# Timing of Changes in Interstitial and Venous Blood Glucose Measured With a Continuous Subcutaneous Glucose Sensor

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**The objective of this study was to use a subcutaneous continuous glucose sensor to determine time differences in the dynamics of blood glucose and interstitial glucose. A total of 14 patients with type 1 diabetes each had two sensors (Medtronic/MiniMed CGMS) placed subcutaneously in the abdomen, acquiring data every 5 min. Blood glucose was sampled every 5 min for 8 h, and two liquid meals were given. A smoothing algorithm was applied to the blood glucose and interstitial glucose curves. The first derivatives of the glucose traces defined and quantified the timing of rises, peaks, falls, and nadirs. Altogether, 24 datasets were used for the analysis of time differences between interstitial and blood glucose and between sensors in each patient. Time differences between blood and interstitial glucose ranged from 4 to 10 min, with the interstitial glucose lagging behind blood glucose in 81% of cases (95% CIs 72.5 and 89.5%). The mean (±SD) difference between the two sensors in each patient was 6.7 ± 5.1 min, representing random variation in sensor response. In conclusion, there is a time lag of interstitial glucose behind blood glucose, regardless of whether glycemia is rising or falling, but intersensor variability is considerable in this sensor system. Comparisons of interstitial and blood glucose kinetics must take statistical account of variability between sensors. *Diabetes* 52:2790–2794, 2003**

Continuous glucose monitoring has been an elusive goal in the management of patients with diabetes, but one that could revolutionize our concept of how diabetes is managed and even defined (1). In 1999 the Food and Drug Administration (FDA) approved a continuous glucose sensor, the Medtronic/MiniMed (Sylmar, CA) continuous glucose monitoring system (CGMS), which utilizes a subcutaneous needle electrode and measures glucose by an amperometric method (2). In 2002 the FDA approved, for use in children, a second device, the GlucoWatch G2 Biographer, manufactured by Cygnus (Redwood City, CA), which also measures interstitial glucose concentration sampled through a process called reverse iontophoresis (3).

Subcutaneous sensors measure the interstitial glucose concentration rather than that of blood. While blood glucose has traditionally been the analyte of choice in defining and managing diabetes, other measures may in fact have even more clinical importance. The effect of hyperglycemia on both the symptoms and complications of diabetes is presumably mediated via interstitial and cellular effects. Thus, blood glucose itself is only a marker for and mediator of glucose effects on tissues.

Glucose, a small molecule of 180 Da, is freely transferred across the capillary endothelium to the interstitium. This process is not mediated by a glucose transporter, but probably by simple transcellular and/or paracellular diffusion (4). The equilibrium kinetics between glucose in the blood and interstitium are not clear, but certainly there is a direct relationship as changes in the interstitial glucose pool are positively correlated with changes in the blood glucose pool (5). Some believe that a significant time difference exists for the equilibration of interstitial and blood glucose (i.e., a "lag time") (6). Biologically, this could reflect the times for diffusion across the transcapillary wall and/or interstitial microconvection (7), although the contribution of each factor is not known. Other questions include the extent to which the sensor's analytic time determines the perceived lag time, whether lag times are consistent or variable, and whether they vary depending on whether blood glucose is rising, peaking, falling, or reaching a nadir.

Such information is important, physiologically and clinically. It bears, for example, on the utility of continuous sensing in providing a real-time indication of clinical hypoglycemia or hyperglycemia. It is also of crucial importance in determining the potential of a sensor in driving a closed-loop insulin delivery system. Too long or too inconsistent a lag time could render interstitial glucose ineffective in controlling insulin delivery.

The objective of this study was to determine whether there is a significant, systematic time difference between blood glucose and interstitial glucose using one CGMS. We also sought to determine whether there is a consistent direction to any time difference throughout each phase of a glucose profile (i.e., as blood glucose rises, peaks, and falls). To do this, we assessed the time differences in the equilibration of blood glucose and interstitial glucose, before and after administering a standardized meal, with

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CGMS, continuous glucose monitoring system; FDA, Food and Drug Administration; IV, intravenous.

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simultaneously measured with frequent intravenous blood sampling and two continuous subcutaneous sensors.

## RESEARCH DESIGN AND METHODS

**Subjects.** Fourteen subjects with type 1 diabetes (8 men and 6 women) were recruited from the diabetes clinic. Mean age was 45 years and mean $HbA_{1c}$ was 8.0% (normal <6.1%). The institutional review board at the Johns Hopkins University School of Medicine (i.e., Joint Committee on Clinical Investigation) approved the protocol, and written consent was obtained from all participants.

**Sensor.** The CGMS sensor was used (2). It is an electrochemical sensor with glucose oxidase immobilized to an electrode. The sensor is attached to a sterile 22-gauge needle that is removed after sensor insertion in the subcutaneous tissue. Interstitial glucose is converted at the glucose oxidase interface to produce hydrogen peroxide, which is oxidized, producing an amperometric signal at the platinum/anodic electrode. The generation of this signal is proportional to the glucose concentration in interstitial fluid. Interstitial glucose is measured every 10 s, and this signal is reported as an average glucose concentration every 5 min. A proprietary software program is designed to eliminate outlier noise during each 5-min interval and to produce a weighted average that reflects interstitial glucose during the interval. A total of 288 average measurements are recorded each day, and the sensor can be worn for 48–72 h, according to the manufacturer. There is no real-time display of glucose values, but a communication station (Com-Station) allows transfer of the stored data in the monitor by infrared pulses through a serial port to an external personal computer for review. A CGMS is designed to measure a range of glucose concentrations from 40 to 400 mg/dl (2).

**Whole blood glucose analyzer.** A glucose analyzer (YSI 2700 Select; YSI, Yellow Springs, OH) was used to measure blood glucose in 25-μl samples. The intra-assay CV was 2% to a maximum of 2.5 mg/dl for the range 25–900 mg/dl.

**Protocol.** The subjects were admitted the previous night to the General Clinical Research Center at the Johns Hopkins Hospital. Two sensors were placed subcutaneously by the clinical investigator into the abdomen within 2 inches of each other. After initialization, a one-point calibration process was performed utilizing a sample of venous whole blood. After an overnight fast, an intravenous (IV) catheter was placed in an antecubital vein at ~0730. The one-point calibration process was repeated, and then whole blood for glucose was sampled every 5 min from the IV catheter for 8 h. The blood glucose was sampled ~2.5 min after the sensor took a reading so as to provide a value relatively close to the sensor's weighted average reading. Each sample was collected into a sodium fluoride tube, and the blood glucose was measured immediately after collection. After a 30-min baseline, subjects took their usual insulin dose and ingested a liquid meal (6 kcal/kg: 50% carbohydrate, 30% fat, and 20% protein) prepared in a metabolic kitchen. The meal was repeated 4 h later.

**Analysis.** Data from both sensors were downloaded via the Com-Station. The Medtronic/MiniMed software package processed the amperometric data, converting it to glucose concentration. A graphing utility then generated a table of summary statistics, a database of all the individual data points, and 24-h and modal-day glucose plots. Glucose data were compared with the blood glucose values from the glucose analyzer.

Smoothing algorithms using a low-order polynomial least squares method were applied to the glucose time curves. The first derivatives of the curves were used to define and quantify the timing of the most rapid rate of rise and peak and most rapid rate of fall and nadir of blood glucose and interstitial glucose. The most rapid rate of rise was defined as the maximum value of the first derivative curve, where simultaneously the second derivative is zero. A peak was noted when the derivative curve passed through zero on the declining arm of the curve. The most rapid rate of fall was the minimum value of the derivative curve, where simultaneously the second derivative is zero. The nadir was defined as when the derivative curve passed through zero on the ascending limb of the curve.

The analysis of the present measurements made use of the zeros of the first and second derivatives of the time-dependent measured signals. For instance, the interstitial glucose concentration, C(t), is related to the measured signal, S(t), through the equation

$$C(t) = A * [S(t) - B]$$

where A and B are slope and offset calibration parameters, respectively. The first and second derivatives of the interstitial glucose concentration are given by the following equations.

$$d/dt\ C(t) = A * d/dt\ S(t) \quad \text{and}$$

$$d^2/dt^2\ C(t) = A * d^2/dt^2\ S(t)$$

It is evident from these equations that the derivatives of C(t) will be nonzero and depend on A whenever S(t) is nonzero. However, the derivatives of C(t) can only be zero when the corresponding derivative of S(t) is zero, and that occurrence is independent of the calibration factor A. Therefore, because the present analysis only makes use of the times when the first or second derivatives are zero, the analysis is independent of the calibration.

The $F$ test was used to verify that the data samples were normally distributed. Student's two-tailed unpaired $t$ tests were used to evaluate statistical significance. Inter-sensor time differences of blood glucose and interstitial glucose were analyzed by comparison of the means. Results were expressed as means ± SD. Statistical significance was defined as $P < 0.05$.

## RESULTS

Twenty-four (of a possible 28) interstitial glucose and 14 blood glucose datasets were analyzed. Four glucose sensors failed, providing no data for analysis. Figure 1*A* illustrates the sensor and blood glucose concentrations in one patient. Figure 1*B* illustrates the first derivatives of the curve in Fig. 1*A*, showing how the rise, fall, peak, and nadir were calculated. The precise time of most rapid rise, peak, most rapid fall, and nadir for interstitial glucose were based on the original amperometric data, not dependent on calculated interstitial glucose. Small changes in glucose were ignored unless they were seen in both blood glucose and interstitial glucose and reached a rate of change of at least $1\ mg \cdot dl^{-1} \cdot min^{-1}$. In the study illustrated, three peaks and two nadirs were analyzed.

Aggregate time differences between sensors in each patient and between sensors and blood glucose are summarized in Table 1. The most rapid rise in blood glucose preceded interstitial glucose in 88% of cases, the peak in blood glucose preceded the peak in interstitial glucose in 71% of cases, the most rapid fall in blood glucose preceded interstitial glucose in 75% of cases, and the nadir in blood glucose preceded the nadir in interstitial glucose in 94% of cases. Thus, in 81% (95% CI 72.5–89.5%) of all situations, blood glucose preceded interstitial glucose.

Therefore, considering each sensor as an independent measure of interstitial glucose and comparing each to the corresponding blood glucose, statistically significant lag times occurred in the rise (10.1 ± 10.1 min, $P < 0.001$), the fall (6.9 ± 8.5 min, $P = 0.017$), and the nadir (9.4 ± 7.7 min, $P < 0.001$). In each case, blood glucose preceded interstitial glucose. The time difference in achieving the peak blood glucose (4.0 ± 10.4 min) approached but did not achieve statistical significance ($P = 0.055$).

But since sensor-to-sensor time variability was considerable, ranging 5.7–7.6 min with a mean of 6.7 ± 5.1 min, the within-individual sensor variation was in many cases greater than the apparent time lag between blood glucose and interstitial glucose. Incorporating this sensor-to-sensor variability within each person into the analyses, the timing of nadir, peak, rise, and fall of interstitial glucose did not significantly differ from that of blood glucose (all $P$ values >0.06). In sum, while there is on average a time lag between blood glucose and interstitial glucose when only individual blood glucose-to-sensor data are considered, the sensor-to-sensor differences (within the same patient) are in the same time range as the blood glucose-to-sensor lag.

## DISCUSSION

Using our analytic approach with a CGMS sensor and considering each measure independently, there are statis-

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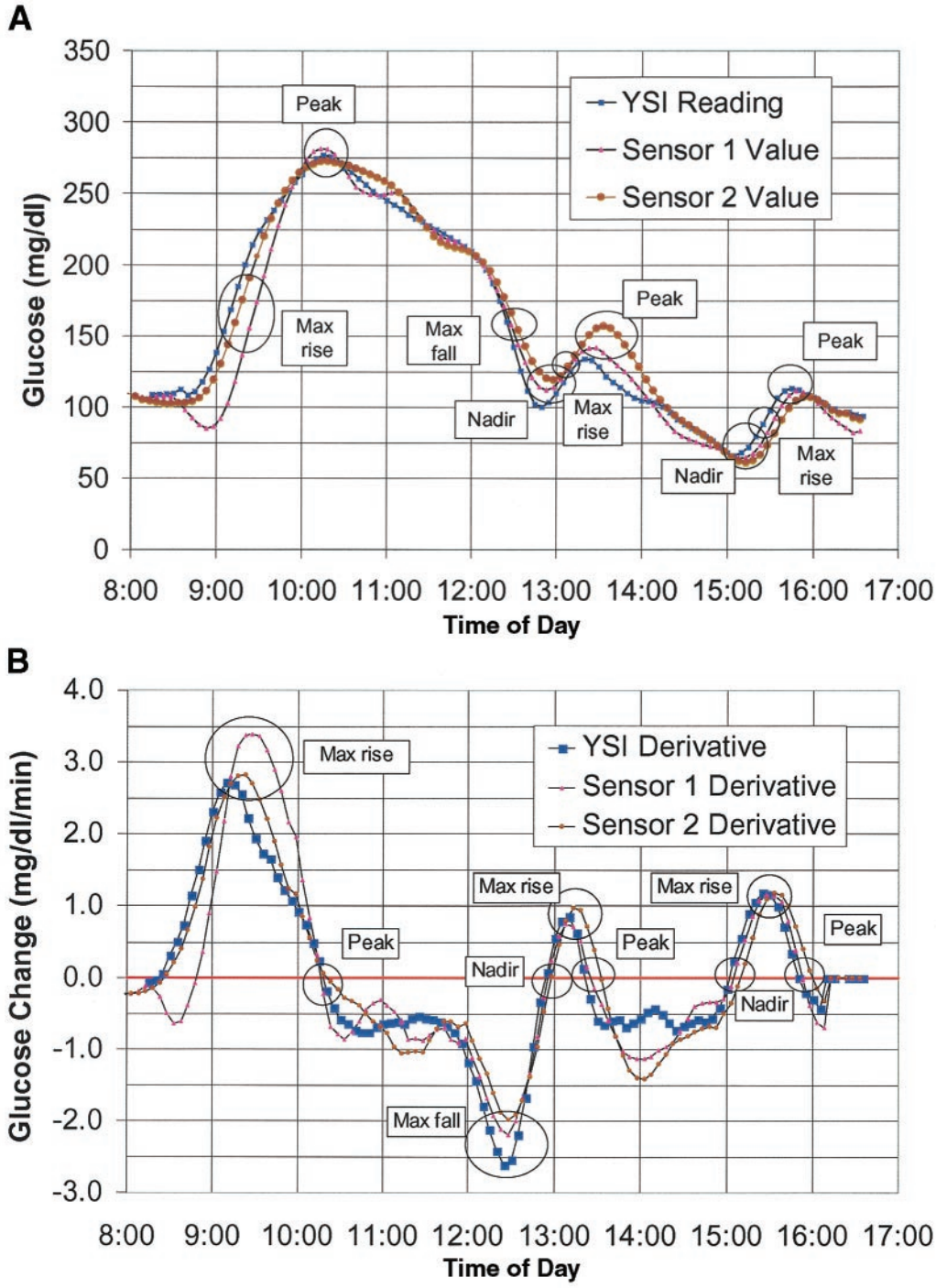


**FIG 1.** ***A*: Glucose concentration by simultaneous venous sampling and two continuous subcutaneous glucose sensors.** ***B*: The first derivatives of the glucose concentration curve from Fig. 1*A*.**

tically significant time differences for the rise, fall, and nadir of glucose between blood glucose and interstitial glucose (the time difference at peak glucose not reaching statistical significance). It is important to note that on average, blood glucose preceded the interstitial glucose at all points in the postprandial glucose curve, irrespective of whether blood glucose is rising, falling, or attaining peak or nadir, with a range of 4–10 min. We found no support for the notion that change in interstitial glucose precedes change in blood glucose when blood glucose is falling.

Furthermore, we found that it is essential to go beyond the simple average blood glucose to interstitial glucose difference by using two sensors on each subject and measuring the differences between these two measures of interstitial glucose, recognizing that theoretically they should be equal, but in practice there would be between-sensor variability even within one subject. We found that the sensors do not react simultaneously and in fact have considerable random time lag between them, the 6.7 ± 5.3 min difference being quantitatively similar to the average apparent interstitial glucose/blood glucose time lag. If that within-subject variance is taken into account, none of the

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individual time differences between blood glucose and interstitial glucose retains significance (although one, for rising blood glucose, was marginally significant).

A large part of the time difference in this system is thus attributable to the individual sensor itself rather than to interstitial glucose/blood glucose dynamics. If there is a relatively large time lag built into the analysis, as is the case with the Cygnus GlucoWatch Biographer (18 min) (8), there is little power to detect a biologic time lag. Analytic time could be minimized if the sensor reported interstitial glucose measurements more frequently than every 5 min. Also, the wide confidence limits indicate that experiments with more subjects could add precision in our measurements. But our data illustrate that intersensor variability must be taken into consideration. It is not clear from the data whether the intersensor variability is constant across the entire glycemic range, but our impression is that the variability fluctuates in a nonpredictable manner.

After insulin administration, the hypothesis has been advanced that cellular glucose utilization would precede and cause a drop in interstitial glucose, followed by a drop in blood glucose—hypoglycohistiosis (low tissue glucose) preceding hypoglycemia (9). This effect has apparently been demonstrated in diabetic rats (10), diabetic dogs (11), and in some patients with type 1 diabetes (9). We found, however, that a drop in interstitial glucose preceded the fall in blood glucose in only 25% of cases. In 75% of cases, interstitial glucose lagged behind blood glucose. Again, this may be due to the operating characteristics of the glucose sensor in the subcutaneous space.

There has been discussion as to whether insulin itself modulates the time differences in the equilibration of interstitial and blood glucose (12). If so, lag times may differ from tissue to tissue, depending on insulin sensitivity and ambient insulin level (13), and in some cases hypoglycohistiosis may precede hypoglycemia. As the catheter of the CGMS system is imbedded in subcutaneous adipose tissue, it may reflect this particular tissue, but our data suggest that the vagaries of the sensors make it unlikely that we could measure variability in insulin action on specific tissues. At any rate, data from Rebrin et al. (14) and Steil et al. (15) using a canine model suggest that insulin does not alter extracellular glucose distribution.

Four sensors failed during this study. In one patient, bleeding was noted at the implantation site. Bleeding has been previously described as interfering with proper subcutaneous sensor function (9). Other sensors showed considerable drift in sensor signal although the reason for the drift is not obvious, possibly reflecting occult hemorrhage/thrombus formation, tissue protein adsorption, localized accumulation of inflammatory cells, or fibrous encapsulation at the sensor-tissue interface (7,16,17). Sensor drift into the hypoglycemic range was noted in several patients, but these "occult" hypoglycemic episodes were not corroborated by concurrent blood glucose measurements (data not shown). This phenomenon has been recently described by other investigators (18). It is to be emphasized that we did not recalibrate sensors in the way recommended for clinical use. Thus, we were rigorously testing time lag kinetics, not clinical accuracy or utility of the sensors.

In conclusion, using the CGMS sensor, a small but

TABLE 1
Time differences between blood glucose (BG) and interstitial glucose (IG) as determined by continuous subcutaneous glucose sensor

| | BG to sensor | | Sensor to sensor | | Is BG-to-sensor time difference zero? (No, if $P < 0.05$) | Is BG-to-sensor time difference comparable to sensor-to-sensor? (Yes, if $P > 0.05$) |
|---|---|---|---|---|---|---|
| | $n$ | Time (min) | $n$ | Time (min) | $P$ | $P$ |
| Time difference in achieving | | | | | | |
| Peak glucose value | 28 | 4.0 ± 10.4 | 26 | 7.6 ± 5.2 | 0.055 | 0.11 |
| Nadir in glucose value | 16 | 9.4 ± 7.7 | 18 | 7.0 ± 6.0 | 0.000 | 0.33 |
| Most rapid rate of rise in glucose | 25 | 10.1 ± 10.1 | 21 | 5.7 ± 4.7 | 0.000 | 0.06 |
| Most rapid rate of fall in glucose | 12 | 6.9 ± 8.5 | 13 | 6.1 ± 4.2 | 0.017 | 0.79 |

Data are means ± SD. $n$, occurrences within the datasets for analysis.

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consistent time difference of 4–10 min was found between blood glucose and interstitial glucose in subcutaneous abdominal tissue. If confirmed, these time differences would have to be taken into account in the design of a closed-loop system. The time differences may not be due to a "lag time," as defined by physiological processes, but rather the response characteristics of the glucose sensor system. These observations are not necessarily applicable to the Cygnus Biographer system.

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