Interstitial glucose concentration and glycemia: implications for continuous subcutaneous glucose monitoring

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Aussedat, B., M. Dupire-Angel, R. Gifford, J. C. Klein, G. S. Wilson, and G. Reach. Interstitial glucose concentration and glycemia: implications for continuous subcutaneous glucose monitoring. Am J Physiol Endocrinol Metab 278: E716–E728, 2000.—The changes in plasma glucose concentration and in interstitial glucose concentration, determined with a miniaturized subcutaneous glucose sensor, were investigated in anesthetized nondiabetic rats. Interstitial glucose was estimated through two different calibration procedures. First, after a glucose load, the magnitude of the increase in interstitial glucose, estimated through a one-point calibration procedure, was 70% of that in plasma glucose. We propose that this is due to the effect of endogenous insulin on peripheral glucose uptake. Second, during the spontaneous secondary decrease in plasma glucose after the glucose load, interstitial glucose decreased faster than plasma glucose, which may also be due to the effect of insulin on peripheral glucose uptake. Third, during insulin-induced hypoglycemia, the decrease in interstitial glucose was less marked than that of plasma glucose, suggesting that hypoglycemia suppressed transfer of glucose into the interstitial tissue; subsequently, interstitial glucose remained lower than plasma glucose during its return to basal value, suggesting that the stimulatory effect of insulin on peripheral glucose uptake was protracted. If these observations obtained in rats are relevant to human physiology, such discrepancies between plasma and interstitial glucose concentration may have major implications for the use of a subcutaneous glucose sensor in continuous blood glucose monitoring in diabetic patients.

The use of the subcutaneous tissue as a site for continuous blood glucose monitoring assumes that the glucose concentration in the interstitial tissue reflects blood glucose level, both under stationary conditions and during glycemic variations (35). We have developed a glucose monitoring system consisting of a needle-type glucose sensor implanted in the subcutaneous tissue (8) and connected to an electronic system (21). The ability of the glucose sensor to measure glucose concentration has been evaluated in vitro and in vivo in rats (3, 24, 25), in dogs (31, 33), and in human volunteers (2, 25, 32). This was done during changes in blood glucose concentration produced by glucose administration into the peritoneal cavity (rat experiments), during an oral glucose tolerance test (trials in nondiabetic human subjects), or after meals and insulin injections (trials in diabetic patients). In these studies, interstitial glucose concentration was estimated from the sensor signal through a two-point calibration procedure taking into account the values of blood glucose concentration and of the sensor signal observed before, and during, the glucose challenge (44). In the course of this evaluation, we observed, in experiments performed both in animals (3, 31, 42) and in humans (36), that the relationship between glycemia and the interstitial glucose concentration, which is actually measured continuously by the glucose sensor, is not simple.

First, during an increase in blood glucose concentration, the sensor signal, which reflects the glucose concentration in the interstitial tissue, lagged 5–10 min behind blood glucose. By contrast, during a decrease in blood glucose after administration of insulin in diabetic rats, the decrease in the subcutaneous glucose concentration preceded that in blood (42); such a phenomenon was also observed after insulin administration in normal dogs (31) and during the secondary decrease in blood glucose concentration of an oral glucose tolerance test performed in a nondiabetic volunteer (36). We hypothesized that a push-pull phenomenon was responsible for this effect. During an increase in blood glucose, the delayed increase in interstitial glucose level was due to the transfer of glucose pushed from blood to the extravascular sector, where the sensor is located. During the decrease in blood glucose, by contrast, the glucose sensor first monitored the local decrease in subcutaneous glucose concentration, which may be due to the effect of insulin on glucose transfer pulled from the interstitial fluid into the surrounding cells (42). This latter phenomenon was also observed by others measuring glucose in the interstitial tissue by a microdialysis technique (41). Second, we observed that after hypoglycemia, the interstitial glucose concentration remained in the hypoglycemic range during the return of glycemia to normal values (3). This protracted decrease in interstitial glucose concentration was also...

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observed by Moberg in nondiabetic patients after an insulin injection (26).

To clarify the relationship between interstitial and blood glucose concentrations, the aim of this work was therefore to compare, in nondiabetic fasting rats, the changes in blood glucose concentration and in interstitial glucose concentration, determined by a subcutaneous glucose sensor, under various conditions: the administration of exogenous glucose or insulin, or of phlorizin, a drug that produces a decrease in blood glucose level through an inhibition of the renal reabsorption of glucose.

**MATERIALS AND METHODS**

Glucose sensor. The preparation of the miniaturized glucose sensor has been described elsewhere (8). Briefly, the sensor consists of a platinum anode covered with Teflon, except for a 2-mm cavity near its extremity, where glucose oxidase is layered; a membrane made of cellulose acetate and Naion is used to screen off interferences such as acetaminophen or ascorbate. The enzyme layer is coated with a solution of 4.3% (wt/wt) polyurethane (Tecoflex S858A, Thermedics), 1.3% (wt/wt) MDX4210 (MED4211, NuSil) with curing agent in 95% (wt/wt) tetrahydrofuran, and 1% N,N-dimethylformamide. The sensor is then cured for 3 days in air and is immersed in 0.1 M PBS for 14 days to facilitate conditioning. A silver/silver chloride cathode, wrapped around the Teflon coating, is used as the reference electrode. The external diameter of the sensor is about 0.35 mm. The in vitro sensitivity of the sensors used in this study, determined in phosphate buffer, was 5.47 ± 0.52 nA/mM glucose, n = 35. The time to reach in vitro 90% of sensor maximal response to a 5 mM increase in glucose concentration was 2.0 ± 0.2 min.

Electronic control unit. The glucose sensor was connected through a cable to an electronic control unit (ECU; École des Mines, Fontainebleau, France), which controls the sensor applied potential and acquires and stores the sensor current. The ECU also performs data processing, including filtering and transforming the sensor output into an estimation of glucose concentration through a calibration procedure described below. Finally, it displays on an LCD the sensor current, the estimate of glucose concentration, or, once the system is calibrated, the estimation of glucose concentration in milligrams per deciliter (3, 21). In these experiments the filter, using mathematical morphology techniques (39), analyzed five consecutive values of the current sampled every 30 s, thus introducing a 2.5-min delay.

Sensor calibration. The sensor output was used to estimate the interstitial glucose concentration by two different methods of in vivo calibration. It is not possible to rely on the in vitro sensor sensitivity because it is well known that the in vivo sensor sensitivity is different when the sensor is implanted in the subcutaneous tissue and cannot be predicted from the in vitro value (16). We first used the two-point calibration procedure developed in our laboratories (44), taking into account two sets of values of plasma glucose concentration (G1 and G2) and concomitant sensor output (I1 and I2) determined before and after a glucose load; the ECU determined each time that the sensor output was stable and triggered an alarm requesting a blood glucose determination. These values of sensor output and of concomitant plasma glucose concentration were entered into the ECU, which calculated an in vivo sensor sensitivity, referred to below as $S_{2P}$, based on this two-point calibration (2P) procedure as $S_{2P} = (I1 - I2)/(G1 - G2)$ and the theoretical sensor output in the absence of glucose ($I_0$) as $I_0 = I1 - G1 \times S_{2P}$, and subsequently transformed the sensor output [$I(t)$] into an estimation of interstitial glucose concentration based on this initial two-point calibration, referred to as $I_{G2P}(t) = [I(t) - I0]S_{2P}$. In this study we also used a second calibration procedure in which the sensor sensitivity ($S$) was simply calculated on the basis of a one-point calibration procedure (1P), referred to below as $S_{1P} = I1/G1$, where I1 and G1 were determined in basal state; subsequently, the estimation by this one-point calibration procedure of interstitial glucose concentration, referred to as $I_{G1P}(t)$, was estimated as $I_{G1P}(t) = I(t)/S_{1P}$.

It must be pointed out that these values of $I_{G2P}$ or $I_{G1P}$ represent only estimations of the real value of interstitial glucose concentration through the chosen calibration procedure. Thus $I_{G2P}$ would be identical to the true value of interstitial glucose if interstitial glucose were identical to plasma glucose concentration both under basal conditions and at the plateau in the current observed after the glucose challenge, which, as will be demonstrated in this study, cannot be true. Nevertheless, this procedure, calibrating the sensor against two values of blood glucose concentration makes it possible, first, to describe the kinetics of interstitial glucose concentration during the calibration procedure itself, i.e., to analyze the delay between the increase in plasma glucose and in interstitial glucose, and second, to detect changes in the relationship between interstitial glucose and plasma glucose during the period after the calibration procedure, because at the end of this initial calibration, plasma glucose and interstitial glucose are, by mathematical construction, identical. Furthermore, this procedure was validated by its ability to detect in a timely fashion insulin-induced hypoglycemia by monitoring glucose concentration with a subcutaneous glucose sensor (3). Similarly, the values of $I_{G1P}$ also represent only an estimation of interstitial glucose concentration through the one-point calibration procedure, which assumes that the sensor response to glucose is linear over the range of experimental values, and that the sensor signal in the absence of glucose is negligible, which has not been experimentally demonstrated. Nevertheless, the one-point calibration procedure can be used to demonstrate different relationships between plasma glucose and interstitial glucose according to the experimental condition.

Experimental procedure. The sensor was implanted, through a 20-gauge cannula, under halothane anesthesia in the interscapular subcutaneous tissue of fasting 250-g male Wistar rats (6 sets of experiments, n = 35 rats) and polarized overnight with a miniaturized 650-mV battery. The next morning the rat was again anesthetized with halothane, and the sensor was connected to the ECU. Throughout the experiment, the animal was warmed under a lamp. To avoid any surgical procedure, no tracheostomy was performed and no catheter was indwelled in these animals. Plasma glucose concentration was determined every 10 min in 50 μl of blood sampled at the tail vein (Bedman Analyzer, Fullerton, CA). In two separate experiments (data not shown), we verified that during the time course of the experiment (6 h), the rectal temperature remained stable between 38 and 39.5°C, the blood pressure measured at the tail artery of the animal at 30-min intervals with a BP Recorder 8005 (Serlabo, Bonneuil/ Marne, France) device was kept within the physiological range (80–120 mmHg), and that finally the 38 50-μl blood samples used in this study to determine blood glucose concentration (sampled in the control experiment from a jugular catheter) had essentially no effect on the rat hematocrit, which remained stable at 40% in one experiment and decreased from 45 to 43% in the other.
Six sets of experiments were performed. Group 1 (n = 6, glucose-glucose): at t = 0 min, i.e., when the ECU had determined that the sensor output was stable, a first intraperitoneal injection of glucose (1 g/kg body wt) was performed and the system was calibrated (two-point calibration procedure) on the basis of the basal and peak in the sensor output. A second injection of glucose (1 g/kg) was performed 3 h after the first glucose injection. Group 2 (n = 7, glucose-insulin-glucose): after the first injection of glucose, used to calibrate the system (two-point calibration procedure), rats received an intravenous injection of insulin (porcine regular insulin, Organon, Puteaux, France, 1.5 U/kg body wt) administered 80 min after the glucose injection. A second injection of glucose was performed 300 min after the first glucose injection. Group 3 (n = 6, insulin-glucose): rats were not given glucose and received an injection of insulin, performed 90 min after the time when the ECU had recognized that the sensor output was stable; an intraperitoneal glucose load was performed at t = 300 min. The system was calibrated (two-point calibration procedure) on the basis of the two sets of sensor output and concomitant plasma glucose concentration determination before and at the nadir of the decrease in the sensor output after insulin injection. Group 4 (n = 6, glucose-phlorizin-glucose): after the first injection of glucose, used to calibrate the system (two-point calibration procedure), rats received an intraperitoneal injection of phlorizin (Sigma, St Louis, MO; 0.6 g/kg, 40% solution in propylene glycol) performed 90 min after the glucose bolus. Phlorizin decreases blood glucose concentration by inhibiting sodium- and energy-dependent glucose transporters at the kidney level (11, 40). Another injection of glucose was performed at t = 300 min. Group 5 (n = 6, phlorizin-glucose): rats received the injection of phlorizin at t = 90 min, and the system was calibrated (two-point calibration procedure) on the basis of the two sets of sensor output, and concomitant plasma glucose concentration was determined before, and at the nadir of, the decrease in the sensor output after phlorizin administration. An injection of glucose was performed at t = 300 min. Finally, group 6 (n = 4, control) consisted of animals in which glyemia and sensor signal were monitored without any intervention during 300 min. Glucose was then injected intraperitoneally to retrospectively calibrate the system (two-point calibration procedure) and to quantify the drift in the sensor response over this period of time. At the end of the experiment, animals were killed with an overdose of pentothal sodium.

Presentation of results and statistics. Because the filter introduced a fixed 2.5-min delay in the sensor output, the sensor output curves were shifted to the left to represent the real time course of the estimation of glucose concentration in interstitial tissue. All data are presented in text and figures as means ± SE, and the statistical significance was assessed by paired Student’s t-test and ANOVA. In each experimental group, ANOVA analysis of the two curves (interstitial glucose and plasma glucose) demonstrated that they were significantly different (P = 0.0001).

RESULTS

Glucose kinetics in plasma and interstitial tissue during a glucose load with interstitial glucose concentration determined on the basis of single- or two-point calibration procedures. Figure 1A represents the results obtained in the three series of animals in which a glucose load was performed at time 0 (groups glucose-glucose, glucose-insulin-glucose, glucose-phlorizin-glucose, n = 19). Plasma glucose increased from 100 ± 4 to 204 ± 5 mg/dl. When interstitial glucose was estimated by the two-point calibration procedure, the increase in IG2P followed that in plasma glucose with a 5 ± 1-min delay. This delay was determined for each experiment as the time necessary for IG2P to reach the value of plasma glucose observed 10 min after the glucose injection. When interstitial glucose was estimated by the one-point calibration procedure performed immediately before the glucose load (IG1P), it increased to 174 ± 7 mg/dl and the ratio between the increments in plasma glucose and IG1P was 75 ± 8% (P < 0.005 vs. 100). Figure 1B presents the data from the glucose load performed at t = 300 min in the control group (n = 4), showing that the results were essentially identical.

Glucose kinetics in plasma and interstitial tissue during two consecutive injections of glucose (Fig. 2). After the first intraperitoneal injection of glucose, plasma glucose concentration (Fig. 2, open circles) increased from 98 ± 5 to 216 ± 10 mg/dl at a rate of 5.61 ± 0.37 mg·dl⁻¹·min⁻¹. Subsequently, from t = 70 min, plasma glucose decreased from 170 ± 6 to 118 ± 8...
mg/dl (at a rate of $0.72 \pm 0.14 \text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$, calculated between 70 and 90 min and of $0.49 \pm 0.04 \text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$, calculated between 70 and 190 min, Table 1). After the second intraperitoneal injection of glucose, plasma glucose concentration (PG) increased at a rate of $2.46 \pm 0.22 \text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ from 118 ± 8 to 170 ± 6 mg/dl. The rate of increase ($P < 0.0005$), the peak value ($P < 0.02$), and the increment ($52 \pm 6$ vs. 118 ± 5 mg/dl, $P < 0.0005$) of PG were significantly lower than after the first glucose administration.

Figure 2A represents the data analyzed through the two-point calibration procedure, making an analysis of the various delays between the two curves possible. During the initial increase in PG after the first glucose load, IG2P followed plasma glucose with a 3.5 ± 1.6 min lag. From the glucose peak, this delay was not observed for 1 h. During this period of time, the decrease in IG2P preceded that in blood for the next 120 min; IG2P was lower than the concomitant value of plasma glucose ($P < 0.05$). This phenomenon is better shown in Fig. 2B, showing the decrease in PG (open circles) and in IG2P (solid line), expressed as a percentage of the values determined at the peak of the glucose load. During the second glucose load, the increments in plasma glucose and in IG2P, estimated from the initial two-point calibration procedure, were essentially identical ($52 \pm 6$ vs. 53 ± 4 mg/dl, NS, ratio $\Delta \text{IG2P}/\Delta \text{PG} = 1.02 \pm 0.04$). IG2P followed plasma glucose with an apparent lag time significantly longer than after the first injection of glucose (9.6 ± 1.4 min, $P < 0.05$). However, when the estimation of IG2P was calculated on a two-point calibration of the sensor, taking into account the basal and peak values of plasma glucose and sensor output observed during the second glucose load, the delay was only slightly, and not significantly, longer (5.6 ± 1.7 min, NS) than during the first glucose challenge.

Figure 2C represents the same data interpreted on the basis of a one-point calibration procedure performed before the first glucose load. During the first glucose load, IG1P (solid line) increased to 186 ± 14 mg/dl, and the ratio between the magnitudes of increase in IG1P and plasma glucose was 73 ± 6% ($P < 0.005$). At time 190 min, when plasma glucose had returned to 118 ± 8 mg/dl, IG1P was 103 ± 6 mg/dl (NS). During the second glucose load, IG1P increased to 142 ± 9 mg/dl, and the ratio between the magnitudes of increase in IG1P and plasma glucose was 71 ± 7% (NS vs. the first glucose load).

Glucose kinetics after insulin administration at the glucose peak reached after a glucose load. Figure 3 presents the results of seven experiments in which exogenous insulin was administered to the animals at the glucose peak after a glucose load. During the first glucose load, plasma glucose increased from 102 ± 6 to 196 ± 6 mg/dl, at a rate of $4.19 \pm 0.17 \text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$.

After the intravenous injection of insulin, plasma glucose decreased within 50 min from 160 ± 11 to 39 ± 3 mg/dl; the rate of decrease was $3.61 \pm 0.42 \text{mg} \cdot \text{dl}^{-1} \cdot \text{min}^{-1}$ (Table 1), which was significantly faster than that observed during the spontaneous decrease in PG observed in the first set of experiments ($P < 0.05$).
injection, the peak in IG 2P value, estimated from the increase in plasma glucose and in IG2P being 4.3.

Table 1. Decrease in plasma and interstitial glucose concentration

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Time, min</th>
<th>Basal, mg/dl</th>
<th>Nadir, mg/dl</th>
<th>ΔPG, mg/dl</th>
<th>ΔPG/min, mg·dl⁻¹·min⁻¹</th>
<th>ΔIG, mg/dl</th>
<th>ΔIG/ΔPG, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-glucose</td>
<td>70–90</td>
<td>170 ± 6</td>
<td>155 ± 6</td>
<td>15 ± 3</td>
<td>0.72 ± 0.14</td>
<td>20 ± 7</td>
<td>133 ± 7</td>
</tr>
<tr>
<td>First estimation</td>
<td>70–190</td>
<td>118 ± 6</td>
<td>118 ± 6</td>
<td>52 ± 5</td>
<td>0.49 ± 0.04</td>
<td>59 ± 4</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>Second estimation</td>
<td>80–100</td>
<td>160 ± 11</td>
<td>118 ± 6</td>
<td>72 ± 8</td>
<td>3.61 ± 0.42a</td>
<td>71 ± 4</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>Insulin-glucose</td>
<td>90–110</td>
<td>89 ± 6</td>
<td>48 ± 4</td>
<td>41 ± 6</td>
<td>2.07 ± 0.32b</td>
<td>33 ± 4</td>
<td>80 ± 4</td>
</tr>
<tr>
<td>Phlorizin-glucose</td>
<td>90–110</td>
<td>100 ± 6</td>
<td>79 ± 5</td>
<td>21 ± 2</td>
<td>1.03 ± 0.10c</td>
<td>24 ± 3</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>Glucose-phlorizin-glucose</td>
<td>90–110</td>
<td>163 ± 9</td>
<td>121 ± 9</td>
<td>42 ± 4</td>
<td>2.11 ± 0.21d,e</td>
<td>49 ± 7</td>
<td>116 ± 11</td>
</tr>
</tbody>
</table>

PG, plasma glucose concentration; IG, interstitial glucose concentration. *P < 0.0001 vs. glucose-glucose group; **P < 0.005 vs. glucose-glucose group; µP = 0.08 vs. glucose-glucose group (first estimation); ¶P < 0.001 vs. glucose-glucose group (second estimation); δP < 0.0002 vs. glucose-insulin-glucose group; *P < 0.02 vs. glucose-insulin-glucose group.

0.0001). Subsequently, PG returned progressively to basal value (84 ± 5 mg/dl at t = 300 min) and, after the second intraperitoneal injection of glucose, plasma glucose concentration increased to 188 ± 10 mg/dl at a rate of 3.44 ± 0.90 mg·dl⁻¹·min⁻¹, which was slightly but not significantly lower than that observed during the first glucose administration (P = 0.39). The increment in plasma glucose was significantly higher than that observed during the second glucose load of the first series of experiments shown in Fig. 2 (104 ± 8 vs. 52 ± 6 mg/dl, P < 0.0005).

Figure 3A represents the data analyzed through the two-point calibration procedure, the delay between the increase in plasma glucose and in IG2P being 4.3 ± 1.0 min (NS vs. the first series of experiments). Here too, during the initial decrease in plasma glucose, this delay was no longer observed. After insulin injection, the decrease in IG2P did not precede, but followed that in plasma glucose, and at 65 ± 10 mg/dl at t = 130 min, which was significantly higher than the plasma glucose nadir (P < 0.01), as already shown (Fig. 3A) by using the two-point calibration procedure. It remained subsequently stable at this level during the recovery in plasma glucose, and at t = 300, when plasma glucose had returned to 84 ± 5 mg/dl, IG2P was still 71 ± 9 mg/dl (P < 0.05).

Glucose kinetics after insulin administration in the basal state. The effect of insulin administered in the basal state is shown in Fig. 4. After the intravenous injection of insulin, plasma glucose decreased from 89 ± 6 to 48 ± 4 mg/dl within 20 min at a rate of 2.07 ± 0.32 mg·dl⁻¹·min⁻¹, which also significantly faster (P < 0.005) than that observed during the spontaneous decrease in plasma glucose observed in the first set of experiments during the second part of the glucose challenge (Fig. 2, Table 1). It then returned progressively to basal value and was 89 ± 8 mg/dl at t = 300 min. After the intraperitoneal injection of glucose, plasma glucose concentration increased to 206 ± 11 mg/dl at a rate of 4.80 ± 0.56 mg·dl⁻¹·min⁻¹. Here again, the increase in plasma glucose was significantly higher than that observed during the second glucose load of the first series of experiments shown in Fig. 2 (118 ± 7 vs. 52 ± 6 mg/dl, P < 0.0001).

Figure 4A gives the results of the two-point calibration procedure, taking into account the values of plasma glucose and sensor output observed before and at the nadir of plasma glucose after insulin injection. Here too, after the administration of insulin, the decrease in IG2P followed that in plasma glucose for 20 min, with
plasma glucose being lower than IG2P at t = 100 min [63 ± 5 vs. 80 ± 7 mg/dl (P < 0.05)]. This is also shown in Fig. 4B, showing that the decrease in IG2P followed that in plasma glucose with a 7 ± 2 min delay.
Subsequently, as in the previous set of experiments, IG2P remained lower than plasma glucose while plasma glucose returned to basal values (76 ± 6 vs. 87 ± 7 mg/dl at t = 290 min, P < 0.05). During the glucose load, IG2P increased from 75 ± 7 to 169 ± 5 mg/dl; the increment in IG2P was lower than that in plasma glucose (94 ± 9 vs. 118 ± 7 mg/dl, P < 0.05, ratio ΔIG2P/ΔPG = 0.80 ± 0.06). When the estimation of IG2P was calculated on a calibration of the sensor, taking into account the basal and peak values of plasma glucose and sensor output observed during the glucose load, the delay was similar to that observed in the previous set of experiments (6.0 ± 1.1 min, NS).

Figure 4C presents the results of the one-point calibration procedure, performed before the insulin injection. After insulin injection, IG1P decreased to reach a nadir of 58 ± 5 mg/dl, which was significantly higher than the plasma glucose nadir (P < 0.0001), similar to the phenomenon observed in the previous set of experiments (Fig. 3C). During plasma glucose recovery to basal value, it increased more slowly than plasma glucose and became lower at 270 min than the concomitant value of plasma glucose (75 ± 9 vs. 85 ± 4 mg/dl, P < 0.05). During the second glucose load, IG1P increased to 148 ± 9 mg/dl and the ratio between the magnitudes of increase in IG1P and plasma glucose was 59 ± 4%, which was similar to that observed during the second series of experiments (Fig. 3, glucose-insulin-glucose group, P = 0.28) and significantly lower than that of the second glucose load of the first series of experiments (Fig. 2) performed without prior insulin administration (P < 0.05).

Glucose kinetics after phlorizin administration in the basal state. The effect of phlorizin administered in the basal state is shown in Fig. 5. After the intraperitoneal injection of phlorizin, plasma glucose decreased from 100 ± 6 to 68 ± 5 mg/dl within 50 min at a rate of 1.03 ± 0.10 mg·dl⁻¹·min⁻¹ (Table 1), which was significantly higher than the rate of decrease in plasma glucose observed in the first set of experiments (P < 0.001). It then returned progressively to basal value and was 89 ± 13 mg/dl at 300 min. After the intraperitoneal injection of glucose, plasma glucose concentration increased to 180 ± 14 mg/dl at a rate of 2.52 ± 0.36 mg·dl⁻¹·min⁻¹.

The data from the two-point calibration procedure is shown in Fig. 5A. After phlorizin administration, IG2P decreased from 98 ± 6 to 69 ± 6 mg/dl, with a very short lag compared with plasma glucose. This 2-min lag is shown in Fig. 5B. At t = 300 min, IG2P was slightly, but not significantly, lower than plasma glucose (79 ± 13 vs. 84 ± 14 mg/dl, NS). After the intraperitoneal injection of glucose, IG2P increased from 79 ± 13 to 143 ± 16 mg/dl, the increment in IG2P being lower than that in plasma glucose (63 ± 10 vs. 91 ± 14 mg/dl, P < 0.05, ratio ΔIG2P/ΔPG = 0.74 ± 0.10). When the estimation of IG2P was calculated on a calibration of the sensor taking into account the basal and peak values of plasma glucose and sensor output observed after the glucose load, the delay was 9.2 ± 2.6 min.

When IG1P was estimated on the basis of a one-point calibration procedure performed before phlorizin injection (Fig. 5C), its decrease was slightly smaller than that of plasma glucose: at time 120 min, plasma glucose
was 73 ± 5, and IG₁P was 79 ± 6 mg/dl (NS). IG₁P remained stable at that level during the recovery of plasma glucose from hypoglycemia, and at time 300 min, the two values were essentially identical (86 ± 11 vs. 89 ± 13 mg/dl, NS). During the glucose load, IG₁P increased to 139 ± 16 mg/dl and the ratio between the magnitudes of increase in IG₁P and plasma glucose was 55 ± 7%, which was lower than that of the second glucose load of the first series of experiments (Fig. 2) performed without prior insulin administration (P < 0.05).

Glucose kinetics after phlorizin administration. After the first intraperitoneal injection of glucose, plasma glucose concentration increased from 100 ± 5 to 208 ± 9 mg/dl at a rate of 4.00 ± 0.47 mg·dl⁻¹·min⁻¹. After the intraperitoneal injection of phlorizin, glycemia decreased within 60 min from 163 ± 9 to 92 ± 9 mg/dl at 150 min at a rate of 2.11 ± 0.21 mg·dl⁻¹·min⁻¹ (Table 1), which was significantly faster (P < 0.0002) than that observed in the absence of phlorizin injection (Fig. 2), but slower (P < 0.02) than that observed after insulin injection (Fig. 3). After the second intraperitoneal injection of glucose, plasma glucose concentration increased from 87 ± 10 to 163 ± 13 mg/dl at a rate of 2.31 ± 0.33 mg·dl⁻¹·min⁻¹. The peak in plasma glucose (P < 0.05), the rate of increase (P < 0.02), and the increment (76 ± 5 vs. 108 ± 9 mg/dl, P < 0.005) were lower than after the first glucose injection.

The results of the two-point calibration procedure are presented in Fig. 6A. During the first glucose load, IG₂P followed PG with a 5.3 ± 1.0-min lag, and again, during the initial decrease in plasma glucose, this delay was no longer observed; this domain was very similar to those described in Figs. 2 and 3. IG₂P decreased also from 167 ± 9 to 81 ± 8 mg/dl (NS) without any lag during the first 20 min after phlorizin administration. In the next 40 min, the decrease in IG₂P was faster than that of plasma glucose (Fig. 6B), a phenomenon similar to that observed in the first group of animals (glucose-glucose group, Fig. 2B). Thereafter, whereas plasma glucose remained stable around 90 mg/dl, IG₂P decreased significantly to reach 54 ± 7 mg/dl at t = 300 min, which was significantly lower than plasma glucose (87 ± 10 mg/dl, P < 0.01). IG₂P increased from 54 ± 7 to 114 ± 17 mg/dl. The magnitudes of the increase in IG₂P and plasma glucose were statistically different (59 ± 10 vs. 76 ± 5 mg/dl, P < 0.05, ratio ΔIG₂P/ΔPG = 0.77 ± 0.10). When the estimation of interstitial glucose was calculated on a calibration of the sensor taking into account the basal and peak values of plasma glucose and sensor output observed during the second glucose load, the delay was only slightly, and not significantly, longer (9.0 ± 3.7 min, NS) than during the first glucose challenge.

Figure 6C presents the results of the one-point calibration method performed before the glucose load. Until phlorizin administration, the results observed were the same as in the previous series of experiments; the increase in IG₁P to 174 ± 8 mg/dl at 30 min was blunted by 69 ± 11% compared with the increase in plasma glucose (P = 0.36 vs. the first series of experi-

Fig. 6. Plasma glucose and interstitial glucose concentration in rats after 2 consecutive administrations of glucose. Phlorizin was administered after first glucose load, when animals were still hyperglycemic (n = 6). A: data analyzed through 2-point calibration procedure. B: decreases in plasma glucose (closed circles) and IG₂P (solid line) expressed as a percentage of values determined before administration of phlorizin. C: data analyzed through 1-point calibration procedure, performed before first glucose administration (cal). *P < 0.05 at least.
Glucose load IG1P increased to 107±6 mg/dl at 300 min, which was significantly lower than the concomitant plasma glucose (P<0.0001). During the second glucose load IG1P increased to 162±11 mg/dl and the ratio between the increase in IG1P and plasma glucose was 58±9%, which was significantly lower than that observed during the first glucose load of this series of experiments (P<0.05) and than that of the second glucose load of the first series of experiments (Fig. 2) performed without prior phlorizin administration (P<0.05).

Sensor output and plasma glucose concentration in the absence of intervention. As shown in Fig. 7A, the current produced by the sensor mirrored the slight changes in glycemic concentration. At t = 300 min, an intraperitoneal injection of glucose produced an increase in plasma glucose from 86±5 to 197±8 mg/dl at a rate of 5.15±0.44 mg·dl⁻¹·min⁻¹, which was not significantly different from increases observed for the first glucose loads in the glucose-glucose and glucose-phlorizin-glucose groups, and slightly, but significantly faster (P<0.02) for the comparison with the glucose-insulin-glucose group. It was twice as fast as the rate of increase observed in animals from the glucose-glucose group (P<0.0002) and in animals previously treated with phlorizin (P<0.001 and P<0.005 for the phlorizin-glucose and glucose-phlorizin-glucose groups, respectively), but not significantly different from the glucose load performed in insulin-treated animals. The sensor output increased from 13.5±1.83 to 26.28±3.58 nA. This glucose load was used to calibrate the sensor by the two-point calibration procedure. The delay between the increases in plasma glucose and IG1P was 6±2 min. From this calibration, it was possible to determine, retrospectively, the glucose concentration in interstitial fluid at t = 0: it was 128±9 mg/dl, slightly higher than the concomitant plasma glucose concentration, which was 110±8 mg/dl (P=0.054). The ratio between the two values was 1.18±0.07%.

The results of the one-point calibration performed at t = 0 min are shown in Fig. 7B. During the glucose load, IG1P increased to 162±12 mg/dl and the ratio between the magnitudes of increase in IG1P and glucose was 72±6%, which was identical to that observed during the first glucose load of the glucose-glucose, glucose-insulin-glucose, and glucose-phlorizin-glucose groups, shown in Fig. 1 (NS), but significantly higher than that observed during the second glucose load of the glucose-insulin-glucose (P<0.05), insulin-glucose (P<0.001), glucose-phlorizin-glucose (P<0.05), and phlorizin-glucose (P<0.005) groups.

Change in the ratio between IG1P and plasma glucose under different experimental conditions. Figure 8A represents the ratio between IG1P and plasma glucose in the control group; the one-point calibration was performed at t = 0 min (open circles), showing that it remained essentially stable until the glucose load. Figure 8B represents the data obtained in the other experimental groups. It illustrates the fact that, first, during an increase in plasma glucose after a glucose load, the delay in the increase in IG1P, as expected, a transient decrease in the ratio IG1P/plasma glucose; second, that during a decrease in plasma glucose, after the administration of insulin or phlorizin at time 80–90 min, the delay in the decrease in IG1P, producing an increase in this ratio, was not observed during the spontaneous decrease in glucose concentration after the initial glucose load (group glucose-glucose), consistent with the data shown in Fig. 2, A and B, obtained by using the other, two-point, calibration procedure; third, that the magnitude of this increase in the ratio of IG1P/plasma glucose was greater in the animals treated by insulin than by phlorizin, and was the highest in the glucose-insulin-glucose group, which exhibited the fastest rate of decrease in plasma glucose (Table 1).

**DISCUSSION**

In this study, we compared the values of plasma glucose concentration and interstitial glucose level determined with a glucose sensor implanted in the subcutaneous tissue. Over the time course of these experiments, the drift in the sensitivity of the sensor
used to determine the interstitial glucose concentration was minimal (Figs. 7 and 8A). Comparison of Fig. 1, A and B, showing essentially similar patterns in plasma glucose and interstitial glucose when the glucose load was performed at t = 0 or 300 min, suggests also that both the physiological preparation and the glucose sensor were stable over the time course of these experiments.

Because the sensor is measuring interstitial glucose concentration, the interpretation of the results must consider the fact that this parameter is the result of different fluxes, under a model shown in Fig. 9. Plasma glucose concentration is the net result of exogenous glucose (Gex) or of endogenous, hepatic, glucose production (Gen), and of glucose elimination by the kidney Gk. Flux Gen is suppressed by insulin and is stimulated by counter-regulatory hormones secreted during hypoglycemia. Gk is stimulated by phlorizin. Interstitial glucose concentration, which is measured by the glucose sensor, is the net result of the input from the vascular blood (flux I) minus the output into the surrounding cells (flux O). Flux O is stimulated by insulin, with this effect being suppressed by catecholamines. We cannot rule out the additional effect of changes in local blood flow around the sensor, occurring for instance in some of these experiments during hypoglycemia, although both an increase (1, 5, 9, 14) and a decrease (19, 20) in subcutaneous blood flow have been described after hypoglycemia.

First, during a glucose load, the increment in interstitial glucose concentration determined through the one-point calibration procedure was found to be blunted by comparison with that in blood. This effect was initially observed for the first glucose load (Figs. 1–3 and 6) and was more pronounced during the second glucose load in those animals in which exogenous insulin was administered before the second glucose load (Figs. 3 and 4). This phenomenon is consistent with an effect of insulin stimulating flux O, thus blunting the increase in interstitial glucose. Incidentally, during the second glucose load performed in the first group of animals (Fig. 2), the increase in plasma glucose concentration was smaller than during the first glucose challenge. This may result from a protracted effect of endogenous insulin secreted during the first glucose load, or more likely of a higher insulin secretion during the second glucose challenge, linked to the memory effect of glucose on insulin secretion (17, 27).

Second, after exogenous insulin administration, the magnitude of the decrease in interstitial glucose concentration estimated through the one-point calibration procedure was less pronounced than that of glycemia during the immediate period after insulin injection and, subsequently, interstitial glucose concentration became lower than glycemia during its recovery to normal range (Figs. 3 and 4). This can also be explained by the following model: during insulin-induced hypoglycemia the decrease in plasma glucose concentration is due to the suppression of endogenous glucose production and the stimulation of peripheral glucose uptake. This results in a decrease in flux I. However, interstitial glucose concentration is the net result of this flux and of peripheral glucose uptake (flux O). It is conceivable that during the hypoglycemic period, catecholamines suppress in part the effect of insulin on flux O, thus preventing interstitial glucose concentration from decreasing.
creasing at the same extent as glycaemia. When plasma glucose concentration returns to the normal range, this catecholamine effect ceases to exist, and the protracted effect of insulin on flux 0 would explain why interstitial glucose concentration finally became lower than glycaemia.

A similar pattern was observed during hypoglycaemia induced by phlorizin administration (Figs. 5 and 6). A protracted decrease in interstitial glucose was observed when glycaemia returned to normal or during the subsequent administration of glucose. This effect was more pronounced when phlorizin was administered at the peak of the first glucose load. In this later case, this can be readily explained by the additive effects of the protracted stimulatory effect of endogenous insulin on flux 0 and the effect of phlorizin on Gs, resulting in a decrease in flux I. Surprisingly, however, in the group of animals in which phlorizin had been administered without prior administration of glucose, the increment in IG2P after the late glucose administration was blunted by 50% by comparison with that in plasma glucose, the results being actually very similar to those observed when the glucose bolus was administered after an injection of insulin. To explain this observation, an increase in plasma insulin after phlorizin administration is unlikely because the hypoglycemic effect of phlorizin should have suppressed endogenous insulin secretion and because phlorizin has been shown to have a suppressive effect on glucose-induced insulin secretion by isolated rat and mouse islets (18, 43). A potentiation of insulin action on flux 0 by phlorizin is also unlikely because phlorizin has been shown to inhibit insulin action on glucose uptake (12, 13, 45). The only explanation for the decrease in IG and the blunted increase in IG after the glucose load, which were observed after phlorizin administration, would therefore be an inhibition of flux I, i.e., of the transfer of glucose from the vascular bed to the interstitial milieu. It has been shown that sodium- and energy-dependent glucose transporters, the target of phlorizin, are present in endothelial cells (6, 7, 22, 23, 28, 29). Consistent with this hypothesis, Quinn et al. (34) observed that during a sharp increase in plasma glucose produced by an intravenous glucose injection, the time required for a subcutaneous glucose sensor to reach its maximum current, corrected for sensor response time, depended on the dose of injected glucose, the delay being longer for the higher doses, suggesting the intervention of a saturable transport mechanism in the transfer of glucose from the blood to the subcutaneous tissue.

Third, the analysis of the changes in IG estimated by the two-point calibration and the data shown in Figs. 2B–6B demonstrates that the rate of decrease in IG is dependent on the experimental conditions. Thus, after a glucose load, IG2P decreased before plasma glucose during the spontaneous return of plasma glucose to basal value (Fig. 2, A and B). The involvement of endogenous insulin in this phenomenon was tested during a decrease in plasma glucose induced by phlorizin administration, which decreases plasma glucose without the intervention of insulin. Here, the decrease in IG2P was even delayed by a few minutes, probably corresponding to the intrinsic response time of the sensor.

The decrease in plasma glucose also preceded that in IG2P when the plasma glucose decrease was produced by an injection of exogenous insulin, either at the peak of a glucose load or in the basal state. It is interesting that, in sharp contrast with the data observed in nondiabetic animals, we have previously observed that, after insulin administration in diabetic rats, it was the decrease in interstitial glucose that occurred first. A first explanation for these discordant results may involve differences in insulin effect on flux 0: these diabetic rats, investigated a few days after diabetes induction by streptozotocin, may have been oversensitive to insulin. Although this seems at first glance to contrast with the well-known insulin resistance present in the diabetic state, we have observed such an unexpected oversensitivity to insulin in diabetic mice a few days after diabetes induction by repeated low doses of streptozotocin (10). It is interesting that in a study published by Schmidtke et al. (38), after insulin administration in rats, the decrease in plasma glucose preceded the decrease in interstitial glucose. Animals used in this later study were, however, old, obese, hyperglycaemic, and presumably insulin resistant. But there is a second possible explanation: after insulin administration, plasma glucose concentration decreased faster than during the spontaneous decrease in IG2P and plasma glucose observed in the glucose-glucose group (Table 1). Thus the fact that the decrease in IG2P followed that in plasma glucose may also be due to an increase in the intrinsic sensor response time. According to Baker and Gough (4) the faster the rate of decrease in glucose concentration, the longer the delay in the sensor intrinsic response to glucose.

Taken together, these results underline the fact that the kinetics of interstitial glucose concentration strongly depends on the physiological status of the animal. This may explain the difficulty of describing unambiguously the relationship between blood and interstitial glucose concentration, which has also been observed by others (30, 38, 41). The present findings are consistent with previous observations made by us and others in animals and in humans, using either an implanted subcutaneous glucose sensor or a micropufusion-based system (15, 16, 37, 41, 46). This suggests that they are not related to the method used in these studies but that they describe the physiological relationship between interstitial glucose concentration and glycaemia. Their relevance is therefore not restricted to this animal model (although extrapolation from studies performed in rats to human physiology requires caution because, for instance, rat adipose tissue is more sensitive to insulin and because the interscapular area may contain high amounts of brown adipose tissue) or to this particular glucose sensor, but should be considered for the design of any continuous glucose monitoring system, invasive or noninvasive, in which the sensing element is not directly implanted in the vascular bed. It may also be argued that these observations
are based on measurement of plasma glucose concentration in venous blood sampled at the tail vein, which may not be representative for body glucose. Whether capillary blood glucose concentration, commonly used by diabetic patients to follow their blood glucose level, may prove to be more accurate as to the similarity with subcutaneous measurement remains to be investigated.

Finally, the fact that this kind of system monitors interstitial, and not blood, glucose concentration, will have to be kept in mind when it is used in the management of diabetic patients. Data presented herein may therefore have paradigmatic significance, leading to a novel approach in the monitoring and interpretation of glucose fluctuations observed in insulin-treated diabetic patients.

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