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Commentary

Can Interstitial Glucose Assessment Replace Blood Glucose Measurements?

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ABSTRACT

Current treatment regiments for individuals depending on exogenous insulin are based on measurements of blood glucose obtained through painful finger sticks. The shift to minimal or noninvasive continuous glucose monitoring primarily involves a shift from blood glucose measurements to devices measuring subcutaneous interstitial fluid (ISF) glucose. As the development of these devices progresses, details of the dynamic relationship between blood glucose and interstitial glucose dynamics need to be firmly established. This is a challenging task insofar as direct measures of ISF glucose are not readily available. The current article investigated the dynamic relationship between plasma and ISF glucose using a model-based approach. A two-compartment model system previously validated on data obtained with the MiniMed Continuous Glucose Monitoring System (CGMS) is reviewed and predictions from the original two-compartment model were confirmed using new data analysis of glucose dynamics in plasma and hindlimb lymph (lymph is derived from ISF) in the anesthetized dog. From these data sets, the time delay between plasma and ISF glucose in dogs was established (5–12 minutes) and a simulation study was performed to estimate the errors introduced if ISF is taken as a surrogate for blood. From the simulation study, the error component resulting from the differences in plasma and ISF glucose was estimated to be < 6% during normal day-to-day use in an individual with diabetes (error component calculated as the standard deviation of the ISF/plasma glucose differences under conditions where the maximal time delay was used). This difference is most likely within the variance between arterial and venous blood glucose. We conclude that the differences between plasma and ISF glucose will not be a significant obstacle in advancing the use of ISF as an alternative to blood glucose measurements.

INTRODUCTION

DRIVEN BY THE DISCOVERY OF INSULIN at the beginning of the last century, the measurement of intracorporal glucose became essential for the successful treatment of diabetes. For several decades, "spot-urine" was checked to estimate hyperglycemic glucose values; still, this did not offer any information in the normo- or hypoglycemic range prompting the focus to shift to the use of whole blood. Methods based on the oxidation of sugars either by cupric or

MiniMed Inc., Sylmar, California.

ferric compounds in alkaline solutions with the formation of certain colors followed by colorimetric quantification were developed in the 1930s. Initially, these methods required several milliliters of blood, but this was reduced to ~0.5 mL by the 1950s. Nonetheless, the methods were generally not specific for glucose because blood contains other reducing compounds. Various protein precipitation and filtering steps were proposed to solve the specificity problem but these methods were time-consuming and impractical for "real time" diabetes therapy. An accurate method that could be performed within 15 minutes on whole blood was proposed in 1959[1] using *O*-toluidine. This method remains in use today for the detection of carbohydrates in protein hydrolysates.[2] High specificity and a tremendous reduction in sample size and assay time came with the introduction of enzymatic methods utilizing glucose dehydrogenase, hexokinase, or glucose oxidase in conjunction with colorimetric, photometric or electro-chemical detection devices. These methods formed the basis for the development of highly accurate laboratory glucose analyzers (e.g., Beckman,[3] YSI[4]) and small inexpensive hand-held meters. With the development of these latter devices, the era of home glucose monitoring based on capillary blood had begun.

ISSUES RELATED TO THE USE OF INTERSTITIAL FLUID AS A REPLACEMENT FOR BLOOD GLUCOSE MONITORING

With the development of accurate glucose assays investigators began to explore the possibility of continuously measuring glucose and reporting the results in real time. Early attempts involved the continuous withdrawal of intravenous blood[5,6]; however, the subcutaneous measuring site rapidly gained attention due to its ease of use and safe accessibility. Today, almost all minimal and noninvasive glucose detection methods, such as iontophoresis[7,8], hypodermic needles[9,10], electrochemical/enzymatic sensors,[11–15] ultrasound,[16] and optical approaches,[17,18] have focused on estimating glucose in the interstitial fluid (ISF) compartment of the skin rather than blood. However, concerns have always existed regarding potential differences in the two sites.[19] Essentially, if the capillary represents a diffusional barrier to glucose, ISF glucose and plasma glucose can be expected to have different dynamic responses and, under some conditions, different steady-state concentrations. (Plasma glucose and blood glucose are related by the hematocrit; for this manuscript, we use plasma glucose as the desired reference.)

Given that plasma and ISF glucose levels may have different characteristics, the question arises as to which glucose level is more advantageous for an individual with diabetes to monitor. It has been argued that during decreasing glucose (e.g., following insulin administration or exercise) ISF glucose may fall in advance of plasma glucose[20–23] and thus provide advanced hypoglycemic detection. Conversely, the level of glucose seen by the brain is more likely to be reflected by plasma glucose and this level is critical for survival.

The issue of which glucose level we wish to monitor is further confounded by the need for *in vivo* calibration. *In vivo* calibration is required for virtually all minimal or noninvasive ISF glucose monitoring methods. That is, even if a method could be calibrated *in vitro* there is no guarantee that the same calibration would hold *in vivo*.[24] Placing a sensor in the subcutaneous tissue means placing it into unknown nonstandard conditions.[25,26] As well, these conditions may change over the time of intended use due to local tissue reactions.[27,28] Currently, most ISF glucose detection methods are calibrated against a *"reference blood glucose reading."* At this point, several questions surface: Should not an "ISF glucose sensor" be calibrated against an "ISF glucose" standard? Is a sensor that is sensing ISF glucose but calibrated against blood glucose providing a measure of "ISF glucose" or a measure of "blood glucose"? What happens if a "standard" or "highly accurate" reference measurement is not available for calibration? And finally, how is a new glucose measurement to be evaluated? In general, it is agreed that a new measurement technique has to be compared to a well-established method to decide whether the new method is in sufficient agreement with the old method.[29] If a sensor is thought to measure ISF glucose then it should rightly be calibrated against a

"highly accurate" measure of ISF glucose and subsequently be evaluated against a well-established method of measuring ISF glucose. The sensor errors would then be defined as:

$$\text{sensor error} = 100\,\frac{\text{ISF Glucose} - \text{Sensor Glucose}}{\text{ISF Glucose}}\,\%\,. \quad (1)$$

Alternatively, if one were to consider an ISF glucose sensor calibrated against a blood glucose reference to be a measure of blood glucose then one would calibrate against a "highly accurate" blood glucose reading and define the error as:

$$\text{sensor error} = 100\,\frac{\text{Blood Glucose} - \text{Sensor Glucose}}{\text{Blood Glucose}}\,\%\,. \quad (2)$$

Clearly, this latter definition is somewhat unfair to the sensor insofar as differences in ISF and blood glucose are interpreted as "sensor errors."

Irrespective of whether an ISF sensor calibrated to blood glucose is "thought" to be an ISF glucose measure or a blood glucose measure (i.e., whether you take equation (1) or (2) as your definition of error) the fact remains that differences in ISF and blood glucose must be understood if the ISF sampling site is to replace blood glucose sampling. To understand the differences, a measure of ISF glucose is needed—a task that has proven to be difficult. Various approaches have been used to directly obtain interstitial fluid. These include implanting capsules,[30] inserting wicks,[31] creating blisters on the skin,[32–34] sampling lymph,[35,36] using ultrafiltration,[37,38] and more recently using hypodermic needles.[9,10] These techniques have produced steady-state estimates of ISF glucose that vary between 50 and 110% of the plasma level with values greater than 100% being reported under basal conditions using lymph.[39] The positive gradient reported with lymph was shown to reverse under hyperinsulinemic conditions (unpublished data G. Steil). The latter fact emphasizes that insulin can change the gradient by increasing glucose uptake from tissues.

Indirect methods also exist to estimate glucose in the ISF. Here "indirect" means that no sample of ISF is obtained. Techniques that fit this description include microdialysis[40–42] and open microperfusion.[43] Generally, these methods need to be calibrated against a reference glucose reading to adjust for variable recovery. To date, the reference has commonly been blood glucose. Importantly, once such a calibration is introduced the gradient between ISF and plasma can no longer be calculated. Although the gradient cannot be calculated, the methods have been used to describe ISF glucose dynamics. However, such reports have to be taken with care in that the identification of time constants is limited by the sampling interval a method allows (≥3 samples must be obtained within the time constant of interest—e.g., to identify a 15-minute time constant samples are needed approximately every 5 minutes). As well, these methods have potential to introduce delays associated with the equilibration rate across the microdialysis membrane per se. Electrochemical sensors have also been used to infer ISF glucose dynamics and represent the bulk of the published data on this topic. There are advantages to this method. Mainly, the devices are typically not limited in sampling frequency. Still, the devices are most often calibrated against plasma glucose and therefore do not provide an absolute ISF glucose level. Further, the devices may have intrinsic delays. With these limitations in mind, the ISF dynamics have been reported to vary widely. Delays of up to 40 minutes have been reported in some studies (for review see Rebrin et al.[44]); while other studies have reported that the change in ISF glucose can occur in advance of the change in plasma glucose.[19,20,22,23] These latter reports have been limited to falling glucose signals. In light of these widely varying observations, considerable confusion remains regarding the dynamic relationship between ISF and plasma glucose.

A THEORETICAL DESCRIPTION OF ISF GLUCOSE DYNAMICS

The theoretical relationship between ISF glucose and plasma glucose can be characterized by considering plasma and ISF as separate "compartments" or "pools." Compartmental models have been extensively used to investi-

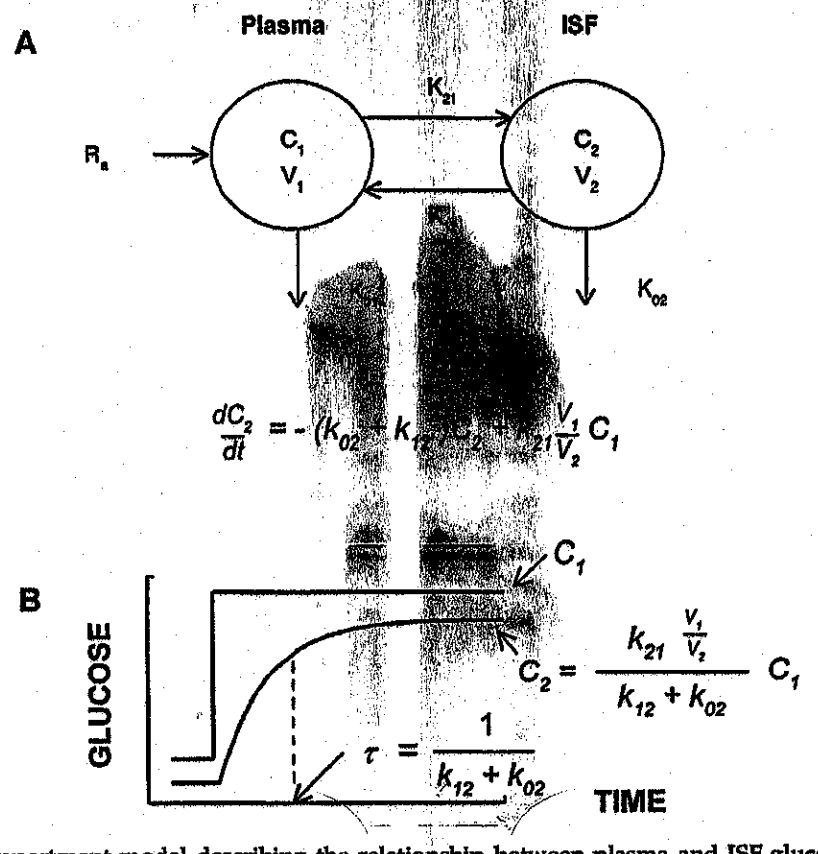

FIG. 1. A: Two-compartment model describing the relationship between plasma and ISF glucose, and B: theoretical response of ISF glucose (C_2) to a step increase in plasma glucose (C_1) demonstrating the dependence of the gradient (C_2/C_1) and time delay (τ) on the diffusion (k_{12} k_{21}) and clearance (k_{02}) parameters.

gate the physiological relationship between plasma glucose and insulin. Often, very high-order models are proposed to describe the glucose dynamics in various tissue beds (splanchnic, muscle, etc.). However, for the purposes of characterizing the ISF seen at a particular sensing site a simple two-compartment model can be used (Fig. 1). For this model, glucose is assumed to enter the ISF space by diffusion across the capillary barrier and to be irreversibly cleared from the tissue bed in proportion to the concentration in that compartment. The rate of clearance from the tissue is determined by k_{02} and the rate of diffusion to the tissue is characterized by k_{21} and k_{12} (for simple diffusion: $k_{21}V_1 = k_{12}V_2$, where V is the volume of the respective pools). If the rate constants are fixed, the relationship between plasma (C_1) and ISF glucose (C_2) can be obtained from the mass balance equation for the ISF glucose pool (see equation of Fig. 1A). Under these conditions, the steady-state glucose concentration in the ISF pool (C_2) is determined by both diffusion of glucose to the pool (k_{12} and k_{21}) and clearance from the pool (k_{02}; Fig. 1B). If no glucose is cleared from the ISF compartment ($k_{02} = 0$) then the plasma and ISF pools have identical steady-state concentrations (*i.e.*, if $k_{02} = 0$ then $C_2 = C_1$). The gradient and time delay is shown in Figure 1B for a step increase in plasma glucose as could be achieved with a hyperglycemic glucose clamp. Here, the time delay (τ) is defined as the time for ISF glucose to reach 63% of the new steady-state ($T_{1/2}$ is related to τ as $T_{1/2} = \tau\ln(2)$). Several conclusions may be drawn from this simple analysis: (1) if the diffusion of glucose to the ISF pool and the fractional uptake of glucose from the pool are constant the gradient and time delay are also constant; (2) if glucose uptake from the ISF pool increases the delay becomes smaller and the plasma: ISF glucose gradient increases; and (3) if diffusion to the ISF pool increases the gradient and time constant both decrease (if there is no diffusion barrier, there is no gradient or time delay).

The model shown in Figure 1 provides insight into the ISF glucose delay and gradient between ISF and plasma. However, we seek to

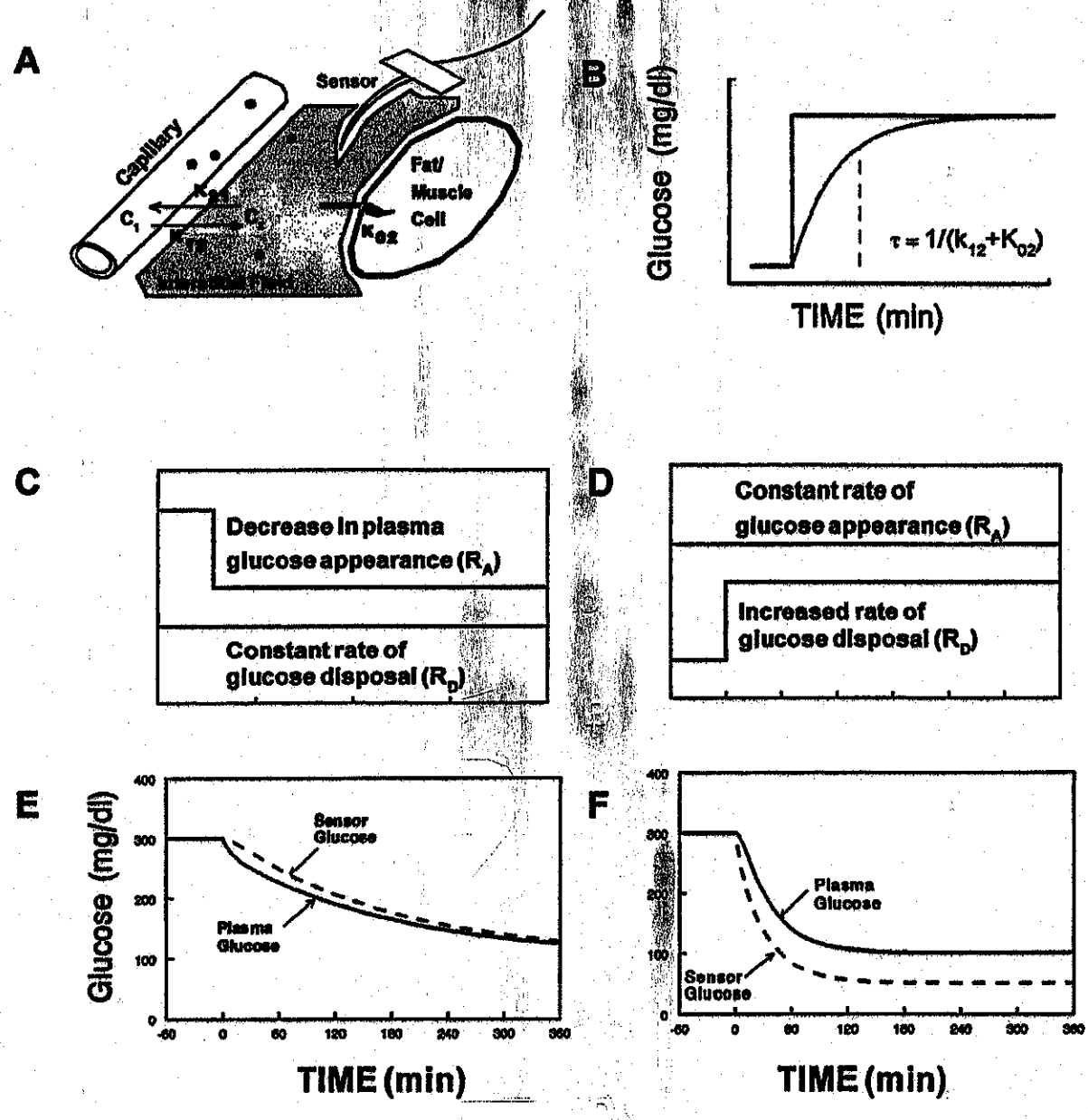

FIG. 2. A: Localized two-compartment description of plasma glucose and sensor current, B: calibrated sensor response to a step increase in glucose demonstrating: (1) the lack of steady-state difference in sensor versus plasma glucose and (2) the existence of the same ISF time delay defined in Fig. 1B, C: and E: simulation of a fall in glucose due to decreased glucose appearance into the plasma pool and the corresponded delay in sensor glucose signal, D: and F: simulation of a fall in glucose due to an increase in glucose uptake in the tissue bed and the corresponding preceding sensor signal.

characterize "sensing devices" that sense ISF glucose while at the same time are calibrated against plasma glucose; for example, an electrochemical sensor inserted into the ISF space as depicted in Figure 2A. Such a sensor produces a current signal (nA) in proportion to the ISF glucose level ($s = \alpha C_2$). If this signal is calibrated against a steady-state plasma glucose level, the calibrated sensor will correctly read that steady-state level. Less obvious is that all the steady-state glucose levels will be correct if the fractional diffusion to and clearance from the ISF pool do not change. Although all steady-state levels are correct, the sensor response will still be delayed by the ISF equilibration time constant (τ) defined in Figure 1. This is demonstrated in Figure 2B for a calibrated sensor under the same conditions as Figure 1.

The simulations of Figures 1 and 2B are over simplified insofar as changes in glucose are usually accompanied by changes in insulin. If insulin changes, and the sensor is exposed to ISF bathing insulin sensitive tissues, then k_{02} k_{21} and k_{12} may not be constant. For example, if insulin stimulates blood flow to the tissue surrounding the sensor, or recruits new capillaries to the tissue,[45] the permeability of the capillary bed can increase (k_{12}, k_{21} increase). If insulin increases glucose clearance from the tissue bed (k_{02} increase) the plasma: ISF gradient will increase and the ISF equilibration time constant (τ) will decrease (described by the equations of Fig. 1). For these conditions the ISF glucose response will be different from that simulated in Figure 1. Using the conceptual model of Figure 1, one can consider three different scenarios for falling glucose: (1) the rate of glucose appearance (RA) into the plasma pool decreases (R_A decreases); (2) the rate of glucose uptake in a compartment remote from the sensor increases (k_{01} increases); and (3) the rate of glucose uptake in the tissue surrounding the sensor increases (k_{02} increases). The first two cases are indistinguishable from the sensor's perspective and for both the sensor signal will lag the plasma signal by the time delay (τ) presented in Figure 1, and no change in the plasma:ISF glucose gradient will occur. The third case represents the only theoretical case where the sensor signal can fall in advance of plasma glucose. Moreover, for this case, a steady-state error will result due to an increase in the plasma:ISF glucose gradient. To visualize this, we simulate the case where plasma glucose decreases from 300 to 100 mg/dl due to a decrease in the R_A (Fig. 2E) and compare it with a similar fall arising from an increase in glucose uptake in the tissue surrounding the sensor (Fig. 2F). For the latter case the sensor signal precedes the fall in glucose and yields a lower steady-state reading.

COMBINING THEORY WITH EXPERIMENTAL DATA

We have previously used the model of Figure 2 to investigate the influence of insulin on subcutaneous ISF glucose using MiniMed's (Sylmar, CA) subcutaneous glucose sensor as part of the continuous glucose monitoring system (CGMS).[44] Specifically, we evaluated in dogs the response of the sensor to step increases in glucose with and without changes in endogenous insulin, and the response during an insulin induced fall in plasma glucose (anal-

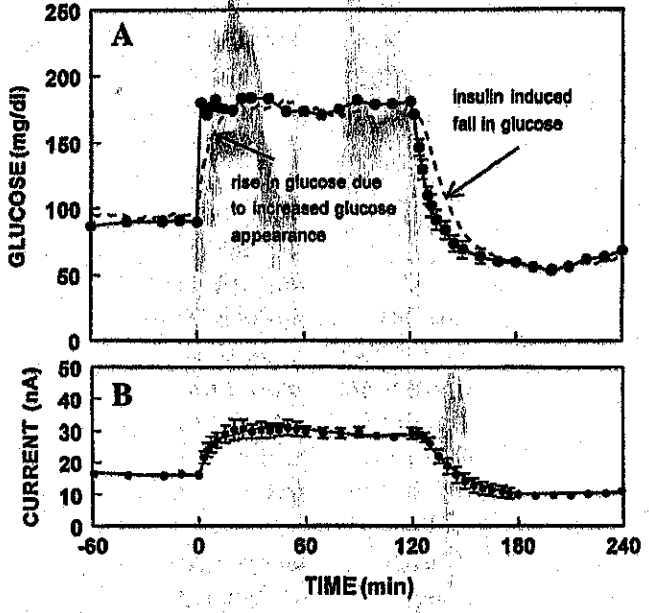

FIG. 3. A: Plasma glucose dynamics (closed circles) during an increase in glucose appearance (0–120 minutes) and an increase in glucose uptake (120–240 minutes) together with a calibrated sensor signal that is uncorrected for the ISF delay (dashed line), and B: sensor current (closed circles) and sensor fit (solid line) during the clamp. Sensor current was fit using the model of Figure 2. (Figure adapted from Rebrin et al.[44])

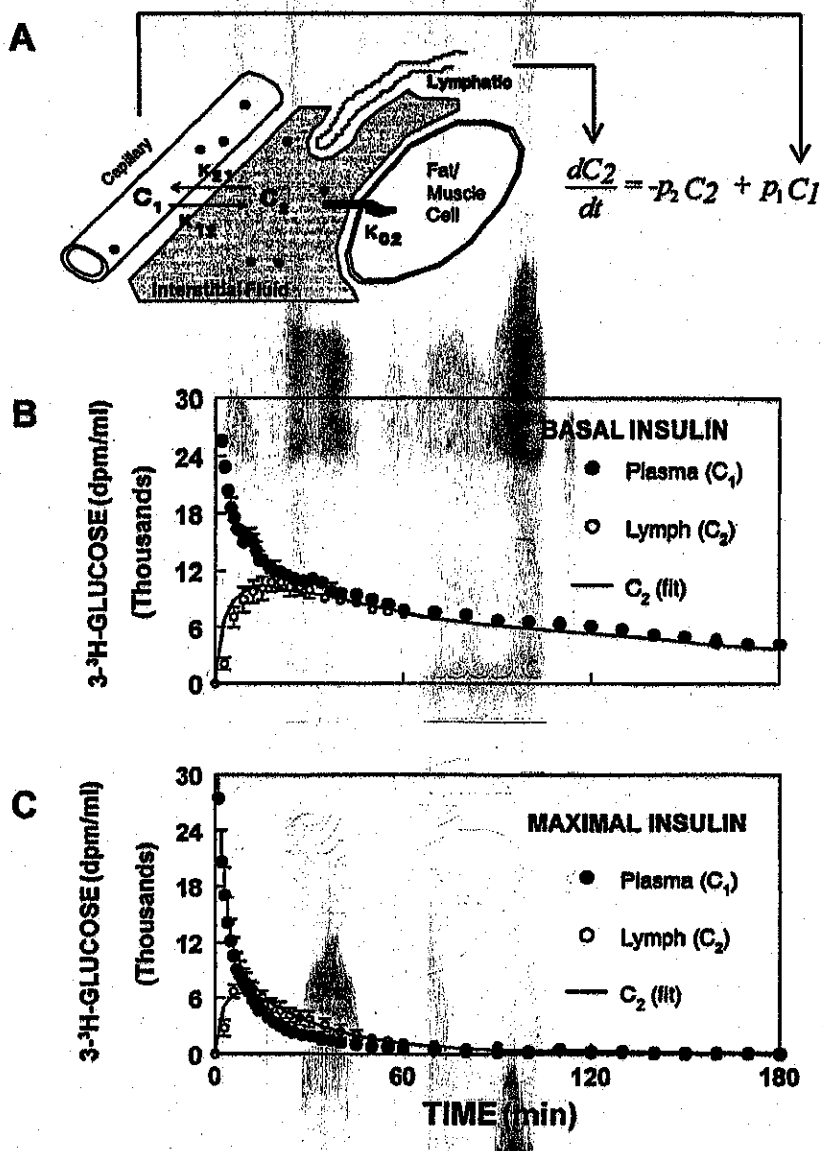

FIG. 4. A: Localized two-compartment description of plasma and lymph ISF, B: plasma (closed circles) and lymph (open circles) 3-^{3}H-D-glucose glucose dynamics during saline infusion together with the model fit (solid line) of the lymph data, and C: glucose tracer dynamics during maximally effective insulin infusion (euglycemic clamps). (Data adapted from reference Steil et al.[36])

ogous to the simulations shown in Figure 2). Results indicated that there was no effect of insulin to alter *in vivo* sensor sensitivity (no change in gradient), and that there was no effect of insulin on the sensor response time (no change in τ). Moreover, the fall in sensor glucose did not precede the fall in plasma glucose during insulin-induced increases in glucose disposal (see Fig. 3). From these data, we concluded that the sensor was not exposed to an insulin sensitive tissue bed and that the sensor delay time (τ) was between 5 and 12 minutes.

As an alternative to implanting a device in the ISF, lymph can be used to more directly assess ISF glucose dynamics. Lymph vessels collect ISF from virtually all tissue beds and transport the fluid back to the vascular space. These vessels can be catheterized and samples obtained roughly every 5 minutes. We have used this fluid to directly study the effect of insulin on glucose dynamics in the ISF surrounding dog hind-limb tissues and have shown that insulin does not increase glucose diffusion to the tissue bed itself[36] (i.e., insulin does not increase k_{21} or k_{12}). This conclusion was based on analysis of L-glucose (a nonmetabolized diffusion marker for glucose) in plasma and lymph under steady-state basal and hyperinsulinemic

conditions. Also presented in the original study were D-glucose kinetics under basal and maximally effective insulin conditions. These kinetics can be used to further validate the plasma: ISF model proposed in Figure 1. That is, D-glucose dynamics in plasma and lymph can be fit to the model shown in Figure 2, where the sensor is replaced by a lymph vessel (Fig. 4A). This allows both the plasma: ISF glucose gradient and the ISF glucose time delay to be obtained from a least squares fit of 3-H^3-glucose (a glucose tracer) under basal (Fig. 4B) and maximally effective insulin conditions (Fig. 4C). The fit of these data confirms that a plasma: ISF gradient exists and that the ISF glucose response is delayed; more importantly though, neither the gradient (C_2/C_1; 0.85 ± 0.06 vs. 0.92 ± 0.06, NS) nor the delay (12.3 ± 2.4 vs. 13.5 ± 2.3 min, NS) were affected by insulin ($p > 0.05$ for both). Note, although the simple model shown fits the glucose data well, analysis in the original study,[36] suggested that hind-limb lymph originates from muscle and a second unknown pool that exhibits a longer delay. To the extent that a slower pool exists, the time constants may actually be overestimated.

WHAT IS THE IMPACT OF ISF DELAY ON SENSOR ACCURACY DURING DAY-TO-DAY USE?

The lymph-glucose kinetic analysis (Fig. 4) and the sensor kinetics under differing insulin kinetics (Fig. 3) suggest that the delay in the ISF glucose is within 5–12 minutes. Further, recent data collected in humans with the MiniMed CGMS indicate the delay (τ) may actually be less than 5 minutes.[46] Although digital filtering techniques exist to compensate for such time delays,[47] we evaluated the sensor error *if the delay is left uncorrected*. To do this, we performed a "simulation study" to estimate the errors if a sensor signal was obtained from an ISF site with a 12-minute delay (delay defined as in Fig. 1). For the simulation study, a reference 3-day glucose profile was first obtained from an individual with diabetes using the MiniMed Continuous Glucose Monitoring System (CGMS; Fig. 5A). The system provides glucose values every 5 minutes; however, for the purposes of the simulation study the 5-minute values were "resampled" at 20-minute intervals (Fig. 5B closed circles). Resampling was performed to provide a smooth reference signal with a known "band-width" (a 20-minute sampling interval limits the bandwidth of the glucose profile to 1.5 cycles/hour). Bandwidth is a common engineering term defining how fast a signal—in this case glucose—can change. All physical systems have bandwidths, for example, the human ear is generally believed to be "band-limited" to 20–20,000 cycles/second. The bandwidth of the glucose system however, has not been accurately measured. Bandwidth can be measured by Fourier analysis or by introducing test signals and measuring the plasma glucose response.[47]

To complete the simulation study, the reference glucose profile (Fig. 5B closed circles) was used to generate an ISF glucose profile using the equations of Figure 1. A "perfect" sensor was then assumed to be implanted in the ISF space and calibrated at a "steady-state" glucose value. This resulted in the sensor profile shown in Figure 5B (solid line) from which a portion (hours 16–22) is expanded in Figure 5C to emphasize the effect of adding the delay. For this data the mean error introduced in the sensor signal was zero (*i.e.*, the sensor is unbiased) and the standard deviation of the error was 3.5% (*i.e.*, 67% of the time the sensor glucose was within 3.5% of the true glucose). However, while the mean error is unbiased the slope of the regression line between plasma glucose and sensor glucose (Fig. 5C) was biased (slope equals 0.94, significantly different from 1, and intercept = 5.65; analysis performed using GraphPad Prizm, San Diego, CA). Further, if the sensor is assumed to be calibrated at random points along the profile (*i.e.*, not at steady-state) the standard deviation of the percent error increases to 5.8%. Under these conditions, the mean error is still zero and the slope and intercept of the regression line are unchanged (slope = 0.94, intercept = 5.65). Thus, it may be concluded that delays—in the absence of any other error—do not bias the mean sensor reading but do bias the regression slope. Bias in the regression slope (slope < 1) means that on average low glucose values are overestimated and high values are

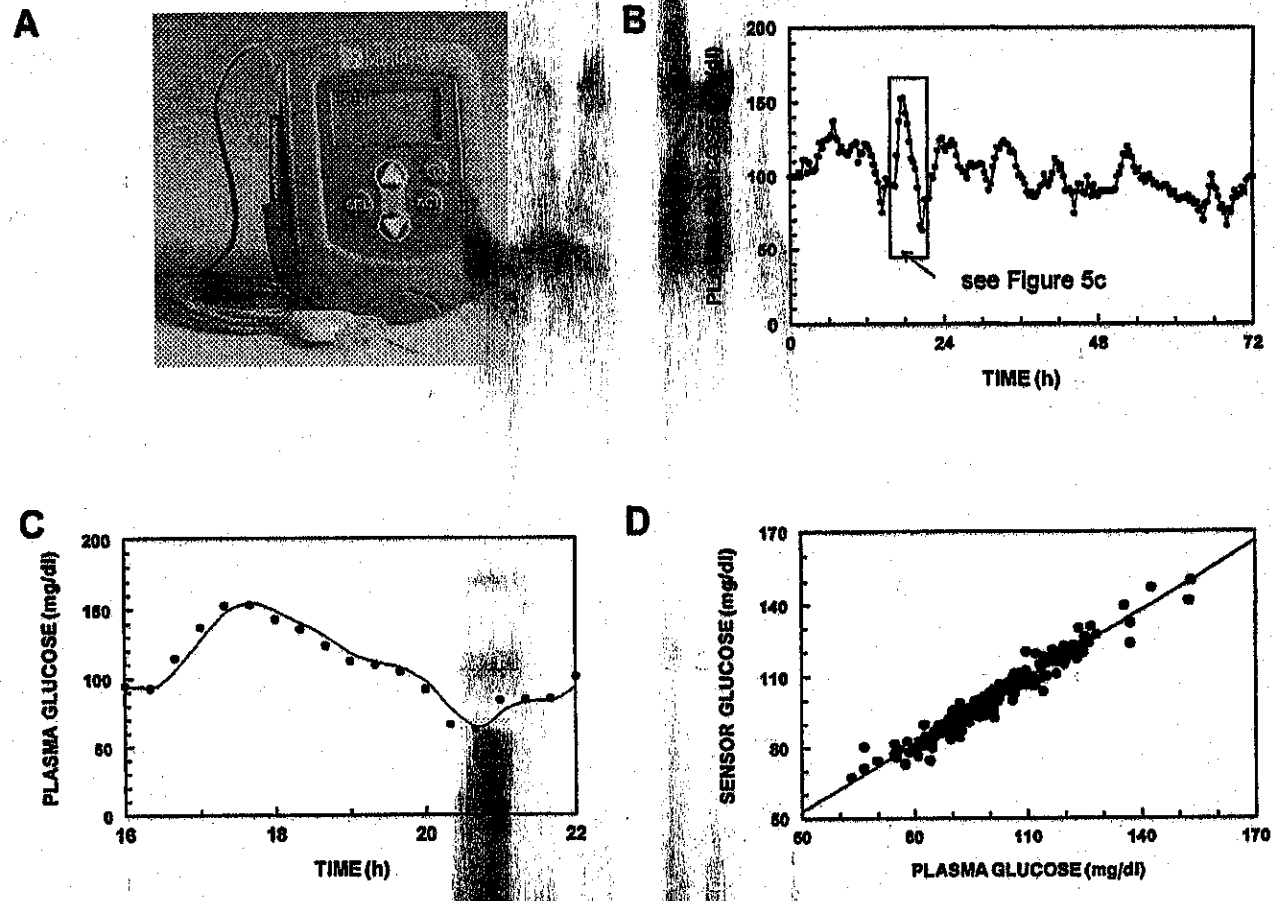

FIG. 5. A: The MiniMed Continuous Glucose Monitoring System. B: Modified plasma glucose dynamics from an individual with Type 1 diabetes (see text). C: Expanded scale demonstrating the delay in the sensor response during rapid glucose kinetics. D: Linear regression of plasma versus sensor glucose.

underestimated (at the point where the regression line intercepts the 1:1 line the two values are identical).

Although an ISF delay introduces an error into a sensor glucose reading the magnitude of the error is relatively small (less than 6% for the simulations presented here). Digital filters that can correct for this error have been proposed by us,[44] and others.[48] However, as the error is most likely within the range of normal arterial-venous blood differences[49] such filters may not be necessary (depending on the application, a digital filter may still improve the overall accuracy). If the combined ISF/sensor delay is longer than 10–15 min—implying that the error will rise above 6%—then digital filter compensation will probably be necessary. These filters, which involve taking a weighted sum of past sensor values, are relatively simple to implement (see Rebrin et al.[44] for details).

CONCLUDING REMARKS

The present review specifically addressed the question "Can interstitial glucose assessment replace blood glucose measurements?" The answer, based on the present review, is yes. Data presented here indicate that the delay in ISF glucose equilibration is most likely less than 10 minutes and the error introduced is no more than 5 or 6%. Nonetheless, while the use of the ISF space as the glucose-measuring site does not appear to be a major problem, it is clear that other issues need to be further investigated. For subcutaneous sensors, these issues include nonstandard measuring conditions, local tissue responses including wound healing, interfering substances, and pathophysiological conditions such as hypoglycemia, ketoacidosis, etc.

From Clark's[50] proposal to modify an elec-

trochemical oxygen sensor for use in continuous glucose monitoring, to the U.S. Food and Drug Administration (FDA) approval of the first commercially available *in vivo* continuous glucose monitoring system (MiniMed Inc.),[51,52] it has taken about 40 years. The original task seemed simple. Glucose-sensing technology was rapidly incorporated into standard laboratory analyzers like the Beckman and YSI.[3,4] Nonetheless, the *in vivo* application of this technology continues to present significant challenges prompting the question put forth by Gough[53]: "Why is it taking so long"? The answer, as stated by Gough, is the need for more focused research with complete presentation of successful and unsuccessful data. Presenting these latter "unsuccessful attempts" would help identify and solve problems in a scientific manner. In the present review, we have argued that ISF can replace blood as the preferred glucose measure and that the relative difference between blood and ISF glucose is small. We believe that many of the new technologies being developed will firmly establish the ISF site as the preferred measuring site.

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