Physiological Differences Between Interstitial Glucose and Blood Glucose Measured in Human Subjects

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OBJECTIVE — This study investigated whether glucose readings from a sensor sampling in interstitial fluid differ substantially from blood glucose (BG) values measured at the same time.

RESEARCH DESIGN AND METHODS — We have evaluated the relationship between BG and glucose extracted from interstitial fluid using the GlucoWatch (Cygnus, Redwood City, CA) biographer, a device that collects glucose from subcutaneous interstitial space through intact skin by application of a low electric current. We evaluated the relative change in the interstitial glucose (IG) signal (IGS) as measured by the biographer versus BG using a normalized two-point sensitivity index (NSI).

RESULTS — The results show that biographer measures of IG differ in time and magnitude from the corresponding BG values. In particular, the biographer values were shifted in time due to instrumental and physiological lag. Results show an average total lag of 17.2 ± 7.2 min for all subjects evaluated. The instrumental lag was 13.5 min, suggesting that physiological lag is ~5 min. In addition, when glucose was increasing, the change in IGS was less than that in BG, while when BG was decreasing, the change in IGS was greater than that in BG.

CONCLUSIONS — Similar results have been reported for other measures of IG, suggesting that differences reflect physiological variation in glucose uptake, utilization, and elimination in blood and interstitial space. This further evidence of the difference between IG and BG should be considered when interpreting glucose measurements from devices that sample interstitial fluid.

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Glucose measured in interstitial fluid (IG) may differ substantially from blood glucose (BG) values measured at the same time. Reach et al. (1) evaluated dynamic changes in IG in rats using an implanted glucose sensor. Their results showed that IG was lower than BG when glucose was increasing, yet IG and BG were similar when glucose was declining. They developed a physiological "push-pull" model to describe these differences. Similar results were obtained when IG was measured by a microdialysis technique and compared with BG (2).

The purpose of this study was to investigate whether these differences observed in interstitial fluid glucose sensors can also be observed using an iontophoretic, transdermal glucose sensor. We have evaluated the relationship between BG and glucose extracted from interstitial fluid using a GlucoWatch (Cygnus, Redwood City, CA) biographer. This is an Food and Drug Administration–approved device used to detect trends and track patterns in glucose levels (3–5). It works through a process called reverse iontophoresis (6), which allows the biographer to collect glucose samples from subcutaneous interstitial space through intact skin by application of a low-level electric current. When current is applied, glucose molecules are pulled through the skin, along with charged molecules (positive and negative ions) and the surrounding medium (water). The ions migrate to gel collection discs placed at the anode (+) and cathode (−) in a single-use AutoSensor. The glucose molecules are then collected in these discs for analysis. The gel collection discs contain the enzyme glucose oxidase. As glucose enters the discs, it reacts with the glucose oxidase in the gel and forms hydrogen peroxide. A biosensor in contact with each gel collection disc detects the hydrogen peroxide generating a current. This current is integrated, producing a signal in units of electric charge, nanoCoulombs (nC). The biographer uses a calibration value entered by the patient to convert the signal into a glucose measurement. The single-point calibration is performed with a traditional BG meter after a 3-h warm-up period.

RESEARCH DESIGN AND METHODS

— Data from existing clinical studies were used for this analysis (5). The studies consisted of diabetic (type 1 and insulin-treated type 2) subjects, each wearing one or two biographers. Informed consent was obtained from all subjects. A total of 59 wear periods were chosen solely on the basis of significant BG excursion throughout the day and sufficient available biographer data to perform time lag analyses. The data were from studies in a controlled clinical setting (28 wear periods) and a "simulated home environment" (31 wear periods). The investigator induced mild hyper- and

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E.K., J.A.T., and M.J.L. are employed by and hold stock in Cygnus, Inc. E.K. and R.O.P. were employed by Cygnus during the research of this article and also hold Cygnus stock.

Abbreviations: BG, blood glucose; IG, interstitial glucose; IGS, interstitial glucose signal; NSI, normalized two-point sensitivity index; T_i, instrumental lag; T_p, physiological lag.

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

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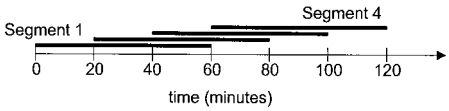

Figure 1—*Overlapping segmentation of the data.*

hypoglycemia during the studies (between 40 and 450 mg/dl).

The 59 biographer wear periods were from 51 unique subjects. There were eight subjects who provided data from two wear periods. There were 22 men and 29 women, and 32 subjects with type 1 diabetes and 19 with type 2 diabetes. The means ± SD of other descriptive statistics were as follows: age 52 ± 12 years, HbA_{1c} (prestudy) 7.7 ± 1.6%, and BMI 28.6 ± 4.6 kg/m^2.

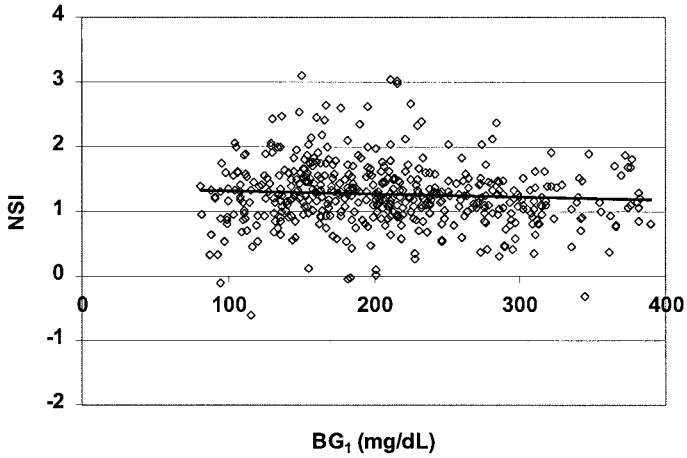

Figure 3— *A plot of NSI versus BG_1 for decreasing glucose values. The regression line (NSI = 1.3821–0.0005 BG_1, r = −0.023, n = 489) is also shown.*

Time lag

The effect of time lag can potentially confound the assessment of interstitial and BG differences. However, when frequent BG and biographer data are available, it is possible to calculate the time lag between the two measurements and eliminate it.

To estimate time lag, IG values were linearly interpolated to yield values every minute. BG readings were taken twice per hour using a HemoCue (Aktiebolaget Leo, Helsingborg, Sweden) analyzer. The time shift required to match the biographer and BG values was then determined using a method similar to cross-correlation analysis, where the biographer glucose data were shifted in time with respect to the BG data until "maximal overlap" was obtained. This analysis yielded an average total lag of 17.2 ± 7.2 min for the biographer glucose relative to the BG value. A similar value was obtained previously (2).

The total lag is a sum of the instrumental and physiological lag. The instrumental lag arises from biographer's measurement method, which produces a glucose value every 20 min by averaging two 10-min values. Each 10-min period consists of 3 min of glucose collection and 7 min of glucose detection. Thus glucose is collected from −20 to −17 min and −10 to −7 min, relative to the biographer value. This leads to an average instrumental lag of −13.5 min. The physiological lag represents the time required for BG to diffuse from capillaries into the interstitial space. The physiological lag associated with the biographer, therefore, is the difference of total lag and instrumental lag. In this case, it was 3.7 min (2), which is in agreement with previously published estimates of time lag between BG and IG (8). For the following analysis, this value was approximated as 5 min. (This analysis used 1-min interpolation of values, so this approximation is unlikely to have affected the results.)

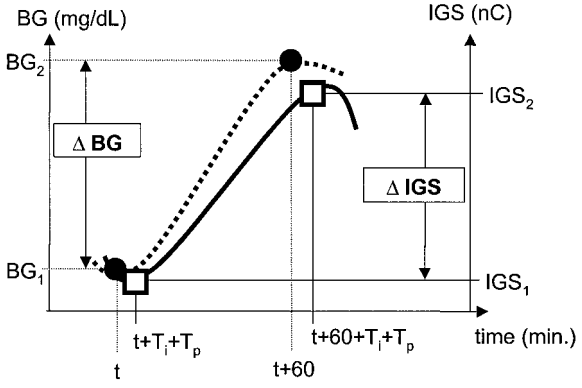

Figure 2— *A schematic representation of the NSI.*

Signal magnitude

To evaluate the relative change in the IGS (in nanoCoulombs) versus BG (in milligrams per deciliter), the normalized two-point sensitivity index (NSI) was calculated using Eq. 1. This index is a measure of how an interstitial sensor responds to a given step-change in BG concentration.

$$\text{NSI} = \frac{\text{Normalized } \Delta\text{IGS}}{\text{Normalized } \Delta\text{BG}} \quad (1)$$

Normalized ΔIGS and ΔBG were calculated using Eqs. 2 and 3, where the subscripts refer to different times.

$$\text{Normalized } \Delta\text{IGS} = \frac{\text{IGS}_2 - \text{IGS}_1}{\text{IGS}_2 + \text{IGS}_1} \quad (2)$$

$$\text{Normalized } \Delta\text{BG} = \frac{\text{BG}_2 - \text{BG}_1}{\text{BG}_2 + \text{BG}_1} \quad (3)$$

To minimize changes in NSI due to signal drift, values were calculated for overlapping 60-min time segments, shown schematically in Fig. 1. This period of time is sufficient for large changes in BG without substantial IGS drift.

Due to the instrumental lag (T_i) and physiological lag (T_p), the IGS values were delayed relative to the corresponding BG value (Fig. 2). The time-delayed value for IGS was determined by linear interpolation at time ($T_i + T_p$). Equations 2 and 3 then become Eqs. 4 and 5, respectively.

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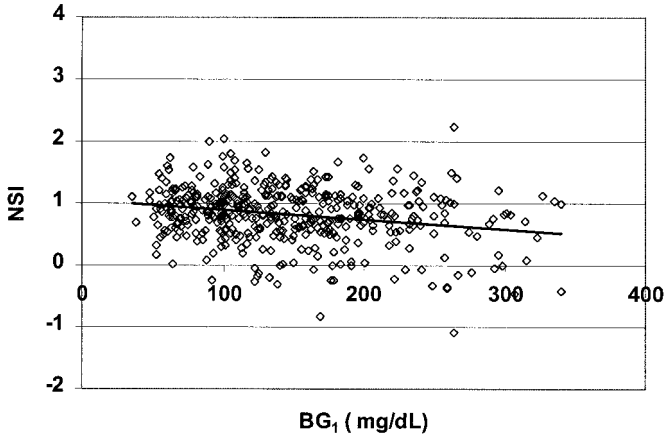

Figure 4— *A plot of NSI versus BG_1 for increasing glucose. The linear regression line is also shown (NSI = 1.05–0.0015 BG_1,* r = −0.22, n = 457*).*

$$\text{Normalized } \Delta\text{IGS} = \frac{\text{IGS}(T + T_i + T_p + 60) - \text{IGS}(T + T_i + T_p)}{\text{IGS}(T + T_i + T_p + 60) + \text{IGS}(T + T_i + T_p)} \quad (4)$$

$$\text{Normalized } \Delta\text{BG} = \frac{\text{BG}(T + 60) - \text{BG}(T)}{\text{BG}(T + 60) + \text{BG}(T)} \quad (5)$$

The NSI was calculated using Eqs. 1, 4, and 5. The NSI is shown schematically in Fig. 2. The NSI values were calculated as a function of BG using 13.5 min for T_I and 5 min for T_p.

Statistical tests of significance were performed on the slopes of the relationship between NSI and BG when BG was increasing and decreasing to determine whether the sensitivity of the IG measurement varied with respect to the level of BG. This was done by determining whether the CI of the slope contained zero. In this case, the relevant CI was used in assigning the appropriate *P* value. Student's *t* tests were also performed to determine whether the mean NSI was different from 1, indicating that the IG sensor sensitivity was different from the BG sensor sensitivity.

RESULTS — The results presented in Fig. 3 show NSI versus BG for declining glucose (ΔBG <0). A linear regression analysis of the data shows no significant dependence of NSI on BG when glucose is decreasing ($P > 0.05$). In addition, the mean value for NSI is >1 (1.27 ± 0.02, $P < 0.01$).

The NSI results for increasing BG are shown in Fig. 4. A linear regression analysis of the data shows a significant decrease of NSI with increasing BG ($P < 0.001$). The results also show that the mean value for NSI is <1 (0.83 ± 0.02, $P < 0.01$). Moreover, the results show that the mean NSI value is significantly lower for increasing glucose than for decreasing glucose ($P < 0.001$), suggesting a different response depending on the direction of glucose change.

The mean and SD for NSI was calculated for various BG ranges for increasing (ΔBG >0) and decreasing (ΔBG <0) BG. A comparison of the mean NSI values shows that the values for decreasing glucose are higher than for increasing glucose at all except the lowest and highest glucose levels. The reversal of trend at the highest glucose level is likely due to the very small sample size, especially for decreasing glucose.

To more easily visualize the data, the regression lines and mean values are plotted in Fig. 5. These results show fundamentally different behavior for increasing and decreasing glucose. For decreasing glucose, NSI is greater than unity for all BG values and decreases slightly with increasing BG. In contrast, for increasing glucose, NSI is less than unity and decreases with increasing BG. Hence, for all determinations made when glucose is decreasing, ΔIGS exceeds ΔBG, while when glucose is increasing, ΔBG exceeds ΔIGS.

The results presented in Fig. 5 show that NSI depends both on BG level and on whether BG is increasing or decreasing. However, they do not indicate whether NSI depends on the rate of change in BG. To examine this, the data were divided into 3 bins of BG and 5 bins of ΔBG, and the mean NSI was calculated for each of the 15 bins. All bins contained at least 17 data points except the bin with the highest BG and largest ΔBG, which contained no points (it is difficult to generate a fast BG increase when the subject's BG is already >280 mg/dl). The mean NSI values are plotted in Fig. 6. The results again show NSI <1.0 for all increasing glucose (ΔBG >30 $mg \cdot dl^{-1} \cdot h^{-1}$) at all BG levels, and the NSI value decreases as BG increases, with a particularly large decline at high BG (280–400 mg/dl) and ΔBG (30–70 $mg \cdot dl^{-1} \cdot h^{-1}$). In contrast, when glucose is decreasing (ΔBG <−30 $mg \cdot dl^{-1} \cdot h^{-1}$), NSI is greater than unity at all BG levels and relatively unchanged with increasing BG.

Using the data from Fig. 6 it is possible to simulate the IG response expected for a sample BG excursion. A simple "trapezoid" input BG pattern was used. To calculate IG, the BG and ΔBG values were determined at each time point on the "trapezoid" input and the appropriate NSI value. The corresponding IG value was estimated by the product of the appropriate NSI and BG values and plotted with a 17-min lag relative to BG. The resultant BG ("trapezoid") and IG points were then fit with a smooth curve (a fourth-order

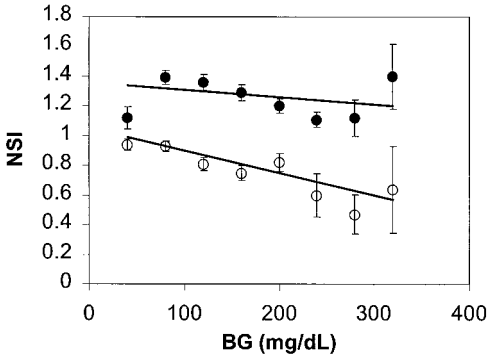

Figure 5— *The mean NSI and SE values versus BG for increasing and decreasing glucose. The regression lines are those shown in Figs. 3 and 4.* ●*, decreasing glucose;* ○*, increasing glucose.*

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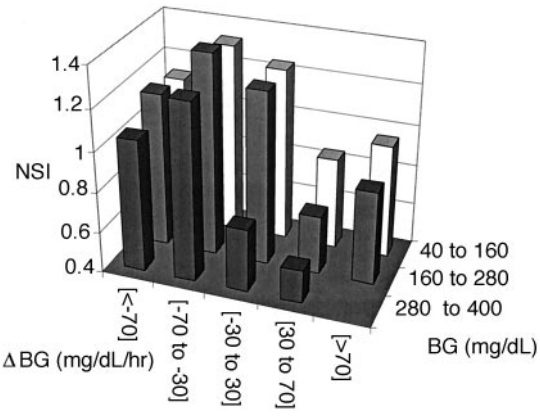

Figure 6— *The mean value of NSI as a function of BG and ΔBG.*

polynomial). The results are shown in Fig. 7.

CONCLUSIONS — The results from a variety of studies show that continuous measurements of IG differ in time and magnitude from corresponding BG values (1,2). The results presented here suggest that the same is true for IG measured by the biographer. The IG values were shifted in time due to instrumental and physiological lag. A cross-correlation analysis showed an average total lag of 17.2 ± 7.2 min for all subjects evaluated. The instrumental lag was 13.5 min, suggesting that physiological lag associated with the biographer is ~5 min.

In addition, the IGS from the sensor (in nanoCoulombs) was compared with BG values (in milligrams per deciliter). The ratio of the 60-min change in signal (after compensating for lag) was compared to the change in BG over 60 min. The ratio of these values, NSI, was then determined as a function of BG for each 20-min measurement cycle during biographer operation. The results demonstrate fundamentally different behavior for NSI depending on whether glucose was increasing or decreasing. In particular, when glucose was increasing, the change in IG signal was less than the change in BG (NSI <1) and decreased with increasing glucose. In contrast, when BG was decreasing, the change in IG signal was greater than the change in BG (NSI >1) but relatively independent of changing BG.

These results show that the IG response differed from BG in both time and magnitude. The greatest difference in magnitude is seen at large BG values, especially when glucose is rapidly increasing. For example, at BG levels >280 mg/dl, the ΔIGS for increasing glucose is only ~60% of the value obtained at low BG.

It is possible that the diminished IG response during increasing BG is due to changes in sensor sensitivity; however, the analysis was performed to minimize this effect. This was done by evaluating IG response over sequential 60-min time periods. While decreased sensor sensitivity is noted over longer times, little change is noted in this short period of time.

A more likely explanation for the results presented here is a physiological-based model proposed by Aussedat et al. (1), who measured IG in rats using an implanted sensor. Their results, like those presented here, showed that as glucose increased, IG was lower than BG. However, as glucose decreased after insulin administration, their data showed much closer agreement between IG and BG. Those results are qualitatively similar to those obtained here with humans. The differences in absolute magnitude and lag between the two studies are small and may reflect differences in glucose measurement (implanted sensor versus a transdermal extract) or the animal species studied.

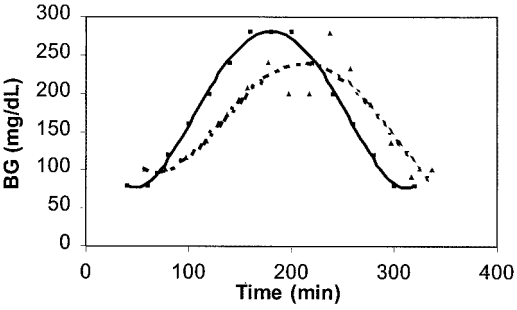

Figure 7— *The simulated relationship between BG "trapezoid" input and IG. Solid line, BG; dashed line, IG.*

Aussedat et al. proposed a multicompartment model of glycemic excursions, with glucose uptake, utilization, and elimination in each compartment varying with insulin and glucose (1). A simplified version of that model is shown in Fig. 8. During glucose uptake (from both exogenous and endogenous sources) the blood compartment concentration increases rapidly. Some of the excess glucose can be eliminated. However, since glucose must be transferred to the interstitial space from the blood, IG is lower and lags behind BG. Furthermore, the faster the rate of glucose increase the greater the difference, while at steady state both compartments equilibrate, and there is little difference.

When hyperglycemia is achieved, exogenous glucose uptake ceases and insulin is administered. Insulin promotes cell uptake of glucose and, hence, IG declines. In addition, insulin inhibits the liberation of endogenous glucose. The BG then also declines because of both elimination (especially at high BG) and transfer to the interstitial space. As a consequence, both IG and BG drop, with the decrease in IG somewhat greater. As BG lowers, elimination and transfer to the interstitial space decrease until equilibrium is approached again.

The data presented in Fig. 6 do not fully agree with some previous results. In this study, for increasing rate of change, there was a "reversal" in the trend of NSI. That is, for rapid increases ($>70\ mg \cdot dl^{-1} \cdot h^{-1}$), the NSI was closer to unity (higher) than for moderate increases (between 30 and 70 $mg \cdot dl^{-1} \cdot h^{-1}$). Conversely, for rapid decreases ($<-70\ mg \cdot dl^{-1} \cdot h^{-1}$), the NSI was lower than that for moderate decreases. Both of these comparisons were significant ($P < 0.001$). It is unclear why this reversal exists; however, it could be the result of rapid decreases in glucose being mediated in tissue beds other than the skin. In this case, the liver might be the primary site for glucose

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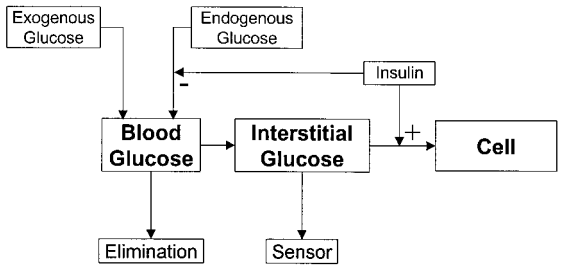

Figure 8— *A model of glucose sensing from interstitial fluid, simplified from Aussedat et al. (1).*

elimination, and the change in skin IG may not be as great relative to blood. Thus, the detailed differences between IG and BG are likely dependent upon many parameters, including insulin sensitivity and vascularization of the sensor site.

In conclusion, the results presented here show that IG differs from BG in both magnitude and timing. These differences are not due to diminished sensor sensitivity. Rather, the differences may reflect physiological variation in glucose uptake, utilization, and elimination in blood, interstitial space, and cells.

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