Journal of Diabetes Science and Technology
Volume 3, Issue 4, July 2009

SYMPOSIUM

Noninvasive Optical Screening for Diabetes

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Abstract

Background:

Advanced glycation end products (AGEs) are implicated in the complications of diabetes. Advanced glycation end products also accumulate in the skin and are sensitive biomarkers for the risk of developing diabetes and related complications. Some AGEs fluoresce and can be measured noninvasively by optical spectroscopy.

Methods:

Noninvasive screening for diabetes has been evaluated in an 18-site study involving a cohort of 2793 subjects meeting American Diabetes Association-based screening criteria. Subjects were measured with a specialized skin fluorimeter and also received traditional blood glucose and glycated hemoglobin tests.

Results:

Retrospective results indicated that the noninvasive technology measuring dermal fluorescence is more sensitive at detecting abnormal glucose tolerance than either fasting plasma glucose or glycated hemoglobin A1C.

Conclusions:

These results suggest that noninvasive measurement of dermal fluorescence may be an effective tool to identify individuals at risk for diabetes and its complications. The noninvasive technology yields immediate results, and since measuring dermal fluorescence requires no blood draws or patient fasting, the instrument may be well suited for opportunistic screening.

J Diabetes Sci Technol 2009;3(4):776-780

Introduction

Protein glycation is a multistage reaction that forms numerous stable adducts and crosslinks known collectively as advanced glycation end products (AGEs). Dermal collagen is a protein that readily undergoes glycation, and because of its long half-life, the level of AGEs in the dermis acts as a long-term integrator of overall glycemia that is insensitive to short- or intermediate-term fluctuations in glycemic control.[1] Advanced glycation end product formation is a part of healthy aging and is accelerated by hyperglycemia.

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Abbreviations: (A1C) glycated hemoglobin A1C, (AGE) advanced glycation end product, (AGT) abnormal glucose tolerance, (AUC) area under the curve, (FPG) fasting plasma glucose, (IF) intrinsic fluorescence, (IFG) impaired fasting glucose, (OGTT) oral glucose tolerance test, (ROC) receiver operator characteristic

Keywords: instrumentation, noninvasive, screening, skin fluorescence

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The relationship between skin AGEs and diabetes and related complications has been well established in multiple studies.[2–6] In addition, clinical studies with the Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications cohorts have shown that specific AGEs—pentosidine and carboxymethyl lysine—and collagen-linked fluorescence are biomarkers of diabetes and are predictive of future complications.[7,8] Skin fluorescence measured noninvasively *in vivo* is correlated to AGE concentrations.[9] An *in vitro* study demonstrated that fluorescence spectroscopy can accurately quantify specific skin AGEs.[10] In addition, researchers have demonstrated that skin AGE fluorescence is correlated with vascular damage and risk of cardiac mortality in patients with type 2 diabetes[11,12] and that skin fluorescence is a predictor for development of microvascular complications, including neuropathy and micro-albuminuria.[13]

Methodology

The measurement of skin fluorescence requires a light source at the appropriate wavelength to induce emission of those AGEs that fluoresce. Also needed is an optical system to couple the excitation light to the patient's skin and collect emitted light and relay it to a detector. The instrument used in this study (SCOUT DS™) is an investigational device that illuminates a small area (0.20 cm^2) of the underside of the forearm with near-ultraviolet, blue and white light for 1.5–3.5 min. Radiant exposure levels are 0.4% of the International Electro-technical Commission ultraviolet light skin exposure limits, and thus there is no significant risk to the skin.

Much of the incident light is absorbed in the skin by melanin and hemoglobin. However, some light is scattered back to the surface of the skin and is detected as diffuse reflectance. Excitation light reaching the dermis can induce fluorescence of the dermal AGEs. The resulting skin fluorescence is measured over the 400–660 nm emission window. Interindividual variation can be substantial, as skin characteristics impacting this measurement vary with race, ethnicity, age, and gender. The determination of intrinsic fluorescence (IF) serves to compensate for these variations. As previously published, IF is computed using both skin fluorescence and reflectance measurements.[10] The IF correction removes spectral distortion due to absorption from melanin and hemoglobin, plus it compensates for light scattering due to skin-layer thickness and collagen organization. The IF is then age corrected to adjust for the accumulation of skin AGEs in normal health.[14]

To make possible the dermal AGE measurement by SCOUT DS, a specialized optical system was designed to capture the fluorescence spectra. A proprietary multi-variate algorithm was developed as the mathematical relationship between the fluorescence signal and screening classification. Upon completing a SCOUT DS measurement, the embedded computer applies the detected fluorescence to the algorithm producing a diabetes risk score. A score of 50 or greater is a positive result, indicative of abnormal glucose tolerance (AGT), and warrants follow-up diagnostic testing.

Results

Previously, the noninvasive skin fluorescence measurement and its classification performance were described in a single site study.[14] In the study reported here, 2793 naïve, at-risk subjects at 18 different sites participated. This was the first phase of a multiphase study and was intended to finalize the calibration characteristics of the investigational SCOUT DS device. Subject inclusion criteria for the study followed the American Diabetes Association's Standard of Care Guidelines.[15] The study protocol was designed as a head-to-head comparison of the noninvasive technology versus fasting plasma glucose (FPG) and glycated hemoglobin A1C (A1C) with the 2-hour 75 g oral glucose tolerance test (OGTT) regarded as screening truth. Although OGTT is inconvenient and has reproducibility issues with an intrasubject coefficient of variation of nearly 17%,[16] it is frequently regarded as the standard reference for diabetes screening.[17,18]

This multisite study was intended to acquire data from a diverse cohort representing the demographics that might be expected in screening of the at-risk population. The demographics of the study participants are shown in **Figure 1**. The top left subplot depicts the cohort ages that ranged from less than 20 years to greater than 80 years in a nearly normal distribution centered on a group aged 50–59 years. The top right pane shows the balance between male (39%) and female (61%) participants. Cohort ethnicity is depicted in the lower left plot. The OGTT results are illustrated in the lower right pane. The classification as normal or AGT was determined based on these OGTT results. An OGTT ≥ 140mg/dl indicates AGT, and a 2-hour plasma glucose value <140mg/dl denotes normal glucose tolerance. For the purposes of this study, serum and plasma glucose are considered identical. In this at-risk population, 23.5% screened positive for AGT (2 hour OGTT ≥ 140 mg/dl). Of those 657 who screened AGT positive, approximately one-quarter (172) had OGTT results of 200 mg/dl or more.

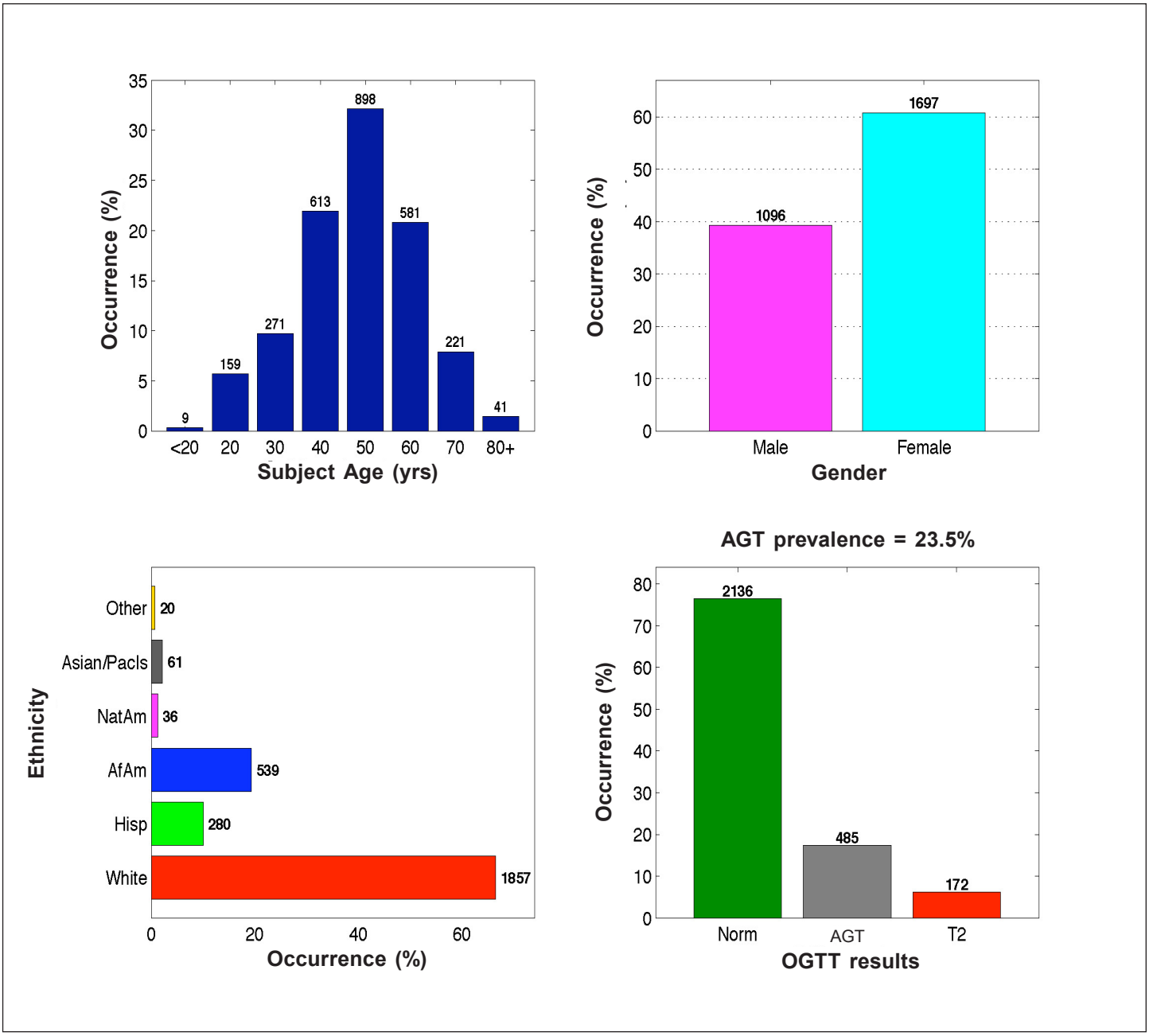

Figure 1. Demographics of 2793 naïve, at-risk subjects in a study examining performance of noninvasive technology versus conventional laboratory tests. Clockwise from the bottom left are distributions of the cohort by ethnicity, age, gender, and prevalence of AGT.

The SCOUT DS results were obtained from predictions based on a calibration model using OGTT values as the reference in a 100-fold cross-validation process. Thus the prediction set is a randomly selected 1% of the subjects, and the calibration model is built using the other 99% of the subjects. This process is repeated over 100 iterations until out-of-sample predictions are made on all subjects. Additionally, this cross-validation process is repeated three times to randomly regroup subjects to further remove any intragroup relationship.

Applying the normal (true negative) versus abnormal (true positive) classification as determined by OGTT, receiver operator characteristics (ROCs) for detection of AGT were generated for FPG, A1C, and SCOUT DS. Receiver operator characteristic curves, describing the relationship between sensitivity and false positive rate (1-specificity) for each test, are plotted in **Figure 2**. Objective assessment of test performance requires the establishment of a clinically relevant specificity for comparing the screening sensitivities of the three tests.

Since each sensitivity-specificity position on a ROC curve is related to a unique test value, that relationship can be reversed to yield a false positive rate (1-specificity) for a given test value. As in the previous single site study,[14] the critical test value for intertest comparison was the FPG value of 100 mg/dl—the lower threshold of impaired fasting glucose (IFG). On the FPG curve in **Figure 2**, derived from the tests performed on the cohort of 2793 at-risk subject reported in this study, the IFG threshold (100 mg/dl) corresponds to a false positive rate of 16.7%. This false positive rate—set by the FPG performance—is denoted as the vertical dashed line in **Figure 2**. At this common false positive rate, the test sensitivities, denoted by symbols in the figure, were 61.0% for SCOUT DS (triangle), 43.5% for FPG (circle), and 45.3% for A1C (diamond). The error bars indicate the 95% confidence intervals for each sensitivity estimate: ±3.9% for SCOUT DS and ±4.0% for both FPG and A1C. The SCOUT DS ROC had an area under the curve (AUC) of 79.4% compared to 70.9% for FPG and 71.5% for A1C. The standard deviation for all three AUC estimates was ±1.4%. The SCOUT DS screening performance advantage in both metrics—sensitivity at a common specificity or AUC—is statistically significant ($p << .05$). The intertest sensitivity margin can also be expressed as a relative sensitivity (relative sensitivity = s_2/s_1-1). In the instance of SCOUT and FPG, the relative sensitivity is approximately 40% (61/43.5-1 = 0.402). The relative sensitivity suggests that SCOUT DS will identify approximately 40% more true positives than FPG at the same false positive rate.

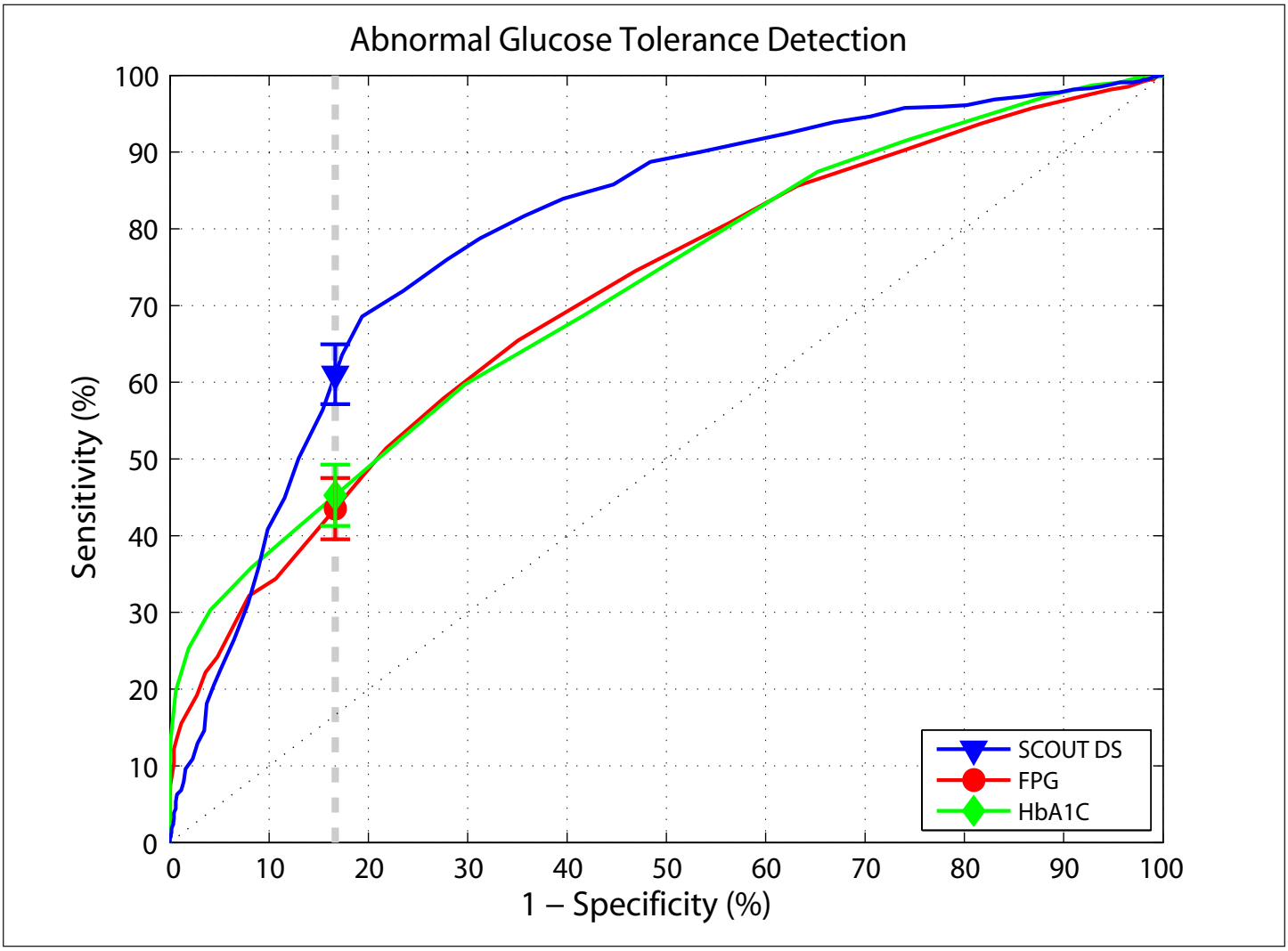

Figure 2. Receiver operator characteristic plots of the performance of SCOUT DS (blue), FPG (red), and A1C (green) tests for screening AGT. The dashed vertical line denotes the FPG false positive rate (16.7%) corresponding to the lower FPG threshold of IFG (100 mg/dl). At that common specificity, symbols denote the sensitivities for SCOUT DS (61.0%; triangle), FPG (43.5%; circle), and A1C (45.3; diamond). Error bars denote the 95% confidence interval for test sensitivities: ±3.9% for SCOUT DS and ±4.0% for both FPG and A1C.

For SCOUT DS compared to A1C, the relative sensitivity is approximately 35%. Test performance metrics are summarized in **Table 1**.

Table 1.
Test Performance Summary

	Threshold	Sensitivity ± confidence interval at 16.7% FPR	Relative sensitivity	AUC ± standard deviation
SCOUT DS	52	61.0 ± 3.9%	—	79.4 ± 1.4%
FPG	100 mg/dl	43.5 ± 4.0%	40%	70.9 ± 1.4%
A1C	5.9%	45.3 ± 4.0%	34%	71.5 ± 1.4%

Conclusions

The noninvasive technology for the measurement of AGEs using dermal fluorescence shows promise as a tool for early detection of AGT. It offers a valuable combination of accuracy and convenience. No blood draws are required, and the results are available quickly while the patient is still in the office. Significantly, it can be used on nonfasting patients. This may make it well suited for opportunistic screening of at-risk individuals. In addition, the superior sensitivity of the noninvasive skin fluorescence test may lead to earlier detection of AGT, enabling early intervention for preventing or delaying the development of diabetes and its devastating complications.

Disclosure:

All authors are employees and option holders of VeraLight, Inc.

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