; $P < 0.05$). The changes in sensor glucose levels during the study were not significantly different than the changes in dialysate glucose concentrations ($P = 0.51$ for group by time interaction).

Sensor glucose levels were also determined when the sensor was calibrated against plasma glucose levels at baseline and at the end of each step of the clamp ("Study calibration" in Table 1). Under conditions that would average out variations in the plasma to interstitial glucose gradients, there was a generalized increase in sensor glucose levels ($P < 0.001$ vs. basal calibration). The sensor was still able to detect the fall in interstitial glucose levels during the euglycemic-hyperinsulinemic step ($P < 0.001$ vs. plasma glucose), but during the hypoglycemic step, sensor and plasma glucose levels did not differ significantly (Table 1).

CONCLUSIONS — The present study was undertaken to examine whether and to what extent changes in plasma insulin and glucose affect interstitial glucose concentrations and, in turn, the results of a glucose-sensing system that measures interstitial glucose levels. To achieve this, we used microdialysis to provide a means to measure changes in interstitial glucose levels directly. Recovery of glucose from the interstitial fluid by equilibrium dialysis is dependent on the dialysis flow rate, characteristics of the membrane, and dimensions of the probe, as well as other factors. We selected a well-established system that under the current conditions of use has been previously shown to result in a >90% recovery of interstitial glucose concentrations (7).

Our findings demonstrated that acute increases in plasma insulin lower interstitial glucose concentrations, even in the face of unchanged plasma glucose levels. Moreover, when plasma glucose was allowed to fall as a result of the insulin infusion, the relative difference in glucose concentrations in these two compartments tended to increase further. The current finding that insulin-induced hypoglycemia increases the interstitial−to−plasma glucose concentration gradient in subcutaneous adipose tissue is consistent with the results of previous hypoglycemic clamp studies in healthy subjects from our laboratory, albeit with a different microdialysis system (5). Although the physiological mechanisms of these effects of insulin have not been established and are likely to be complex (8–10), enhanced uptake of interstitial glucose by adipocytes that is not fully compensated for by increased delivery of glucose from the microcirculation undoubtedly plays an important role.

Calibration of the sensor against fasting plasma glucose and basal insulin levels resulted in baseline sensor glucose values that were nearly identical to plasma glucose concentrations. During the euglycemic-hyperinsulinemic phase of the study, the reduction in interstitial glucose concentrations was detected by the sensor and reported as a 20% fall in plasma glucose values. Thereafter, sensor glucose levels remained 20% below plasma values during the hypoglycemic step and 15% lower than plasma levels during recovery from hypoglycemia. Thus, as we hypothesized, increases in the plasma−to−interstitial fluid glucose gradient induced by insulin may cause the sensor to overestimate the fall and underestimate the degree of recovery from hypoglycemia. Similar discrepancies between plasma and interstitial glucose concentrations during acute changes in plasma glucose and insulin levels have been reported in rats (11). It should be noted, however, that the CGMS is usually calibrated against glucose levels that are distributed throughout the day and, consequently, at widely varying plasma glucose and insulin levels. To address this issue, we reassessed sensor function when the system was calibrated against plasma glucose levels at baseline and at the end of each step of the clamp procedure. Under these conditions, in which variations in plasma−to−interstitial glucose gradients and any potential downward drift of sensor outputs are averaged out, differences between plasma and sensor glucose levels were substantially reduced. Indeed, with this calibration, there were no significant differences between plasma and sensor glucose levels during the hypoglycemic step.

In studies that have used the CGMS to monitor glycemic control in intensively treated type 1 diabetic patients, frequent and prolonged asymptomatic hypoglycemia has often been observed, especially during the night (1). Consequently, a primary aim of this study was to validate the accuracy of the sensor in detecting hypoglycemia. Our results demonstrated that in healthy nondiabetic subjects, the CGMS tracks changes in plasma glucose very well and with an accuracy that is comparable to that of conventional meter methods. Preliminary reports from two other studies that used the hypoglycemic clamp to validate CGMS glucose levels in human subjects found only modest discrepancies between plasma and sensor glucose levels (12,13). Furthermore, the study by Kerr et al. (12) showed a similar lag in sensor readings during recovery from hypoglycemia. The effect of varia-

tions in plasma insulin and glucose on CGMS performance in patients with type 1 diabetes remains to be determined.

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