

Skin Autofluorescence Increases Postprandially in Human Subjects

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ABSTRACT

Background: Skin autofluorescence (SAF) is a property used for the noninvasive assessment of skin advanced glycation end products (AGEs) and concentration of redox-regulated fluorophores. SAF was shown to closely mirror cardiovascular risk and to constitute a more sensitive parameter for diabetes screening than fasting glucose and hemoglobin A1c. It has also been suggested that SAF measurement is independent of fasting status. Our study was designed in order to test whether SAF changes postprandially.

Methods: We have investigated 21 Caucasian subjects (10 healthy subjects, 11 subjects with type 2 diabetes mellitus). SAF was measured in the fasting state, as well as 2 and 4 h following a meal with a medium AGE content.

Results: Two hours postprandially, SAF significantly increased by 10.2% in the whole group, by 11.6% in the group of individuals with diabetes, and by 8.7% in healthy subjects (for all measurements $P < 0.05$ vs. baseline).

Conclusions: SAF increases postprandially in individuals with diabetes mellitus and in healthy subjects. Therefore, we suggest that measurements of SAF should be performed in the fasting state in order to increase sensitivity and specificity of the method for assessing cardiovascular risk and diabetes screening.

BACKGROUND

DIABETES MELLITUS is accompanied by an increased cardiovascular risk,¹ and hyperglycemia and hyperlipidemia have been suggested to play an important role in the development of diabetes complications.² Some of the most important pathomechanisms mediating these effects seem to be the generation of advanced glycation end products (AGEs)³ and oxidative stress (OS).⁴

AGEs are a heterogeneous group of moieties resulting from nonenzymatic glycation of pro-

teins, lipids, and nucleic acids.⁵ Assessment of AGE residues^{6–8} and OS⁹ comprises laborious methods and provides insights into protein damage and glycemic control in diabetes. These techniques require specialized equipment and high analytical standards.

In an attempt to find an easier and more accessible method for assessing AGEs and OS, the technique of measuring skin autofluorescence (SAF) noninvasively has been developed. It has been suggested that SAF reflects more trustworthily the long-term AGE exposure,¹⁰ which is increased in people with diabetes.¹¹ Spectro-

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scopic measurements of dermal AGE are based on the autofluorescence property of some AGEs.¹² Still, SAF has been suggested to reflect not only skin AGE concentration, but also the concentration of redox-regulated fluorophores,¹³ and some authors have questioned the specificity of the method for the assessment of skin AGE.¹⁴ Nevertheless, AGEs and OS are closely interrelated, and both play a major role in the development of diabetes complications.¹⁵ SAF has been shown to correlate with the degree of diabetic neuropathy¹⁶ and to be a good predictor of mortality in populations at risk,^{12,17} and a quantitative diabetes risk score according to SAF measurements has been proposed.¹¹ Recently, it has been suggested that SAF represents a more sensitive tool for screening for diabetes in the general population than fasting glucose or hemoglobin A1c.¹¹ The article also suggested that SAF measurement is independent of the fasting status of subjects. Nevertheless, it can be hypothesized that blood AGE and redox-regulated fluorophores from skin microvessels contribute to SAF in addition to skin-bound compounds. In that case, postprandial AGE and OS increase¹⁸ might influence SAF.

Therefore, our study aimed at investigating whether postprandial status has an influence on SAF measurements.

SUBJECTS AND METHODS

Twenty-one subjects (age, 46 ± 22 years; body mass index, 26.6 ± 5.5 kg/m² [median \pm interquartile range]; male/female ratio, 13/8; smokers/nonsmoker ratio, 2/19; 10 healthy individuals, 11 with type 2 diabetes) were investigated after approval of institutional review board and individual written consent. All subjects were Caucasian with white skin and a good skin reflection ($>10\%$).¹² Each subject served as his or her own control. The renal function was preserved (estimated creatinine clearance, >50 mL/min) in all people with diabetes mellitus. Data in healthy individuals were not available, but none had a known renal disease.

SAF was assessed by the AGE Reader (AGE-R) (Diagnoptics BV, Gröningen, The Nether-

lands). The method has been described in detail elsewhere.¹⁶ In brief, the AGE-R illuminates 1 cm² of a skin surface isolated from further light influence, with an excitation light source between 300 and 420 nm. The light from the skin is measured with the spectrometer (model PC-1000 fiber optic spectrometer, Ocean Optics, Dunedin, FL) in the 300–600 nm range, using a 200- μ m glass fiber (Farnell, Leeds, UK). Autofluorescence is defined as the average light intensity per nanometer in the range between 420 and 600 nm, divided by the average light intensity per nanometer in the range between 300 and 420 nm (autofluorescence). Skin reflection measurements were corrected against a white standard to maximally reduce influences of skin pigmentation and redness.

Blood samples were analyzed for serum glucose (Architect ci8200 Analyzer, Abbott Diagnostics, Wiesbaden, Germany).

Measurements were performed after an overnight fast and smoking cessation, as well as at 2 and 4 h following a usual, high fat meal (whole-grain bread, 90 g; butter, 20 g; Gouda cheese, 45% fat [20 g]; Camembert cheese, 60% fat [30 g]; tomato, 50 g; containing 27% carbohydrates, 59% fat, and 14% proteins; 594 kcal) with a calculated AGE content of 8.518 kU.¹⁹ For the assessment of AGE concentration, Goldberg et al.¹⁹ tested foods for their content in a common AGE marker, *N*-carboxymethyllysine, using an enzyme-linked immunosorbent assay based on an anti-*N*-carboxymethyllysine monoclonal antibody. Lipid and protein AGEs are expressed in units of AGEs. Cautions regarding these measurements have been emphasized by some authors.²⁰ The AGE amount of the meal can be classified as medium.²¹ At every time point (baseline, 2 h, and 4 h) three SAF measurements were performed at the volar forearm, and the values were averaged. Intraindividual variance assessed by measurements on another day in fasting state (two measurements, 2 h apart from each other, while only water intake was allowed) was less than 4.5%, in agreement with previously published data.¹²

Statistical analyses

The group was first analyzed as a whole. A two-way analysis of variance was performed

for the analysis of SAF as a function of time. After the test was found significant, a paired *t* test was performed to compare SAF at baseline with the values at 2 h and at 4 h. The same sequence of analyses was then performed separately for the healthy group and the group of people with diabetes.

SAF is expressed in arbitrary units (AU). Data are median \pm interquartile range unless stated otherwise. The level of significance was $P = 0.05$.

RESULTS

In the whole group SAF significantly increased at 2 h by 10.2%: 1.97 ± 0.62 AU at baseline, 2.17 ± 0.62 AU at 2 h ($P < 0.01$ vs. baseline), and 2.16 ± 0.72 AU at 4 h ($P < 0.01$ vs. baseline). At 2 h an increase in SAF was noted

in 19 of 21 subjects. A decrease in SAF at 2 h occurred in one subject in the diabetes group (-0.01 AU) and in one subject in the healthy group (-0.02 AU). Values remained significant for the whole group after adjustment for SAF variance.

In the group of individuals with diabetes (Fig. 1A) and in the group of healthy subjects (Fig. 1B), the SAF increases at 2 h were 11.6% and 8.7%, respectively (both $P < 0.05$ vs. baseline).

In individuals with diabetes mellitus, serum glucose was 136 ± 34 mg/dL at baseline, 154 ± 52 mg/dL at 2 h ($P < 0.05$ vs. baseline), and 109 ± 25 mg/dL at 4 h ($P < 0.05$ vs. baseline). A significant correlation ($r = 0.599$, $P < 0.05$) was noted between changes in SAF and blood glucose at 2 h in this group. In healthy subjects serum glucose was 78 ± 16 mg/dL at baseline, 83 ± 22 mg/dL at 2 h, and 74 ± 19 mg/dL at 4 h ($P =$ not significant vs. baseline).

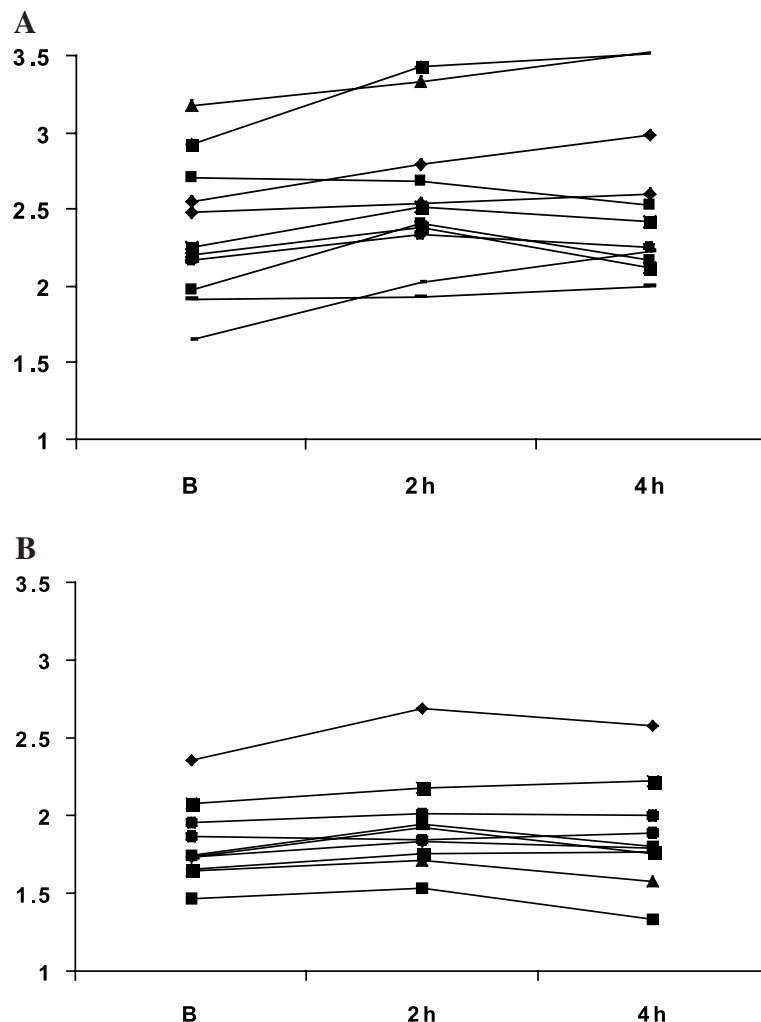


FIG. 1. SAF values (in AU, y-axis) at baseline (B) and at 2 h and 4 h after the meal: (A) in all diabetes patients and (B) in all healthy subjects. Median values \pm interquartile range were as follows: in individuals with diabetes ($n = 11$), 2.25 ± 0.55 (B), 2.51 ± 0.37 (2 h) ($P < 0.05$ vs. B), and 2.42 ± 0.59 (4 h) ($P < 0.05$ vs. B); in healthy subjects ($n = 10$), 1.73 ± 0.26 (B), 1.88 ± 0.21 (2 h) ($P < 0.05$ vs. B), and 1.79 ± 0.21 (4 h). The bold lines represent the median values.

DISCUSSION

The main finding of our study is that in a Caucasian population (both those with diabetes and those that were healthy) SAF increases postprandially following a usual meal with a medium AGE content. Following a meal with a high AGE content, a more pronounced increase in AGE and OS²¹ and thus in SAF can be postulated. Moreover, SAF did not fully recover after 4 h, suggesting that this is a more prolonged effect.

At 2 h postprandially, SAF increased by 10.2% in the group as a whole, and this increase remained significant after adjustment for the intrinsic variation of SAF (less than $\pm 4.5\%$).

Our population consisted of a middle-aged group of individuals with type 2 diabetes (age, 55 ± 12 years) with altered postprandial regulation and increased AGE and OS load and a young group of healthy subjects (age, 33 ± 5.5 years) with unaltered mechanisms for postprandial regulation⁹ and supposed to have a low AGE and OS load.^{22,23} People with diabetes and healthy subjects can be regarded as the extremes of a cohort subjected to screening for diabetes. SAF increase at 2 h occurred in both groups.

Forearm skin has a fair vascularization, and we suggest that the contribution of blood AGEs and redox-regulated fluorophores to the total SAF cannot be neglected. We have previously shown that postprandially, a vasodilatation of microcirculation and an acute increase in serum AGE and substances modified by OS occur.²¹ By these mechanisms, the amount of blood AGE and redox-regulated fluorophores directed towards the skin increases postprandially, thus explaining the SAF increase.

Recently, a very interesting study was published by Maynard et al.¹¹ suggesting SAF as a more sensitive method for diabetes screening than fasting plasma glucose and hemoglobin A1c. Still, the authors reported that not all subjects have been investigated in the fasting state, with some measurements being performed also following an oral glucose tolerance test. Commercially available oral glucose tolerance tests contain AGEs¹⁹ that can be rapidly absorbed into the circulation.²⁴ Furthermore, an oral glucose tolerance test can increase OS²⁵ and blood AGE

by hyperglycemia²⁵ and endogenous generation of alpha-dicarbonyls²⁶ (e.g., methylglyoxal). Indeed, following an oral glucose tolerance test in healthy subjects, SAF increased by a comparable extent like in our study (over 7% [A. Smit et al., Groningen, The Netherlands, unpublished data]). Moreover, in our study SAF and blood glucose excursions at 2 h were significantly correlated in individuals with diabetes.

SAF could represent an easy-to-use and non-invasive tool for screening people for undetected diabetes or assessing cardiovascular risk, but we suggest that investigations should be performed in the fasting state and in a timely manner apart from any exposure that can increase glycemia, AGE load, or OS. This includes not only meals or oral glucose tolerance tests, but also smoking.²⁷ This approach might improve the sensitivity and specificity of the method.

LIMITATIONS OF THE STUDY

We did not aim at comparing groups directly; therefore they were not matched.

We did not measure blood AGEs or parameters of OS, since the investigation of substances that contribute to SAF or mechanisms influencing postprandial SAF exceeds the purpose of our study. Therefore we are unable to state which of the mechanisms (increase in AGE or in OS) have contributed more to the effects seen. However, the main message of our study—namely, that postprandial status influences SAF—remains unaltered.

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