Skin Intrinsic Fluorescence and Age-Related Macular Degeneration: The Beaver Dam Eye Study

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Purpose. To determine if skin intrinsic fluorescence (SIF), a noninvasive measure of advanced glycation endproducts and oxidative stress in skin is associated with AMD.

Methods. SIF was measured with the SCOUT DS skin fluorescence spectrometer in a cross-sectional cohort study of 969 persons aged 68 to 102 years from the 1181 who participated in the 25-year follow-up examination in the Beaver Dam Eye Study (BDES) in 2014 to 2016. The SCOUT DS skin fluorescence spectrometer uses five light-emitting diodes, centered at 375 nm to 456 nm. AMD was assessed by grading of digital color 45° stereoscopic fundus photographs of the macula using the Wisconsin Age-Related Maculopathy grading scheme. Analyses included logistic regression with generalized estimating equations to account for correlation between the eyes of a person.

Results. There were data for 1827 eyes for analyses. Early AMD was present in 22% and late AMD in 4% of the eyes. While adjusting for age, sex, smoking status, and history of cardiovascular disease, there were no significant associations of any SIF measure with any AMD or exudative AMD. SIF01 (odds ratio per 1 SD difference on the log scale, 95% confidence interval) (1.66, 1.00–2.74, $P = 0.05$) and SIF03 (1.81, 1.16–2.81, $P = 0.008$) were associated with geographic atrophy.

Conclusions. There was a suggestive relationship of two SIF measures, SIF01 and SIF03, using different correction factors from the excitation centered at 375 nm, with the prevalence of geographic atrophy in the BDES. Longitudinal follow-up is indicated to assess a temporal relationship.

Keywords: advanced glycation endproducts, age-related macular degeneration, risk factors, skin intrinsic fluorescence, cohort study

Skin intrinsic fluorescence (SIF) is a measure of accumulation in the skin of advanced glycation endproducts (AGEs) (e.g., crossline and pentosidine), as well as products of metabolism and oxidative stress (e.g., nicotinamide adenine dinucleotide hydride [NADH] and flavin adenine dinucleotide [FAD]).[1] SIF measurements have been shown to be directly correlated with AGEs measured in biopsies of the same skin site where the measurements were taken.[2,3] SIF has been shown to be elevated in many conditions, including cardiovascular disease, chronic kidney disease, and diabetes.[2–5] We examine the cross-sectional associations of SIF with any and late AMD, adjusting for age and other factors to investigate whether SIF is associated with AMD in a well-defined cohort of persons who participated in the Beaver Dam Eye Study (BDES). Study of the relationship of SIF to AMD may provide insights into the pathogenesis of AMD and prospective study of the relationship may elucidate new interventions to treat AMD and also provide information regarding the use of SIF to quantify the risk of developing AMD.

Materials and Methods

Subjects

The study group for this investigation consisted of 1181 persons from the original cohort of the general population in the BDES who participated in the 25-year follow-up examination from 2014 to 2016.[6–10] Of these 1181, there were 1036 (88%) with SIF measurements. Sixty-seven of these were excluded due to lack of data for AMD.

Procedures

Informed consent conforming to the tenets of the Declaration of Helsinki was obtained from participants before each examination, and institutional review board approval was obtained from the Human Subjects Committee of the University of Wisconsin-Madison. The study operations were performed in adherence to the Health Insurance Portability and Accountability Act.

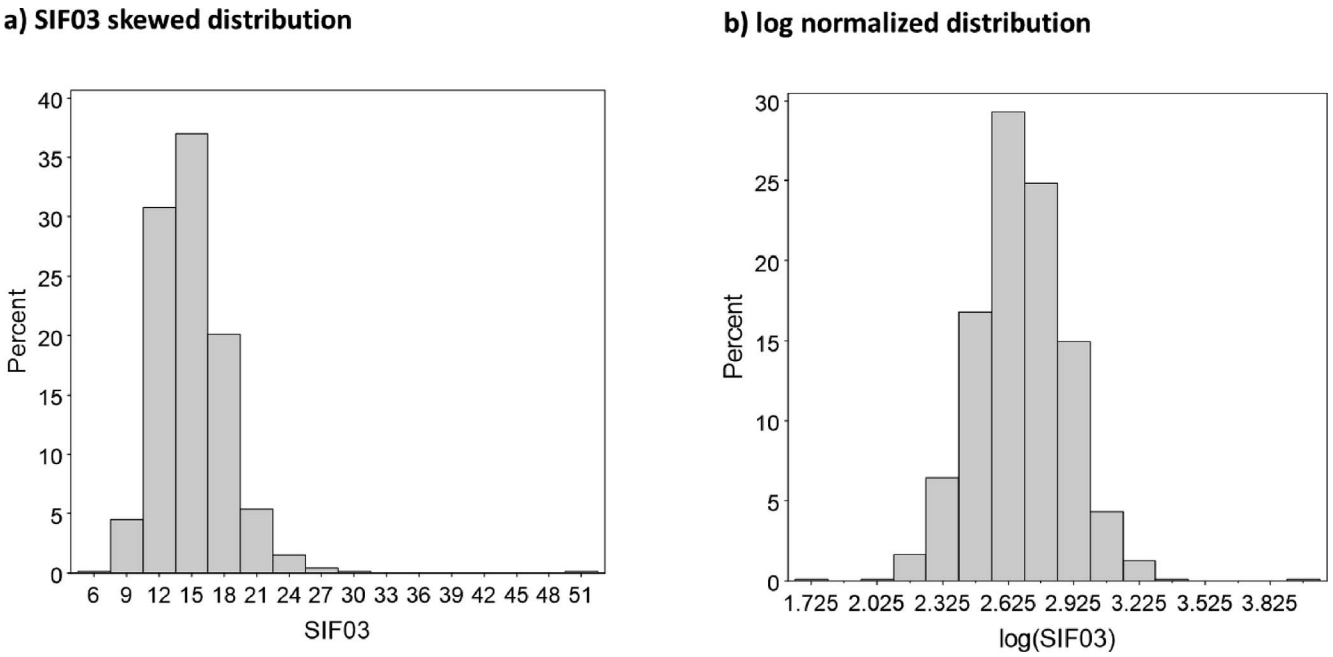

FIGURE 1. Figure showing (a) skewed distribution for SIF03 and (b) after log normalization.

Measurements

The study evaluations were conducted by trained examiners. The pertinent parts of the ocular and physical examinations for this investigation included measuring blood pressure, dilating the pupils, and taking 45° stereoscopic digital color fundus photographs of two standard fields (modified Early Treatment of Diabetic Retinopathy Study fields 1 and 2).[11–13] Participant medical history was obtained and included whether subjects smoked and the medications they used in the past year. An aliquot of whole blood was used to determine HbA1c level with nonporous ion exchange HPLC on the Tosoh Automated HPLC Analyzer HLC-723G8 (Tosoh Bioscience Inc., South San Francisco, CA, USA, and Tokyo, Japan) which is standardized by the National Glycohemoglobin Standardization Program. For each eye, the maximum grade for each of the lesions of AMD, in the macular grid, was determined using the Wisconsin Age-Related Maculopathy classification scheme.

The SCOUT DS skin fluorescence spectrometer (VeraLight, Inc., Albuquerque, NM, USA) was used to measure skin pigmentation and SIF from the underside of the left forearm approximately 3 inches from the elbow. Skin fluorescence was excited with light-emitting diodes (LEDs) centered at 375, 405, 416, 435, and 456 nm, with the resulting emission measured over the 435- to 655-nm spectral range. These emission ranges were selected to excite a variety of skin fluorophores related to AGE formation, oxidative stress, and metabolic activity. A white LED was used to measure skin reflectance over the 435- to 655-nm spectral range.

The intrinsic fluorescence correction uses the measured skin reflectance at both the excitation wavelengths and the emission wavelengths to mathematically adjust the measured fluorescence for optical distortion due to absorption of light from melanin and hemoglobin, as well as light scattering.[14–18] As shown in the equation, the measured fluorescence (F_{xm}) is divided by reflectance from both the excitation LED (R_x) and the white LED (R_m), which are further scaled by dimensionless exponents k_x and k_m to yield the intrinsic fluorescence (f_{xm}).

$$f_{xm} = \frac{F_{xm}}{R_x^{k_x} R_m^{k_m}}$$

For each of the five excitation LEDs, three different adjustment exponent pairs (k_x, k_m) were used, resulting in 15 SIF measurements (see Supplementary Tables S1, S2 for SIF measurements) for each person. In addition, the autofluorescence ratio was calculated from LED1 by setting k_x to 1 and k_m to 0, effectively scaling the measurement fluorescence (F_{xm}) by just the reflectance at the excitation wavelengths. These intrinsic correction coefficients were selected in the development of this methodology in a population with diabetes. They reduce intrasubject measurement variance and by compensating for skin color (melanin, hemoglobin content) and light scattering while maximizing the associations with diabetic microvascular complications. SIF measurements that reflect a higher k_m weight than k_x weight have been shown to reduce intraindividual measurement variations (especially in older individuals with age spots and wrinkles) and be less influenced by melanin content in those with darker skin. The low wavelength (375 nm) from LED1 excites fluorescent AGEs, NADH, and FAD. The 375-nm LED (SIF1, SIF2, and SIF3) is likely the most important for detecting AMD because it has the strongest fluorescence from AGEs and FAD. The highest wavelength (456 nm) from LED5 excites fluorescence from collagen cross-links and FAD.[19] Additional details on the intrinsic correction and tissue characteristics have been reported elsewhere.[15,19]

Duplicate SIF measures were obtained from each study subject, with the mean of the measurements used for analysis. SIF values are skewed and not normally distributed. To normalize the distribution, the SIF values were logarithmically transformed for statistical analyses, for example, from a mean of 14.94 and SD of 3.22 as measured to a mean of 2.68 and SD of 0.20 as transformed logarithmically and illustrated in Figure 1.

Definitions

Age in years was defined as the participant's age at the time of the current examination. Systolic and diastolic blood pressures

Table. Comparison of Participant Characteristics for Those Included and Excluded in Analyses of SIF With AMD in the BDES

	Included, $n = 969$		Excluded, $n = 212$		
Characteristic	***n***	**Mean (SD) or % With Condition**	***n***	**Mean (SD) or % Without Condition**	***P****
Age, y	969	78.0 (6.4)	212	81.3 (7.6)	<0.001
Sex, % male	969	42.7	212	27.4	<0.001
BMI (kg/m^2)	957	30.9 (5.9)	197	29.7 (6.9)	0.14
Systolic BP, mm Hg	961	134.7 (19.6)	147	131.9 (19.6)	0.11
Diastolic BP, mm Hg	961	72.0 (9.9)	147	68.9 (9.3)	0.03
Hypertension, %	966	79.4	190	87.4	0.04
Ever smoke, %	969	48.2	212	47.2	0.73
Cardiovascular disease, %	969	17.0	209	19.1	0.61
Diabetes, %	883	24.0	121	34.7	0.01
HbA1c, %	890	5.9 (0.8)	99	6.0 (0.9)	0.57
Any AMD, %, worse eye	921	21.4	116	30.2	0.23
Late AMD, %, worse eye	921	4.7	116	11.2	0.04
SIF03 (log)	969	2.7 (0.2)	67	2.7 (0.3)	0.66
SIF15 (log)	969	0.3 (0.2)	67	0.4 (0.3)	0.54

BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin A1c.
* *P* value is after adjustment for age.

were defined as the average of two measurements taken with the Dinamap ProCare 300 (GE Healthcare, Waukesha, WI, USA). Diabetes was defined based on self-reported presence of diabetes with treatment and/or HbA1c >6.5%. Smoking history (never, past, current smoker) was obtained from the question "have you smoked 100+ cigarettes in your lifetime?" and the question "do you currently smoke?" Cardiovascular disease history was from self-reported presence of angina, myocardial infarction, or stroke.

Drusen area, type, and largest size, as well as the presence of increased retinal pigment or depigmentation were determined. No AMD and early and late AMD were defined using the 3 Continent Consortium definition.[20] Early AMD is defined as presence of any drusen with pigmentary abnormalities, or large drusen (>125 um) in the absence of pigmentary abnormalities. Late AMD is pure geographic atrophy (GA) in the absence of exudative macular degeneration or exudative macular degeneration with or without GA present.[20] If there was no evidence of subretinal new vessels or intravitreal anti-VEGF treatment for it, but the eye had a history of having been treated for subretinal new vessels with intravitreal anti-VEGF injection, that eye was defined as having exudative AMD. Any AMD was defined by the presence of early or late AMD.

Statistical Analysis

All outcomes were binary. Logistic regression models were fitted to the data using generalized estimating equations to account for the correlation between the eyes. All models were adjusted for age, gender, smoking history, and cardiovascular disease history. Additional adjustments for diabetes status, chronic kidney disease, and skin tone were considered, but were not associated with AMD and not confounders of the SIF and AMD associations, so were not part of the final models. SAS version 9 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

Results

Of the 1181 persons who participated in the sixth examination phase of the BDES, there were 1036 with SIF measurements of whom 969 also had graded images for AMD. Selected characteristics for those who provided information for the analyses at the time of the examination (included) and those persons who did not (excluded) are given in the Table. Those excluded were more likely to be older, female, and to have hypertension, diabetes, and late AMD.

Early AMD was present in 22% and late AMD in 4% of the eyes. For subtypes of late AMD, 2.5% had exudative AMD and 1.6% had pure GA. While adjusting for age, sex, smoking status, and cardiovascular disease history, there were no significant associations of any SIF measure to the presence of any AMD (Fig. 2a), but there were suggestive associations with the presence of late AMD (Fig. 2b), specifically no associations with exudative AMD (Fig. 2c), and some associations with pure GA (Fig. 2d). There were no statistically significant associations of any of the SIF measures with the presence of soft or large drusen or of pigmentary abnormalities (data not shown). There was an association of SIF03 with late AMD (odds ratio [OR] per 1 SD difference on the log scale: 1.48, 95% confidence interval [CI] 1.07–2.05, $P = 0.02$; Fig. 2b). SIF01 (OR per 1 SD on log scale: 1.66, 95% CI 1.00–2.74, $P = 0.05$) and SIF03 (OR 1.81, 95% CI 1.16–2.81, $P = 0.008$) were associated with GA (Fig. 2d). Significance remained after further adjustment for diabetes status and chronic kidney disease (data not shown).

Discussion

We had hypothesized that AGEs as measured by SIF were associated with AMD. We did not find associations of SIF with any AMD or exudative AMD. This may be because AGEs are not causally associated with any AMD or that the AGEs detected by SIF are different from AGEs involved in the pathogenesis of AMD.

We found associations of SIF01 and SIF03 (both from the lowest LED wavelength) with pure GA in persons 68 to 102 years of age participating in the BDES. Both the SIF01 and SIF03 are from the 375-nm light, which creates the strongest fluorescence for AGEs, NADH, and FAD. Although SIF02 is also from this same wavelength, the correction factors may be more affected by melanin variations (such as age spots).

Few other studies have examined SIF with AMD. A case-control study found the SIF score was significantly higher in 73 patients with neovascular AMD than in 31 age-matched

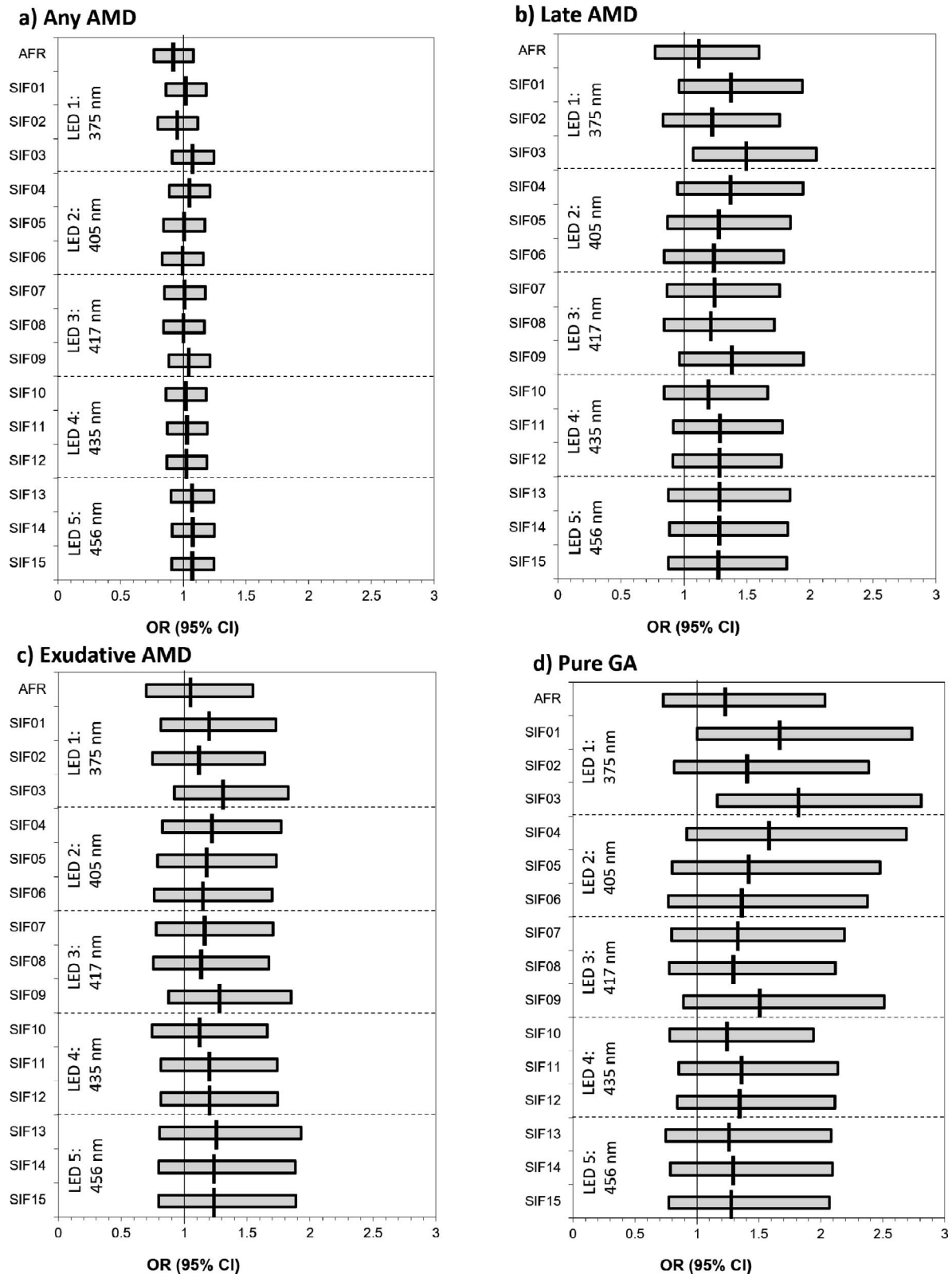

Figure 2. SIF associations (ORs and 95% CIs per 1 SD, on log scale) after adjustment for age, sex, smoking, and cardiovascular disease with (**a**) any AMD, (**b**) late AMD, (**c**) exudative AMD, and (**d**) pure GA.

controls (2.57 vs. 2.23 units, $P = 0.002$).[21] To our knowledge, no significant associations of SIF measures to GA have been reported. Data from most proteomic studies have not shown relationships of AGEs with AMD.[22] A large population-based cohort, the Age, Gene/Environment Susceptibility–Reykjavik Study did not find a cross-sectional relationship of carboxymethyl lysine (CML) with early or late AMD.[23] They did not measure SIF in this study.

The SIF measurements have not been widely evaluated in a general population, especially for ocular measures. SIF has been shown in other studies to be associated with cardiovascular disease, kidney disease, and diabetes.[2–5] We also find strong associations ($P < 0.001$) of each SIF measure with these outcomes. For example, SIF03 is associated (OR, 95% CI) with diabetes (1.85, 1.53–2.22), cardiovascular disease (1.43, 1.19–1.73), and chronic kidney disease (1.64, 1.39–1.94). Because these outcomes are more common than late AMD, there is greater power to detect associations. The associations are still much stronger than what we observe for AMD. It is possible that the effect of some AGEs in the skin, which is what SIF measures, are associated with the more systemic conditions, but may not reflect the effect of AGEs in the retina. Why we find an association with pure GA, which has very low power, but not exudative AMD or any AMD is not clear.

Our study had strengths and weaknesses. Its strengths include the use of standard fundus photographic images of the macula and standard protocols for grading AMD. The uniformity of skin tone in our population reduces concerns of the effect of melanin levels in the skin on the SIF measures. Its weaknesses include the possibility of selective participation (e.g., by age, diabetes status, selective mortality) and the study's cross-sectional design limiting the ability to examine antecedent-consequent relationships; SIF may not be specific enough to reflect AGEs, and there is limited power for detecting associations with late AMD. Despite the low prevalence of pure GA, the association with SIF was strong enough to reach statistical significance with this endpoint, although as noted above this was not a primary endpoint and may be a chance finding. The associations with exudative AMD and with other SIF measures are weaker, but in a similar direction.

Conclusions

We found suggestive associations of SIF measures from skin excitation centered at 375 nm, where there is strong fluorescence from AGEs and FAD, with pure GA. There is a need to replicate these findings and also to examine the relationship of SIF markers with the incidence of late AMD, specifically for pure GA.

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