Transcutaneous Glucose Measurement Using Near-Infrared Spectroscopy During Hypoglycemia

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OBJECTIVE — To analyze a transcutaneous near-infrared spectroscopy system as a technique for in vivo noninvasive blood glucose monitoring during euglycemia and hypoglycemia.

RESEARCH DESIGN AND METHODS — Ten nondiabetic subjects and two patients with type 1 diabetes were examined in a total of 27 studies. In each study, the subject's plasma glucose was lowered to a hypoglycemic level (~55 mg/dl) followed by recovery to a glycemic level of ~115 mg/dl using an intravenous infusion of insulin and 20% dextrose. Plasma glucose levels were determined at 5-min intervals by standard glucose oxidase method and simultaneously by a near-infrared spectroscopic system. The plasma glucose measured by the standard method was used to create a calibration model that could predict glucose levels from the near-infrared spectral data. The two data sets were correlated during the decline and recovery in plasma glucose, within 10 mg/dl plasma glucose ranges, and were examined using the Clarke Error Grid Analysis.

RESULTS — Two sets of 1,704 plasma glucose determinations were examined. The near-infrared predictions during the fall and recovery in plasma glucose were highly correlated (r = 0.96 and 0.95, respectively). When analyzed during 10 mg/dl plasma glucose segments, the mean absolute difference between the near-infrared spectroscopy method and the chemometric reference ranged from 3.3 to 4.4 mg/dl in the nondiabetic subjects and from 2.6 to 3.8 mg/dl in the patients with type 1 diabetes. Using the Error Grid Analysis, 97.7% of all the near-infrared predictions were assigned to the A-zone.

CONCLUSIONS — Our findings suggest that the near-infrared spectroscopy method can accurately predict plasma glucose levels during euglycemia and hypoglycemia in humans.

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It has been established that maintaining near-euglycemia in patients with type 1 diabetes can prevent and/or delay the development of long-term complications (1). The achievement of plasma glucose levels within a narrow range requires frequent and regular glucose monitoring, especially in patients with insulin-requiring diabetes. Intensive insulin treatment used in such patients, however, is associated with more frequent episodes of hypoglycemia. The Diabetes Control and Complications Trial demonstrated that episodes of biochemical and symptomatic hypoglycemia were increased during intensive therapy, and the risk of severe hypoglycemia was increased threefold (2). In addition, frequent episodes of mild biochemical or symptomatic hypoglycemia are now believed to lead to an increased risk of severe hypoglycemia (3–6).

Frequent and regular self-monitoring of blood glucose may be cumbersome, and several other methods to measure blood glucose using less invasive or noninvasive techniques have been proposed. As a noninvasive method of blood glucose monitoring, near-infrared spectroscopy (NIRS) is clinically appealing not only because it could enable more glucose measurements to be easily performed but also because such a technique might have more widespread use than invasive sensors. The NIRS technique involves illumination of a vascular tissue component with a broad-band energy emitted by a source through a fiberoptic bundle. Energy is scattered, absorbed, and transmitted in all directions within the measurement volume and then collected by the detection fiber optic. The spectra that are detected represent the molecular vibrations of many biological constituents. In order for NIRS to be applicable to glucose sensing, the spectral signature of glucose must be different from that of other molecules, and at the same time the signal-to-noise ratio must be high enough to permit differentiation between signals generated by glucose and those generated by other chemical constituents of the tissue. The NIR spectra obtained by absorption and/or reflection are processed (using multivariate processing algorithms) in order to construct a calibration model capable of relating the spectral information to the concentration of plasma glucose measured by standard means (7,8); that is, the calibration model is used to predict plasma glucose levels. Several reasons prompted the present study. First, we wished to examine the performance of a novel dual-beam NIRS system in vivo. Second, there is a scarcity of data that validate the NIRS as a noninvasive glucose monitoring method in vivo, in particular during clinically relevant fluctuations in plasma glucose. Third, early recognition of blood glucose concentration at or near the hypoglycemic range may prevent severe hypoglycemia (4). Finally, an advantage of NIRS is that signal noise (or measurement error) is largely independent of the concentration of the analyte, and acceptable performance at lower plasma

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Abbreviations: NIRS, near-infrared spectroscopy.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

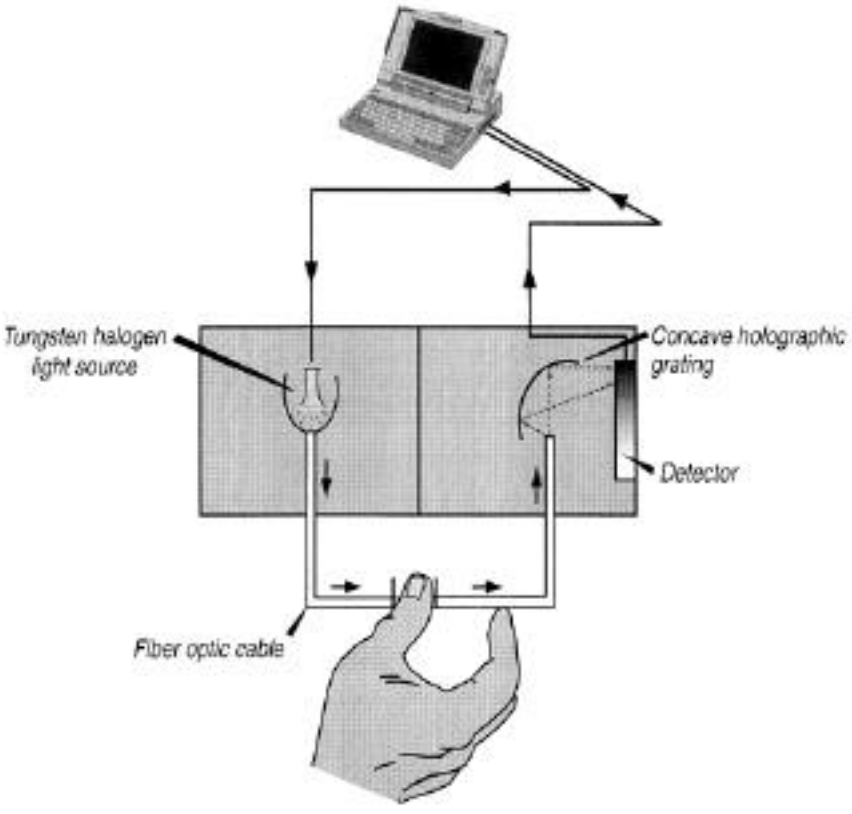

Figure 1—*Schematic representation of the NIRS system.*

glucose concentrations can be extrapolated to the hyperglycemic state as well.

We used NIRS to predict the plasma glucose concentration values during a fall and recovery in plasma glucose induced by insulin infusion. The calibration models that we used allowed reliable NIRS prediction of plasma glucose in each subject, demonstrating feasibility of obtaining meaningful spectral data in euglycemic and hypoglycemic ranges of plasma glucose.

RESEARCH DESIGN AND METHODS

Study subjects

We studied 10 nondiabetic subjects (4 women and 6 men) and 2 women with type 1 diabetes. The average age of all subjects was 30.8 ± 1.2 years (mean ± SEM), and BMI was 24.2 ± 0.4 kg/m^2. The subjects were otherwise in good health and took no medications (other than insulin in those with diabetes). Informed consent was obtained after explanation of the purpose and potential risks of the study in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

Experimental protocol

The patients with type 1 diabetes were admitted to the General Clinical Research Center on the evening before the day of the study. Long-acting insulin was withheld, and the patients received an overnight intravenous infusion of regular human insulin (Humulin Regular, Lilly, Indianapolis, IN) according to an algorithm based on hourly blood glucose measurements. Studies in all subjects were begun at 0800 (t = 0) after an overnight fast. A small polyethylene catheter was inserted into an antecubital vein for infusion of insulin and 20% dextrose (Abbott, North Chicago). A second catheter was inserted in the contralateral forearm in a retrograde fashion for blood sampling. This arm was heated (60°C) in order to obtain arterialized venous blood.

The experimental protocol consisted of an infusion of regular human insulin at a dose of 0.4 mU · kg^{-1} · min^{-1} to induce a ~0.3–0.4 mg · dl^{-1} · min^{-1} fall in plasma glucose to ~55 mg/dl. Intravenous 20% dextrose was given intermittently to maintain the above rate of fall in plasma glucose. After achievement of the hypoglycemic nadir, the insulin infusion was stopped and 20% dextrose was infused to obtain a recovery in plasma glucose at a rate of ~0.4 mg · dl^{-1} · min^{-1} up to a value ~115 mg/dl.

The duration of each study was 325 min. For each study, we obtained two sets of glucose determinations, one by standard chemometric technique (Beckman Glucose Analyzer; Beckman, Fullerton, CA) and one composed of NIRS-predicted values, with a total of 62–64 determinations per study.

Analytical procedures

Plasma glucose concentrations were measured at 5-min intervals, and the rates of fall and recovery in the plasma glucose concentration were adjusted using 20% dextrose infusion. Plasma glucose was measured in duplicate with a glucose analyzer (Beckman) using the glucose oxidase method. The resulting plasma glucose determinations served as reference values for the NIRS calibrations and for the correlation of NIRS predictions versus reference. Simultaneously with blood withdrawal, NIR spectral data were obtained in 40-s segments. The NIRS system was composed of a 42-W tungsten-halogen source, a fiber-optic interface placed on the ventral aspect of the subject's thumb, and a spectrophotometer sensitive to 400–1,700 nm (BioNIR, New York). In these experiments, the sample and reference NIR spectra were recorded separately during each 40-s measurement sequence and stored in a microcomputer controlling the spectrophotometer. This dual-beam system collected both sample and reference spectra during the same measurement interval, thereby providing internal stability and sensitivity of the measurement. Light is launched into the optical fiber using direct illumination from a reflector-type projector lamp. Dispersion is accomplished by a concave holographic grating. Light is collected after striking the thumb, which is in contact with the sensor fiberoptic bundle using an InGaAs parallel channel array (Fig. 1). Because the data can be markedly influenced by variation in skin-sensor interface, the pressure of the probe against the skin is controlled using a mechanical holding receptacle.

Computation of the spectral data was performed on a microcomputer using MATHLAB for MS Windows. To extract the spectral data, we first ratioed the sample and reference data and then applied a linearizing function. Initial analysis was performed using the ratio of the negative logarithm of the sample to the reference measurement, based on the exponential Beer-Lambert relationship. These methods

require a training set of representative data that covers the range of all the variables expected in future measurements. This training set consisted of primary measurements of plasma glucose and the associated spectral data. Based on this training data set, a mathematical relationship was developed between the spectral data and the plasma glucose using partial least squares analysis (9). The above mathematical modeling of spectral information allows rejection of data that does not fit the model. The number of factors ranged between 10 and 27 and was used based upon statistical significance to avoid over-fitting of data. Outliers were identified by spectral characteristics alone (e.g., Mahalnobis distance, spectral residuals levels, and the temporal characteristics of the signal during measurement) and averaged 4.2%.

To predict plasma glucose values during the experimental protocol, we first used the plasma glucose determination by the reference method (Beckman analyzer) and the parallel NIRS readings to construct a calibration model. Applying a NIRS reading to the subjects' calibration model enabled us to predict the respective plasma glucose. By applying the NIRS readings during the whole protocol to the calibration model, we predicted the plasma glucose values for each parallel reference determination. Because the same reference values were used for the calibration model and then for the correlation (reference plasma glucose versus NIRS predictions), we predicted plasma glucose values at specific levels that were masked or not taken into the calibration model calculations.

The correlation of the reference plasma glucose versus NIRS predictions was performed for the overall study as well as separately during the decline in plasma glucose from euglycemia to the hypoglycemic nadir and during the recovery from hypoglycemia back to euglycemia. In addition, in 21 studies we masked 6–10 reference results chosen randomly from each study (masked values). The NIRS predictions were correlated to those values. We also compared the correlation between the masked values and the overall unmasked results.

The correlation of the above data was examined using the Error Grid Analysis (10) to quantify and describe the clinically acceptable and dangerous blood glucose estimations. Finally, to observe whether there is a constant error in the NIRS predictions at different glycemic levels, we examined the model during the fall in plasma glucose, during hypoglycemia, and during recovery in all studies with data divided into 10 mg/dl segments. We compared the absolute mean difference (plasma glucose by reference, glucose prediction by NIRS) at different segments. The 10 mg/dl segment analysis was performed separately for the nondiabetic subjects and the patients with diabetes.

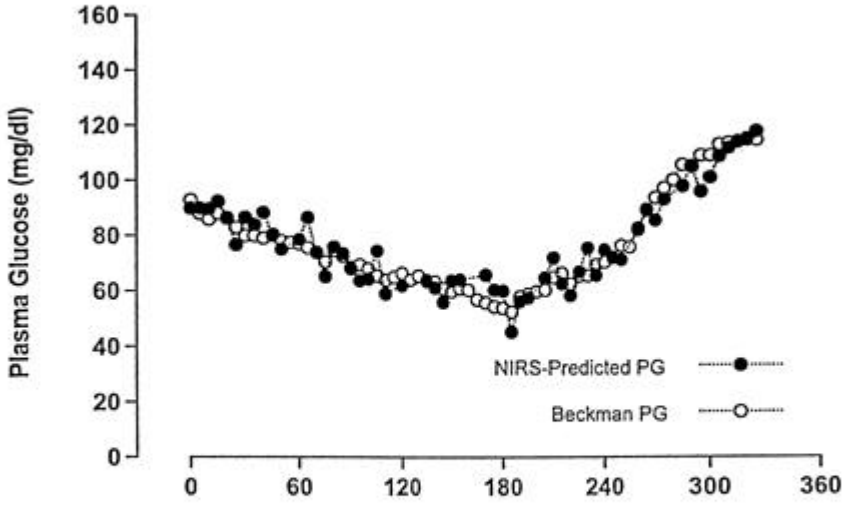

Figure 2—*Relationship between plasma glucose measured by a glucose analyzer and values predicted by NIRS during a representative study in a nondiabetic subject.*

The data presented in the text and figures are given as means ± SEM. Comparisons between groups were done using the single factor analysis of variance. The Pearson linear correlation (*r*) was used to examine the two data sets (11).

RESULTS — We performed 27 studies in 12 subjects (22 in nondiabetic and 5 in type 1 diabetic subjects). A total of 1,704 glucose determinations were performed, of which 1,384 were in nondiabetic subjects and 320 in type 1 diabetic patients. The discrepancy in the proportions of samples between nondiabetic subjects and patients with diabetes reflects the number of repeat studies performed in the same individual. There was no bias introduced into these analyses by participation of subjects in repeat studies because the data in each study were calibrated on a unique set of results. A total of 183 reference determinations were masked (149 in the nondiabetic group and 34 in the type 1 diabetic patients).

Data from a representative study in a nondiabetic subject during an experimental protocol is shown in Fig. 2. Overall, in all experiments, the average plasma glucose at $t = 0$ was 100 ± 4 mg/dl, the rate of fall to hypoglycemia was 0.32 ± 0.03 mg · dl^{-1} · min^{-1}, and the plasma glucose at the hypoglycemic nadir was 55 ± 3 mg/dl. The average rate of rise during recovery was 0.41 ± 0.04 03 mg · dl^{-1} · min^{-1}, and the final plasma glucose at recovery was 114 ± 3 mg/dl. When examined separately, the average plasma glucose in the two women with diabetes at $t = 0$ was higher (130 ± 4.2 mg/dl, $P < 0.05$) and the rate of decline to hypoglycemia was somewhat greater (0.45 ± 0.03 03 mg · dl^{-1} · min^{-1}, $P < 0.05$). Plasma glucose concentrations in the masked data set ranged between 52 and 136 mg/dl for all subjects. The NIRS predictions during the fall in plasma glucose and during recovery in all studies (Fig. 3) were highly correlated with the reference. The correlation coefficient (*r*) was 0.96 for the unmasked data during the fall in plasma glucose (Fig. 3*A*). During the recovery, both values were again highly correlated ($r = 0.95$, Fig. 3*B*). The masked data analysis showed similar results: $r = 0.94$ during the fall in plasma glucose and 0.96 during recovery (Figs. 3*C* and *D*, respectively).

Using Error Grid Analysis, 97.7% of the NIRS predictions were assigned to the A-zone (acceptable); the remainders (2.3%) were located in the B-zone (Fig. 4).

The absolute difference between the reference plasma glucose and NIRS deter-

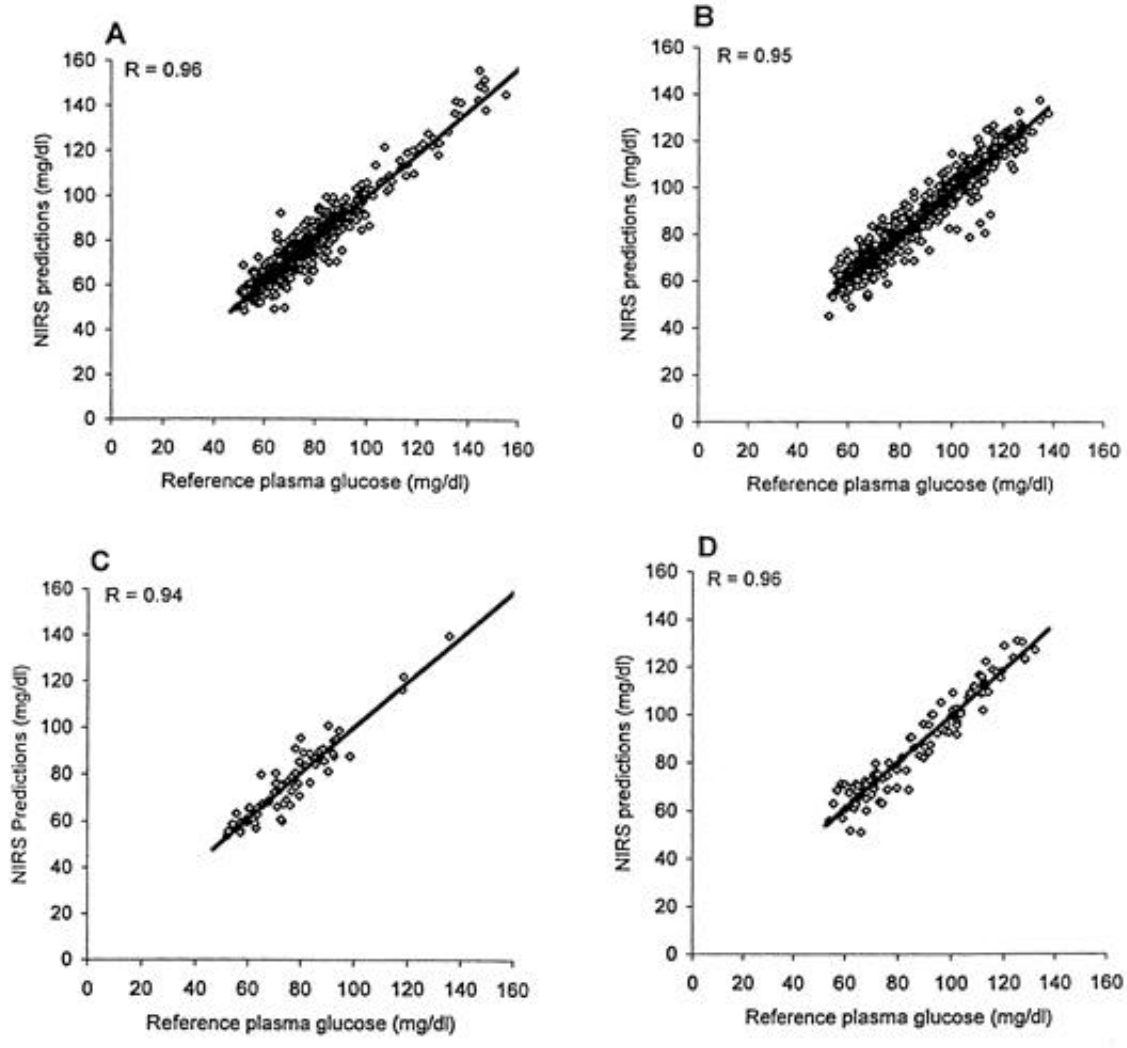

Figure 3—*The unmasked values for the NIRS predictions versus reference (Beckman) during the fall* (A) (n = *829) and recovery* (B) (n = *692) in plasma glucose. The masked values are represented in* C (n = *75) and* D (n = *108), respectively.*

minations was calculated during the fall and rise (recovery) in plasma glucose. The data were analyzed in 10 mg/dl segments as well as separately for the nondiabetic subjects and the patients with type 1 diabetes. During each 10 mg/dl segment, we calculated the mean plasma glucose concentration determined by the reference method and the corresponding predictions by NIRS. In the nondiabetic subjects, the absolute difference was essentially constant across different segments, ranging from 3.3 mg/dl during the first segment (euglycemia at t = 0) to 4.4 mg/dl during the recovery period at a plasma glucose concentration averaging 85 mg/dl. As expected, the proportion of this error averaged 3.4% at euglycemia and was the highest (6.8%) at the hypoglycemic nadir (56.6 mg/dl). In the type 1 diabetic patients, the absolute difference ranged from 3.8 to 2.6 mg/dl, respectively, and the ratio was 4.05 and 5.4% at euglycemia and the nadir, respectively (Fig. 5).

CONCLUSIONS — In the present study, we have demonstrated the feasibility of obtaining NIRS data that correlate highly with and predict plasma arterialized venous glucose concentrations in the physiological range and in the hypoglycemic range in human subjects. Furthermore, in these experiments in which plasma glucose was controlled by exogenous insulin and glucose infusion, we were able to simulate the rates of decline and rise in plasma glucose observed clinically; under such conditions, the NIRS data remained reliable in calibrations developed for individual subjects.

Noninvasive approaches to glucose measurement are appealing because they may facilitate more frequent self-monitoring of glucose in people with diabetes, especially among children for whom current forms of self-monitoring are cumbersome. However, noninvasive monitoring using NIRS is not currently approved by the U.S. Food and Drug Administration, in large part because of lack of reliability of the determinations in the ranges of plasma glucose commonly observed in diabetes (12,13). In particular, other systems using NIRS have not demonstrated sufficient precision and reproducibility in euglycemic and hypoglycemic ranges to be safely used for diabetes management by people with insulin-requiring diabetes. In addition, NIRS systems should ideally be capable of rapid responses to track plasma glucose during hypoglycemia and recovery, and the ability of existing systems to demonstrate

such response capability has not been reported.

The near-infrared region of the electromagnetic spectrum spans the end of the visible spectrum (wavelengths ~780 nm) to the beginning of the infrared bands at 2,500 nm. Absorptions in the near-infrared range are associated with the overtone and combination bands of the fundamental molecular vibrations of O-H, N-H, and C-H groups that are observed in the mid-infrared range (14). Therefore, most biologic molecules possess unique absorption signatures, and thus NIRS has become an established technique in the estimation of carbohydrate content of plant substances (14), for in vivo study of body composition (15), and for hemoglobin oxygen saturation (16). Though plasma glucose is one component in a highly complex matrix of water, proteins, lipids, and polysaccharides, the acquired signal can be extracted in vitro (14,17) and in vivo (12,13,18). For in vivo glucose monitoring applications, NIRS measures an averaged signal from a volume of heterogeneous tissue that includes blood and interstitial fluid as well as glucose in intracellular compartments of muscle, fat, and connective tissue (12,13). However, the plasma glucose compartment is the predominant source of the spectral information.

Optical glucose measurement techniques have relied on calibration of the noninvasive method with simultaneous chemometric measurements using various algorithms (12,13,18) but have failed to show requisite sensitivity in the hypoglycemic range perhaps because of interference from the strong absorbance characteristics of water. In vitro, Pan et al. (17) demonstrated the reliability of a series of calibration models for glucose over the range from 1 to 20 mmol/l by use of partial least squares regression analysis of near-infrared spectra. The addition of lipid or protein did not result in prediction bias despite the fact that these matrix components possess strong absorption bands that overlap and overshadow the glucose bands. Similar techniques have been published for in vivo NIRS systems with lower correlation especially during hypoglycemia (18,19). A superior quality of NIRS predictions in the present study may be attributed to a more sensitive NIRS system used, differing methods for spectral data processing, or differences in study design.

In our study, the deviation of values predicted by NIRS from the measured chemometric values in arterialized venous plasma glucose was examined over the euglycemic to hypoglycemic ranges of plasma glucose from ~115 to ~50 mg/dl. In both nondiabetic subjects as well as patients with diabetes, the absolute variance of NIRS determinations averaged 2–4 mg/dl across this range. The fact that the deviation of NIRS values from the chemometric measurements is relatively constant across a wide plasma glucose range is an inherent feature of the spectroscopic techniques used. Consistent with this constant absolute error, we observed an inverse relationship of the relative error of NIRS with plasma glucose, ranging from 2.2 to 6.8%.

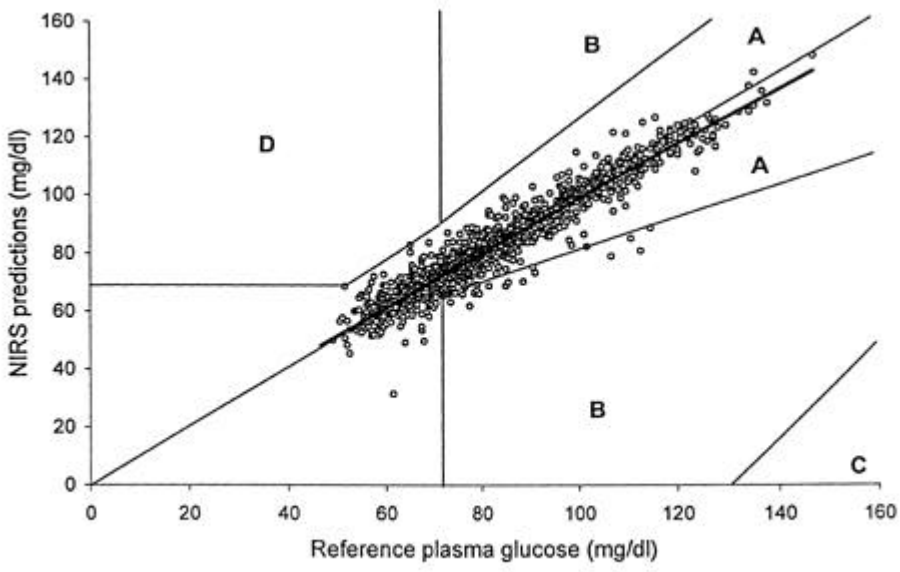

Figure 4—*Error Grid Analysis for all determinations (n = 1,704); 97.7% of the values falls in the clinically acceptable A-zone, 2.3% in the B-zone, and no values in C- or D-zone. It should be noted that the grid is modified based on the lower ranges of plasma glucose tested.*

More conventional analyses of the relationship between NIRS and chemometric measurements were also performed. Linear regression of the data, not surprisingly, yielded very strong coefficients when the data from all studies were combined because these analyses included NIRS values that were included in the original calibration sets used in each individual. Another indicator of the accuracy of the NIRS technique is reflected in the analysis of masked data in which the NIRS values were not included in the original calibration set. The correlation coefficients for these regressions were still very strong, with *r* ranging from 0.94 to 0.96. Finally, when the data were analyzed using the Error Grid approach used by Clark et al. (11), the majority of the NIRS results were in the clinically acceptable A-zone (97.7%). In addition, 2.3% matched the B-zone and none of the predictions fell into the unacceptable C- or D-zones.

The major advantage of NIRS detection is that it is completely noninvasive. Our approach involved extraction of information from spectra collected directly from a probe placed on the skin. Previous studies have used transmission through an ear lobe, finger web (20), and forearm or finger cuticle (18). The system we describe has the capability of obtaining reliable spectra from a surface probe placed at the subject's thumb. However, it should be noted that variations in the spectra obtained from reflected NIR light might be a result of pressure differences of the interface to the skin. Furthermore, the small absorption signal produced by glucose requires attention to the stability and noise characteristics of a particular instrument. The feasibility of accurate prediction of plasma glucose concentrations during hypoglycemia is a promising feature of NIRS, because reliable and rapid glucose determinations are critical at these plasma glucose levels. In addition, during hyperglycemia the glucose spectral signal is stronger, and thus NIR detection will remain as accurate (M.M., Y.A., H.S., unpublished observations). As for the ability of the NIRS method to measure glucose levels independently, we have described earlier the importance of a reliable calibration model in order to generate predictions. Once the calibration model was available, NIRS predictions were performed for the respective data points. The

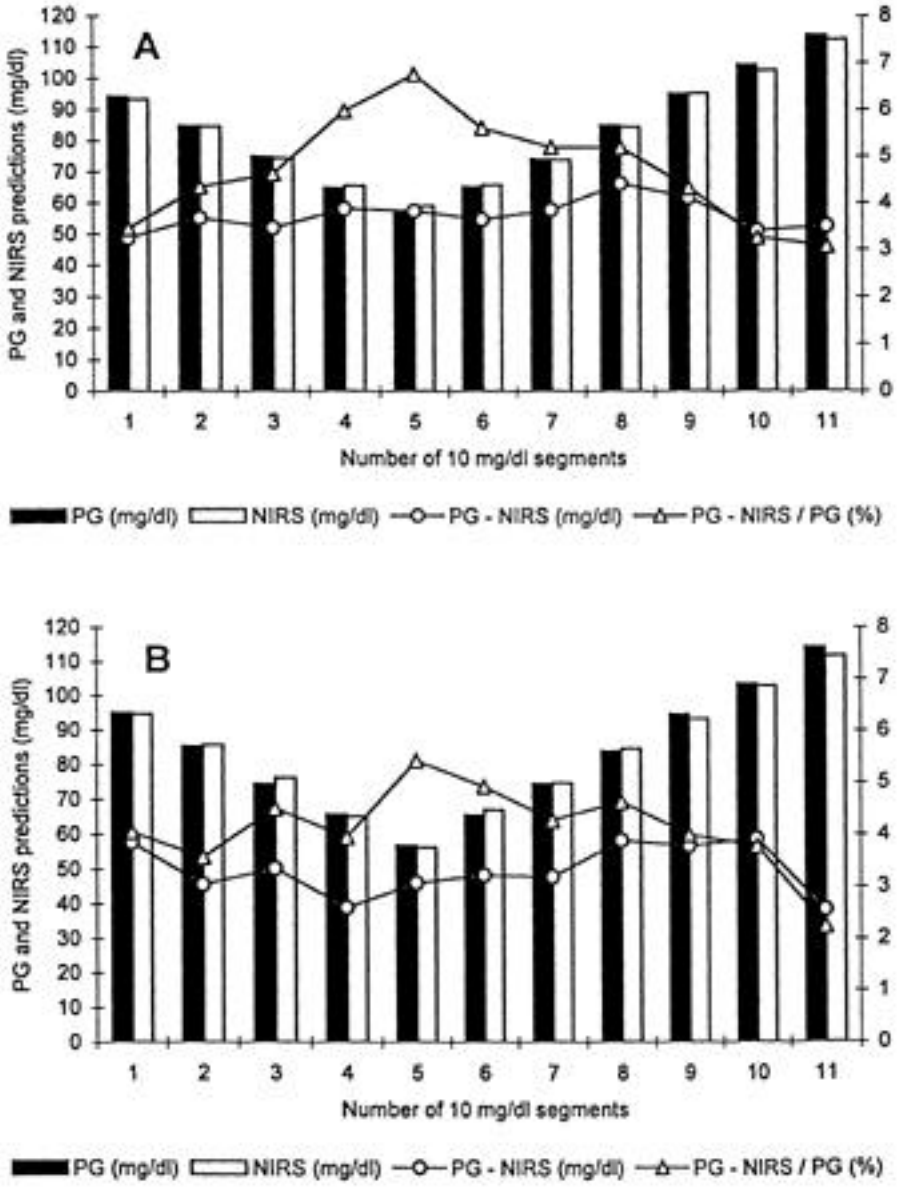

Figure 5—*Plasma glucose concentrations during hypoglycemia and recovery in nondiabetic subjects* (A) *and patients with type 1 diabetes* (B). □, *Reference;* ■, *NIRS;* ○, *absolute difference between plasma glucose and NIRS predictions for each plasma glucose range bracket;* △, *the difference as per - cent of plasma glucose.*

masked data that was not used in the calibration model calculation represent completely independent predictions, which correlate highly with the respective reference plasma glucose.

A number of challenges remain before we can apply such a noninvasive technique clinically. Future studies will be designed with the purpose to broaden the applicability of the NIRS calibration in order to produce a universal calibration set that could be used in clinical settings. Important considerations in future studies will include identification and analysis of key factors affecting the accuracy of NIRS. Although we have observed no trends so far, studies should include a systematic comparison of the reliability of NIRS prediction with respect to skin color, gender, body fat, temperature, hydration status, etc. Finally, studies must be performed in nonresearch settings relevant to day-to-day diabetes management. To conclude, we believe that this is the first NIRS system to demonstrate the technical feasibility of highly accurate plasma glucose prediction in both euglycemia and hypoglycemia.

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