

Subcutaneous glucose predicts plasma glucose independent of insulin: implications for continuous monitoring

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Rebrin, Kerstin, Garry M. Steil, William P. Van Antwerp, and John J. Mastrototaro. Subcutaneous glucose predicts plasma glucose independent of insulin: implications for continuous monitoring. *Am. J. Physiol. 277 (Endocrinol. Metab. 40): E561–E571, 1999.*—The present study investigated the relationship between blood and subcutaneous interstitial fluid (ISF) glucose by employing an amperometric glucose sensor specifically developed for 3-day continuous glucose monitoring. The apparent sensor sensitivity and ISF glucose equilibration delay were estimated on separate days during hyperglycemic clamps in four dogs in which insulin was either suppressed with somatostatin, allowed to change, or increased with an exogenous infusion. A 2-h sensor “settling-in” period was allowed before the clamps. During insulin deficiency, the sensor sensitivity and ISF glucose delay were 0.23 ± 0.03 nA per mg/dl and 4.4 ± 0.8 min. Sensitivity was not affected by increases in endogenous (0.30 ± 0.04 vs. 0.28 ± 0.04 nA per mg/dl) or exogenous insulin (0.18 ± 0.01 vs. 0.16 ± 0.01 nA per mg/dl) nor was the delay (3.3 ± 1.2 vs. 5.7 ± 1.1 and 9.2 ± 2.6 vs. 12.3 ± 1.7 min; $P > 0.05$ for all). Sensor glucose accurately predicted plasma glucose without correcting for delays <10 min ($r > 0.9$ for all), whereas for longer delays a digital corrective filter was used ($r = 0.91$ with filter). We conclude that the relationship between blood and ISF glucose is not affected by insulin and that delays in ISF glucose equilibration can be corrected with digital filters.

continuous glucose sensor; subcutaneous tissue; interstitial fluid

THE MANAGEMENT of blood glucose in patients with diabetes is based primarily on patients monitoring their own glucose levels. Patients typically perform self monitoring of blood glucose with capillary samples obtained from skin punctures limited to a few samples that can be taken each day. Thus glucose sensors that continuously monitor glucose have long been sought as a way to achieve better overall control of blood glucose. For this purpose, the subcutaneous space is an ideal site for the sensor, as it is easily and safely accessed by both patients and physicians. However, subcutaneous glucose sensors do not measure plasma or blood glucose but rather the glucose in the interstitial fluid (ISF) surrounding the sensor. The relationship between “blood” and “ISF” glucose is still poorly understood. Interstitial glucose concentrations have been reported to be from 20 to 110% of the plasma values, and changes in ISF glucose have been reported both to fall in

advance of changes in plasma glucose (37, 39) and to be delayed by as much as 45 min (21, 22); a summary of these studies is provided in Table 1. In analyzing these data, we found that implantation site specifics such as tissue glucose utilization, blood flow, prevailing insulin levels, local tissue reactions, as well as device specifics such as size, intrinsic sensitivity, lag time, and sampling frequency, all have to be considered (26, 27).

The gradient and dynamic relationship between plasma and ISF glucose can be described by assuming plasma and ISF to be separated by a capillary barrier as shown in Fig. 1. Complete equations describing such a model have been published elsewhere (35). Basically, if the capillary is a significant barrier to glucose, then changes in ISF glucose will be related to changes in plasma glucose by the rate of glucose diffusion across the capillary (diffusion coefficient: $D = k_{21}V_1 = k_{12}V_2$; ml/min) and by the rate of glucose clearance from the ISF surrounding the sensor ($k_{02}V_2$; ml/min). If glucose uptake in the tissue immediately surrounding the sensor is negligible (i.e., if $k_{02} = 0$), then steady-state plasma and ISF glucose levels will be equal and deviations will only exist during transient glucose changes. However, if glucose uptake from the surrounding tissues is not negligible, a steady-state gradient will exist that will affect the apparent in vivo sensor sensitivity. Furthermore, if glucose uptake in the region surrounding the sensor is sensitive to insulin, then the gradient will change with insulin availability. Dynamically, changes in sensor glucose will lag behind changes in plasma glucose if the change in plasma glucose is due to a change in the rate of appearance into the plasma space, for example, a change in glucose appearance after meals or a change in endogenous glucose production. Conversely, if the change in plasma glucose is due to an increase in glucose uptake from the sensor surrounding ISF space, then sensor dynamics will lead to changes in plasma glucose. Importantly, changes in glucose due to uptake in an insulin-sensitive tissue site remote from the sensor will also result in a delayed sensor signal.

Some investigators have studied the delay and gradient with subcutaneous glucose sensors per se (Table 1). Implanted amperometric glucose sensors cannot, however, be used to directly evaluate the magnitude of a gradient because the sensors are calibrated against plasma glucose. That is, sensor current (nA) is related to blood glucose (mg/dl) by the overall in vivo sensor sensitivity (change in sensor current divided by change in ISF glucose, all times plasma-glucose divided by ISF-glucose). Calibrations performed this way correct for the plasma-to-ISF glucose gradient. The sensors could, of course, be precalibrated in vitro but the sensor

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Table 1. Summary of findings characterizing interstitial glucose dynamics vs. blood-plasma dynamics (delay, gradient) published by various authors applying different methods

Species	Test	Measurement (tissue)	Delay	Gradient	Ref.
Rabbits, baboons	basal	Guyton capsules (sc)		49–77%	41
Rabbits	glucose infusion	electro-chem sensor (sc)	20 min		15
Dogs (normal, diabetic)	glucose and insulin infusion	wick technique (sc)		100%	11
Dogs (normal, diabetic)	insulin, food, OGTT	ultrafiltration (sc)		90%	13
Dogs (normal, anesthetized)	glucose injection	thoracic duct lymph	10 min	105%	3
Dogs (normal)	glucose infusion	electro-chem sensor (sc)	25/6 min (rise/fall)		10
Dogs (diabetic)	glucose and insulin injection	electro-chem sensor (sc/muscle)	5–15/5–10 min	64/83%	32
Rats (diabetic)	glucose infusion and insulin injection	electro-chem sensor (sc)	5–8/–15 min (rise/fall)	<50%	39
Rats (normal, anesthetized)	insulin injection	electro-chem sensor (sc)	25 min		30
Rats (normal, anesthetized)	glucose injection	electro-chem sensor (sc)	5–12 min		25
Pigs (normal, anesthetized)	glucose and insulin injection	electro-chem sensor (sc)	12 min	20%	8
Human (healthy)	OGTT glucose clamp	microdialysis (sc)	2/8 min		14
Human (healthy)	OGTT insulin injection	electro-chem sensor (sc)	0–40/0–15 min (rise/fall)		22
Human (healthy)	glucose clamp	microdialysis (sc)	20–45 min		21
Human (healthy, diabetic)	monitoring, glucose clamp	microdialysis (sc)	12/2.3 min (rise/fall)	78%	37
Human (diabetic)	OGTT	microdialysis (sc)	4.4/8.2 min (rise/fall)		36
Human (healthy)	glucose infusion	ultrafiltration (adipose)		~50%	1
Human (healthy)	glucose infusion	microdialysis (adipose)		~84%	29
Human (healthy)	glucose clamp	transdermal iontophoresis	20 min		40
Human (healthy, diabetic)	random	hypodermic cannula		Δ 1.1 mM	38
Human (diabetic)	meal	hypodermic cannula	10 min	110%	31
Human (healthy)	insulin infusion	microdialysis (adipose/muscle)	18/30 min	Δ -2/-1.2 mM	4
Human (healthy)	basal and glucose clamp	microdialysis (adipose/muscle)		78/63%	19
Human (healthy)	basal	microdialysis (muscle)		80%	17
Human (diabetic)	monitoring	microdialysis (adipose)		87–101%	20
Human (healthy)	basal and glucose clamp	open-flow microperfusion (adipose)		60–75%	6
					28

OGTT, oral glucose tolerance test; electro-chem, electrochemical.

sensitivity may change once placed in the in vivo environment. Although the in vivo calibration prevents the direct assessment of the plasma-to-ISF gradient, changes in the gradient can be inferred from changes in the apparent sensor sensitivity. Delays in the ISF glucose equilibration can also be assessed with subcutaneous sensors, although the glucose sensor may also have an intrinsic delay (diffusion of glucose through the

membrane). Again, the sensor delay can be identified in vitro, but the in vitro delay may not be identical to the in vivo delay. Note, that the delay in ISF glucose equilibration and the delay in sensor response per se are both identifiable¹ from sensor current-plasma glucose data. Thus implanted glucose sensors such as those in the present study can identify any change in the plasma-to-ISF gradient and all time delays.

Delays in either ISF glucose equilibration or the sensor per se can introduce errors into the calculated sensor glucose values. These errors can be corrected with digital filters (see APPENDIX), providing both delay and gradient are constant. Increases in the gradient, such as might occur with increases in insulin, would result in lower ISF-glucose for the same plasma glucose and consequently introduce an error in the calculated plasma-blood glucose reading. An increase in glucose clearance from, or diffusion to, the ISF space would decrease the delay and consequently introduce transient sensor reading errors in the presence of a compensatory digital filter tuned to a longer delay. A decrease

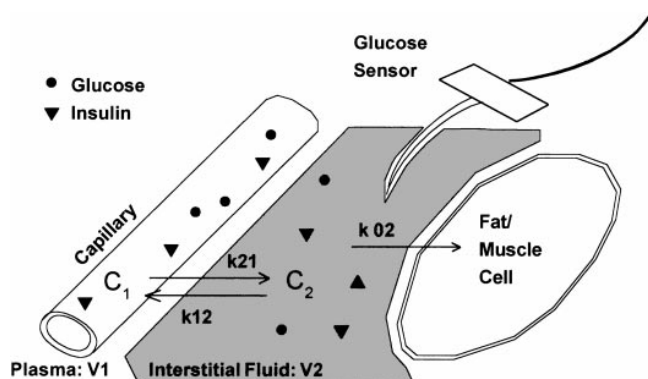


Fig. 1. Model describing plasma (C_1) and interstitial fluid (ISF; C_2) glucose kinetics assuming glucose equilibrates by diffusion ($D = k_{21}V_1 = k_{12}V_2$) and is cleared from ISF by tissue surrounding the sensor (clearance = $k_{02}V_2$), where V_1 and V_2 represent plasma volume and ISF distribution volume seen by the sensor, respectively.

¹ If, for example, there was a 10-min delay in ISF glucose and 5-min delay in the sensor, then a second order dynamic system with time constants of 5 and 10 min would be identifiable rather than a first order system with a single 15-min time constant.

in the ISF glucose delay is probably not serious in that it would result in an advanced notice of a glucose fall; however, an increase in delay would obviously result in a delayed hypoglycemic warning. An increase in delay would occur with decreased glucose diffusion to the sensor site or decreased glucose uptake from the ISF immediately surrounding the sensor. The latter process would be mainly associated with increases in glucose (decreased insulin) and thus not affect a hypoglycemic alarm *per se*.

In any case, changes in sensor glucose readings due to changes in the plasma-to-ISF glucose gradient and delays in ISF glucose equilibration must be understood if subcutaneous glucose sensing is to become a surrogate for blood glucose measurement. The present study was therefore conducted to address three physiological questions: 1) does insulin affect the apparent *in vivo* sensor sensitivity through changes in the plasma-to-ISF glucose gradient? 2) Is there a delay between plasma and ISF-sensor glucose? 3) If the delay exists, is it affected by insulin? Changes in plasma glucose were induced by exogenous glucose infusions both in the absence and presence of endogenous changes in insulin (hyperglycemic clamps). In a separate experiment, plasma glucose was decreased with an exogenous insulin infusion, whereas the rate of exogenous glucose appearance was held constant. Under the conditions of this latter clamp, the normalization of plasma glucose is mainly, if not entirely, due to increased glucose uptake and is the only condition in which the sensor signal might be expected to precede the change in plasma glucose.

METHODS

Animals. The study was performed in four mongrel dogs (27.6 ± 1.5 kg). Dogs were bred and raised under standard kennel conditions at the Veterans Affairs Medical Center/West Los Angeles vivarium. Each of the four dogs underwent four experiments in which four sensors were inserted in the neck area (64 sensors in total). Experiments in the same dog were separated by at least 7 days, and animals were used for studies only if judged to be in good health as determined by weight stability, body temperature, hematocrit, and leukocyte count. The experimental protocol was approved by the Animal Research Committee of the Veterans Affairs Medical Center/West Los Angeles.

Sensors. Each sensor is an amperometric device designed and developed by MiniMed for 3 days of continuous subcutaneous use. The sensor is produced by semiconductor technology and consists of an electroenzymatic three-electrode cell in which a constant potential (0.6 V) is maintained between the working electrode and the reference electrode. The principle is based on the generation of hydrogen peroxide from glucose and oxygen via the enzyme glucose oxidase (2, 5, 9, 18, 32). An ammeter detects the current generated due to the oxidation of hydrogen peroxide at the working electrode. The sensor is enclosed in a flexible polymer tubing with a side window exposing the active electrode area that is covered by a polyurethane membrane. The membrane is glucose diffusion limiting and defines a linear relationship between the glucose concentration in surrounding fluid and the sensor current in the range of 20–400 mg/dl. *In vitro* precision is within 5% in the range of 50–350 mg/dl, and the *in vitro* sensor response time is <1.5 min (τ_{90} ; time to reach 90% steady state). The

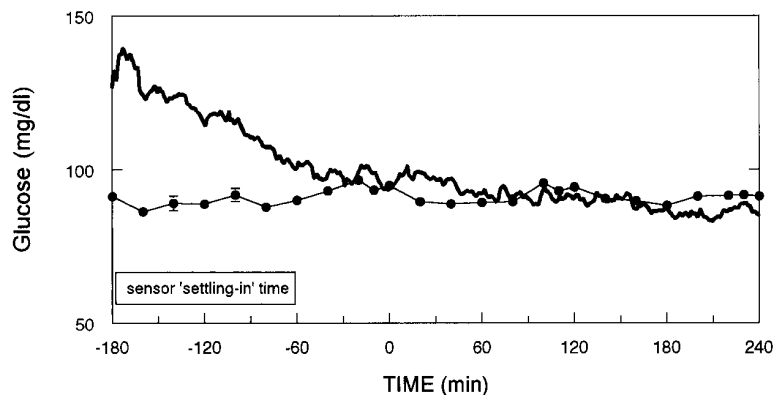
tubing containing the sensor is embedded in a split (half) 22-gauge needle that is removed after the sensor is inserted into the subcutaneous space. The sensor signal is transmitted to a Holter-style monitor via a cable, and the data are stored for later downloading. The sensor signal is acquired every 10 s with the average value stored in memory every minute. Glucose sensors are packed individually and sterilized in accordance with the Association for the Advancement of Medical Instrumentation Guideline for Electron Beam Radiation Sterilization of Medical Devices. All components that pass into subcutaneous tissues or contact the skin meet the appropriate requirements of ISO 10993–1 (Biological Evaluation of Medical Devices).

Initially, the sensor “settling-in” time was compared in sensors that were implanted either with or without a precalibration *in vitro* ($n = 18$). As no obvious differences were observed, subsequent sensors ($n = 46$) were all calibrated *in vitro* before implantation in the animal. All sensors ($n = 64$) used *in vivo* were calibrated *in vitro* after the experiments. *In vitro* calibration was performed with three glucose solutions (~ 100 , ~ 200 , ~ 400 mg/dl) and buffer (0 mg/dl), all at 37°C .

Protocol. After an overnight fast (12 h) animals were equipped with catheters inserted into peripheral veins for infusion of substances (glucose, insulin, and somatostatin as required) and blood sampling. After the animals were shaved and their skin was antiseptically cleaned, four sensors were inserted subcutaneously in the neck area and fixed with sutures on wings (instead of adhesive as proposed for human application). Each animal underwent four experiments: a control experiment used to evaluate sensor stability and settling-in time (no exogenous glucose) plus three hyperglycemic clamp protocols. In the first hyperglycemic clamp protocol (*protocol 1*), endogenous insulin release was prevented with somatostatin ($0.8 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Three hours after sensor placement, glucose was elevated to ~ 180 mg/dl for 2 h with a variable glucose infusion, and after the infusion was stopped, glucose was allowed to return to basal and the dynamics were recorded for a further 2 h. For this protocol, changes in plasma glucose are solely due to changes in the rate of glucose appearance with little or no change in fractional glucose uptake. In a second protocol (*protocol 2*), insulin was allowed to change normally in response to the hyperglycemia; if the sensor is surrounded by insulin-sensitive tissues such as fat or muscle, then the increase in insulin should increase the plasma-to-ISF glucose gradient and decrease the tissue equilibration time. Under these conditions, the falling edge sensor signal ($120 < t < 240$ min) would be expected to have a decreased sensitivity (increased plasma-to-ISF gradient) and a shorter delay (faster glucose clearance from the ISF compartment) compared with the rising edge dynamics ($0 < t < 120$ min). In the third protocol (*protocol 3*), glucose was again raised to ~ 180 mg/dl and endogenous insulin was allowed to change; however, in this protocol glucose was normalized to basal with an intravenous exogenous insulin bolus-infusion ($2 \text{ U bolus} + 1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ infusion) without changing the exogenous rate of glucose infusion. For this case, the initial rise in plasma glucose was due to a change in the rate of appearance of glucose, whereas the fall in glucose was due to the increase in glucose uptake. If the sensor is exposed to the ISF in an insulin-sensitive tissue site, then the sensor signal should precede the plasma glucose dynamics during an insulin-induced fall in glucose. Experiments were randomized by dog and protocol with the exception of *protocol 3*, which was performed last.

Hyperglycemic clamps. Clamps were performed as follows: after sensor implantation and 2 h of settling-in and 1 h of basal monitoring, plasma glucose was rapidly raised to ~ 180

Fig. 2. Plasma glucose (line with ●) and sensor glucose (solid line) during settling-in time ($-180 \leq t \leq 60$ min) and experimental period ($-60 \leq t \leq 420$ min).



mg/dl with a primed variable rate glucose infusion (20% glucose). The priming bolus was based on an assumed glucose distribution space of 16.25% of total body weight (25% extracellular space of which 65% is immediately accessible to glucose). Samples for blood were drawn between 2 and 20 min and the sensor current was recorded every minute. Catheters for blood sampling were flushed with heparinized saline (30 U/ml) to maintain patency. Blood samples (0.2 ml for glucose; 1.5 ml for insulin) were taken in heparinized tubes and immediately centrifuged, and the supernatant (plasma) was separated. Plasma was either used for immediate glucose determination (YSI, Yellow Springs, OH) or stored (-20°C) for later determination of insulin (RIA, Diagnostic Products, Los Angeles, CA).

ISF glucose-sensor signal dynamics. The sensor signal dynamics were characterized to allow for a gradient and delay between plasma and interstitial glucose as shown in Fig. 1. To estimate the gradient and delay the mass balance equation for the ISF pool was first obtained as

$$\frac{dC_2}{dt} = -(k_{02} + k_{12})C_2 + k_{21}\frac{V_1}{V_2}C_1 \quad (1)$$

where C_1 and C_2 are plasma and ISF glucose kinetics, respectively (see Fig. 1).

From Eq. 1 the plasma-to-ISF glucose gradient and the ISF equilibration time constant (delay) are

$$C_2 = \frac{k_{21}\frac{V_1}{V_2}}{k_{12} + k_{02}}C_1; \quad \tau = \frac{1}{k_{12} + k_{02}} \quad (2)$$

The ISF equilibration time constant² (τ) reflects a delay in ISF glucose relative to changes in plasma glucose. Sensor current (nA) was then formulated as a function of plasma glucose by assuming sensor current is proportional to the concentration of glucose in the ISF compartment [i.e., sensor current (s) = αC_2]. Substituting this relationship into Eq. 1 and combining terms yields

$$\frac{ds}{dt} = -p_2s + p_3C_1 \quad (3)$$

where $p_3 = k_{21}V_1/V_2\alpha$ and $p_2 = k_{12} + k_{02}$ are identifiable from plasma glucose and sensor current data. Both parameters were identified by nonlinear least-squares analysis with a

Marquardt-Levenberg algorithm with inverse-variance weighting (7). For this analysis, plasma glucose (C_1) was taken as the independent variable and sensor current (s) was assumed to be the dependent (C_1 was assumed to be known without error). The apparent sensor sensitivity and ISF delay were subsequently calculated as p_3/p_2 [nA/(mg/dl)] and $\tau = 1/p_2$ (min), respectively. Sensor glucose was calculated a posteriori both with and without correction for the delay as described in the APPENDIX.

Statistical analysis. Data are reported as means $\pm$ SE. Differences in parameters were evaluated with paired t -tests. For all in vivo calibrations, agreement with the measured plasma glucose was evaluated as the mean and standard deviation of the differences or by correlation of plasma glucose with sensor glucose. Correlation coefficients (r values) were calculated with and without delay correction, and statistically significant changes were evaluated by paired t -test after Fisher's Z-transformation.

RESULTS

In vitro sensitivities and settling-in time. Of the 16 sensors implanted for the purpose of evaluating the settling-in time, 3 sensors had to be removed from the analysis due to technical difficulties during the experiment (broken lead or nonproper tissue placement). For the remaining sensors, ~ 2 h were required to reach a stable sensitivity (0.393 ± 0.024 vs. 0.364 ± 0.029 nA/(mg/dl), $P = 0.103$; hour 3 vs. hour 5). This period, defined as the settling-in time, was followed by a 5-h period during which both plasma glucose [91.1 ± 2.8 mg/dl; coefficient of variation = $4.0 \pm 0.5\%$] and sensor current (35.3 ± 2.5 nA; coefficient of variation = $10.3 \pm 1.6\%$) were relatively stable. For the calibrated signal (Fig. 2; sensor calibration based on the average-current/average-glucose during the 5-h stable period), the sensor signal can be seen to rapidly fall during the settling-in period. We note that while the sensor sensitivity was not statistically different ($P = 0.103$) during hour 3 vs. hour 5, it nonetheless decreased 7% [from 0.393 to 0.354 nA/(mg/dl)]. This was due to a greater fall in sensor current (11%; 37.3 ± 2.5 vs. 33.2 ± 2.7 nA; $P = 0.016$) than in plasma glucose (4%; 94.4 ± 3.1 vs. 90.8 ± 2.4 ; $P = 0.07$); however, whether the fall in current was due to an insufficient settling-in or due to a decrease in sensitivity is not known. In vitro, the postimplantation sensitivity was actually higher than the preimplantation sensitivity [0.35 ± 0.05 vs. $0.20 \pm$

² The time constant is related to the half-time according to $\tau = t_{1/2}/\ln(2)$.

0.02 nA/(mg/dl), $P < 0.01$] with the estimated in vivo sensitivity between the pre- and postimplantation in vitro values (0.25 ± 0.02 nA/(mg/dl); $n = 46$; control experiments + clamps).

Protocol 1: hyperglycemic clamps without changes in insulin. After the settling-in time, sensor current and plasma glucose (Fig. 3A) were both stable (22.6 ± 2.3 nA and 61.6 ± 7.6 mg/dl, respectively, -60 to 0 min). During the clamp period, glucose was elevated to 235.6 ± 16.2 mg/dl, resulting in a sensor signal of 74.1 ± 9.9 nA (60 – 120 min). After the clamp period, glucose slowly returned toward basal but was still elevated after 2 h (123.6 ± 19.0 mg/dl, 180 – 240 min), presumably due to the relative suppression of insulin by somatostatin (Table 2). Sensor current mimicked this pattern and was well fit by the sensor calibration equation (Eq. 1). No differences were observed in the sensitivity when the profile was separately assessed during the rising and falling phases (Table 3). The estimated delay time tended to be longer when assessed separately for the rise and fall compared with that observed for the entire curve (Table 3); however,

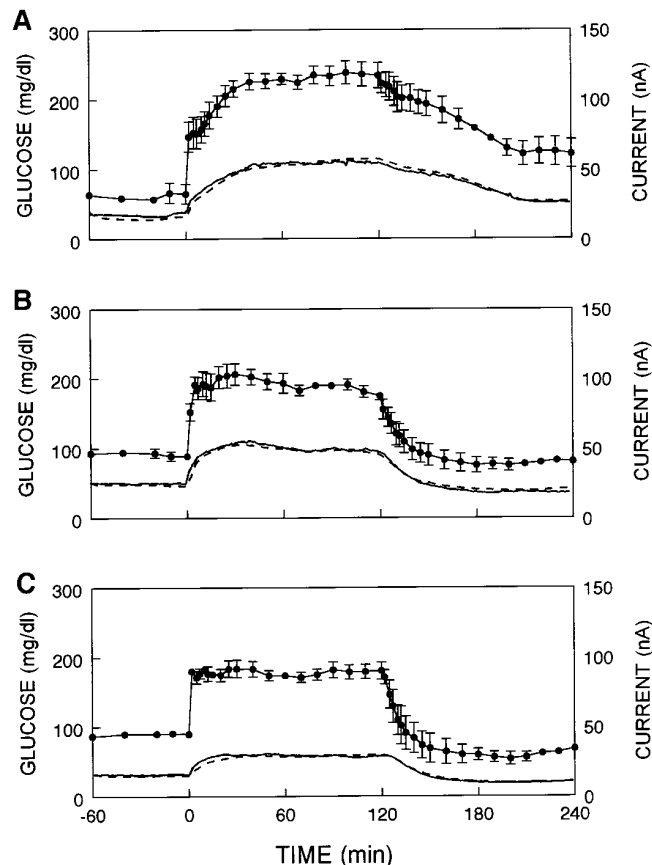


Fig. 3. Plasma glucose (●; left axis), sensor signal (solid line; right axis), and model fit (dashed line; right axis) during hyperglycemic clamps in absence of changes in endogenous insulin (A), hyperglycemic clamps with changes in endogenous insulin (B), and hyperglycemic clamps with changes in endogenous insulin ($0 < t < 180$) but with plasma glucose renormalized to basal with exogenous insulin ($180 < t < 240$). Sensor signal model fit was performed with mass balance equation (Eq. 3) derived from Fig. 1.

Table 2. Insulin concentration in plasma at end of each experimental period for three clamp protocols and control experiments

	Average Insulin Concentration, μ U/ml		
	Basal I ($-30 < t < 0$)	Hyperglycemic clamp ($90 < t < 120$)	Basal II ($210 < t < 240$)
Control	3.4 ± 0.5	2.3 ± 0.3	3.8 ± 0.9
Protocol 1	1.6 ± 0.1	4.8 ± 2.6	3.5 ± 1.6
Protocol 2	3.7 ± 1.0	17.3 ± 6.3	2.8 ± 0.5
Protocol 3	3.3 ± 0.5	15.5 ± 4.6	46.7 ± 9.8

Values are means $\pm$ SE; $n = 4$. Protocol 1, hyperglycemic clamp without changes in insulin; protocol 2, hyperglycemic clamp allowing insulin to change; protocol 3, hyperglycemic clamp with additional exogenous insulin.

this difference did not achieve statistical significance ($P > 0.05$).

Protocol 2: hyperglycemic clamps allowing endogenous insulin secretion. As in protocol 1, sensor current and plasma glucose (Fig. 3B) were both stable (21.2 ± 1.8 nA and 92.4 ± 6.0 mg/dl, respectively) before starting the clamp. Glucose was elevated to 184.3 ± 6.0 mg/dl, and sensor current was increased to 47.0 ± 1.2 nA during the clamp. In contrast to protocol 1, however, glucose returned to near basal concentration within 20 min of terminating the exogenous glucose (79.9 ± 4.4 mg/dl plasma glucose and 20.0 ± 1.1 nA sensor current). The rapid fall in plasma glucose can be attributed to the elevation in plasma insulin (insulin increased ~ 5 times over basal; Table 2). With the increase in plasma insulin, one might have anticipated a faster equilibration time due to increased glucose clearance from the ISF compartment and a decreased sensitivity due to changes in the plasma-to-ISF glucose gradient. However, when the time delay and sensitivity were separately estimated for the rise ($-30 < t < 60$) and the fall ($90 < t < 180$), neither parameter was different [sensitivity = 0.30 ± 0.04 vs. 0.28 ± 0.04 nA/(mg/dl)]; ($\tau = 3.3 \pm 1.0$ vs. 5.7 ± 1.1 min; $P > 0.05$ for both). Thus the sensor current was well fit by the ISF model without separate assessment of rise and fall.

Protocol 3: hyperglycemic clamps with exogenous insulin. Again, sensor current and blood glucose (Fig. 3C) were stable before starting the clamp (18.4 ± 1.3 nA and 89.6 ± 4.7 mg/dl, respectively). Glucose was elevated to 180.2 ± 10.6 mg/dl during the clamp, and sensor current was increased to 33.3 ± 3.0 nA. Unlike protocol 1 and protocol 2, however, in this protocol plasma glucose was normalized back to basal with an exogenous insulin infusion. During the renormalization period, the glucose infusion was maintained at the steady-state clamp value (i.e., maintained at 11.4 ± 1.8 mg $\cdot$ min $^{-1}$ $\cdot$ kg $^{-1}$). Sensor sensitivity was not affected by the administration of exogenous insulin [0.18 ± 0.01 vs. 0.16 ± 0.01 nA/(mg/ml); rising vs. falling edge; $P > 0.05$], suggesting that insulin did not increase the plasma-to-ISF glucose gradient. Sensitivities did tend, however, to be lower for protocol 3 than for either protocol 1 or 2 (Table 3). The lower sensitivity observed

Table 3. Sensor sensitivities and time constants (lag time) estimated from Eq. 3 in each of 3 hyperglycemic clamp protocols

Protocol	In Vivo Sensitivity, nA per mg/dl			Time Constant ISF vs. Plasma Glucose, min		
	All	Rise	Fall	All	Rise	Fall
1	0.23 ± 0.03	0.25 ± .03	0.22 ± .03	4.4 ± 0.8	6.2 ± 1.5	7.8 ± 1.6
2	0.28 ± .04	0.30 ± .04	0.28 ± .04	5.3 ± 1.0	3.3 ± 1.2	5.7 ± 1.1
3	0.17 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	9.7 ± 1.2	9.2 ± 2.6	12.3 ± 1.7

Values are means ± SE. ISF, interstitial fluid.

in *protocol 3* was, however, likely due to lower sensitivities of the sensors themselves (*protocol 3* was the last protocol performed in the study with a new batch of sensors). That the sensors per se had lower sensitivities can be inferred by comparing the in vivo sensitivity during the rise in *protocol 2* [0.30 ± 0.04 nA/(mg/ml)] vs. the rise in *protocol 3* [0.18 ± 0.01 nA/(mg/dl)]; *protocol 2* and *3* have identical in vivo conditions during the rise period.

The exogenous insulin infusion used in *protocol 3* resulted in the most rapid fall of plasma glucose observed in any of the protocols (glucose fell ~71 mg/dl in 10 min). Renormalization of glucose with exogenous insulin represents the most likely situation in which the fall in sensor current might be expected to precede the fall in plasma glucose. Sensor current did not, however, fall in advance of plasma glucose; rather, the falling edge current was delayed 12.3 ± 1.7 min relative to plasma, the longest delay observed in the study (Table 3). No difference in delay time was observed when the rising edge was compared with the falling edge (9.2 ± 2.6 vs. 12.3 ± 1.7 min rise vs. fall; $P > 0.05$). Although not statistically significant, the longer delay time during the falling edge resulted in systematic errors in the sensor fit during the rise (Fig. 3C). Although there was no difference in the delay times observed during the rise compared with the fall, the delays did tend to be longer for this protocol than for *protocol 1* or *2*. The longer delays may have been due to the lower sensitivities observed for the sensors used in this protocol (Table 3). Delay time estimated for the rise in *protocol 3* (9.2 ± 2.6 min) was longer than that observed for *protocol 2* (3.3 ± 2.6 min; $P > 0.05$) despite the fact that the in vivo conditions during the rise were identical, suggesting that the sensor per se increased the delay.

In vivo calibration and estimation of plasma glucose. To evaluate the influence of the delays (3–14 min for all sensors) on the calibrated sensor glucose signal, the sensor glucose profile (G) was calculated both with (Eq. A3) and without ($G = b \times s$; where b is a calibration coefficient and is equal to p_2/p_3) delay correction. In the absence of any change in insulin (*protocol 1*), sensor glucose followed the plasma glucose profile without correction for delay (Fig. 4A, dashed line). Introducing delay correction resulted in a small improvement in the mean fit (Fig. 4A, solid line); however, the average r values with and without delay correction were not different (0.90 ± 0.023 vs. 0.92 ± 0.014 ; $P = 0.84$). Allowing for changes in endogenous insulin (*protocol 2*)

did not markedly affect sensor performance, and the calibrated signal again mimicked the plasma profile well without delay correction (Fig. 4B). Adding delay correction yielded improvements during the rising and falling edges (Fig. 4B), although the average r values were statistically not different (0.91 ± 0.02 vs. 0.88 ± 0.028 , $P = 0.14$, with vs. without delay correction). However, in *protocol 3*, delay correction did result in more accurate sensor dynamics (Fig. 4C). When glucose was elevated in the presence of changes in endogenous insulin and the hyperglycemia was normalized with exogenous insulin, both the rising and falling edge sensor glucose dynamics were delayed relative to plasma glucose dynamics. Adding delay correction virtually removed the delay in sensor signal (Fig. 4C), and the r

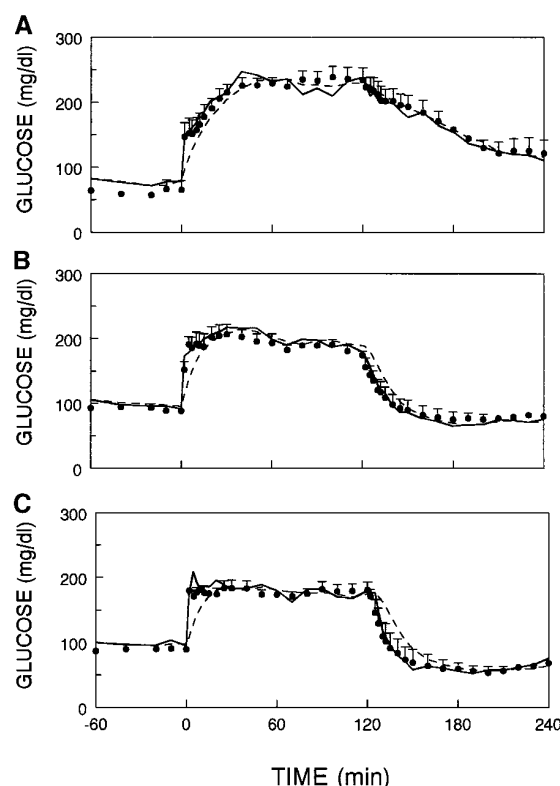


Fig. 4. Plasma glucose (●) together with calibrated sensor glucose either with (solid line) or without (dashed line) correction for delay during hyperglycemic clamps in absence of changes in endogenous insulin (A), with changes in endogenous insulin (B), and during clamps in which glucose was renormalized to basal with exogenous insulin (C).

values were significantly improved (0.91 ± 0.016 vs. 0.82 ± 0.021 , $P < 0.001$). Although the delay correction improved the correlation, it also introduced a slight “overshoot” during the rising edge. This overshoot results from correcting for a time lag greater than actually present. That is, the filter corrected for a 9.7 ± 1.2 -min delay, estimated from all the data, although the delay during the rise was only 9.2 ± 2.6 min (Table 3).

DISCUSSION

The present study investigated the use of subcutaneous glucose sensors for estimating plasma glucose concentrations. It was hypothesized that if a plasma-to-ISF glucose gradient existed it would introduce delays into the sensor signal and that both the gradient and the delay could be affected by changes in insulin. Delays or changes in the gradient would introduce errors to the sensor glucose signal. Results from the study with dogs indicated, however, that ISF can be used to accurately estimate plasma glucose levels irrespective of changes in plasma insulin and without correction for time delay. Changes in plasma glucose resulting from alterations in the rate of glucose appearance (iv glucose infusion) in the absence or presence of changes in endogenous insulin were closely mimicked by sensor glucose dynamics (Fig. 4, A and B, dashed lines) as were changes in plasma glucose due to exogenous insulin infusion (Fig. 4C). The latter case (Fig. 4C, falling edge), in which the fall in plasma glucose is solely due to an increase in glucose uptake, is the most obvious experimental situation in which the sensor signal might theoretically be expected to precede plasma glucose dynamics. Although a sensor delay of ~ 5 – 10 min was observed both with and without changes in endogenous insulin, the delay was not affected by insulin (Table 3) and correcting for it did not dramatically improve sensor accuracy (Fig. 4, A and B, solid line). A slightly longer delay of ~ 12 min was observed during normalization of glucose with exogenous insulin; however, this delay could be compensated for with a simple digital filter (Fig. 4C, solid line).

Subcutaneous glucose sensors such as those used in the present study cannot be used to measure a plasma-to-ISF glucose gradient per se because the sensor is calibrated against plasma glucose. Nonetheless, an increase in the plasma-to-ISF glucose gradient will appear as a decrease in the apparent sensor sensitivity. It was originally hypothesized that an increased plasma-to-ISF gradient might occur during high insulin concentrations due to stimulated tissue glucose uptake at the sensor implantation site. However, when the sensor sensitivity was separately assessed under differing insulin levels (Table 3, rising vs. falling dynamics), no change in sensitivity was observed, indicating that insulin did not increase the gradient to a degree that would affect sensor accuracy. That the estimated delay was not decreased during periods of hyperinsulinemia also supports this contention that there was no significant increase in glucose uptake at the sensor site. It should be mentioned that the study was performed with an electrochemical-enzyme-based glucose sensor

known to lose sensitivity in vivo over longer time periods. Both a loss of in vivo sensor sensitivity and an increased glucose gradient would result in lower overall sensitivity values. On average, in vivo sensitivity showed a tendency but did not significantly change during the protocols (Table 3) as confirmed by the control experiments (Fig. 2). Clearly, the postimplantation in vitro sensitivity of the sensors per se was not decreased. Long-term studies have to be performed to address in vivo stability issues specifically for this sensor.

Still, a more direct measure of the plasma-to-ISF glucose gradient and dynamics is difficult to obtain because the ISF cannot be sampled directly. Through application of the so-called wick technique, plasma and ISF glucose values have been determined to be nearly identical (11), studies with dialysis capsules (41) or ultrafiltration (13, 29) have previously reported that ISF glucose concentrations are 10–55% lower than the corresponding plasma levels, and microdialysis has indirectly, i.e., after calibration, revealed ISF levels $\sim 15\%$ lower (16, 20). Some authors reported (29) varying values pending on duration of device insertion with high recovery immediately after implantation, supposedly due to capillary damage, and $\sim 50\%$ ISF values several hours later or the opposite, lower glucose recovery shortly after implantation and high recovery ($\sim 84\%$) after 5–9 days (40). ISF fluid sampled with hypodermic cannulas has indicated higher ISF glucose levels (4) in both normal and diabetic humans. Thoracic-duct lymph glucose from conscious dogs has also been found to be both higher than plasma and to have delayed dynamics (3). Thoracic-duct lymph is, however, derived from ISF originating in both muscle and the liver (34), and the liver may have a higher concentration of glucose because it is a site of endogenous glucose production. Nonetheless, in a subsequent study with lymph derived from hindlimb muscle of dogs, plasma and ISF glucose (23) were not significantly different under either basal or moderate hyperinsulinemic clamp conditions. A gradient was, however, observed during supraphysiological insulin levels; (unpublished observations from Steil et al.; Ref. 33). Thus, although supraphysiological insulin levels can increase the gradient, it is unlikely that physiological changes in insulin dramatically alter it (27). This conclusion is supported by the present study in which we found no evidence of decreased sensor sensitivity at high physiological insulin levels during either sustained hyperglycemia or during hyperinsulinemia induced by exogenous insulin infusion (Table 3).

Although we did not observe any change in sensitivity during increased insulin, small delays in the sensor current were observed during all clamps. Whereas the gradient per se cannot be identified with a sensor calibrated against plasma glucose, the sensor can be used to identify delays in the system. In the present study, delays of 3–14 min were detected. That this delay was considerably longer than the in vitro delay of the sensor ($\tau_{90} < 1.5$ min) supports the contention that the delay was due to ISF glucose equilibration. Microdi-

alysis studies support that an ISF delay exists, but many studies have reported the delay to be considerable longer than those reported in the present study (Table 1). However, it is difficult to accurately assess time delays with microdialysis because equilibration across the dialysis membrane can itself introduce delays (1, 4, 12, 21, 37). Furthermore, the longer sampling interval required by microdialysis (15 min) does not allow the assessment of time constants shorter than 30–45 min. Although lymph could potentially be used to more accurately assess ISF glucose dynamics (3), most lymph studies have focused on insulin kinetics during euglycemic clamps and thus offer limited insight into glucose dynamics.

In contrast to studies in which a delay in ISF glucose was observed, some studies have reported that changes in ISF glucose precede changes in plasma glucose during falling plasma glucose values. In theory, such a preceding signal could be due to increased insulin-dependent glucose disposal in the tissue immediately surrounding the sensor. In the present study, the sensor signal did not precede the plasma signal during a rapid fall in glucose due to increased insulin (Fig. 3C; $120 < t < 240$ min). In fact, this fall was detected with a lag time of 12.3 ± 1.7 min, the longest observed in the study. That the sensor delay was not decreased during this period strongly supports the contention that the sensor was not measuring ISF originating from insulin-sensitive tissues. Interestingly, this delay (12.3 min) was also associated with the fastest rate of fall in glucose (glucose fell ~ 70 mg/dl in 10 min during this infusion); however, we do not believe these two observations are causally related. Rather, we believe the increased delay time observed during *protocol 3* was due to the lower in vitro sensor sensitivity exhibited by sensors in that study (Table 3). This hypothesis is supported by the fact that the delay in glucose response was longer in *protocol 3* compared with *protocol 2* even when the identification was performed on the rising edge alone (*protocol 2* and *3* have identical in vivo conditions during the rising edge). The longer delay time observed during the *protocol 3* rise suggested that the sensor per se can contribute to the delay dynamics and that sensors with lower sensitivities may introduce a further delay.

Irrespective of the cause for the longer delay observed during *protocol 3*, it is clear that this was the only protocol to benefit substantially from time-lag correction. Time-lag correction was performed in the present study with a two-point moving average filter with weighted coefficients (see APPENDIX). This filter virtually eliminated the 12-min lag in sensor glucose observed during the fall in glucose brought about with exogenous insulin (Fig. 4C) and significantly improved the correlation coefficient (0.91 ± 0.016 vs. 0.82 ± 0.021 , $P < 0.001$). Correcting for shorter lags (< 10 min; *protocol 1* and *2*) yielded improvements in sensor accuracy, but those did not reach statistical significance. The greater improvement in sensor accuracy based on the 12-min delay correction compared with the improvements seen with delays < 10 min may have

been affected by the faster glucose dynamics observed during the exogenous insulin infusion. The delay identified by Eq. 2 is, of course, not affected by the rate of change of glucose if the parameters themselves are not affected by changes in insulin or glucose. However, the errors introduced by any given delay depend very much on the rate of change in glucose. That is, for any given delay, the errors will be larger for rapid changes in glucose compared with slow changes. Therefore, it is possible that both the longer delay and the faster glucose dynamics contributed to the improvement in sensor accuracy observed in *protocol 3*, and it is also possible that during the slower day-to-day glucose dynamics observed in normal individuals, correcting for even a 12-min delay may not be necessary. Conversely, correcting for even a 10-min delay may be relevant for the more rapid falls in glucose that can occur in “brittle” diabetes during hypoglycemic episodes. Human subjects with diabetes will ultimately need to be studied to resolve this issue.

Overall, sensors used in the present study appeared to have a faster response time than those used by Schmidtke et al. (30) in a study to detect transient differences in blood and ISF after insulin injection. In the Schmidtke study, delays up to 25 min were reported with onset times up to 9 min (the delay lag time was defined as the difference between lowest blood and lowest subcutaneous reading after insulin injection, and the onset time was defined as the initial delay in sensor response). Note that the identification strategy used by Schmidtke et al. was slightly different from that used in the present study. In the present study, both the sensitivity (p_3/p_2) and delay ($1/p_2$) were identified from plasma glucose-sensor current data, whereas in Schmidtke et al., the sensitivity was calculated from a “one-point calibration” (sensitivity = blood glucose-sensor current under basal conditions) after which the sensor current was converted into units of ISF glucose, and a single parameter representing the mass transfer delay was identified. Strictly speaking, this procedure only yields the true ISF glucose level if the initial plasma-to-ISF gradient is one (i.e., if no gradient exists at the time the one-point calibration is performed). The filter used by Schmidtke et al. also differs from that used in the present study in that Schmidtke et al. used a deconvolution integral and we used a finite-impulse-response filter (24). Nonetheless, important for both approaches is that the relationship between plasma and ISF glucose is not affected by dynamic changes in insulin.

In summary, the present study indicated that, in dogs, plasma glucose estimation based on subcutaneous ISF glucose sensing accurately mimics plasma glucose across a wide range of glucose profiles irrespective of changes in plasma insulin. The data support the contention that the plasma-to-ISF glucose gradient does not increase during physiological changes in insulin. Delays in ISF glucose equilibration observed in the present study were typically < 10 min, and while such delays can be corrected with digital filters, the correction is unlikely to be necessary for the slower glucose

dynamics observed in day-to-day human use. The absence of an effect of insulin to alter the apparent sensor sensitivity and delay will, of course, need to be verified in humans.

APPENDIX

Subcutaneous glucose sensors measure glucose in ISF. If the fractional clearance rate of glucose from the ISF surrounding the sensor (k_{02} in Fig. 1) is constant, then changes in ISF glucose will be delayed relative to changes in plasma glucose (C_1). This delay results in a sensor current equation (derived in METHODS)

$$\frac{ds}{dt} = -p_2s + p_3C_1 \quad (A1)$$

From this relationship the estimated sensor glucose can be calculated as

$$\begin{aligned} \hat{C}_1(n) &= \frac{\frac{ds(n)}{dt} + p_2s(n)}{p_3} = \frac{\frac{s(n) - s(n-1)}{dT} + p_2s(n)}{p_3} \\ &= b_1s(n) + b_2s(n-1) \end{aligned} \quad (A2)$$

where $b_1 = p_2/p_3 + 1/[dT \cdot p_3]$ and $b_2 = 1/(p_3 \cdot dT)$. For this approximation, the derivative $ds(t)/dt$ has been replaced with the first backward difference in time [dT being the increment in time between samples $s(n)$ and $s(n-1)$]. Although this approximation compensates for the delay in ISF glucose equilibration, the difference approximation is very sensitive to noise. The correction and sensitivity to noise can be demonstrated by simulating a fall in plasma glucose from ~200 to 100 mg/dl in the presence of a 12-min ISF glucose delay (Fig. 5). The corrected sensor signal (solid line; Eq. A2) follows the true plasma glucose signal (closed circles; 10 min intervals) without any observable errors, whereas the uncorrected signal [dashed line; $\hat{C}_1 + b_1s(n)$, where $b_1 = p_2/p_3$] lags plasma glucose during the entire fall. Although the correction is essentially perfect in the absence of noise, the addition of even a small amount of noise (0.75% noise) dramatically degrades the calibrated sensor signal (Fig. 5B). This sensitivity to noise can, however, be reduced by introducing a three-point moving average to the derivative term as follows

$$\begin{aligned} \hat{C}_1 &= \frac{[s(n) + s(n-1) + s(n-2)]/3 - [s(n-1) + s(n-2) + s(n-3)]/3}{dT} + p_2s(n) \\ &= b_1s(n) + b_3s(n-3) \end{aligned} \quad (A3)$$

where $b_1 = [p_2 + 1/(3 \cdot dT)]/p_3$ and $b_3 = 1/(3 \cdot p_3 \cdot dT)$. Eq. A3, which filters the derivative but not the sensor signal per se, does not increase the computational requirement because the terms involving $s(n-1)$ and $s(n-2)$ cancel. This modification dramatically reduces the sensitivity to noise (Fig. 5C). Note, that for this approximation the three-point moving average was applied to the derivative but not to the signal. Filtering the signal per se could be accomplished by introducing a moving average to the term $p_2s(n)$ in Eq. A3. Filtering the sensor signal would have resulted in 3rd-order equation for calibration without correction for delay and a 4th-order

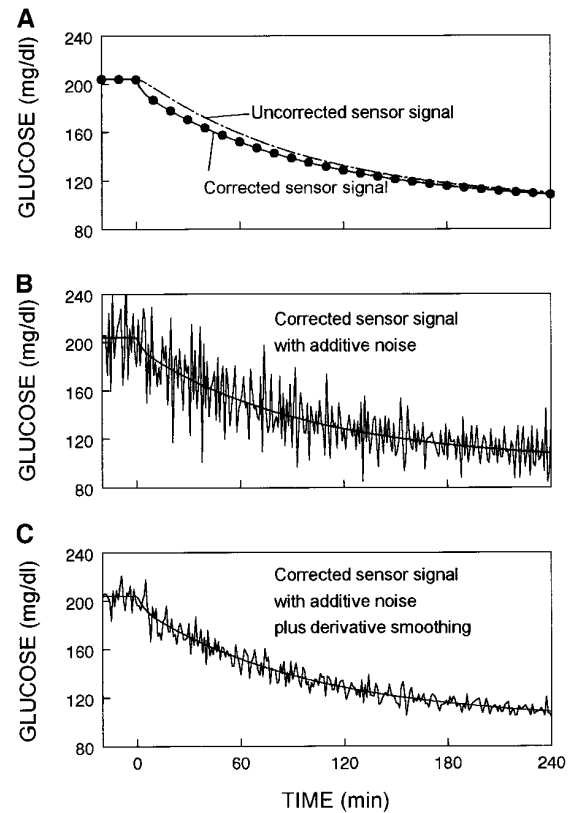


Fig. 5. A: plasma glucose dynamic (●) shown every 10 min together with sensor glucose (solid line) with Eq. A3. B: plasma glucose dynamic (smooth line) with sensor glucose (line) with Eq. A3 but with 0.75% Gaussian random noise applied to sensor signal. C: plasma glucose dynamic (smooth line) together with sensor glucose (line) with Eq. A4 with same noise as B.

equation with correction for delay. In general, finite difference equations that express the current "output" as a weighted sum of past "inputs" are known as finite-impulse-response filters for which the optimal coefficients $b_1 \dots b_n$ can be obtained by least squares. Such filters are known as Wiener filters (24) and can optimally correct for delays and suppress noise (eliminate all frequency components above the glucose

band-width). The full development of such a filter is, however, beyond the scope of the present study.

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