OBJECTIVE — To evaluate the precision and accuracy of a new advanced prototype of a noninvasive blood glucose monitor across a wide range of serum glucose concentrations.

RESEARCH DESIGN AND METHODS — An advanced handheld noninvasive glucose monitor prototype was calibrated and tested using patients recruited by the General Research Center of the University of Connecticut Health Center. The monitor, developed by Infratec, uses principles of thermal emission spectroscopy. The noninvasive measurement of tympanic membrane glucose concentration was calibrated to the serum glucose concentration using 432 paired measurements from 20 subjects with insulin-requiring diabetes. This calibration was subsequently tested (results of power analyses) in a blind fashion with 126 paired measurements from six diabetic subjects who require insulin.

RESULTS — In vivo measurements demonstrated the reproducibility of the methodology of the noninvasive glucose monitor. Based on the calibration model, predicted glucose concentrations for six subjects were as follows (for 126 data points): SD = 32 mg/dl, mean absolute relative error (%MARE) = 11.0, with a correlation coefficient of \( r = 0.87 \). Noninvasive glucose results were also compared with laboratory reference measurements using an error-in-variables method. Clark error grid analysis showed that 100% of the measurements fell within zones A and B (90% in zone A and 10% in zone B). The SD for all noninvasive measured concentrations was 27 mg/dl, %MARE was 8.6, and the correlation coefficient was \( r = 0.94 \).

CONCLUSIONS — This first independent clinical study of an advanced noninvasive blood glucose monitor demonstrated the reproducibility of the methodology of the noninvasive glucose monitor across a wide range of serum glucose concentrations.

RESEARCH DESIGN AND METHODS

Noninvasive glucose monitor technology

The technology used for the glucose measurement device is based on the discovery that natural mid-IR emission from the human body, especially from the tympanic membrane, is modulated by the state of the emitting tissue. Spectral emissivity of human IR radiation from the tympanic membrane consists of spectral information of the blood analyte. This can be directly correlated with the blood analyte concentration (for example, the glucose concentration). Tympanic membrane thermometers, currently widely used at home and in the hospital environment, determine temperature by using total energy from a wide spectral range of human body emission, which is usually contained between 8 and 14 \( \mu \)m. In the case of this instrument, the spectral signatures (e.g., of glucose) contained in such broadband IR energy emission are used to perform constituent composition and concentration analysis. The device is a very sensitive portable handheld filterometer.
The human body is an excellent blackbody emitter of mid-IR light at precisely the right spectral region. The spectral characteristic of thermal emission is influenced by the individual's tissue composition and analyte concentrations. Kirchhoff's law confirms that for the entire body at the same temperature and for the same wavelength, absorptivity is equal to monochromatic emissivity.

Sensors for analyte measurements should have the required sensitivity and selectivity, sterilizability, and long-term stability. Spectroscopic sensors can meet all of these requirements. Among the various spectral regions, mid-infrared spectroscopy offers enhanced sensitivity and selectivity because of the information content of the fingerprint region. The selectivity of this technology is based on the same principle as the selectivity of the absorption spectroscopy method for analyte measurements. Glucose has very well-defined spectral features in the fingerprint IR region as shown (for example, in the studies by Heise et al. [7] and Vonach et al. [10] and in our plasma thermal emission spectra plots Fig. 2).

In a simple experimental arrangement, one can demonstrate that emission from glucose can be detected using room temperature detectors in a filter-based setup. A specially designed nondispersive filter-based spectrometer (Fig. 1) performed absorption and emission measurements. A filter spectrometer has the advantage of simplicity, a high signal-to-noise ratio, high throughput, and low cost. The rotatable filter wheels with characteristic transmission curves, when placed in the light path, generate variable pass bands with low resolution. As a filter, we used a circular variable IR filter, segment #3, made by the Optical Coating Laboratory, with a transmission band from 7.7 to 14.1 µm. IR radiation was collected by an IR wave-guide made from a tube, with a gold-plated inner surface, which directed IR radiation into a variable filter in very close proximity. On the other side of the filter, a thermopile detector (Perkin Elmer/Heineman model TPS 434) was placed at the optical axis of the wave-guide very close to the filter surface. The combination of wave-guide diameter, opening hole of the detector, and the dimension of its sensitive area performed like a slit in the standard spectrometry with <0.2-µm spectral resolution. Useful spectral data information was found in the range of 9–13 µm, owing to the combination of the edge effect on the ends of the filter and resolution due to slit width. At least six spectra were averaged, and the six-point smoothing Fourier procedure was used to remove fast noise from the spectrum. Both transmission and emission spectra (after correction for instrument background thermal emission) were divided by blackbody theoretical radiation intensity values resulting in values proportional to the absorptivity and the monochromatic emissivity. Measured changes in monochromatic emissivity were in the range of 10⁻⁴, still above the noise limit of the system. As an example, Fig. 2A shows the IR thermal (at 41°C) emission spectra (top curve) of glucose in a KBr (potassium bromide) tablet with a transmittance spectrum (lower curve) for comparison. Even with these poor quality spectra, one can observe corresponding bands of glucose absorption, e.g., a main band at 9.6 µm, a band at 10.9 µm (corresponding to the 914 cm⁻¹ vibrational state of glucose), and a weaker band around 12 µm. One can notice a typical mirror image between the transmission and emission spectrums. The pure glucose IR spectrum points out fundamental glucose signature spectral bands.

Thermal IR emission characteristics of different glucose concentrations in water and human plasma solutions were measured. To our knowledge, this is the first time such measurements were reported. The results for human plasma IR emission at 37°C are shown on Fig. 2B. This figure emphasizes two important features: first, it shows the spectral region of interest and, second, it presents experimental proof of the thermal emission detection ability of current room temperature IR detectors. Deconvolution shows bands sensitive and nonsensitive to glucose concentration changes in human plasma. For viewing clarity, the spectra are up-shifted along the vertical axis. Results from deconvolution in the inserted table show peak intensity changes versus glucose concentration. Again, one can observe in the emission the corresponding bands of glucose absorption, e.g., a main band at 9.8 µm, a band at 10.9 µm (corresponding to the 914 cm⁻¹ vibrational state of glucose), and a weaker band around 11.9 µm.

Thermal radiation from the human body contains information about spectral characteristics of the object and is determined by absolute body temperature as well as by the properties and states of the emitting body tissues. Thus, one can conclude that blood spectral characteristics with different contents of glucose (or other analytes) will change the emissivity of the tympanic membrane and make it possible to measure the concentration of glucose in blood.

The tympanic membrane is known to be in an excellent position to measure body temperature because it shares its blood supply with the hypothalamus, the center of core body temperature regulation. A tympanic thermometer measures the integral intensity (over all wavelengths) of IR thermal radiation. A sensor.
inserted in the ear canal can obtain a clear view of the membrane and its blood vessels to measure the amount of IR radiation the membrane emits. When compared with the theoretical blackbody radiation described by Planck’s and Kirchhoff’s laws, this IR radiation is spectrally modified by the tissue composition. Thus, IR radiation has spectral characteristics of, for example, blood in the tympanic membrane.

In this instrument, spectral characteristics of various constituents of blood were separated using analytical chemistry spectroscopy methods. The instrument relies on the use of IR filters placed in front of IR detector windows. One filter passes radiation through the thermal emission bands with glucose signatures and is placed in one of the IR detector windows, while the other IR detector window is covered by a filter capable of passing radiation that does not include emission bands characteristic of the analyte at wavelengths in the range of interest. A comparison of radiation intensity between the two detector windows (as shown on Fig. 3) provides a measurement that is proportional to the analyte concentration and can be correlated with the concentration of BG.

Figure 3 shows a simplified diagram of the instrument. The instrument optically receives IR radiation from the object target, such as a tympanic membrane. The detecting system consists of an optical IR filter set and a thermopile detector sensitive in the IR region of human body radiation. One of the sensing elements is covered by an IR filter sensitive to IR glucose signature, while an appropriate filter that does not have spectral bands characteristic to the measured analyte covers the other sensing area. In our prototype design, a so-called quasi-isosbestic point at about 8.5 μm for reference emission intensity measurements and 9.6 μm for glucose signature measurements was used. Spectrally modified IR radiation from the tympanic membrane illuminates both windows. The difference of the radiation intensity between the two radiation paths provides a measure proportional to the analyte concentration.

**Clinical study designs**
The University of Connecticut Institutional Review Board approved the study, and all subjects gave written informed consent before participating. A total of 5 women and 26 men with insulin-requiring diabetes, ranging in age from 18 to 75 years, were enrolled. Two enrolled subjects were excluded from testing. One had poor venous access and the other had a serum glucose concentration >400 mg/dl at the beginning of the study and required medical treatment. Paired glu-
Cose measurements of the first 23 subjects were used to calibrate the noninvasive monitor measurement of tympanic membrane glucose concentration with the serum glucose concentration from an antecubital vein. This calibration was subsequently tested by comparing the tympanic membrane glucose concentrations to the serum glucose concentrations in six subjects.

On the morning of the study, subjects continued their usual medications but did not take insulin or eat breakfast. Each subject’s ear canal was examined to verify that the tympanic membrane was clear of cerumen. In four subjects, warm water irrigation was used to remove cerumen occluding the tympanic membrane. An intravenous line was placed in an antecubital vein and kept open with 0.45% saline. At 0 min and at every 10 min for a total of 210–250 min, 3 ml blood was drawn for measurement of serum glucose concentration. A measurement of tympanic membrane glucose concentration was made immediately after phlebotomy was completed. The subject’s usual long-acting insulin was administered at 0 min, a carbohydrate-consistent diet breakfast was administered at 30 min, and the patient’s usual insulin bolus was delivered at 90 min. For the subjects not normally using an insulin bolus, the bolus was determined by the supervising physician in an attempt to normalize the serum glucose concentration by the end of the study. The supervising physician was aware of the results of the serum glucose measurements and made clinically appropriate interventions for serum glucose concentrations >400 mg/dl or <60 mg/dl. Ambient temperature was maintained between 18 and 25°C. The subject’s oral temperature, room temperature, and room humidity were recorded at 0 min and at every 30 min until the end of the study. The range of room relative humidity during the experiments was between 20 and 60%. Measurements of IR ear temperature were made 2 min after each glucose measurement. The nurse performing the measurements was blind to the tympanic membrane glucose concentrations.

A total of 432 paired data points from 20 subjects were used for calibration of the tympanic membrane glucose concentration with the serum glucose concentration. A trained nurse made the tympanic membrane glucose concentration measurements for 19 of these subjects. Four subjects were trained and performed the tympanic membrane glucose concentration measurements on themselves. Two monitors were available for use. In the calibration mode, the primary monitor was used for 16 subjects. The backup monitor was used for four subjects.

The results from three subjects were not included in the calibration analysis. The results from one subject who performed his own measurements and from two other subjects (with measurements performed by a nurse) were rejected because the readings did not meet the criteria set for noninvasive instrument data acceptability (see DATA ANALYSIS METHODS). The primary monitor was used for these three subjects. After calibration of the noninvasive device, the study protocol was performed in a blind prospective fashion on six subjects all using the primary monitor. The observer reporting the tympanic membrane glucose concentration and the observer receiving the report of the serum glucose concentration were blind to the results of the complementary measurement. All reported data points were included in the data analysis of the six subjects studied prospectively.

**Data analysis methods**

The studies of the noninvasive glucose monitor were divided into two parts. In the first part, the monitor was calibrated using a nonlinear regression model with data from the first group of subjects. In the second part, the prospective studies were performed for validation of the calibration and the method used.

In the first part of the experiment, the observer reporting the tympanic membrane glucose concentration had access to both the invasive and noninvasive measurements. Data from 23 subjects (511 paired data points) were collected for monitor calibration purposes. During the analysis of calibration data, it was found that for three subjects, >50% of measurements (22–25 measurements were performed on a single subject) had an instrument error when compared with none or less than a few errors for other subjects. Instrument error was indicated if a signal from the monitor detector was not smooth (the internal value of a signal from the detector changes >20% for some of 60 subsequent measured data points per single insertion of the monitor into the subject’s ear canal) or the detector signal was out of range as defined in the dependence of the ambient temperature and room humidity. The cluster of errors for these three subjects suggested a fundamental error owing to medical (e.g., potential other analytes spectral interference that will require specially designed studies or a not straight or well-straightened ear canal) or measurement procedure (the ear canal was not...
sealed properly by the measurement tip of the instrument, the measurement tip was not placed along the ear canal and the tympanic membrane axis, or an operator technique error occurred in handling the measurements). These three subjects were eliminated as data input for calibration. For final calibration, 20 subjects were used (a total of 432 instrument error-free paired data points), with instrument error indicated for 13 data points (2.9% of 445 total points). Based on the calibration results, power analysis of the above data was performed by a University of Connecticut Health Center statistician, who indicated that four to six subjects are needed to produce a predictive mode of measurements for validation of calibration for the noninvasive glucose monitor.

In the second part of the experiment, fully predictive measurements were performed. The observer reporting the tympanic membrane glucose concentration was blind to the results of the laboratory glucose concentrations. The estimates by both invasive and noninvasive methods were made independently. For the six subjects, the instrument indicated four measurement errors (3.1% of a total of 130 paired data points). Once an estimate was made and reported, no points were discarded. This second part aimed at demonstrating the reproducibility of the methodology once calibration of the glucose monitor was established.

A final statistical analysis of the results from the noninvasive BG monitor studies was made using an error-in-variables method (also called “orthogonal regression”). Ordinary least-squares regression assumes that only the y coordinate measurements are associated with random measurement errors. It is often the case that uncertainties in data lie with both the x and y coordinates. This is the case where both x and y are observed quantities and hence are known to have errors. The error-in-variables model takes measurement errors for both sets of measurements into account. Such models include Deming (20), the method of Passing and Bablok (21), and orthogonal regression. The Deming method requires specification of the ratio between squared SDs for two observed quantities but does not allow using different SDs over the range of x or y measured quantities. Most of the orthogonal regression procedures distribute error equally over x and y coordinates. In this analysis of noninvasive monitor studies, the orthogonal regression method based on an algorithm described by Reilly et al. (22) was used. The above model allows one to introduce measurement error for both axes over the whole range of measured quantities.

### Laboratory serum glucose measurements

Laboratory serum glucose concentration was determined by the oxygen consumption rate method using an oxygen electrode (Synchron LX20 Instrument; Beckman Instruments, Brea, CA). The laboratory measurement SDs supplied by the Clinical Laboratory at the University of Connecticut Health Center are as follows: for quality control level 1 (QC1) with a mean BG of 61 mg/dl, SD = ±2 mg/dl; for QC2 with a mean BG of 120 mg/dl, SD = ±4 mg/dl; and for QC3 with a mean BG of 373 mg/dl, SD = ±11 mg/dl. Relative error over the whole range of glucose concentrations was about ±3.3%.

### RESULTS

None of the subjects dropped out of the study once it was initiated. There was no significant pain or discomfort associated with the noninvasive measurement off the tympanic membrane every 10 min.

As described in RESEARCH DESIGN AND METHODS, in the first part of the experiment, the noninvasive glucose monitor was calibrated using a nonlinear regression model for data from the first 20 subjects (432 paired data points). The calibration SD was 37.4 mg/dl, and the mean absolute relative error (%MARE) was 13.05, with a correlation coefficient of \( r = 0.89 \). If one applied Passing and Bablok’s (21) method of comparison for calibration results for the regression line intercept of 25.3 (mg/dl) and the slope of 0.89, the 95% CIs of 16.35 and 0.85–0.93, respectively, would be found. Based on this model, the next six subjects’ predicted glucose concentrations were (for 126 data points) as follows: SD = 32 mg/dl, %MARE = 11.6, with a correlation coefficient of \( r = 0.87 \). Correlations between invasive (laboratory) and noninvasive glucose concentrations are shown in Fig. 4. Figure 4A shows data from calibration for 20 subjects, and Fig. 4B shows the prediction correlation for six subjects.

### Noninvasive measurement data analysis

For the final statistical analysis of noninvasive BG measurements, there are 558 paired (laboratory and noninvasive glucose concentrations) data points. The cumulative correlation between the invasive (laboratory) and noninvasive glucose concentrations are as follows: SD = 36.2 mg/dl, %MARE = 12.7, with a correlation coefficient of \( r = 0.89 \). For this case, errorless invasive glucose measurements were assumed. In the Clark error grid (23), the clinically accurate zone A contains 81% of noninvasive data, and the clinically acceptable zone B contains 19% of the data. One data point was close to zone D — a clinically significant error.

In the next two cases, orthogonal regression is used to introduce errors associated with the laboratory serum glucose method.
Case 1. The relative error of laboratory versus “true” glucose data were set at ±3%, as supplied for laboratory data from quality control measurements over the whole sampling range of serum glucose concentrations. Figure 5A shows a plot of resulting noninvasive measurements with SD = 26 mg/dl, %MARE = 9.5, and a regression coefficient of \( r = 0.94 \). In the clinically accurate zone A, there is 89.4% of noninvasive data; in the clinically acceptable zone B, there is 10.6% of data. Figure 5C shows a plot of laboratory versus “true” glucose data, with SD = 6.2 mg/dl, %MARE = 1.5, and a regression coefficient of \( r = 0.997 \). Both plots show quality control invasive serum glucose calibration data.

Case 2. In case 2, no arbitrary distribution of error for laboratory versus “true” glucose data was assumed. The orthogonal regression program calculated the best least-squares fit between laboratory and noninvasive measurements. The results are shown in Fig. 5B, with an SD = 27 mg/dl, %MARE = 8.6, and a regression coefficient of \( r = 0.94 \). In the clinically accurate zone A, there is 90.3% of noninvasive data; in the clinically acceptable zone B, there is 9.7% of data. Figure 5D shows a plot of laboratory versus “true” glucose data, with SD = 15.5 mg/dl, %MARE = 2.7, and a correlation co-

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**Figure 5**—Plot of correlations between “true” serum glucose invasive concentrations and noninvasive glucose concentrations for case 1 (A) and case 2 (B) and corresponding plots of correlations between invasive (laboratory) serum glucose concentrations and “true” serum glucose concentrations for case 1 (C) and case 2 (D).
Efficient of $r = 0.98$. In the clinically accurate zone A, there is 97.5% of invasive measurements, and there is 2.5% in the clinically acceptable zone B. One can notice a qualitative difference in the correlation plot between the case when arbitrary distribution of error for laboratory versus “true” glucose data were applied (Fig. 5C) and the case with no such assumption (Fig. 5D). In the last case, most of the laboratory versus “true” glucose concentrations lie on the identity line: 77% within 1 SD (relative error 3.3%), 86% of points within 2 SDs (relative error 6.6%), 97.5% of points deviating from this line <20% (zone A), and ~2.5% points deviating >20% (they exist in zone B). These results were calculated statistically, indirectly from orthogonal regression, and do not necessarily indicate that laboratory glucose data possess such errors.

Deming regression (20) has been widely adopted in clinical medicine because unlike least-squares linear regression, which only allows for imprecision in the response variable $y$, imprecision can be present in both the variables $x$ and $y$. The results for this noninvasive data were calculated using the Analyze-it Clinical Laboratory Module for Microsoft Excel (24) and are shown in Table 1. The ratio of variance equal to 0.057 was taken from the imprecision of $SD_1 = 6.2$ and $SD_2 = 26$, as in the above orthogonal analysis.

Passing and Bablok’s (21) method of comparison is also useful when imprecision occurs in both variables. However, the imprecision need not be normally distributed and can have a nonconstant variance over the sampling range. Its only restriction is that the ratio of the imprecision $x/y$ must be equal to the slope squared. Another useful feature of the procedure is that the regression line is not unduly biased by extreme values. Results calculated using available software (24) are shown in Table 1.

**CONCLUSIONS** — Clinical studies were performed using a new advanced handheld prototype of a noninvasive BG monitor based on TES. Patients with type 1 and type 2 diabetes were tested using this device. A total of 20 subjects were used for device calibration. Six subjects (sample size as determined by a power analysis) were evaluated for validation in a full predictive “blind” for patients and operators mode. Measurements demonstrated the reproducibility of the methodology of the noninvasive glucose monitor.

Statistical analysis of the noninvasive monitor studies data was performed using error-in-variables methods such as Deming (20), Passing and Bablok (21), and orthogonal regression with parameter estimation (22). The Deming regression did not give any improvement for intercept and slope of linear regression even when used with the best-assumed ratio of SD for laboratory and noninvasive glucose concentrations. Results from the Passing and Bablok method of comparison for slope and intercept of linear regression are comparable with orthogonal regression with parameter estimation. The orthogonal regression made it possible to calculate other statistical parameters. The SD was 27 mg/dl, %MARE was 8.6, and the regression coefficient was $r = 0.94$. Clarke error grid (23) analysis showed that 100% of the measurements fell within zones A and B. In the clinically accurate zone A, there was 90% of noninvasive data; in the clinically acceptable zone B, there was 10% of the data.

In the statistical analysis of the results, significant improvement of statistical parameters was shown when error of the standard laboratory reference invasive measurements was taken into consideration. The error in laboratory measurements as an additive quantity is increasing the real error of noninvasive results.

This truly noninvasive prototype based on thermal emission in the mid-IR spectral region has shown glucose measurements with clinically acceptable accuracy without the necessity of individual daily calibration. For comparison to another noninvasive monitor, one can refer to data reported for the U.S. Food and Drug Administration–approved (March 2001) GlucoWatch system (Cygnus), which requires a 3-h warm-up period and individual calibration every 12 h (25).

The first clinical study with the new technology based on TES is promising, although the number of patients tested is small. This handheld noninvasive BG monitor will improve the lives of people with diabetes.

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**References**