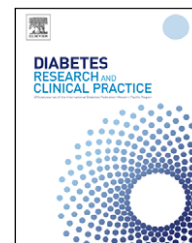


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# Skin autofluorescence in type 2 diabetes: Beyond blood glucose

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## ABSTRACT

**Background and aim:** Skin autofluorescence (AF), which has been proposed as a measure of tissue content of advanced glycation end-products, is a predictor of health outcomes in diabetic patients. Aim of this study is the assessment of parameters associated with increased AF in a sample of type 2 diabetic patients.

**Methods:** AF was determined in a consecutive series of type 2 diabetic 92 patients aged  $69.1 \pm 12.4$  years. Univariate and multivariate correlations with several clinical and chemical parameters were assessed.

**Results:** A significant ( $p < 0.01$ ) correlation of AF was found with age ( $r = 0.33$ ) and HbA1c ( $r = 0.34$ ). After adjusting for age and HbA1c, micro- or macrovascular complications of diabetes were associated with higher AF. Furthermore, a higher AF was found in patients with metabolic syndrome ( $2.7 \pm 1.0$  AU versus  $2.2 \pm 0.7$  AU;  $p < 0.05$ ). Waist circumference, triglyceride, and HDL cholesterol showed a significant correlation with AF after adjustment for age and HbA1c (adj.  $r = 0.30, 0.29$ , and  $-0.27$ ; all  $p < 0.05$ ).

**Conclusions:** Skin autofluorescence in type 2 diabetic patients is associated not only with degree of hyperglycaemia and age, but also with adiposity and metabolic syndrome.

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## 1. Introduction

A simple, non-invasive method for the assessment of skin autofluorescence has recently been proposed as a measure of accumulation of advanced glycation end products (AGEs). This method, which utilizes the fluorescent properties of AGEs [1], provides a measure which shows a fair correlation with collagen-linked fluorescence and AGEs content in skin biopsies [1,2]. Therefore, the assessment of skin autofluorescence could have some advantage over the measurement of plasma AGEs, which are less reproducible and less well related to tissue contents of AGEs [3].

The formation of AGEs, which is increased in diabetic patients as a function of hyperglycaemia [2,4], contributes to the pathogenesis of cardiovascular disease and microvascular complications of diabetes [5,6]. In fact, a higher skin autofluorescence is associated with nephropathy, neuropathy, and cardiovascular disease in cross-sectional studies [2,7,8]. Furthermore, elevated skin autofluorescence, which is associated with higher tissue AGE content, is a predictor of mortality in diabetes [7] and in end-stage renal disease [2]. In recipients of renal transplants, skin autofluorescence predict the decline of renal function in longitudinal observations [9].

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In diabetic patients, a direct correlation of skin autofluorescence with HbA1c has been reported in several studies [1,2,7,8]. Other parameters correlated with skin autofluorescence include age [1,2,7,8] and duration of diabetes [7,8,7]. Smoking, which is associated with increased production of reactive glycation products [10], is also associated with higher skin autofluorescence [8,9].

There is some evidence that some other factors could be involved in the determination of skin autofluorescence, independent of age, smoking habits, and blood glucose. In fact, higher body mass index [7,8] and triglyceride levels [7], and lower HDL cholesterol [8] have also been reported to be associated with increased skin autofluorescence. These data suggest that the metabolic syndrome (MS) could be associated with elevated skin autofluorescence.

Aim of this cross-sectional study is the assessment of the association of skin autofluorescence with components of MS in type 2 diabetic patients, after adjusting for other factors influencing AGE formation, such as age and degree of hyperglycaemia.

## 2. Materials and methods

### 2.1. Study population

A consecutive series of 92 Caucasian type 2 diabetic patients referring to our outpatient clinic, who provided their informed

consent, was enrolled in the study. The characteristics of the sample are summarized in Table 1.

A complete medical history with detailed information on duration of diabetes, any current pharmacological treatment, main associated cardiovascular risk factors, self-reported smoking habits and alcohol intake, and other relevant medical conditions was collected. Self-reported alcohol consumption of more than two drinks a day was used as cut-off to define alcohol abuse. All patients underwent a physical examination, during which their weight, height, and blood pressure were recorded. A standard electrocardiogram was recorded.

### 2.2. Laboratory procedures

A blood sample was drawn in the morning, after overnight fast, for the determination of HbA1c and lipid profile. Creatinine, total cholesterol, HDL cholesterol, and triglyceride were measured with an automated method (Aeroset, Abbott Laboratories), while HbA1c was measured with high-pressure liquid chromatography (Menarini Diagnostici, Italy; upper limit of normal range 6.2%). Microalbuminuria was determined from a 24-h urine sample (upper limit of normal: 20 µg/min).

### 2.3. Diagnostic criteria

Patients were considered hypertensive if they were on antihypertensive medication and/or if blood pressure was  $\geq 140/90$  mmHg [11]. Renal failure was defined as serum

**Table 1 – Principal characteristics of the sample enrolled**

|                            | All                 | Metabolic syndrome  |                      |
|----------------------------|---------------------|---------------------|----------------------|
|                            |                     | No                  | Yes                  |
| Number (women, %)          | 92 (39.1)           | 38 (26.3)           | 54 (48.1*)           |
| Age (years)                | 69.1 $\pm$ 12.4     | 69.0 $\pm$ 15.1     | 69.2 $\pm$ 10.3      |
| Duration diabetes (years)  | 12.3 $\pm$ 10.7     | 13.9 $\pm$ 12.1     | 11.3 $\pm$ 9.5       |
| HbA1c (%)                  | 7.6 $\pm$ 1.4       | 7.3 $\pm$ 1.4       | 7.8 $\pm$ 1.4        |
| BMI (kg/m <sup>2</sup> )   | 29.1 $\pm$ 6.1      | 26.5 $\pm$ 5.0      | 30.9 $\pm$ 6.2**     |
| Skin AU                    | 2.5 $\pm$ 0.9       | 2.2 $\pm$ 0.7       | 2.7 $\pm$ 1.0*       |
| Total cholesterol (mg/dl)  | 187.9 $\pm$ 42.6    | 190.2 $\pm$ 36.1    | 187.1 $\pm$ 45.1     |
| Triglyceride (mg/dl)       | 155.0 [97.5; 214.0] | 137.0 [98.0; 218.0] | 169.0 [97.0; 211.0]* |
| Retinopathy (%)            | 8.7                 | 2.6                 | 14.8*                |
| Neuropathy (%)             | 30.4                | 18.4                | 38.9*                |
| Stroke/TIA (%)             | 14.1                | 15.8                | 13.0                 |
| Ischemic heart disease (%) | 28.3                | 13.2                | 38.9**               |
| Diabetic foot ulcers (%)   | 23.9                | 18.4                | 27.8                 |
| Malignancies (%)           | 9.0                 | 6.3                 | 11.1                 |
| Microalbuminuria (%)       | 19.6                | 15.8                | 22.2                 |
| Renal insufficiency (%)    | 10.9                | 2.6                 | 16.7*                |
| Hypertension* (%)          | 65.2                | 39.5                | 83.3*                |
| Alcohol abuse (%)          | 5.4                 | 7.9                 | 3.7                  |
| Current smokers (%)        | 9.8                 | 5.3                 | 13.0                 |
| Antihypertensives (%)      | 60.9                | 31.6                | 81.6**               |
| Statins (%)                | 23.9                | 18.4                | 27.8                 |
| Antiaggregants (%)         | 47.8                | 34.2                | 57.4*                |
| Metformin/TZD (%)          | 55.4/5.4            | 47.4/7.9            | 61.1/3.7             |
| Insulin secretagogues (%)  | 37.0                | 38.0                | 36.5                 |
| Insulin (%)                | 35.9                | 28.9                | 40.7                 |

Data are expressed as mean  $\pm$  S.D., median [quartiles], or percent. \* $p < 0.05$ , \*\* $p < 0.01$ : metabolic syndrome yes vs. no; AU: autofluorescence; TIA: transient ischemic attack; #defined as blood pressure  $\geq 140/90$  mmHg or antihypertensive drugs; TZD: thiazolidinediones.

creatinine  $>1.5$  mg/dl, and microalbuminuria as albumin excretion rate  $>20$   $\mu$ g/min in at least two consecutive 24-h urine samples. Ischemic heart disease was diagnosed when patients reported previous myocardial infarction, angina (or when ECG showed unequivocal signs of current or previous infarction or ischemia according to Minnesota coding system) [12]. A history of stroke or transient ischemic attack was used to make the diagnosis of cerebrovascular disease. Self-reported alcohol consumption of more than two drinks a day was used as cut-off to define alcohol abuse.

The diagnosis of MS was made following either the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATPIII) [13] or the International Diabetes Federation (IDF) criteria [14]. According to both sets of criteria MS is present when at least three of five conditions (abdominal adiposity, hyperglycaemia, reduced HDL-cholesterol, elevated triglyceride, and increased arterial blood pressure) are satisfied, but IDF criteria established lower thresholds for abdominal adiposity and hyperglycaemia, and considered elevated waist circumference as a mandatory condition for the diagnosis.

#### 2.4. Skin autofluorescence

Skin autofluorescence was measured using the AGE Reader I (DiagnOptics BV, Groenigen, The Netherlands), as previously described [1]. Briefly, the instrument illuminates a  $1\text{ cm}^2$ -surface of the skin of the arm, guarded against surrounding light, with a wavelength of 300–420 nm. Light from the skin is measured with a spectrometer in the 300–600 nm range. Autofluorescence is calculated as the average light intensity per nanometer in the 420–600 nm range divided by the average light intensity per nanometer in the 300–420 nm range. The mean of three measurements 5 min apart was used for calculations.

#### 2.5. Statistical analysis

Statistical analysis was performed using SPSS 12.0.1. Data are expressed as mean  $\pm$  S.D. or median [quartiles], depending on their normal or non-normal distribution. Student's unpaired *t*-test was applied for comparison of means of normally distributed parameters. Correlations were explored using Pearson's or Spearman's methods, whenever appropriate.

Multiple linear regression, after transformation of categorical in dummy (0/1) variables, and stepwise logistic regression were used for multivariate analysis.

### 3. Results

Mean skin autofluorescence in the patients studied was  $2.46 \pm 0.95$  AU. A significant correlation of autofluorescence was found with age and HbA1c ( $r = 0.33$  and  $0.34$ ; both  $p < 0.01$ ). A significantly higher skin autofluorescence was observed in patients with microalbuminuria, chronic renal insufficiency, diabetic neuropathy, arteriopathy of lower limbs, current foot ulcers, diabetic retinopathy, and ischemic heart disease, as well as in smokers (data not shown). After adjusting for age and HbA1c, all those conditions retained a significant association with a higher skin autofluorescence, except smoking status, microalbuminuria, diabetic retinopathy and ischemic heart disease, which only showed a non-significant trend (Table 2).

Patients with microvascular complications (retinopathy, microalbuminuria, or renal insufficiency) showed a significantly higher HbA1c than the rest of the sample ( $8.4 \pm 0.9\%$  versus  $7.4 \pm 1.4\%$ ,  $p < 0.01$ ). The age-adjusted correlation of HbA1c with skin autofluorescence did not reach statistical significance in patients with or without microvascular complications (adj.  $r = 0.29$  and  $0.16$ , respectively). A higher HbA1c ( $7.9 \pm 1.3\%$  versus  $7.3 \pm 1.4\%$ ,  $p < 0.05$ ) was also observed in patients with macrovascular complications (ischemic heart disease, cerebrovascular disease, or arteriopathy of the lower limbs); after adjustment for age, HbA1c was significantly correlated with skin autofluorescence in patients with (adj.  $r = 0.47$ ,  $p < 0.01$ ), but not in those without (adj.  $r = -0.01$ ), macrovascular disease. In patients with neuropathy, who showed a HbA1c of  $7.9 \pm 1.4\%$  (versus  $7.4 \pm 1.3\%$  of the rest of the sample;  $p = 0.06$ ), HbA1c was significantly correlated with skin autofluorescence after adjustment for age (adj.  $r = 0.51$ ,  $p < 0.01$ ), while such a correlation was not observed in non-neuropathic patients (adj.  $r = -0.01$ ).

Patients with MS had higher mean skin autofluorescence than the rest of the sample ( $2.7 \pm 1.0$  AU versus  $2.2 \pm 0.7$  AU;  $p < 0.05$ ). A significantly higher autofluorescence was associated with high waist circumference  $2.6 \pm 1.0$  versus  $2.1 \pm 0.8$  ( $p < 0.05$ ), hypertriglyceridaemia  $2.9 \pm 0.8$  versus  $2.3 \pm 0.9$

**Table 2 – Association of skin autofluorescence with different diabetic complications**

| Diabetic complications      | OR               | HR               |                 |
|-----------------------------|------------------|------------------|-----------------|
|                             |                  | Age              | Age, HbA1c      |
| Microalbuminuria            | 1.6 [1.0; 2.7]*  | 1.5 [0.9; 2.6]   | 1.4 [0.8; 2.5]  |
| Chronic renal insufficiency | 2.9 [1.5; 5.9]** | 3.0 [1.4; 6.3]** | 2.4 [1.1; 5.4]* |
| Neuropathy                  | 2.8 [1.6; 5.1]** | 2.4 [1.3; 4.3]** | 2.1 [1.1; 3.8]* |
| Retinopathy                 | 2.1 [1.1; 4.2]*  | 2.1 [1.1; 4.1]*  | 2.1 [0.9; 4.6]  |
| Arteriopathy of lower limbs | 2.7 [1.5; 4.9]** | 2.3 [1.3; 4.1]** | 1.9 [1.1; 3.6]* |
| Ischemic heart disease      | 1.8 [1.1; 2.9]*  | 1.7 [1.1; 2.9]*  | 1.6 [0.9; 2.7]  |
| Stroke/TIA                  | 1.6 [0.9; 2.8]   | 1.4 [0.8; 2.6]   | 1.4 [0.7; 2.7]  |
| Current foot ulcers         | 4.4 [2.1; 9.0]   | 3.7 [1.8; 7.7]   | 3.4 [1.6; 7.3]* |

Data are expressed as unadjusted odds ratio (OR), or adjusted hazard ratio (HR [95% CI]) for each unit of increment of skin autofluorescence.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; TIA: transient ischemic attack.

( $p < 0.01$ ), low HDL-cholesterol  $3.2 \pm 1.2$  versus  $2.3 \pm 0.8$  ( $p < 0.01$ ), but not with elevated blood pressure ( $2.6 \pm 1.0$  versus  $2.2 \pm 0.7$ ;  $p = \text{ns}$ ), as defined by NCEP.

Skin autofluorescence showed a correlation with BMI ( $r = 0.23$ ;  $p < 0.05$ ) and waist circumference ( $r = 0.22$  and  $0.27$  in women and men, respectively; both  $p < 0.05$ ), which retained statistical significance after adjustment for age, sex, and HbA1c (adj.  $r = 0.30$  and  $0.33$  for BMI and waist circumference, respectively; both  $p < 0.01$ ). The age-, sex- and HbA1c-adjusted correlation of BMI with skin autofluorescence was statistically significant in patients without MS (adj.  $r = 0.30$ ;  $p = 0.049$ ), but not in those with MS (adj.  $r = 0.13$ ). A significant correlation was also observed with triglyceride (Fig. 1), which was maintained after adjusting for the same

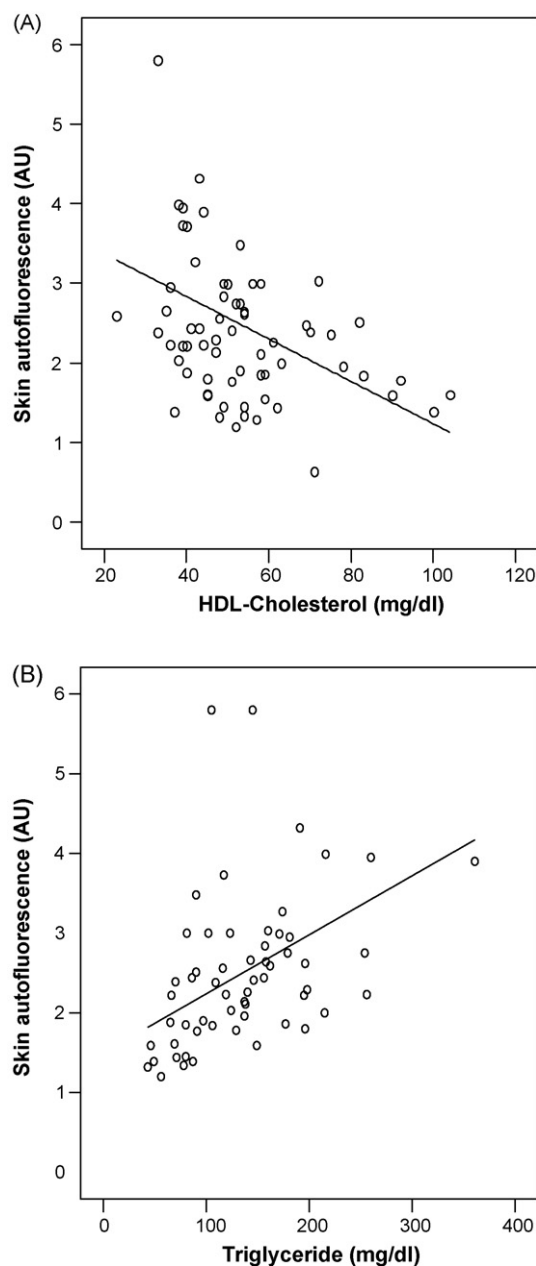
confounders (adj.  $r = 0.29$ ;  $p < 0.01$ ). Conversely, skin autofluorescence was inversely correlated with HDL cholesterol (Fig. 1), even after adjustments ( $r = -0.27$ ;  $p < 0.05$ ). The correlation of skin autofluorescence with triglyceride and HDL cholesterol retained statistical significance when waist circumference was added as a covariate, together with the confounders reported above, in a multiple linear regression model (adj.  $r = 0.27$  and  $-0.25$  for triglyceride and HDL cholesterol, respectively;  $p < 0.01$  and  $0.05$ , respectively). Similar results were obtained with a model in which BMI replaced waist circumference (data not shown).

#### 4. Discussion

The formation of AGEs follows the glycation of proteins. For this reason, AGEs content in tissues is higher when blood glucose, and therefore protein glycation, are increased. In fact, skin autofluorescence, which is a measure of AGE content in the skin, is higher in diabetic subjects in comparison with normoglycaemic individuals [1,2,7,8]; in diabetic patients, skin autofluorescence is higher in those with a greater degree of hyperglycaemia, i.e. with higher HbA1c levels [1,2,7,8]. The present study confirmed the presence of a weak correlation of skin autofluorescence with HbA1c, while no association was found with microvascular complications of diabetes after adjustment for confounders. Interestingly, the correlation of HbA1c with skin autofluorescence was more evident in patients with chronic complications of diabetes; this suggests that skin AGEs could be a marker of hyperglycaemia in a longer time span than HbA1c. Alternatively, it is possible that other factors, beyond hyperglycaemia, contributing to the pathogenesis of diabetic complications, could lead to increased skin AGE formation. It should also be considered that glycated proteins could be more exposed to oxidative damage, leading to diminished clearance and turnover. The reported increase of skin autofluorescence with age [1,2,7,8], which was confirmed in the present sample, could be explained by a lower turnover of skin proteins in older subjects. The higher autofluorescence in smokers [8,9] could be due to the oxidative stress induced by tobacco use, which favours the formation of AGEs [10].

The association of increased skin autofluorescence with components of MS, observed in the present study, is less obvious. A correlation of autofluorescence with measures of adiposity has already been reported in type 2 diabetic patients [7,8]. Furthermore, higher skin autofluorescence has been associated with higher blood pressure [9], low HDL cholesterol [8], and elevated triglyceride [7]. Furthermore, circulating AGEs have been reported to be increased in association with MS [15]. We observed a relevant increase in skin autofluorescence in patients with MS, which could not be explained by differences in age or HbA1c. It should be considered that the correlation of autofluorescence with triglyceride and HDL cholesterol was stronger than that with HbA1c or age. Furthermore, this correlation was maintained after adjusting for measures of adiposity, and therefore it could not be attributed to excess fat mass. This association deserves to be further investigated in larger samples of patients.

The causal relationships between skin autofluorescence, AGE formation, adiposity and MS are speculative. Treatment



**Fig. 1 – Correlation between skin autofluorescence and lipid parameters (panel A: HDL-cholesterol,  $r = 0.44$ ; panel B: triglyceride,  $r = 0.54$ ).**



with orlistat has been shown to reduce circulating AGEs [16], suggesting that excess body fat, via inflammatory mediators and/or oxidative stress [17], could facilitate AGE formation. On the other hand, it has been speculated that AGEs contribute to the pathogenesis of insulin resistance and related metabolic abnormalities [18]. Our data confirm that adiposity is independently associated with elevated skin autofluorescence. However, the association of triglyceride and low HDL cholesterol with skin autofluorescence cannot be entirely accounted for by waist circumference or BMI, suggesting that other mechanisms, independent of fat mass, could be present. Furthermore, the possibility that other factors, different from AGEs, and related to MS, contribute to skin autofluorescence cannot be completely ruled out.

It should also be considered that fluorescent AGEs, which are measured by the AGE reader used in the present study, are only a fraction of total AGEs. The relationship of fluorescent with nonfluorescent AGE compounds, such as hydroimidazolones, which are present in much higher levels in the skin of diabetic patients, has not been extensively studied so far.

In conclusion, skin autofluorescence in type 2 diabetic patients is associated not only with degree of hyperglycaemia, age, and smoking status, but also with adiposity and metabolic syndrome.

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