

# Calibration of a subcutaneous amperometric glucose sensor implanted for 7 days in diabetic patients

## Part 2. Superiority of the one-point calibration method

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### Abstract

Calibration, i.e. the transformation in real time of the signal  $I(t)$  generated by the glucose sensor at time  $t$  into an estimation of glucose concentration  $G(t)$ , represents a key issue for the development of a continuous glucose monitoring system. **Objective:** To compare two calibration procedures. In the one-point calibration, which assumes that  $I_0$  is negligible,  $S$  is simply determined as the ratio  $I/G$ , and  $G(t) = I(t)/S$ . The two-point calibration consists in the determination of a sensor sensitivity  $S$  and of a background current  $I_0$  by plotting two values of the sensor signal versus the concomitant blood glucose concentrations. The subsequent estimation of  $G(t)$  is given by  $G(t) = (I(t) - I_0)/S$ . **Research design and methods:** A glucose sensor was implanted in the abdominal subcutaneous tissue of nine type 1 diabetic patients during 3 ( $n = 2$ ) and 7 days ( $n = 7$ ). The one-point calibration was performed a posteriori either once per day before breakfast, or twice per day before breakfast and dinner, or three times per day before each meal. The two-point calibration was performed each morning during breakfast. **Results:** The percentages of points present in zones A and B of the Clarke Error Grid were significantly higher when the system was calibrated using the one-point calibration. Use of two one-point calibrations per day before meals was virtually as accurate as three one-point calibrations. **Conclusion:** This study demonstrates the feasibility of a simple method for calibrating a continuous glucose monitoring system. ● 2002 Published by Elsevier Science B.V.

**Keywords:** Glucose sensor; Calibration; Subcutaneous tissue; Type 1 diabetes; Continuous glucose monitoring; Interstitial glucose concentration

### 1. Introduction

Continuous glucose monitoring may represent a major advance in diabetes therapy, as it would decrease the risk of severe hypoglycemia and therefore help to implement intensified insulin therapy in the framework of the DCCT recommendations (The DCCT Research Group, 1993). In a first configuration, the glucose sensor is indwelled in the vascular bed (direct blood

glucose monitoring); even though such systems have been successfully evaluated in a limited number of dogs for several months (Armour et al., 1990), such an approach requires the invasive access to a major blood vessel, and the safety of the procedure will certainly represent a limitation to its large-scale use. This was the rationale for proposing a second configuration (indirect blood glucose monitoring), in which the sensor measures glucose in another compartment, such as the subcutaneous tissue (Shichiri et al., 1982), the peritoneal cavity (Velho et al., 1988), in a liquid extracted from the subcutaneous tissue by iontophoresis (Garg et al., 1999), or microdialysis (Sternberg et al., 1995).

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Whatever the method used for monitoring glucose concentration in diabetic patients, the calibration of the system represents a crucial step. It consists first in the determination of the parameters describing the relationship between the sensor output and the concomitant blood glucose concentration, and secondly in the subsequent use of these parameters to transform in real time the sensor output into an estimation of blood glucose concentration. These parameters can be determined either on the basis of a single blood glucose measurement (one-point calibration), or of two measurements (two-point calibration), or even on the basis of the a posteriori analysis of all the determinations performed during a calendar day, this last method being however not compatible with blood glucose monitoring achieved in real time (Velho et al., 1988; Csoregi et al., 1994; Mastrototaro, 1999; Aussedat et al., 2000).

In the *one-point calibration* procedure, the sensor sensitivity  $S$  is determined from a single blood glucose determination as the ratio between the concomitant sensor current  $I$  and the blood glucose concentration  $G$ . Subsequently, the glucose concentration can be estimated at any time from the current  $I$  as  $G(t) = I(t)/S$ . The *two-point calibration* consists first in the determination of two parameters: the sensor sensitivity  $S$ , expressed in nA/(mg/dl), and the intercept  $I_0$ , with the sensor output (current, expressed in nA) axis, i.e. the theoretical sensor output, which would be observed in the absence of glucose. These parameters  $S$  and  $I_0$  are subsequently used to transform at any time the sensor output  $I(t)$  into an estimation of glucose concentration  $G(t)$ , as  $G(t) = (I(t) - I_0)/S$ . A method for determining  $S$  and  $I_0$  takes into account, during a change in blood glucose concentration  $G$  from  $G1$  to  $G2$ , the change in the sensor current  $I$  from  $I1$  to  $I2$ :  $S = (I2 - I1)/(G2 - G1)$ , and  $I_0 = I1 - (S \times G1)$ .

The fact that the calibration procedure will be ultimately performed by the patient, and must be as user-friendly and efficient as possible, represents a strong case for a one-point calibration procedure. However the use of the one-point calibration procedure assumes that the in vivo background current, referred to below as  $I_0$ , is negligible (it might be possible to use a one-point calibration procedure if  $I_0$  were constant, but this would need to use a two-point calibration procedure to determine its value).

In the companion paper, we showed that a measurement error on the current, linked for instance to electric noise, and/or on capillary blood glucose measurement, related to the well known  $\pm 10\%$  error of the commercial glucose meters (Poirier et al., 1998), contributed to the generation of a value of  $I_0$  which could be either positive or negative. The two-point calibration procedure, in addition to the fact that it is time consuming, has indeed the disadvantage of using two estimations of glucose

concentration and sensor output, each adding to the measurement errors. This unexpected finding prompted us to test the hypothesis that  $I_0$  can be considered negligible, i.e. that it is possible to use a one-point calibration procedure.

## 2. Materials and methods

### 2.1. The continuous glucose monitoring system

The miniaturized enzymatic sensor and the electronic control unit used in this study, as well as the design of the evaluation performed in nine type 1 diabetic volunteers, are described in detail in the companion paper.

### 2.2. Sensor calibration

The one-point calibration procedure was performed a posteriori by taking into account the capillary blood glucose concentration determined immediately before meals, whatever their level, and the concomitant sensor output, as described above. This procedure was performed either once per day (before breakfast, the value of glycemia and sensor output being those used in the two-point calibration procedure), twice per day (before breakfast and dinner), or three times per day (before breakfast, lunch and dinner).

The two-point calibration procedure was performed in real time the morning following sensor implantation, as previously described (Aussedat et al., 1997): the ECU was programmed to trigger an alarm when the sensor output was estimated to be stable (less than 1% variation) over eight consecutive measurements. Capillary blood glucose concentration was determined and the patient made his (her) usual insulin injection. If blood glucose was lower than 150 mg/dl, he (she) had breakfast immediately. If blood glucose was higher than 150 mg/dl, he (she) was asked to wait until the capillary glucose had fallen to a stable lower level. A second capillary blood glucose measurement was performed when the ECU recognized that the sensor output had reached a second plateau. The two sets of values of capillary blood glucose concentration and concomitant sensor output were used to calibrate the sensor (two-point calibration procedure) as described above. For the other days of the trial, the procedure was repeated a posteriori by taking into account the capillary blood glucose concentration observed immediately before breakfast, and that observed during the next plateau of sensor output following breakfast, and the sensor outputs corresponding to these two blood glucose determinations.

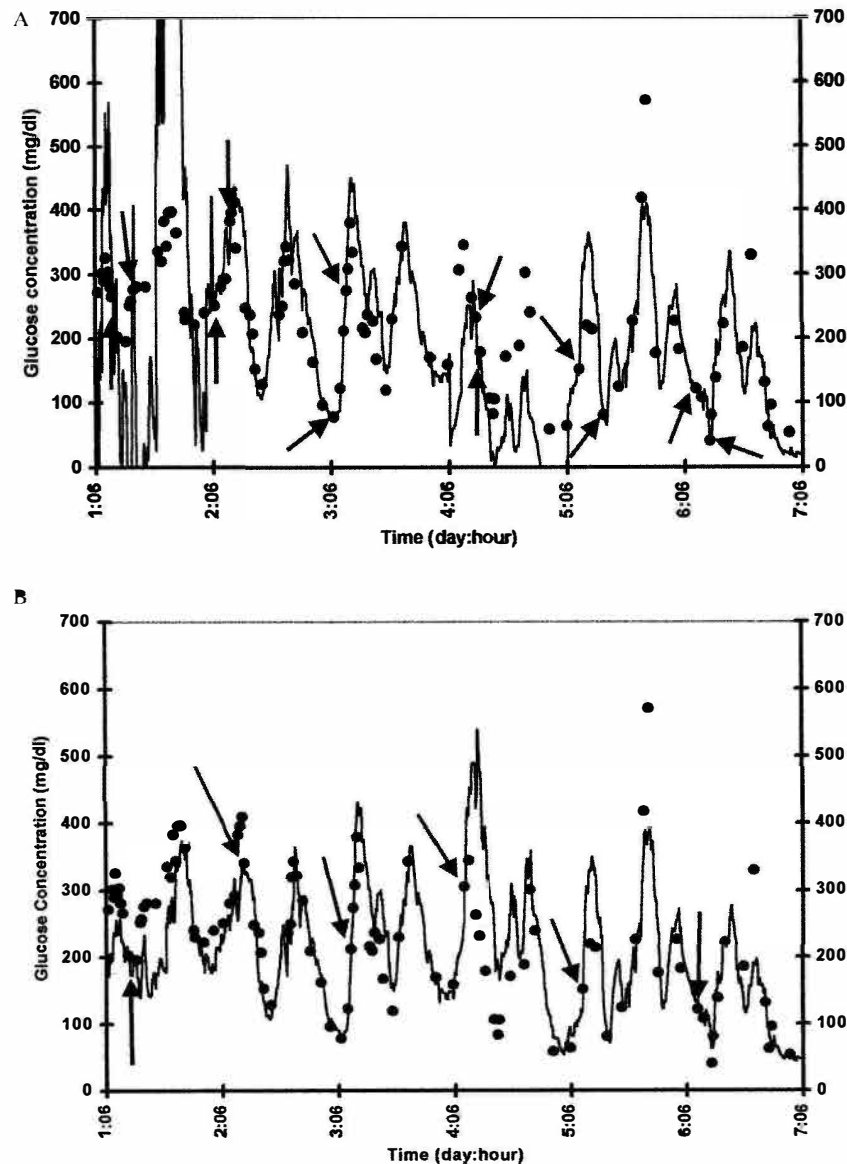


Fig. 1. (A) Continuous monitoring of glucose concentration by a subcutaneous glucose sensor (continuous line) and capillary blood glucose concentration (closed circles) during 7 days. The sensor was calibrated once per day during breakfast using the two-point calibration procedure (arrows). (B) Same data analyzed through the one-point calibration procedure taking into account the value of capillary blood glucose concentration and sensor output determined before breakfast (arrows).

### 2.3. Data analysis

We developed software (WinEcuDraw), working in the Windows environment, which made it possible to represent graphically the sensor output, the Glucotrend data, to perform interactively the different calibrations, and to plot on the same screen the Glucotrend data and the continuous glucose monitoring as estimated by the system from each different calibration condition. The software integrates the Clarke Error Grid Analysis (Cox

et al., 1997), providing the percentages of data present in the different zones of the grid when the estimation of glucose concentration by the system is plotted versus the reference (here the Glucotrend) method. This analysis represents a method to assess not only the accuracy, but also the clinical safety of a glucose monitoring system. All data in text and figures are presented as mean  $\pm$  S.E.M., and the statistical significance of the data was determined by ANOVA analysis and subsequent two-tailed student unpaired *t*-test.

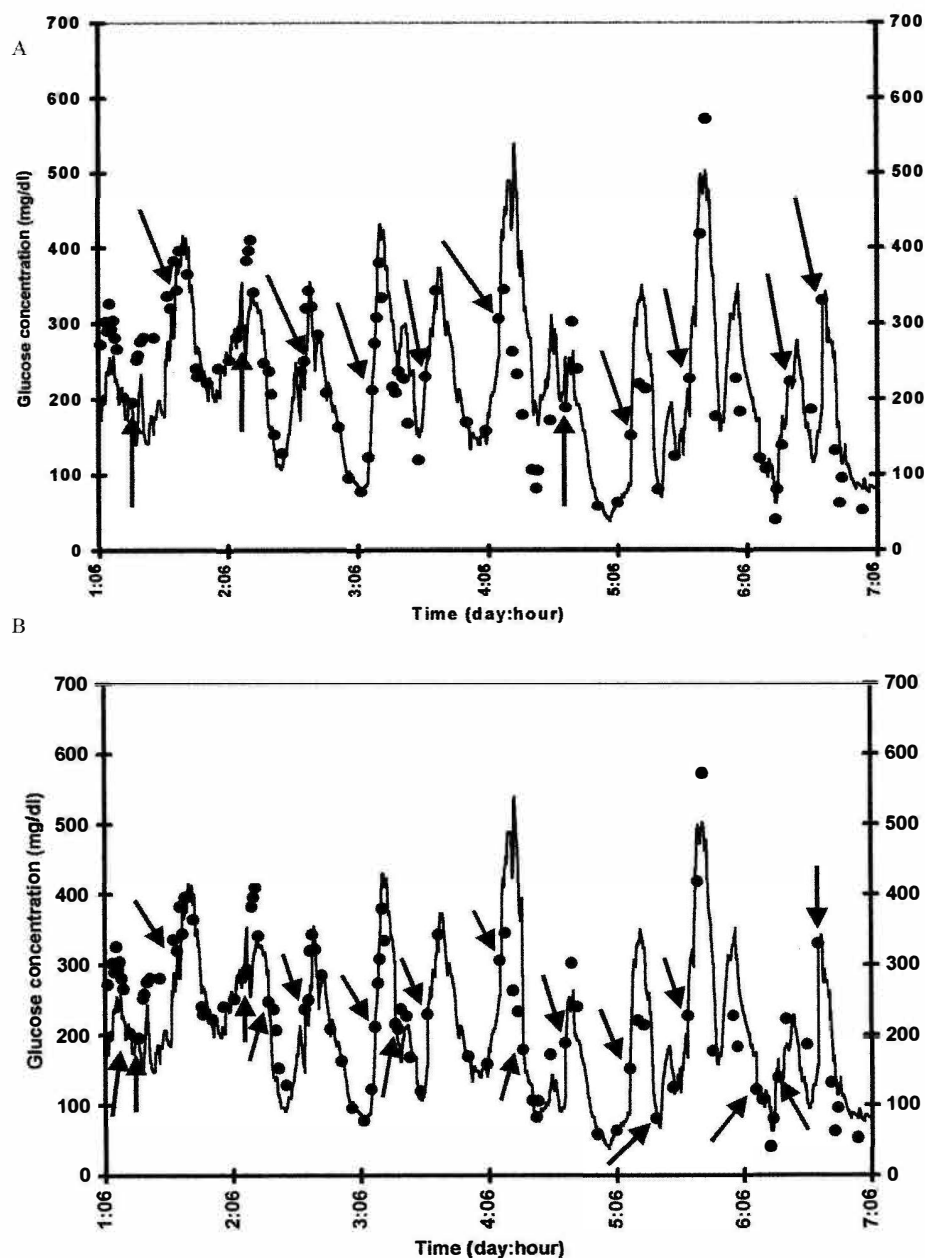


Fig. 2. (A) Same data analyzed through the one-point calibration procedure performed twice per day, before breakfast and dinner (arrows). (B) Same data analyzed through the one-point calibration procedure performed three times per day, before breakfast, lunch and dinner (arrows).

### 3. Results

#### 3.1. Comparison between two-point and one-point calibration performed once per day, and between one-point calibration performed once, two times or three times per day before meals

Fig. 1 compares, for one of the trials, the data of the two-point (A), and of the one-point (B), calibration procedure performed once per day during breakfast, and Fig. 2 represents the data obtained when the one-point calibration was performed twice per day, before break-

fast and dinner (A), or three times per day, before breakfast, lunch and dinner (B). As shown in these figures, the one-point calibration yielded more accurate data and the results were improved by performing two one-point calibrations per day, with no major improvement by the addition of a third one-point calibration performed before lunch. The two-point calibration procedure (Fig. 1A) yielded erratic results with zero and sub-baseline responses at times and off the scale spikes at other times. This was not observed by using the one-point calibration procedure (Fig. 1B; Fig. 2A,B). The Error Grid Analysis for this trial indicated that the

Table 1

Percentage of points in Zones A and B for each individual trial, determined using the two-point calibration procedure (TP) or the one-point calibration procedure performed once (OP 1 ×), twice (OP 2 ×) or three times per day (OP 3 ×)

| Trial no. | TP   | OP 1 × | OP 2 × | OP 3 × |
|-----------|------|--------|--------|--------|
| 1         | 60.3 | 87.7   | 93.6   | 93.6   |
| 2         | 79.6 | 88.3   | 86.2   | 88.6   |
| 3         | 57.8 | 93.8   | 93.8   | 96.3   |
| 4         | 79.7 | 78.7   | 83.7   | 86.8   |
| 5         | 74.5 | 79.8   | 83.7   | 84.1   |
| 6         | 74.6 | 82.8   | 89.5   | 92.9   |
| 7         | 81.9 | 83.3   | 85.5   | 85.8   |
| 8         | 85.0 | 89.6   | 89.3   | 91.4   |
| 9         | 83.3 | 78.9   | 87.2   | 85.2   |

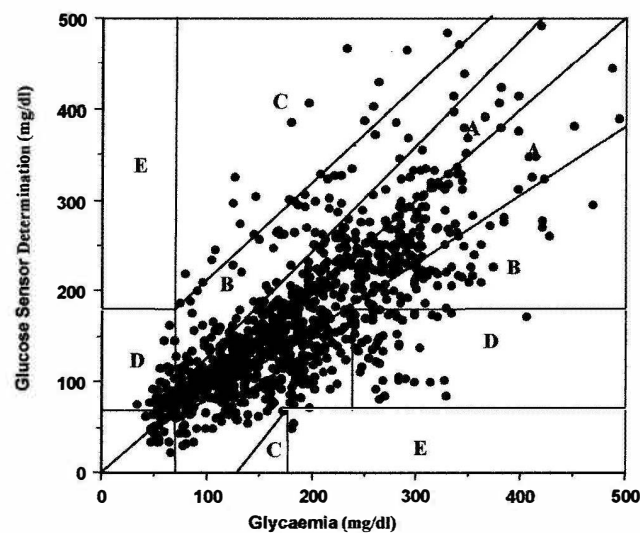


Fig. 3. Error grid analysis plotting the results of the continuous monitoring system (one-point calibration procedure performed twice per day) versus the concomitant estimation of capillary blood glucose concentration determined with a Glucotrend meter.

percentages of points in zones A, B, C, D and E of the grid were 41, 39, 11, 5, 4%, respectively (two-point calibration); 53, 35, 5, 7, 0% (one-point calibration once a day); 58, 29, 8, 6, 0% (one-point calibration twice a day); and 67, 22, 5, 6, 0% (one-point calibration three times a day). Table 1 represents the percentages of points present in zones A and B for each individual trial, and Fig. 4 represents the percentages of points present in zones A and B on different days. The erratic results yielded by the two-point calibration procedure were especially observed during day 1, which may suggest a lower precision in the determination of sensor output shortly after sensor implantation.

The superiority of the one-point calibration procedure is supported by the Error Grid Analysis performed on the data of the nine trials. Fig. 3 represents the analysis of all the values observed during these trials (7 days,  $n = 7$ , and 3 days,  $n = 2$ ,  $n = 986$  values) of the one-point

Table 2

Percentage of points in the different zones of the Error Grid, for the two-point calibration performed once a day (TP), the one-point calibration procedure performed once (OP 1 ×), twice (OP 2 ×) or three times a day (OP 3 ×)

|       | TP         | OP 1 ×                  | OP 2 ×                    | OP 3 ×                      |
|-------|------------|-------------------------|---------------------------|-----------------------------|
| A     | 43.4 ± 2.8 | 42.4 ± 4.2              | 49.4 ± 5.3                | 48.9 ± 5.0                  |
| B     | 31.8 ± 2.4 | 42.4 ± 3.3              | 38.6 ± 4.9                | 40.5 ± 4.5                  |
| A + B | 75.2 ± 3.3 | 84.8 ± 1.8 <sup>a</sup> | 88.1 ± 1.3 <sup>b,d</sup> | 89.4 ± 1.4 <sup>c,e,f</sup> |
| C     | 18.0 ± 3.7 | 4.3 ± 1.3               | 3.6 ± 1.4                 | 3.3 ± 1.3                   |
| D     | 4.9 ± 0.7  | 9.2 ± 1.2               | 6.9 ± 1.4                 | 6.1 ± 1.3                   |
| E     | 1.9 ± 0.6  | 1.7 ± 1.1               | 1.4 ± 1.0                 | 1.2 ± 1.1                   |

<sup>a</sup>  $P = 0.06$  vs. TP.

<sup>b</sup>  $P = 0.017$  vs. TP.

<sup>c</sup>  $P = 0.012$  vs. TP.

<sup>d</sup>  $P = 0.023$  vs. OP 1 ×.

<sup>e</sup>  $P = 0.002$  vs. OP 1 ×.

<sup>f</sup>  $P = 0.05$  vs. OP 2 ×.

calibration performed twice per day, and Table 2 compares the percentages of values observed in the different zones of the grid for the different calibration conditions ( $n = 9$  trials, mean ± S.E.M.). This a posteriori analysis indicates that the percentages of points present in zones A and B of the error grid were significantly higher when the system was calibrated using the one-point calibration procedure. This is also shown on Fig. 4, representing the percentages of values in zones A and B, for each day following sensor implantation, demonstrating the advantage of calibrating the system twice a day, with no major effect of a third calibration before lunch. Table 2 indicates that the percentages of values present in zones D and E were higher (although not significantly) for the one-point calibration performed once a day ( $P = 0.077$  vs. two-point calibration procedure). This was no longer observed when the one-point calibration procedure was performed at least twice a day (NS vs. two-point calibration procedure:  $P = 0.039$  and  $0.80$ , comparing the two-point calibration to the one-point calibration performed twice or three times a day, respectively).

Recalibrating the sensor at least once per day was thus compatible with continuing estimation of glucose concentration over this period of time, in spite of the changes in the sensor sensitivity observed during the implantation period, shown for each individual trial in Table 3.

#### 4. Discussion

In this study, we compared the performances of two methods for calibrating a continuous glucose monitoring system. A glucose sensor was implanted for 7 days in seven, and for 3 days in two diabetic patients, in the abdominal subcutaneous tissue. Table 3 demonstrates

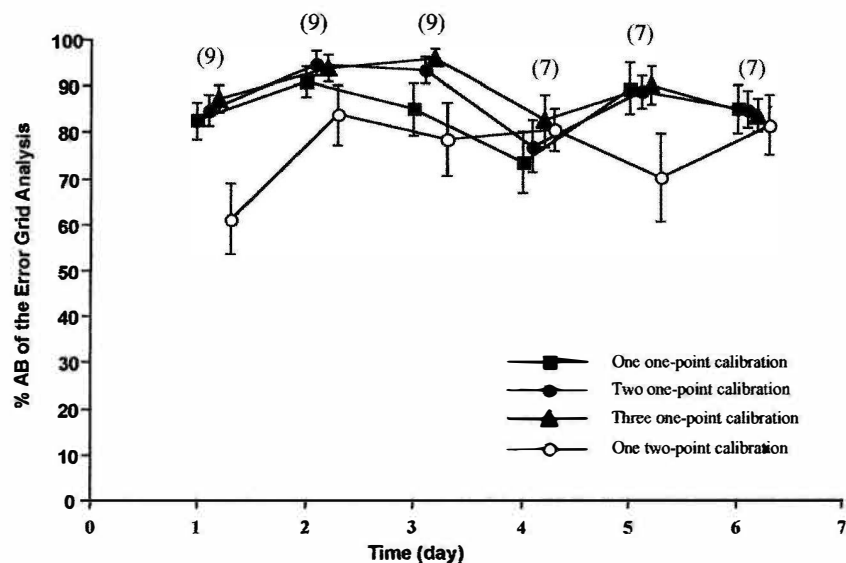


Fig. 4. Comparison of the percentages of points present in zones A and B of the error grid during the 7 days of the trial ( $n = 9$ , mean  $\pm$  S.E.M.). Subcutaneous glucose concentration was determined using the two-point calibration procedure once per day (open circles) or the one-point calibration performed once (squares), twice (closed circles) or three times (triangles) per day, and compared to the concomitant estimation of capillary blood glucose concentration.

that the sensor sensitivity was far from constant, which makes recalibration of the system mandatory. Incidentally, the sensor sensitivity was found either to decrease or increase over the time course of the study, suggesting different mechanisms (fouling or damage of the outer membrane). The sensor was therefore calibrated every day by using either a simple one-point calibration procedure assuming that the sensor output is directly proportional to blood glucose concentration ( $I_0$  being negligible), or by using a two-point calibration procedure. Not only was the one-point calibration procedure compatible with an accurate estimation of blood glucose concentration but in addition, it yielded significantly better results than the two-point calibration procedure (Tables 1 and 2). The results were slightly improved by performing the one-point calibration twice per day, with no major advantage of adding a third calibration before lunch (Tables 1 and 2 and Fig. 2).

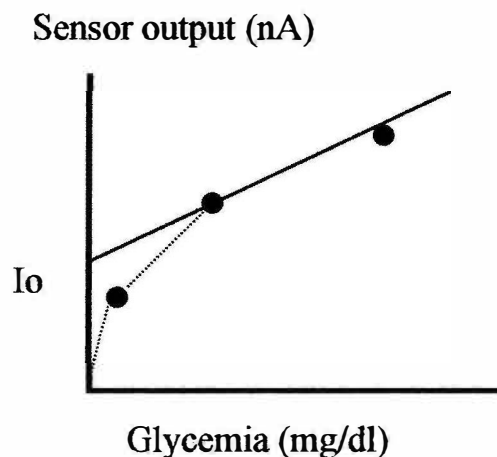


Fig. 5. If the sensor response to an increase in glycemia is not linear, plotting the sensor output versus glycemia will result in a non negligible  $I_0$ . See text for the discussion of the lack of linearity of the sensor response to glucose.

Table 3

Sensor sensitivity (nA/100 mg per dl) determined as the ratio of the sensor output and the capillary blood glucose concentration determined before breakfast

| Day | Patient no. |       |      |      |      |      |      |      |       |
|-----|-------------|-------|------|------|------|------|------|------|-------|
|     | 1           | 2     | 3    | 4    | 5    | 6    | 7    | 8    | 9     |
| 1   | 11.26       | 10.32 | 3.84 | 3.19 | 4.26 | 1.23 | 5.48 | 4.72 | 4.9   |
| 2   | 27.61       | 11.32 | 5.14 | 1.56 | 3.62 | 2.21 | 7.52 | 2.93 | 8.2   |
| 3   | 28.98       | 10.93 | 7.51 | 2.39 | 8.22 | 4.48 | 6.41 | 3.43 | 11.74 |
| 4   | 21.38       | 8.71  |      | 0.87 | 9.79 | 3.24 | 5.43 | 5.83 |       |
| 5   | 15.46       | 7.12  |      | NA   | 5.86 | 3.35 | 9.35 | 6.69 |       |
| 6   | 11.69       | 7.90  |      | 0.26 | 6.44 | 9.31 | 9.54 | 4.78 |       |

The sensor was implanted on day 0 at 6 p.m. The duration of the trial was 7 days, except for patients 3 and 9.

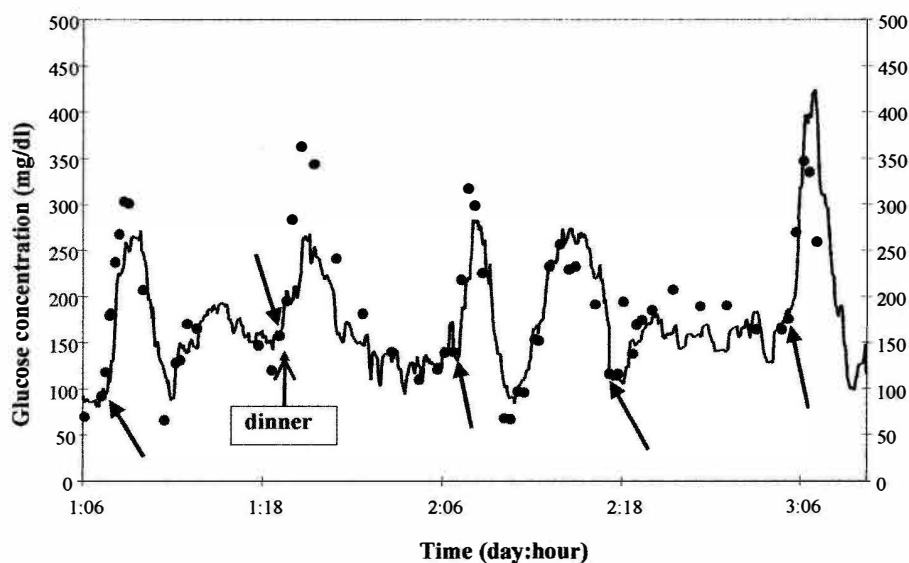


Fig. 6. Continuous monitoring of glucose concentration by a subcutaneous glucose sensor (continuous line) and capillary blood glucose concentration (closed circles) during 3 days. The sensor was calibrated twice per day before breakfast and dinner using the one-point calibration procedure (arrows). Note that especially during dinner of day 1, the increase in subcutaneous glucose concentration, determined by the glucose sensor is blunted by comparison to the increase in capillary blood glucose concentration.

The better results obtained with the one-point calibration procedure (Table 1) are compatible with the fact that with the two-point calibration procedure, the accuracy of the estimation of  $S$  and  $I_0$ , and subsequently of the estimation of glucose concentration, can be compromised by measurement errors. In the companion paper, we demonstrated that they can indeed produce artifact non-zero values of  $I_0$ , either positive or negative.

There is however another reason which can explain that a specific sensor can apparently produce a non negligible value of  $I_0$ : a non linear response of the current to a change in blood glucose concentration (Fig. 5). This lack of linearity can be observed under two circumstances: (1) the intrinsic sensor response (the increment in the sensor output per unit increase in glucose concentration) is not linear; (2) the increase in interstitial glucose concentration is blunted, due for instance to the effect of insulin on glucose uptake from interstitial fluid to the surrounding cells (Schaupp et al., 1999; Aussedat et al., 2000). Given the fact that the sensor monitors interstitial, and not blood, glucose concentration, and that on Fig. 5, the current is plotted against blood, and not interstitial, glucose concentration, this apparent absence of linearity between the increase in the sensor output and blood glucose concentration is due, here, not to the sensor per se but to the physiology of glucose transfer kinetics.

Fig. 6 represents a typical example of such a phenomenon: following the dinner of day 1, the increase in glucose concentration monitored in the subcutaneous tissue was less marked than that of the capillary blood glucose measurements. This phenomenon, which was observed on several other occasions (not shown), may

be due to the effect of insulin pulling the glucose from the interstitial fluid into the surrounding cells, similarly to what we have observed consistently in a study performed in non-diabetic rats (Aussedat et al., 2000). Furthermore, as shown in Fig. 3, the results of the one-point calibration procedure performed twice per day demonstrate that a significant number of glucose estimations were present in the lower zones B and D of the Error Grid. Some of these underestimations of glucose concentration during hyperglycemia may be explained by this phenomenon. Interestingly, similar data are observed when measuring glucose concentration by another subcutaneous glucose sensor (MiniMed, Gross and Mastrototaro, 2000) or by iontophoresis (GlucoWatch, Garg et al., 1999; Tierney et al., 2000).

This last finding also has important implications for the design of the one-point calibration procedure: obviously, this procedure can yield accurate data only if it is performed when the discrepancy between blood and interstitial glucose concentrations is absent or very small. Indeed, the sensor can only be calibrated against blood glucose concentration, while it monitors interstitial glucose concentration. From our experience of glucose monitoring in rats (Thomé-Duret et al., 1996; Aussedat et al., 1997, 2000), these discrepancies are observed both following an increase in blood glucose concentration, as shown above, or during the recovery from hypoglycemia, the glucose concentration in interstitial tissue remaining low, an observation also made by others in human diabetic patients using a microdialysis system (Moberg et al., 1997). In this study, the one-point calibration of the system was performed before meals, a condition supposed to minimize such situations, but it is

possible that this phenomenon was responsible for some of the observed differences between subcutaneous glucose concentration monitored by the system and the concomitant capillary blood glucose level. Determining the optimal timing of the one-point calibration certainly remains a major challenge in this field, which will require the analysis of large data sets. These considerations are relevant for any system monitoring glucose concentration not directly in blood, including indwelled subcutaneous glucose sensors, microdialysis or iontophoresis-based systems, and non invasive technologies.

Finally, calibrating the sensor only twice per day by using the simple one-point calibration procedure was found to yield accurate continuous glucose monitoring over 7 days, in spite of changes in the sensor sensitivity. Whether this strategy will be considered acceptable by diabetic patients remains to be demonstrated, taking into account the fact that two or three blood glucose measurements performed before meals may provide them with accurate continuous glucose monitoring, especially during the night, and therefore pave the way to a hypoglycemic alarm system. Nevertheless, it will remain important to keep in mind that discrepancies between blood and subcutaneous glucose concentrations may occur physiologically and that it may be safe to check capillary blood glucose concentration before taking potentially dangerous decisions. It is also obvious that the definition and the validation of algorithms using the information, provided in real time by continuous subcutaneous glucose monitoring, to optimize insulin therapy, will represent a novel challenge in diabetes care.

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