Noninvasive Type 2 Diabetes Screening

Superior sensitivity to fasting plasma glucose and A1C

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OBJECTIVE — This study compared the performance of a novel noninvasive technology to fasting plasma glucose (FPG) and A1C tests for detecting undiagnosed diabetes and impaired glucose tolerance.

RESEARCH DESIGN AND METHODS — The design was a head-to-head evaluation in a naïve population. Consented subjects received FPG and A1C tests and an oral glucose tolerance test (OGTT). Subjects were also measured by a noninvasive device that detects the fluorescence of skin advanced glycation end products. A total of 351 subjects participated.

RESULTS — Subjects with 2-h OGTT values ≥140 mg/dl defined the positive screening class. A total of 84 subjects (23.9% prevalence) screened positive. The performances of the noninvasive device, FPG, and A1C were evaluated for sensitivity and specificity against this classification. At the impaired fasting glucose threshold (FPG =100 mg/dl), the FPG testing sensitivity was 58% and the specificity was 77.4%. At that same specificity, the sensitivity for A1C testing was 63.8%, while the noninvasive testing sensitivity was 74.7%. The sensitivity advantage of the noninvasive device over both blood tests for detecting diabetes and precursors was statistically significant ($P < 0.05$).

CONCLUSIONS — The noninvasive technology showed clinical performance advantages over both FPG and A1C testing. The sensitivity differential indicated that the noninvasive device is capable of identifying 28.8% more individuals in the OGTT-defined positive screening class than FPG testing and 17.1% more than A1C testing. The combination of higher sensitivity and greater convenience—rapid results with no fasting or blood draws—makes the device well suited for opportunistic screening.

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The U.S. is facing a dangerous epidemic in type 2 diabetes. Of the estimated 20.6 million individuals with diabetes, ~30% are undiagnosed (1). Another 54 million people have some form of pre-diabetes, and many will progress to frank diabetes within 3 years (1–3). Numerous studies have shown that with early detection and effective intervention, diabetes can be prevented or delayed (2–7). In patients with diagnosed diabetes, other studies have shown that glucose control can lower the incidence of complications (8,9).

Diagnosis is typically initiated during a physical exam with a primary care physician. However, current screening methods for type 2 diabetes and pre-diabetes are inadequate due to their inconvenience and inaccuracy. Specifically, the most widely applied screening test in the U.S., fasting plasma glucose (FPG) testing, has convenience barriers in the form of an overnight fast and a blood draw. FPG also suffers from poor sensitivity (40–60%) contributing to late diagnoses (10). In fact, about one-half of diabetic patients present with one or more irreversible complications at the time of diagnosis (11,12). A more accurate and convenient screening method could dramatically improve early detection of type 2 diabetes and its precursors, facilitating interventions that can prevent or at least delay the development of type 2 diabetes and its related micro- and macrovascular complications.

Several studies, including the DCCT (Diabetes Control and Complications Trial) and EDIC (Epidemiology of Diabetes Interventions and Complications Study), have demonstrated that elevated skin advanced glycation end products (AGEs) are biomarkers of diabetes, are highly correlated with the complications of diabetes, and are predictive of future diabetic retinopathy and nephropathy (13–15). Individuals with diabetes accumulate skin AGEs faster than individuals with normal glucose regulation (16). Thus, skin AGEs constitute a sensitive summary metric for the integrated glycemic exposure that the body has endured.

However, until the recent development of novel noninvasive technology to measure AGEs, a punch biopsy was required to quantify skin AGE levels. This method, Spectroscopic measurement of dermal AGEs (SAGE), measures skin fluorescence due to AGEs in vivo and provides a quantitative diabetes risk score based on multivariate algorithms applied to the spectra (17). SAGE does not require fasting and creates no biohazards. It automatically compensates for subject-specific skin differences caused by melanin, hemoglobin, and light scattering. The measurement time is approximately 1 min, thus providing an immediate result.

The concept of quantifying dermal AGEs noninvasively was successfully tested in a previous in vitro study. In that work, concentrations of a well-studied fluorescent AGE, pentosidine, were accurately quantified in a porcine dermis model by noninvasive fluorescence spectroscopy (18). Subsequently, an early noninvasive prototype was evaluated in a

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Abbreviations: AGE, advanced glycation end product; AUC, area under the curve; EER, equal error rate; FPG, fasting plasma glucose; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; PCA, principal-components analysis; ROC, receiver-operator characteristic; SAGE, Spectroscopic measurement of dermal AGE.

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

Table 1—*Summary of study demographics*

Age (years)					Sex					Race/ethnicity				
	n	NGT	AGT	Prevalence (%)		*n*	NGT	AGT	Prevalence (%)		*n*	NGT	AGT	Prevalence (%)
21–30	17	15	2	11.8	Male	128	100	28	21.9	Caucasian	187	149	38	20.3
31–40	52	44	8	15.4	Female	223	167	56	25.1	Hispanic	128	92	36	28.1
41–50	99	75	24	24.2	Total	351	267	84	23.9	African Am	11	10	1	9.1
51–60	88	71	17	19.3						Native Am	17	11	6	35.3
61–70	65	41	24	36.9						Asian	3	2	1	33.3
71–80	22	14	8	36.4						East Indian	1	1	0	0.0
≥81	8	7	1	12.5						Other	4	2	2	50.0

Data are (*n*) unless otherwise indicated. Prevalence (%), prevalence of abnormal glucose tolerance (AGT), determined by AGT/*n*. Am, American; NGT, normal glucose tolerance.

diabetic versus normal (case-control) human subject study, demonstrating that SAGE could accurately classify disease in a case-control population (19). This led to the premise of the current work: We hypothesize that SAGE can detect undiagnosed diabetes and pre-diabetes with sufficient performance to serve as a screening tool.

RESEARCH DESIGN AND METHODS

— The present study is a direct comparison of SAGE, FPG, and A1C assessed using the 2-h oral glucose tolerance test (OGTT) to determine truth (i.e., the "gold standard"). The threshold for impaired glucose tolerance (IGT), 2-h OGTT ≥140 mg/dl, delineated the screening threshold for abnormal glucose tolerance. A subject is classified as having abnormal glucose tolerance if they screen positive for either IGT (OGTT 140–199 mg/dl) or type 2 diabetes (OGTT ≥200 mg/dl). The abnormal glucose tolerance group encompasses all subjects needing follow-up and diagnostic confirmation. The study was conducted in a naïve population—subjects who had not been previously diagnosed with either type 1 or type 2 diabetes.

To demonstrate superior sensitivity at 80% power with 95% CI, an abnormality in 80 subjects was required (20). At that prevalence and for a projected SAGE sensitivity of 68%, the power calculations yield a 95% CI for test sensitivity of 57.8–78.2%.

Study subjects were selected from individuals who responded to flyers and newspaper advertising. Subjects were recruited until the target prevalence of abnormal glucose tolerance was comfortably achieved. Selection criteria were one or more risk factors for diabetes per the American Diabetes Association standard-of-care guidelines (21). Individuals with a previous diagnosis of diabetes were excluded. Ages in the cohort ranged between 21 and 86 years, while the ethnic and racial composition mirrored the demographics of Albuquerque, New Mexico. The cohort demographics are summarized in Table 1. The study protocol was approved by the University of New Mexico School of Medicine Human Research Review Committee. When recruiting concluded, 84 subjects with abnormal glucose tolerance had been identified within a cohort of 351 participants.

Subjects were asked to fast overnight for a minimum of 8 h before participation. All provided informed consent. Blood was drawn from subjects for clinical chemistry tests. The glucose assays were run on a Vitros 950 clinical chemistry analyzer, while the A1C assay was performed on a Tosoh G7 HPLC. (The assays adhered to internal standard operating procedures: *CHEM-081: Glucose, Serum, or CSF by Vitros Slide Technology* or *HEM-003: Hemoglobin A1C, Tosho G7*.)

The prototypical SAGE instrument is a table-top apparatus. The subject sits in a chair beside the instrument and rests his/her left forearm in an ergonomically designed cradle. A custom fiber-optic probe couples output from near-ultraviolet and blue light–emitting diodes to the subject's volar forearm and collects the resulting skin fluorescence and diffuse reflectance. The sequentially illuminated light-emitting diodes have peak wavelengths at 375, 405, 420, 435, and 460 nm. The optical radiation emitted from the skin is dispersed in a modified research-grade spectrometer and detected by a charge-coupled device array.

The optical exposure from SAGE was compared with the International Electrotechnical Commission (IEC) ultraviolet skin exposure limits (22). Skin exposure from the screening device was 250 times smaller than the exposure limit. Hence, the risk of skin erythema or other damage due to optical radiation from the SAGE is negligible.

Melanin and hemoglobin are optical absorbers at the wavelengths of interest; they reduce light amplitude and distort the skin's spectral characteristics. In addition, subject-specific tissue characteristics such as wrinkles, dermal collagen concentration and organization, and hair follicles scatter light in the skin. Previous studies developed techniques that were applied in the prototype instrument to mitigate the impact of skin pigmentation, hemoglobin content, and light scattering on the noninvasive measurement (18). Also, skin AGEs accumulate naturally over time in all people. An algorithm compensated for patient age to remove this trend. Principal-components analysis (PCA) was applied to the spectra from 267 subjects with normal glucose regulation with ages ranging 22–85 years. PCA reduces the dimensionality of the dataset, transforming the fluorescence spectra into eigenvalues and eigenvectors (23). Linear regression determined the age-related slope of the eigenvalues. The age dependence is then removed from all spectra to compensate for subject age. The pigmentation- and age-corrected spectra comprise the "intrinsic" dermal fluorescence spectra.

Linear discriminant analysis was applied to the intrinsic spectra to assess noninvasive disease classification performance (24). In this method, the intrinsic dermal fluorescence spectra were first decomposed by PCA. From the resulting spectral scores, multidimensional spectral distances were determined. These distances (Mahalanobis distances) represent the ef-

fective distance of each spectra with respect to the normal (D0) and abnormal (D1) groups. From the difference between the distances (D1 − D0), posterior probabilities ranging from 0 to 100 are computed. A posterior probability—the SAGE output value—represents a likelihood metric for that subject belonging to the abnormal class.

During each SAGE session, subjects were measured three times, lifting and replacing their arm into the cradle between measurements. In addition, subjects were tested by SAGE in two sessions in order to assess any effect due to subject fasting status. The first SAGE session always occurred in a fasting state. Approximately 60% of the study cohort received both FPG testing and an OGTT during a single visit. For the remaining group, the OGTT was administered on a subsequent day. For all subjects, their second SAGE session occurred at least 1 h after ingestion of the glucose load, near the anticipated peak of the acute blood glucose level due to the OGTT glucose bolus. Subject convenience dictated whether they participated in one or two visits. In all cases, subjects were in a nonfasting state during their second SAGE session. In principle, SAGE should be independent of fasting status since AGE concentration is not influenced by acute blood glucose levels. SAGE dependence on fasting status was evaluated by comparing classification performance stratified by first versus second session.

Artifacts in the fluorescence spectra arising from subject movement or poor contact with the optical probe were identified by objective spectral outlier metrics. This quality control step rejected <10% of the dataset as "spectral outliers." SAGE values (linear discriminant analysis [LDA] posterior probabilities) were determined for all clean spectra. Thus, the SAGE classification output contains multiple values per subject, and the subsequent analysis includes this inherent measurement uncertainty. The redundant measurements also enable computation of the intrasubject variance.

To quantitatively assess the impact of skin coloration on the noninvasive classification performance, subject skin pigmentation was objectively quantified from diffuse reflectance measurements and classified into light and dark subgroups. Noninvasive disease classification performance was then evaluated for each subgroup.

The screening performance of FPG and A1C tests and SAGE were assessed by comparing their respective sensitivities at a relevant clinical threshold. An appropriate comparative threshold for screening is the FPG threshold for impaired fasting glucose (IFG). All three tests were evaluated at the specificity corresponding to this FPG value (100 mg/dl).

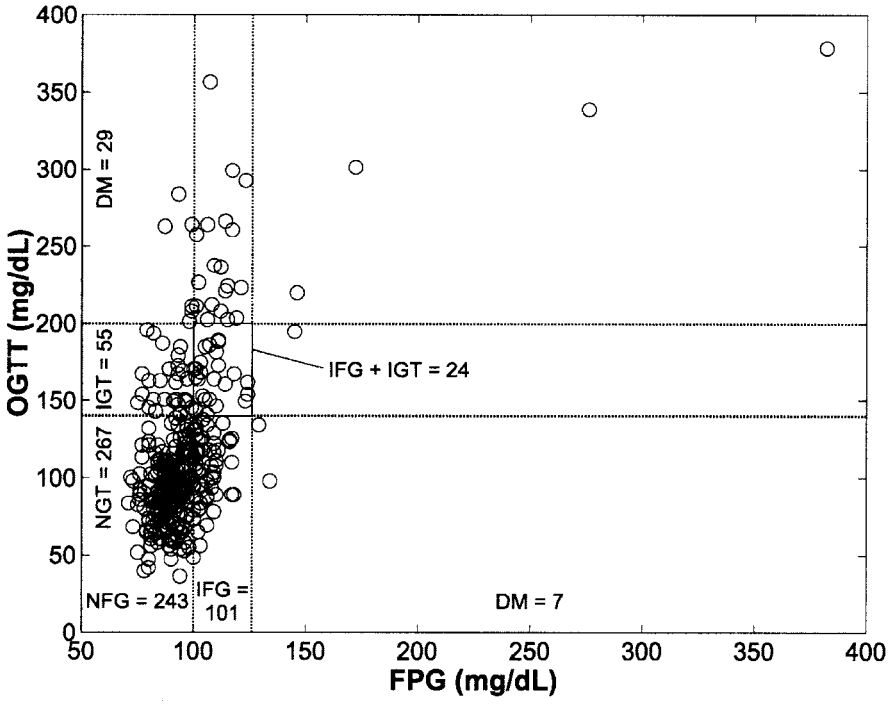

Figure 1—*Comparison of blood glucose screening tests: OGTT vs. FPG* (n = *351*). *Dashed lines delineate screening state categories (e.g., NFG, normal fasting glucose; IFG; etc.). Category counts lay inside their respective axis. The solid box denotes the overlap of IFG and IGT categories* (n = *24*).

RESULTS — The OGTT identified abnormal glucose tolerance in 84 of the 351 subjects (23.9% prevalence). Of the 84 subjects with abnormal glucose tolerance, IGT was found in 55 subjects and frank type 2 diabetes in 29 subjects. Prevalence of abnormal glucose tolerance by age, sex, and ethnicity is provided in Table 1. The table also details the specific numbers of subjects with normal and abnormal glucose tolerance in these demographic categories. A comprehensive comparison of OGTT and FPG screening categorization is presented in Fig. 1.

Using the normal versus abnormal classification determined by OGTT, the receiver-operator characteristics (ROCs) for FPG and A1C testing and SAGE were computed. The IFG threshold of 100 mg/dl corresponds to an FPG specificity of 77.4%, the critical specificity for comparing the tests. At 77.4% specificity, the FPG testing sensitivity was 58.0%, the A1C testing sensitivity was 63.8%, and SAGE sensitivity was 74.7%. The test values corresponding to the critical specificity were 100 mg/dl for FPG, 5.8% for A1C, and 50 for SAGE. The ROC plots are shown in Fig. 2, and test performance is summarized in Table 2.

The 95% CI for SAGE sensitivity was 65.4–84%. Thus, the sensitivity differences between SAGE and both FPG and A1C tests are statistically significant ($P < 0.05$). The actual CI differs from that estimated by the power calculations in RESEARCH DESIGN AND METHODS, since the study found higher prevalence and increased SAGE sensitivity at the IFG-defined critical specificity. The absolute sensitivity advantage of the noninvasive device compared with FPG and A1C were 16.7 and 10.9 percentage points, respectively. The relative sensitivity advantage for SAGE versus FPG testing was 28.8%, and for A1C testing the relative advantage was 17.1%. These values estimate the additional fraction of subjects with abnormal glucose tolerance that is detected by SAGE but missed by the conventional blood tests.

Alternatively, the tests can be compared via their equal error rate (EER), which is the point toward the top-left corner of the respective ROCs where sensi-

Table 2—*Summary of test performances*

Test	Sensitivity	Threshold	SAGE sensitivity advantage	
SAGE	74.7%	50	Absolute	Relative
FPG	58.0%	100 mg/dl	16.7%	28.8%
A1C	63.8%	5.8%	10.9%	17.1%

Comparison of sensitivities for SAGE, FPG, and A1C for detecting abnormal glucose tolerance. The FPG threshold for IFG (100 mg/dl) set the critical specificity (77.4%) for this comparison. Thresholds for each test at the critical specificity are indicated. The *right section* notes the performance advantage of SAGE over the two blood-based tests in terms of absolute and relative sensitivity.

tivity and specificity are equal. The SAGE EER was 24.1% (sensitivity = specificity = 75.9%), while the EER for A1C and FPG tests were 27.7 and 32.2%, respectively.

The general performance metric of area under the curve (AUC) shows a statistically significant advantage ($P < 0.05$) for SAGE (AUC 79.7%) versus FPG testing (72.1%). The AUC values for SAGE (79.7%) versus A1C testing (79.2%) were not statistically separable. The Hoorn coefficient of variation of SAGE, quantifying the intersession reproducibility of the noninvasive instrument, was 9.4%.

SAGE performance was assessed for high and low melanin concentration subgroups that were divided by their measured skin diffuse reflectance. At the IFG threshold noted above (critical specificity 77.4%), sensitivity for detecting abnormal glucose tolerance in subjects with lighter skin was 70.1%, while in those with darker skin it was 82.1%. Compared with the results for the entire cohort, the performance for subcohorts stratified by skin melanin content are not statistically different; i.e., SAGE sensitivity is not impaired by intersubject skin melanin variations.

Classification performance was also stratified by subject fasting status. SAGE sensitivity for first session values (fasting) was 78.4%, while the sensitivity for second session values (nonfasting) was 72.7%. The session-stratified sensitivities are not significantly different from those of the full cohort. Alternatively, the correlation coefficient between fasting and nonfasting SAGEs was $r = 0.87$ ($P < 0.001$). Consequently, SAGE performance is independent of the ambient blood glucose level.

CONCLUSIONS — SAGE significantly outperforms FPG and A1C testing for detection of abnormal glucose tolerance. SAGE identified ~29% more individuals with undiagnosed abnormal glucose tolerance than FPG testing and ~17% more than A1C testing. In addition, SAGE provides rapid results and does not require fasting or blood draws, factors that are convenience barriers to opportunistic screening.

The low sensitivity for detection of abnormal glucose tolerance with FPG testing reported here is not unexpected. A review of studies of FPG screening for undiagnosed diabetes has found that sensitivities ranged from 40 to 65% (10). Since negative screening results are not subject to confirmatory testing, the large false-negative rate for FPG testing is a latent problem and contributes to the growing number of undiagnosed cases of type 2 diabetes.

The results presented here are consistent with the pathogenesis of abnormal glucose regulation, in which excessive postprandial glucose levels accelerate accumulation of skin AGEs, although fasting levels may remain normal. Since dermal AGEs represent the integrated damage due to hyperglycemia, noninvasive measurement of these biomarkers is a promising means for early detection of abnormal glucose regulation.

Given the increasing worldwide prevalence of type 2 diabetes and prediabetes, a move to earlier detection and treatment is necessary to help mitigate the diabetes epidemic. In the U.S., if current trends continue, the prevalence of diabetes is expected to more than double by 2025 and affect 15% of the population (25). The recent estimate of $135 billion for annual diabetes-related health care costs in the U.S. means that the cost of the diabetes epidemic threatens to overwhelm the nation's health care system (26).

Fortunately, once detected, diabetes is now more treatable than ever. Large

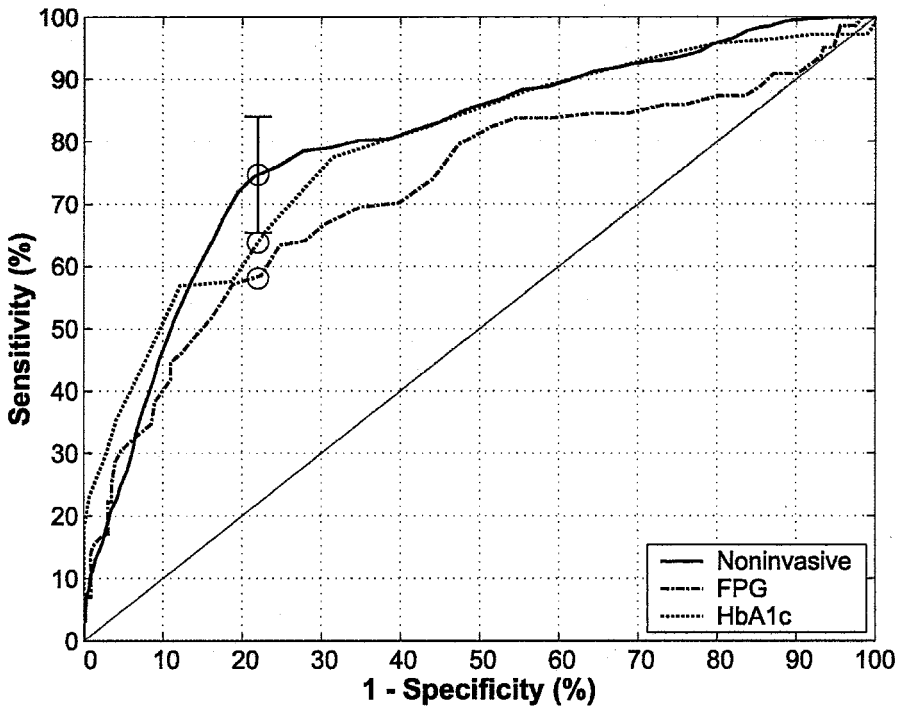

Figure 2—*Test performances for detecting abnormal glucose tolerance compared via ROC plots. ○, test performance at the critical specificity corresponding to the IFG threshold. The error bars on the SAGE measurement indicate 95% CI in sensitivity.*

clinical studies such as the DCCT (Diabetes Complications and Control Trial) and UKPDS (UK Prospective Diabetes Study) have shown that tight control of glucose levels has significant health benefits for those with established diabetes (8,9).

Moreover, if pre-diabetes is detected and treated, progression to frank type 2 diabetes can be delayed or prevented. The DPP (Diabetes Prevention Program), FDPS (Finnish Diabetes Prevention Study), and DREAM (Diabetes Rduction Assessment With Ramipril and Rosiglitazone Medication) trials have shown that it is possible to prevent or at least delay the development of type 2 diabetes in patients with pre-diabetes (3–5). This may be accomplished with aggressive diet and exercise modification and/or therapeutics such as metformin (DPP) and rosiglitazone (DREAM trials).

The combination of accuracy and convenience of SAGE make it well suited for opportunistic screening and earlier detection of diabetes and pre-diabetes. This noninvasive technology is a promising tool to facilitate early intervention for preventing or delaying the development of diabetes and its devastating complications.

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