Noninvasive Glucose Monitoring
Comprehensive Clinical Results

Janet A. Tamada, PhD
Satish Garg, MD
Lois Jovanovic, MD
Kenneth R. Pitzer, DVM, MS
Steve Fermi, MS
Russell O. Potts, PhD
and the Cygnus Research Team

Context Intensive diabetes management using frequent blood glucose measurements to guide therapy has been shown to significantly improve short- and long-term outcomes. Development of a device that makes possible frequent, automatic, painless, and accurate measurements of glucose would facilitate intensive management.

Objective To determine the accuracy of the GlucoWatch automatic glucose biographer (Cygnus Inc) compared with that of serial blood glucose measurements.

Design Multicenter comparative study of the GlucoWatch biographer and the HemoCue blood glucose analyzer (Aktiebolaget Leo) performed between August 29 and October 17, 1998. Participants wore up to 2 biographers during the 15-hour study session and performed 2 fingersticks per hour for comparative blood glucose measurements. The biographers were calibrated with a single HemoCue measurement after a 3-hour warm-up period. Diet and insulin were manipulated to produce a broad glycemic range during the study.

Setting Controlled clinical environment at 2 diabetes centers and 3 contract research organizations in the United States.

Participants A total of 92 subjects (mean [SD] age, 42.1 [15.1] years; 59.8% women) with type 1 or 2 diabetes requiring treatment with insulin.

Main Outcome Measures Mean error, mean absolute error, correlation, slope, and intercept using Deming regression, and clinical significance of differences between biographer readings and blood glucose measurements using the Clarke error grid.

Results Results showed close tracking of blood glucose over a range of 2.2 to 22.2 mmol/L (40-400 mg/dL) for up to 12 hours using a single point calibration. The biographer readings lagged behind serial blood glucose values by a mean of 18 minutes. An analysis of 2167 data pairs shows a linear relationship (r = 0.88; slope = 1.03; intercept = −0.33 mmol/L [−6 mg/dL]) between biographer readings and serial glucose measurements. The mean absolute error between the 2 measurements was 15.6% (mean error [SD], −0.07 [1.82] mmol/L [−1 (33) mg/dL]), and 96.8% of the data fell in the therapeutically relevant regions of the error grid analysis.

Conclusion These results demonstrate close agreement between GlucoWatch biographer readings and blood glucose measurements using repeated fingerstick blood samples. The automatic, frequent, and noninvasive measurements obtained with the biographer provides more information about glucose levels than the current standard of care.

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SELF-MONITORING OF BLOOD GLUcose (BG) is a critical part of managing diabetes. However, present procedures for obtaining such information are invasive, painful, and provide only periodic measurements. Development of a painless and automatic approach would represent a significant improvement in the quality of life for people with diabetes. In addition, results from the Diabetes Control and Complication Trial Research Group,[1] UK Prospective Diabetes Study,[2] and Kumamoto trials[3] showed that a tight glucose control regimen, which uses frequent glucose measurements to guide the administration of insulin or oral hypoglycemic agents, leads to a substantial decrease in the long-term complications of diabetes; however, there was a 3-fold increase in hypoglycemic events.[1] Moreover, as many as 7 BG measurements per day were not sufficient to detect a number of severe hypoglycemic and hyperglycemic events.[4]

The GlucoWatch automatic glucose biographer (Cygnus Inc, Redwood City, Calif) provides a means to obtain painless, automatic, and noninvasive glucose measurements. The device provides up to 3 readings per hour for as long as 12 hours after a single BG measurement for calibration.

A monitoring system that provides automatic and frequent measurements could provide detailed information on glucose patterns and trends that might identify opportunities for improved BG control. Automatic readings also provide the opportunity for an alarm to be sounded in response to values below a user-selected alert level or

Author Affiliations: Cygnus Inc, Redwood City, Calif (Drs Tamada, Pitzer, and Potts and Mr Fermi); Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver (Dr Garg); and Sansum Medical Research Institute, Santa Barbara, Calif (Dr Jovanovic). The members of the Cygnus Research Team are listed at the end of this article.
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Corresponding Author and Reprints: Russell O. Potts, PhD, Cygnus Inc, 400 Penobscot Dr, Redwood City, CA 94063 (e-mail: russ_potts@cygn.com).

Figure 1. GlucoWatch Biographer on a Subject's Arm

as a result of rapid declines in the measured glucose values. These alarms could provide a method to reduce the risk of hypoglycemia and make intensive therapy for diabetes safer and acceptable to more patients.

METHODS

Noninvasive Glucose Extraction and Detection

The noninvasive method described here extracts glucose through the skin using an applied potential (a process known as iontophoresis) and measures the extracted sample using an electrochemical-enzymatic sensor. Iontophoresis is a technique whereby a constant, low-level electrical current (0.3 mA/cm^2 in these studies) is conducted through the skin between an anode and cathode.[5] Because of the applied potential, sodium and chloride ions from beneath the skin migrate toward the cathode and anode, respectively. Uncharged molecules, including glucose, are also carried along with the ions by convective transport (electro-osmosis). This electro-osmotic flow causes glucose to be transported across the skin.[6] The skin has a negative charge at neutral pH and so there is greater net transport to the cathode. As a result, glucose is preferentially extracted at the cathode.[6,7]

The feasibility of iontophoretic glucose extraction has been demonstrated both in vitro[6] and in human subjects.[7,8] In feasibility studies with human subjects, glucose transport correlated well with BG in a linear manner; however, the sensitivity (ie, the amount of glucose extracted compared with the BG) varied among individuals and skin sites. A single point calibration was found to compensate for this variability. The results of a feasibility study using 5 subjects with diabetes in 12 uses showed a mean correlation coefficient of 0.89 and a mean absolute error (MAE) of 13% for the comparison of extracted glucose to BG values obtained using a self-monitoring BG device.

In the biographer, the concentration of extracted glucose is measured by a biosensor. An amperometric, electrochemical-sensing chemistry was chosen as the most suitable for this application. The biological selectivity element in this biosensor is the enzyme glucose oxidase (GOx), which catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2). The reaction can be summarized as follows: glucose + O_2 → gluconic acid + H_2O_2.

The H_2O_2 is detected via an electrocatalytic oxidation reaction at a platinum-containing working electrode in the sensor, where an electric current is produced, and oxygen regenerated as follows: $H_2O_2 \rightarrow O_2 + 2H^+ + 2$ electrons.

Thus, for every glucose molecule extracted, 2 electrons are transferred to the measurement circuit, if we assume that all of the peroxide generated can be electrochemically oxidized. The magnitude of the resulting electric current is correlated with the amount of glucose collected through the skin. The operating principles of the sensor are described in detail elsewhere.[9]

The biographer is shown in FIGURE 1. Extraction and detection are achieved by means of 2 hydrogel pads placed beneath the device and against the skin. Each hydrogel pad is composed of an aqueous salt solution in a cross-linked polymer containing glucose oxidase. The side of each pad away from the skin is in contact with a separate electrode assembly, which contains 2 sets of iontophoretic and sensing elements. Two such electrode sets are required to complete the iontophoretic circuit. During operation, 1 iontophoretic electrode is cathodic (negatively charged) and the other anodic, enabling the passage of current through the skin. The iontophoretic time interval is adjusted to minimize skin irritation and power requirements and yet extract sufficient glucose for subsequent detection. The optimal time for extraction of glucose is about 3 minutes.

A sensing electrode is located at both the iontophoretic anode and cathode. Thus, there are 2 sensing electrodes, sensors A and B. These sensing electrodes are composed of a platinum composite and are activated by applying a potential of 0.3 to 0.8 V (relative to a silver–silver chloride reference electrode).

Note that current is generated at both electrodes, even though glucose is primarily collected at the cathode. The anode signal is due, in large part, to ascorbic and uric acids. Ascorbate and urate are known to react directly with a platinum electrode and produce a signal that interferes with many self-monitoring BG devices. During iontophoresis these anions collect only at the anode, while glucose is found primarily at the cathode. Furthermore, because of the size exclusion properties of the skin,[5] proteins (eg, hemoglobin) that may interfere with self-monitoring BG devices are not present. Hence, the unique ion and size-selective nature of iontophoretic extraction substantially reduces interference in the measurement of glucose.

The current (milliamperes) used in iontophoresis potentially interferes with detection of the low current (nanoamperes) generated at each sensor. Consequently, the iontophoretic and sensing electrodes are not activated at the same time. Instead, iontophoresis proceeds for 3 minutes to collect an adequate amount of glucose. Iontophoresis is then stopped, and the sensing electrodes are activated for 7 minutes. This period was chosen so that all of the glucose would be converted to H_2O_2 and so that all of this peroxide would diffuse to the platinum electrode and subsequently oxidize to generate a current. Thus, all extracted glucose and H_2O_2 are consumed during this half

cycle. The iontophoresis polarity is reversed and the half cycle is then repeated.[10] Thus, if sensor A is the cathode during the first half cycle, sensor B is the cathode during the second half cycle. The combined full cycle requires 20 minutes, and the combined cathode sensor data from each half-cycle is a measure of the glucose extracted. This 20-minute cycle is repeated throughout operation of the biographer. This procedure creates a "moving average" type of measurement that is conceptually different from the BG values obtained with current self-monitoring BG devices.

Calculation of Glucose Value

The biographer contains sensors to measure skin temperature and conductance. The latter is directly related to the amount of sweat on the skin's surface. Since the glucose in sweat can confound the measurement, if the skin conductance exceeds a predetermined threshold, the measurement for that cycle is skipped. An alarm is sounded for both sweating and for hypoglycemia because the former is often associated with the latter. Similarly, since temperature directly affects the sensor operation, if the temperature or time rate of change of the temperature exceeds predetermined thresholds, the entire cycle is skipped. Finally, because the current vs time characteristics of the sensor signal conform to theoretically predicted and empirically measured values, predetermined thresholds were also established for these measurements. If these thresholds are exceeded, the entire cycle is skipped. Through the use of these predetermined screens, spurious data are skipped, eliminating aberrant glucose measurements.

The current vs time data are then integrated to yield a net charge (Q). The charge from 2 successive cathode half-cycles are added ($Q_A + Q_B$) and used as the signal for subsequent glucose calculation. To calibrate the biographer, a single blood measurement (obtained with a self-monitoring BG device) is used. A calibration factor is then determined.

Calibration Factor

The calibration factor ($BG/[Q_A + Q_B + 2000]$) is then used to convert all subsequent charge measurements into glucose values. An offset of 2000 nCoulombs is an empirically derived constant required to obtain the best correlation between the biographer readings and the reference BG values.

To achieve consistent readings, an equilibration period is required after the biographer is applied to the skin and iontophoresis is initiated. Three hours is required to achieve full equilibration among all subjects. Thus, calibration occurs 3 hours after application. Glucose measurements are then obtained for the subsequent 12 hours.

An algorithm containing the screen thresholds and signal processing parameters[11] was programmed into external software. In brief, the algorithm uses a mixture of experts method with input variables of the biosensor signal, the BG value at calibration, the elapsed time since calibration, and a combination of BG value at calibration, signal, and the offset constant described above. The optimized parameters were obtained by minimizing the difference between biographer readings and comparative BG values in a subset of the devices. These fixed parameters were then applied to data from the remaining biographers. In this manner, the algorithm parameters were tested in an "out of sample" population. Upon completion of the clinical study, the data from each biographer were downloaded through a serial interface. The downloaded data were subsequently analyzed on a personal computer using the fixed algorithm and optimized parameters.

Study Design

Biographers were applied to the forearm of subjects, who were individuals diagnosed as having type 1 or 2 diabetes requiring insulin therapy. The trials were performed at 2 diabetes centers (Barbara Davis Center, University of Colorado, Denver; Casa Blanca Medical Center, Mesa, Ariz) and 3 contract research organizations (East Bay Clinical Trial Center, Concord, Calif; Orlando Clinical Research, Orlando, Fla; and National Clinical Research, Richmond, Va, following institutional review board approval. All subjects signed informed consent forms required by the respective institutional review boards. Subjects remained indoors during the study and were not permitted to smoke. Subjects with skin disease at the site of application were excluded. Clinical personnel applied and removed the biographer and performed the calibration.

Up to 3 biographer measurements were obtained per hour. In addition, subjects provided 2 fingerstick capillary blood samples per hour, and the glucose concentration was determined using a BG analyzer (HemoCue; Aktiebolaget Leo, Helsingborg, Sweden). To account for the time difference between measurement technologies, the protocol was set up to compare a BG measurement with the biographer value obtained 15 minutes later. Following calibration with a BG value at 3 hours, measurements were continued for 12 hours, yielding a maximum of 23 paired measurements (excluding the calibration point) of the biographer and BG values. Diet and insulin were manipulated to produce a broad glycemic range.

Skin irritation was evaluated on all subjects immediately after removal of the biographer. The skin sites were scored for erythema and edema using a modified Draize scale.[12]

Statistical Analyses

Paired values from the biographer and the BG analyzer were compared by means of a series of standard metrics. Mean error (ME) is defined as the mean, over all data pairs, of the difference between the biographer value and the comparative BG value obtained with the BG analyzer. The ME was determined for all paired values and for 4 data subsets based on the comparative BG range. The MAE is the mean of the absolute value of the difference between the biographer and the comparative BG value divided by the comparative value. Linear regression values (slope and intercept) were obtained using a Deming re-

gression method that takes into account variance in the comparative method. The variance ratio between the 2 techniques (biographer/BG analyzer) was estimated to be approximately 2. The correlation coefficient was obtained by a least squares regression method.

The same metrics were used to assess agreement between 2 biographers worn simultaneously by a subset of subjects. In this case, the variance ratio used for the Deming regression was 1. The coefficient of variation of the difference between the 2 biographers was also determined for various ranges of glucose.

The Clarke error grid approach[13] was used to assess the clinical significance of differences between the biographer readings and the BG measurements. The error grid is a commonly used method to compare 2 devices for measuring BG. This analysis divides a correlation plot of the experimental method results vs the comparative method results into regions of error based on therapeutic utility. Region A includes all paired values with the comparative measurement above 3.9 mmol/L (70 mg/dL); absolute error for the experimental method was less than 20%. Region A also includes all paired values where both results are in the hypoglycemic range (<3.9 mmol/L [<70 mg/dL]). Values in this region are clinically accurate and lead to correct treatment decisions. Region B represents values that deviate from the reference by more than 20% but would lead to benign or no treatment. Values that fall in regions C, D, or E lead to progressively more dangerous potential outcomes. In summary, values in regions A and B are clinically acceptable, whereas values in regions C, D, and E represent clinically significant errors.

To measure the accuracy of trend information, the change in BG ($\Delta BG/\Delta t$) over the 20-minute period of each measurement was compared with the corresponding change in the biographer ($\Delta Bio/\Delta t$) value.

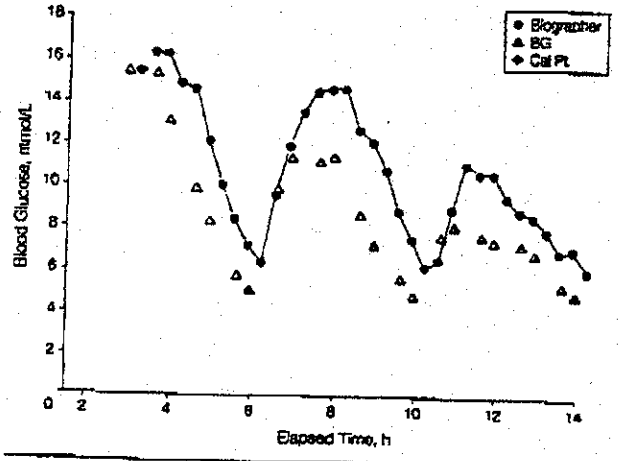
RESULTS

The study consisted of 92 subjects wearing a total of 155 biographers (63 subjects wore 2 devices). All subjects were aged 18 years or older (mean [SD], 42.1 [15.1] years). The population consisted of both men (40.2%) and women (59.8%) from a broad ethnic cross section (white, African American, and Hispanic), with mean (SD) body mass index of 27.8 (5.4) kg/m^2. Of the 155 biographers, 46 were randomly selected to optimize the algorithm parameters used to convert the electrochemical signal into a glucose measurement.[11] These fixed parameters were then applied to data from the remaining 109 devices.

Results obtained with 1 device from the group of 109 test biographers are shown in FIGURE 2. These results show close tracking of biographer and BG values throughout the study. The results also show that the biographer reading lags behind the corresponding BG value. Using a cross-correlation (or "frameshift") approach to determine the characteristic lag time for each individual subject resulted in a mean (SD) value of 18 (10) minutes.

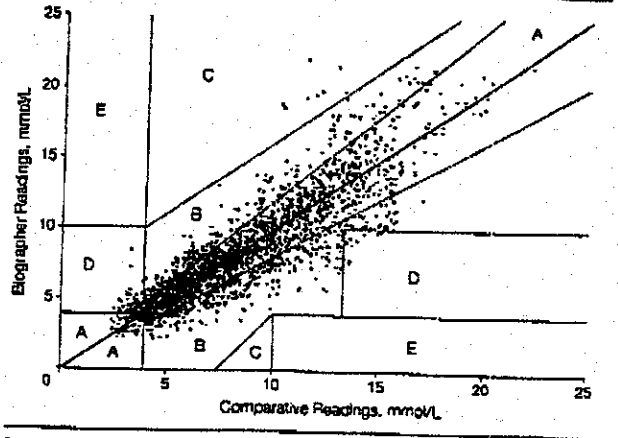
The total of 109 biographers had a maximum of 2507 (109 × 23) possible data pairs (BG and biographer values), not including the calibration points. Of those 2507 data pairs, 2354 were available for analysis. The unavailable pairs (153) were primarily due to biographer shutoff and missing BG values, as well as BG or biographer values outside the range tested (2.2-22.2 mmol/L [40-400 mg/dL]). Of the remaining 2354 data pairs, 7.9% (187) were removed (skipped) by the predetermined threshold screens pro-

Figure 2. Glucose Concentration vs Elapsed Time for 1 Subject as Measured by the Biographer and Reference Blood Glucose (BG) Methods



Cal Pt indicates calibration point. To convert millimoles per liter to milligrams per deciliter, multiply by 18.

Figure 3. Comparison of All Paired Data by Error Grid Analysis



Region A includes all paired values with the comparative measurement above 3.9 mmol/L (70 mg/dL) and absolute error for the experimental method less than 20% and all paired values where both results are in the hypoglycemic range (<3.9 mmol/L [<70 mg/dL]). Region B represents values that deviate from the reference by more than 20% but would lead to benign or no treatment. Values that fall in regions C, D, or E lead to progressively more dangerous potential outcomes.

grammed into the algorithm. On average, therefore, about 1 measurement every 3 hours was removed due to these screens.

The results for the 2167 data pairs remaining after screening are shown in FIGURE 3. These data show a plot of the biographer measurements vs the comparative method. The data yield a correlation coefficient (r) of 0.88, a slope near unity (1.03), and an intercept near 0 (−0.33 mmol/L [−6 mg/dL]). For all 2167 data pairs, an MAE of 15.6% was observed, while the ME and SD were −0.07 mmol/L (−1 mg/dL) and 1.82 mmol/L (33 mg/dL), respectively.

The ME and SD for biographer readings as a function of BG range are shown in the TABLE. These results show less variation (as measured by SD) at low BG than that seen in any other range.

The correlation data are also superimposed on an error grid in Figure 3. The results show that 70% of the biographer data fall in region A and 96.8% fall in the combined A and B regions. By contrast, no data fall in the E region, while 3.2% are found in the C and D regions. Thus, more than 96% of the measurements obtained with the biographer fall in the therapeutically relevant regions of the error grid.

For 894 data pairs available to calculate $\Delta BG/\Delta t$ and $\Delta Bio/\Delta t$, the mean difference (SD) between the corresponding BG and biographer values was 0.01 (0.91) mmol/L (0.2 [16] mg/dL). A regression analysis of the same $\Delta BG/\Delta t$ vs $\Delta Bio/\Delta t$ data showed that the 2 measures were highly correlated ($r = 0.76$; $P>.99$) with an intercept near 0 and slope near unity (data not shown).

Among the 109 biographers in the test set, 31 subjects wore 2 devices at the same time. These paired biographers provide a measure of the precision, or agreement between independent biographer measurements. A linear regression analysis of biographer 1 vs biographer 2 yields a slope of 1.03, an intercept of −0.24 mmol/L (−4 mg/dL), and an r of 0.93. The difference (SD) between the 2 biographers, averaged over all data, was −0.04 (−1.34) mmol/L (−0.7 [24] mg/dL). In addition, the coefficient of variation of the difference ranged from a high of 8.4% at glucose levels below 5.6 mmol/L (100 mg/dL), to 5.2% for all glucose values above 13.3 mmol/L (240 mg/dL).

After removal of the biographers, mild skin irritation was noted at the site of iontophoresis. In all cases, the irritation resolved within 3 to 7 days.

Table. Biographer Mean Error (ME) and SD by Blood Glucose Range*

Blood Glucose Range, mmol/L	Total Points	ME, mmol/L	SD, mmol/L
Full Range	2167	−0.07	1.82
≤3.9	116	0.68	0.97
>3.9 to ≤10	1324	−0.03	1.36
>10 to ≤13.3	469	−0.25	2.42
>13.3	258	−0.35	2.61

*To convert millimoles per liter to milligrams per deciliter, multiply by 18.

COMMENT

One of the greatest potential advantages of the biographer is the ability to determine glucose patterns and trends. The results of this study demonstrate that for all subjects and glucose ranges studied, there is close agreement between both the absolute BG measurements and the direction and rate of change. Hence, the biographer closely tracks changes in glucose. Although this study compared biographer results with as many as 23 fingerstick BG tests in a 12-hour period, the relevant clinical comparison is between the biographer and the periodic tests (typically 1-4 per day) that constitute the current standard of care.

It is important to note that the protocol for this study of biographer accuracy allowed comparative BG measurements to be obtained in a window of ±5 minutes. During this 10-minute period, the BG may change by as much as 1.5 mmol/L (27 mg/dL) (data not shown). This source of variation is independent of the biographer and results in an overestimation of the error. In addition, no statistical comparison of paired results can account for the pattern-recognition benefits of the frequent, automatic information provided by the biographer.

Of particular concern for any glucose measuring device is the accuracy at low glucose values. The biographer results at low BG seen in this study (Table) compare favorably with published results for self-monitoring BG devices. For example, Trajanoski et al[14] evaluated 5 self-monitoring BG devices and reported values for the SD of ME of up to 0.5 mmol/L (9 mg/dL) at BG less than 3.9 mmol/L (70 mg/dL). Similarly, Brunner et al[15] evaluated ME in self-monitoring BG devices and found SDs up to 0.45 mmol/L (8 mg/dL) in the hypoglycemic range and up to 1.5 mmol/L (27 mg/dL) in the hyperglycemic range (>10 mmol/L [>180 mg/dL]).

Error grid values similar to the biographer results for the combined A and B regions have been published for commercially available self-monitoring BG devices.[13,15] In addition, similar frequencies of the C and D region results were observed for self-monitoring BG devices tested in these trials. For example, Brunner et al[15] studied 6 different devices and found region D values ranging up to 8% at glucose levels below 3.9 mmol/L (70 mg/dL).

The method used to measure biographer precision in this study is an underestimate since, of necessity, different biographers at different skin sites were used. Nevertheless, the coefficient of variation results are similar to values that have been reported for self-monitoring BG devices using spilt samples.[16] In addition, the biographer precision results show a mean difference and SD similar to values obtained for replicate measures of blood samples.[16]

Taken together, the results of this study and the published data on performance of self-monitoring BG devices show that the accuracy of the biographer compares well with currently available devices. The close agreement between biographer readings and BG measurements also demonstrates that subcutaneous glucose is an appropriate measure of the glycemic state. A similar conclusion has been derived from studies of subcutaneous glucose made with implanted devices.[9,17-19] In fact, because glucose utilization oc-

curs in peripheral tissue, not the blood, it has been suggested that subcutaneous glucose may be the better measure to guide therapy.[18,20]

The study results demonstrate an important lag between the BG measurements and the biographer readings. In addition, qualitative review of the tracking plots shows that the lag varies depending on whether glucose levels are rising or falling. Similar trends have been observed in continuous measurements of subcutaneous glucose obtained with implanted sensors or microdialysis devices,[4,16-20] and are associated with mass transfer between the blood and peripheral tissue. However, with the biographer the primary component of the lag time is the 20-minute measurement cycle and resulting "moving average" nature of the readings. This effect can be accommodated in clinical use by reviewing a series of results before making a management decision and setting the low glucose alert level somewhat above the level at which treatment of hypoglycemia is needed.

As seen in this study, iontophoresis does cause transient mild skin irritation. It is expected that this can be managed by rotating the wearing site as needed until the irritation is resolved. The number of sites required will depend on individual patient response and the frequency with which the device is used. Both the volar and dorsal surfaces of both forearms can be used to provide multiple sites at which the device may be worn.

The frequent, automatic, and noninvasive measurements obtained with the biographer provide access to previously unavailable information about glucose levels. With this information, both patients with diabetes and health care professionals may be able to make better decisions about all aspects of diabetes management. Detection of impending hypoglycemia and hyperglycemia will allow early treatment of these frequent complications of diabetes, and thus, potentially provide greater confidence for patients to pursue more aggressive control of their disease.

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