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## Article : Complications

# Non-invasive measures of tissue autofluorescence are increased in Type 1 diabetes complications and correlate with a non-invasive measure of vascular dysfunction.

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## Abstract

**Objective** To determine if ocular and skin autofluorescence, reflecting advanced glycation end-products, and vascular stiffness correlate in non-diabetic and Type 1 diabetic subjects and if levels differ by diabetes status.

**Research design and methods** Patients with Type 1 diabetes ( $n = 69$ , 19 with and 50 without vascular complications) and 60 subjects without diabetes (control) had ocular and skin autofluorescence and pulse-wave analysis performed in the fasted state. Correlations between measures within groups used the Pearson or Spearman correlation-coefficient and measures between groups were compared by ANOVA.

**Results** Lens and skin autofluorescence correlated in control ( $r = 0.58$ ,  $P = 0.0001$ ) and in Type 1 diabetes ( $r = 0.53$ ,  $P = 0.001$ ). Corneal autofluorescence correlated with lens ( $r = 0.53$ ,  $r = 0.52$ ,  $P = 0.0001$ ) and skin autofluorescence ( $r = 0.34$ ,  $P = 0.01$  and  $r = 0.49$ ,  $P = 0.00001$ ) in control and Type 1 diabetes respectively. In Type 1 diabetes, small and large artery elasticity correlated inversely and systemic vascular resistance correlated positively with skin autofluorescence (all  $P = 0.001$ ), and with lens and corneal autofluorescence (all  $P < 0.03$ ). In Type 1 diabetes tissue advanced glycation end-products correlated with C-reactive protein and inversely with the estimated glucose disposal rate and with circulating advanced glycation end-product levels. Relative to non-diabetic subjects, lens, corneal and skin fluorescence were increased (all  $P < 0.001$ ) and small artery elasticity was decreased in diabetes ( $P = 0.04$ ). Lens, corneal and skin autofluorescence were greater (all  $P = 0.0001$ ) in patients with Type 1 diabetes with complications compared to those without complications, but small artery elasticity did not differ significantly.

**Conclusions** Ocular and skin autofluorescence and vascular stiffness correlate in non-diabetic and Type 1 diabetes subjects and are increased in Type 1 diabetes.. Tissue advanced glycation end-products correlate with vascular risk factors, including circulating advanced glycation end-products.

**Key words** diabetes, glycation, tissue fluorescence

**Abbreviations:** AGEs, Advanced Glycation End-Products;

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## Introduction

Even with tight glycaemic, blood pressure and lipid control vascular complications still occur at unacceptably high rates in diabetes. Additional therapies are needed and, given the slow development of vascular damage, surrogate end points are required to facilitate clinical trials and clinical practice. Tests to predict high complication-risk patients are required to target therapies and to test and monitor interventions. As advanced glycation end-products (AGEs) promote vascular damage and anti-AGE drugs are available or in development [1], clinically applicable tools to assess tissue AGE burden are merited.

Advanced glycation end-products are a family of compounds formed by reactions between glucose, lipids and protein or derived from food and smoking [2]. Adverse AGE effects include defective vasodilatation, vascular leakage, inflammation, foam cell formation, pro-thrombotic and sclerotic effects. The levels of AGEs are usually higher in diabetes *per se*, in the presence of vascular complications and can predict diabetes complications [3]. Increased skin collagen AGEs, measured biochemically from skin biopsies, have been demonstrated with aging [4] and in Type 1 diabetes and its complications [5]. In the Diabetes Complications and Control/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort skin collagen AGEs predicted renal, retinal and arterial damage [6,7]. Improved aortic compliance in elderly non-diabetic subjects [8] by 'AGE breaker' drugs support that AGE-targeted interventions ameliorate vascular damage. We previously demonstrated increased AGEs in serum and haemoglobin of Type 1 diabetes subjects and predictive power for abnormalities of pulse-wave analysis and renal function [9]. As AGEs are difficult to remove from long-lived tissues and complications develop over years, longer-term AGE measures and non-invasive assessments are preferable. Ocular and skin autofluorescence and pulse-wave analysis may be suitable.

Tissue AGE measures by non-invasive ocular and skin fluorescence readers have been validated against biochemical AGE levels [2,10], and pulse-wave analysis has been validated against brachial artery flow-mediated dilatation [11,12]. Previous studies have demonstrated abnormalities of each of these parameters in human diabetes [3,4,13,14] but, to date, there are no studies reporting relationships between tissue autofluorescence or AGE measures in various tissues and non-invasively measured vascular health. Therefore, to guide instrument choice and facilitate comparisons we conducted a cross-sectional study of non-diabetic subjects (control) and people with Type 1 diabetes (Type 1 diabetes), with and without vascular complications. The primary outcome of interest is the inter-relationships between the measures by the various devices. We also compared ocular and skin AGE measures, circulating AGE levels and vascular health measures within and between groups.

## Methods

### Subjects

The study was approved by the Ethics Committee and each subject gave written informed consent. Patients were recruited from St. Vincent's Hospital and controls were recruited from a data-base of volunteers from the general community. Type 1 diabetes and non-diabetes groups were matched for age, sex, blood pressure, BMI and renal function. Exclusion criteria were end-stage renal disease, inflammatory conditions, pregnancy or breast feeding and current or recent (< 3 months) surgery, infective illness, myocardial infarction, stroke, cardiac arrhythmia, diabetic ketoacidosis or anti-oxidant vitamin supplementation. Only three Type 1 diabetes patients were excluded because of recent illnesses and over 90% of subjects asked agreed. Control subjects had no current or past history of diabetes, no known cardiovascular or renal disease and had normal fasting glucose and renal function. A history and examination was performed and complication status verified by treating clinicians. Subjects were evaluated with all three devices after an overnight fast before medication or smoking. Blood and a mid-stream urine were collected after the non-invasive measures.

### Tissue AGEs

The ocular fluorescence of anterior ( $F_a$ ) and posterior ( $F_p$ ) pole lens capsule and corneal fluorescence were measured using an ocular fluorimeter (Fluorotron, Ocumetrics, San Jose, CA, USA) with an anterior segment adaptor. Data were processed using EUROEEYE software (version 4.6; van Best and Larsen, Leiden, The Netherlands; Copenhagen, Denmark) to correct for loss of light and expressed as nanograms of fluorescein equivalents. The lens transmission index ( $T$ ) was calculated using:

$$T = \sqrt{\frac{F_p}{F_a}}$$

The mean of triplicate measures in both eyes was used in data analysis.

The skin fluorescence of unscarred inner forearm was measured by a Skin AGE Reader (DiagnOptics, Groningen, The Netherlands), and the mean of three measures, expressed in arbitrary units, from each arm used. Correlation between left and right arms was  $r = 0.92$ ,  $P = 0.00001$ .

## Non-invasive measures of vascular health

Pulse wave analysis, including large and small artery elasticity and systemic vascular resistance was performed on rested supine subjects (Pulse Wave™ CR-2000; Hypertension Diagnostics Inc., Eagan MN, USA), as described elsewhere [11] and the mean of three measures over 5–10 min used. Inter-measurement CVs for large and small artery elasticity were 7.1% and 4.9%, respectively.

## Estimated glucose disposal rate

The estimated glucose disposal rate was calculated using the method of Williams *et al.* [15].

## Clinical chemistry and vascular risk factors

The Clinical Chemistry Department of St Vincent's Hospital determined renal and liver function, lipids, glucose, HbA<sub>1c</sub>, full blood count and erythrocyte sedimentation rate, and analysed urine for cell count, microalbumin/creatinine ratio and culture. Blood for the research laboratory was centrifuged (2000 g, 10 min, 4°C) and serum and red blood cell pellet aliquots were stored at –86°C until analysis.

### Inflammation

C-Reactive protein was measured by high-sensitivity immunonephelometry (Dade Behring, Marburg, Germany). Intra- and inter-assay coefficients of variation (CVs) were 4.6% and 4.3%, respectively.

### Circulating fluorophores

Low molecular weight fluorophores were quantified by fluorescence spectroscopy as described elsewhere [9] with intra- and inter-assay CVs of 4.7% and 6.4%, respectively. Haemoglobin-AGEs were quantified by fluorescence spectroscopy as described elsewhere [16] with modifications. Briefly haemoglobin from lysed red blood cells was diluted (1 mg/ml protein). Globin was precipitated with cold 1% HCl in acetone, washed with ethyl acetate and diluted (1 mg/ml protein). Separate red blood cell lysate and globin were injected into a flow Waters high-pressure liquid chromatography system with 474 fluorescence and 486 absorbance (ultraviolet) detectors. Fluorescence was monitored at 308/345 nm and absorbance at 280 nm and fluorescence/ultraviolet ratio results expressed as arbitrary units (AU), with inter- and intra-assay CVs of 9% and 5%, respectively.

## Statistics

Data were analysed by STATISTICA, version 8.0. StatSoft, Inc. (2008). Differences between groups were tested using Student's *t*-test, ANOVA or  $\chi^2$  tests. Non-normally distributed variables (corneal fluorescence, triglycerides, urinary albumin/creatinine, serum creatinine, hs-C-reactive protein (CRP), low molecular weight fluorophores, erythrocyte sedimentation rate and glomerular filtration rate) were log-transformed. Associations between variables were tested by Pearson correlation coefficients. Dimensionless Z-scores for tissue fluorescence measurements were used for comparison of concordance. Relationships between tissue autofluorescence and pulse-wave analysis were also calculated after controlling for age, duration of diabetes and concurrent HbA<sub>1c</sub>.

## Results

### Subject characteristics

The subject characteristics are shown in Table 1, including all Type 1 diabetes, and subdivided according to vascular complications. Microvascular complications are defined as previous pan-retinal laser for proliferative diabetic retinopathy ( $n = 17$ ), and/or increased albuminuria confirmed on at least two of three timed urine collections ( $n = 14$ ). Twelve Type 1 diabetes had both retinopathy and nephropathy. Four subjects had clinically evident macrovascular disease, all of whom also had a microvascular complication. More Type 1 diabetes smoked. Total and LDL-cholesterol were lower and fasting glucose and HbA<sub>1c</sub> levels were higher in Type 1 diabetes. When divided by complication status those with complications had longer duration of diabetes, were heavier, had worse renal function, poorer glycaemic control, were more insulin resistant (based on estimated glucose disposal rate) and were less likely to be smokers. Results (not shown) did not change substantially if statistically adjusted for smoking or if smokers were excluded.

## Correlation between ocular and skin autofluorescence

### Patients without diabetes

Anterior and posterior pole lens capsule autofluorescence were correlated ( $r = 0.93$ ,  $P < 0.000001$ ), and correlated with corneal autofluorescence ( $r = 0.53$ ,  $r = 0.48$ , both  $P = 0.0001$ ), and skin autofluorescence ( $r = 0.58$ ,  $r = 0.60$ , both  $P = 0.00001$ ). Corneal and skin autofluorescence were correlated ( $r = 0.34$ ,  $P = 0.01$ ).

### Patients with Type 1 diabetes

Anterior and posterior pole lens capsule autofluorescence were correlated ( $r = 0.80$ ,  $P < 0.000001$ ), and correlated with corneal autofluorescence ( $r = 0.52$ ,  $r = 0.58$ , respectively, both  $P = 0.00001$ ) and skin autofluorescence ( $r = 0.53$ ,  $r = 0.56$ , respectively, both  $P = 0.0001$ ). Corneal and skin autofluorescence were correlated, ( $r = 0.49$ ,  $P = 0.00001$ ). Similar levels and significance between the calculated Z-scores of the measurements were observed in control and diabetic subjects (results not shown). In a Bland–Altman plot analysis the mean difference between anterior lens capsule fluorescence and skin fluorescence Z-scores was 0.06 (95% CI  $-0.17$ – $0.29$ ) in controls and  $-0.001$  ( $-0.22$ – $0.22$ ) in Type 1 diabetes (both  $P > 0.05$ ). The difference (95% CI) between corneal and skin fluorescence Z-scores in controls and in patients with Type 1 diabetes was 0.09 ( $-0.20$ – $0.38$ ) and  $-0.02$  ( $-0.24$ – $0.20$ ) respectively (both  $P > 0.05$ ).

### Patients without diabetes and Type 1 diabetes combined

Anterior and posterior pole lens capsule autofluorescence were correlated ( $r = 0.88$ ,  $P < 0.000001$ ), and correlated with corneal autofluorescence ( $r = 0.56$  and  $r = 0.60$ , respectively, both  $P < 0.000001$ ), and skin autofluorescence ( $r = 0.66$ ,  $r = 0.67$ , both  $P < 0.000001$ ). Corneal and skin autofluorescence were correlated ( $r = 0.54$ ,  $P < 0.000001$ ).

## Relationship between tissue autofluorescence and vascular health

As shown in Fig. 1a, skin autofluorescence correlated (inversely) with small artery elasticity in both control patients and patients with Type 1 diabetes (both  $r = -0.52$ ;  $P = 0.001$ ), with large artery elasticity in Type 1 diabetes patients only ( $r = -0.42$ ;  $P = 0.001$ ), and was positively correlated with systemic vascular resistance in both control patients and patients with Type 1 diabetes ( $r = 0.33$ ,  $P = 0.01$  and  $r = 0.45$ ,  $P = 0.001$ , respectively). After adjusting for subjects' age, duration of diabetes and glycaemic control ( $HbA_{1c}$ ) skin autofluorescence correlated (inversely) with small artery elasticity in both control and Type 1 diabetes groups (both  $r = -0.32$ ,  $P = 0.01$ ), with large artery elasticity in patients with Type 1 diabetes only ( $r = -0.26$ ;  $P = 0.04$ ), and was positively correlated with systemic vascular resistance in the Type 1 diabetes group ( $r = 0.29$ ,  $P = 0.02$ ). As shown in Fig. 1b, anterior and posterior pole lens capsule autofluorescence were also correlated with small artery elasticity, large artery elasticity and systemic vascular resistance in both groups on univariate analysis, but not when adjusted for age, duration of diabetes and  $HbA_{1c}$ . Corneal autofluorescence was correlated with small artery elasticity, large artery elasticity and systemic vascular resistance only in the Type 1 diabetes patients. After adjustment for age, duration of diabetes and  $HbA_{1c}$  corneal autofluorescence was (inversely) correlated with large artery elasticity in both the control group and in the Type 1 diabetes group (both  $r = -0.27$ ,  $P = 0.04$ ).

In combined control and Type 1 diabetes groups skin autofluorescence correlated with small artery elasticity ( $r = -0.53$ ,  $P < 0.000001$ ), large artery elasticity ( $r = -0.22$ ,  $P = 0.01$ ) and systemic vascular resistance ( $r = 0.37$ ,  $P = 0.000007$ ). Anterior and posterior pole lens capsule autofluorescence were also inversely correlated with small artery elasticity ( $r = -0.47$  and  $r = -0.46$ , respectively, both  $P < 0.000001$ ), large artery elasticity ( $r = -0.17$ ,  $P = 0.05$ ; and  $r = -0.19$ ,  $P = 0.03$ ) and were positively correlated with systemic vascular resistance ( $r = 0.36$  and  $r = 0.40$ , respectively, both  $P < 0.00002$ ). Corneal autofluorescence correlated with small artery elasticity ( $r = -0.33$ ,  $P = 0.00007$ ), large artery elasticity ( $r = -0.24$ ,  $P = 0.04$ ) and systemic vascular resistance ( $r = 0.23$ ,  $P = 0.007$ ). After adjustment for age, duration of diabetes and  $HbA_{1c}$  skin autofluorescence correlated with small artery elasticity ( $r = -0.29$ ,  $P = 0.0009$ ) and large artery elasticity ( $r = -0.25$ ,  $P = 0.005$ ). Corneal autofluorescence was (inversely) correlated with small artery elasticity and large artery elasticity (both  $r = -0.20$ ,  $P = 0.02$ ).

## Correlation between tissue autofluorescence, age, diabetes duration and concurrent $HbA_{1c}$

### Patients without diabetes

Anterior and posterior pole lens capsule autofluorescence correlated with age ( $r = 0.76$  and  $r = 0.84$  respectively, both  $P = 0.001$ ) and with  $HbA_{1c}$  ( $r = 0.54$  and  $r = 0.55$ , both  $P = 0.001$ ). Corneal fluorescence correlated with age ( $r = 0.30$ ,  $P = 0.02$ ) only and skin autofluorescence correlated with age ( $r = 0.73$ ,  $P = 0.001$ ) and  $HbA_{1c}$  ( $r = 0.38$ ,  $P = 0.004$ ).

## Patients with Type 1 diabetes

Anterior and posterior pole lens capsule autofluorescence, respectively, correlated with age ( $r = 0.68$  and  $r = 0.58$ , both  $P = 0.001$ ) and duration of diabetes ( $r = 0.77$  and  $r = 0.69$ , both  $P = 0.001$ ) but not with HbA<sub>1c</sub>. Corneal fluorescence correlated with age ( $r = 0.25$ ,  $P = 0.02$ ) and duration of diabetes ( $r = 0.43$ ,  $P = 0.001$ ) and skin autofluorescence correlated with age ( $r = 0.46$ ,  $P = 0.001$ ), duration of diabetes ( $r = 0.63$ ,  $P = 0.001$ ) and HbA<sub>1c</sub> ( $r = 0.29$ ,  $P = 0.02$ ).

## Relationships between tissue autofluorescence, vascular health and circulating AGEs in diabetes

Plasma low molecular weight fluorophore levels were not increased in Type 1 diabetes (Table 1). In Type 1 diabetes low molecular weight fluorophore levels correlated with skin and corneal fluorescence ( $r = 0.40$ ,  $P = 0.02$  and  $r = 0.38$ ,  $P = 0.04$ , respectively) and with systemic vascular resistance ( $r = 0.56$ ,  $P = 0.001$ ).

Haemoglobin-AGE levels were similar in Type 1 diabetes and control groups. The Globin fraction haemoglobin-AGE levels were significantly higher in the Type 1 diabetes group than in the control group (Table 1). In the Type 1 diabetes group haemoglobin-AGE levels correlated with anterior pole lens capsule autofluorescence ( $r = 0.38$ ,  $P = 0.02$ ), lens transmission index ( $r = -0.56$ ,  $P = 0.0002$ ) and skin fluorescence ( $r = 0.48$ ,  $P = 0.002$ ). Globin fraction haemoglobin-AGE levels in the Type 1 diabetes group correlated with the lens transmission index only ( $r = -0.38$ ;  $P = 0.01$ ).

## Correlates of tissue AGEs with inflammation and with insulin sensitivity in diabetes.

In the Type 1 diabetes group there were significant univariate correlations between tissue autofluorescence and inflammation, as reflected by serum C-reactive protein levels, corneal (but not lens) autofluorescence ( $r = 0.43$ ,  $P = 0.02$ ) and skin autofluorescence ( $r = 0.55$ ,  $P = 0.002$ ). These correlations remained significant after adjustment for age, diabetes duration and HbA<sub>1c</sub>.

In the Type 1 diabetes group estimated glucose disposal rate, a measure of insulin sensitivity, inversely correlated with skin autofluorescence ( $r = -0.49$ ,  $P = 0.001$ ), corneal fluorescence ( $r = -0.37$ ,  $P = 0.005$ ), and anterior ( $r = -0.53$ ,  $P = 0.001$ ) and posterior pole lens autofluorescence ( $r = -0.46$ ,  $P = 0.0003$ ) on univariate analyses.

## Increased tissue autofluorescence in diabetes

Age-adjusted results are shown in Fig. 2. Lens (Fig. 2a), corneal (Fig. 2b) and skin autofluorescence (Fig. 2c). Relative to patients without diabetes the lens and corneal autofluorescence were significantly higher in both Type 1 diabetes subgroups, and were significantly higher in Type 1 diabetes with vascular complications vs. patients with Type 1 diabetes without vascular complications. Skin fluorescence followed the same pattern.

## Pulse-wave analysis

Small artery elasticity was significantly lower in all Type 1 diabetes patients (with or without vascular complications) compared with control ( $7.43 \pm 0.36$  vs.  $8.68 \pm 0.45$  ml/mmHg  $\times$  100,  $P = 0.04$ ) and was significantly lower in Type 1 diabetes with vascular complications ( $6.27 \pm 0.83$  ml/mmHg  $\times$  100,  $P = 0.02$ ) than in control. Small artery elasticity did not differ significantly between the control group and the Type 1 diabetes without vascular complications group, or between Type 1 diabetes with vascular complications group and the Type 1 diabetes without vascular complications group. Large artery elasticity was similar in Type 1 diabetes and control patients ( $17.90 \pm 0.70$  vs.  $16.86 \pm 0.73$  ml/mmHg  $\times$  10;  $P = \text{NS}$ ) and in Type 1 diabetes with vascular complications vs. Type 1 diabetes without vascular complications ( $16.97 \pm 1.35$  vs.  $18.11 \pm 0.84$  ml/mmHg  $\times$  10,  $P = \text{NS}$ ).

## Discussion

We report for the first time the relationships between non-invasive measures of ocular and skin autofluorescence and vascular health. While each of these devices has been previously used to study people with diabetes [10,17,18] none have applied more than one technique, and most studies have been in patients with Type 2 diabetes. In general, without adjustment for age, diabetes duration and glycaemia, autofluorescence in one site correlated well with that in other tissues and with lower vascular elasticity, reflecting vascular stiffness. In diabetes, tissue autofluorescence also correlated with levels of C-reactive protein and some circulating AGE levels, and inversely with estimated glucose disposal rate, a measure of insulin sensitivity. After adjustment for age, diabetes duration and HbA<sub>1c</sub> skin autofluorescence was more strongly associated with vascular health, assessed by pulse-wave analysis, than ocular autofluorescence. Relative to control patients without diabetes all three tissue autofluorescence measures were increased and small artery elasticity decreased in Type 1 diabetes. Lens, corneal and skin fluorescence were all significantly higher in Type 1 diabetes with vascular complications vs. Type 1 diabetes without vascular complications, but small artery elasticity did not differ significantly. We report major results for the control and Type 1 diabetes groups separately as relationships between the measures may be changed by diabetes status or drugs. For example, angiotensin-



converting enzyme inhibitors and 'statins', used more commonly in diabetes complications, can improve small artery elasticity in weeks [19,20]. In general the relationships between the non-invasive measures in different groups were similar.

Non-invasive measures of tissue autofluorescence and vascular stiffness may be suitable for clinical research and clinical practice. Skin autofluorescence, measured using the same device as in this study, correlates with skin biopsy collagen fluorescence and specific (fluorescent and non-fluorescent) AGE levels [10,17]. Meerwaldt *et al.* [10] demonstrated that skin autofluorescence was higher in patients with Type 1 diabetes than in patients without diabetes and correlated this with age, glycaemia and serum creatinine. Skin autofluorescence was an independent predictor of Type 2 diabetes microvascular complications [14], mortality in dialysis patients (with and without diabetes) [17], and of cardiovascular death in the general population [21]. Such non-invasive studies in Type 1 diabetes are lacking. Recently Chabroux *et al.* [22] reported skin fluorescence measurements in Type 1 diabetes and found correlations with age, diabetes, duration, glycaemia and diabetes complications. Our findings, while predominantly aimed at describing relationships between the output of the various non-invasive devices, inform and extend previous and future studies as they include other tissue sites, a non-invasive measure of vascular function and circulating AGE measures.

Relationships between tissue AGEs and vascular health, particularly using non-invasive measures in people with diabetes are not fully elucidated. In a rat model of diabetes and islet cell transplantation lens autofluorescence, aorta and tail AGEs correlated and were increased in diabetes [23]. In a cross-sectional study of non-diabetic end-stage renal disease patients and healthy subjects skin autofluorescence, using the same device as in the present study, correlated with brachial artery pulse wave velocity [24]. In another study Conway *et al.* [25] demonstrated correlation between skin fluorescence and coronary artery calcification in patients with 40 years of Type 1 diabetes. The results of the present study, in a group of younger Type 1 diabetes patients with normal or only mildly impaired renal function, are in accord with that study. We have used a functional measure of vascular status rather than vascular calcification, which occurs late in vascular damage. In addition, we demonstrate correlations between vascular status (by pulse-wave analysis) and ocular autofluorescence, correlations between lens and corneal autofluorescence, and between ocular and skin autofluorescence.

In the present study we identified significant correlations between circulating low molecular weight fluorophores and haemoglobin-AGE levels and tissue fluorescence and vascular health in diabetes; in agreement with this, Tan *et al.* [26] reported a correlation between flow-mediated dilatation and serum AGEs in Type 2 diabetes. In contrast, in another high vascular risk group of systemic lupus erythematosus patients, skin autofluorescence was elevated relative to healthy subjects, but was not correlated with plasma levels of AGEs or its soluble receptor (sRAGE) [27]. The difference in half-lives of tissue AGEs (likely years) and circulating AGEs (likely days to months), different methodologies and study subjects likely contribute to these contrasting observations.

Our results for Type 1 diabetes patients, which include both lens and corneal autofluorescence, demonstrate correlations with age, diabetes duration, HbA<sub>1c</sub> (for corneal autofluorescence) and with vascular dysfunction, and demonstrate increased ocular autofluorescence in Type 1 diabetes *per se*, with higher levels in diabetic patients with complications versus those without complications. Others have also demonstrated increased ocular autofluorescence in Type 1 diabetes [28], and correlations with diabetes duration and glycaemia. In a human twin study lens autofluorescence related to age, smoking, glycaemia and genetic factors [29] and over 13-months lens autofluorescence increased in over 60% of people [30]. Lens autofluorescence correlates well with biochemically quantified lens AGEs in patients [13,30]. The ability to utilize corneal autofluorescence as a measure of tissue AGEs is particularly relevant in the assessment of patients who have had cataract removal and lens implants. Once adjusted for age, diabetes duration and HbA<sub>1c</sub> the correlations between ocular autofluorescence and pulse-wave analysis were less strong than for skin autofluorescence, which may reflect different influences of these parameters on the tissues measured.

While in clinical practice ocular and renal status should be assessed by ophthalmoscopy, blood and urine renal markers it is interesting to note the increased ocular and skin autofluorescence measures in association with concurrent complication status. We suggest that prospective studies of tissue autofluorescence and pulse-wave analysis for the vascular complications of diabetes are merited. These non-invasive devices may prove useful in clinical research and practice to identify high (or low) complication risk-patients, as a surrogate end point in clinical trials and for monitoring AGE-related therapies. Our study results and relative instrument costs may facilitate instrument choice and study comparisons.

## Competing interests

Nothing to declare

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**FIGURE 1** Tissue autofluorescence correlates with vascular health. (a) Correlations between skin fluorescence and measures of vascular health (SAE, small artery elasticity; LAE, large artery elasticity ; SVR, systemic vascular resistance) determined by pulse wave analysis in patients without diabetes subjects (control), Type 1 diabetes without vascular complications (T1DM CX[-]) and Type 1 diabetes with vascular complications (T1DM CX[+]). Solid line, all Type 1 diabetes; dashed line, control. There was no statistically significant difference in the regression line slopes of the Type 1 diabetes and control groups. (b) Similar correlations were noted for other tissue autofluorescence measures (lens capsule anterior and posterior poles and cornea) in control and Type 1 diabetes.

**FIGURE 2** Comparison of tissue autofluorescence (FL). (a) Lens capsule anterior pole autofluorescence. (b) corneal autofluorescence; (c) Skin autofluorescence in patients without diabetes (control), Type 1 diabetes with vascular complications (T1DM CX[+]) and Type 1 diabetes without vascular complications (T1DM CX[-]). Measurements, done in triplicate, are shown as mean and standard error of value of age adjusted bilateral measures. Lens and corneal autofluorescence are expressed in nanograms of fluorescein equivalents and skin autofluorescence in arbitrary units.

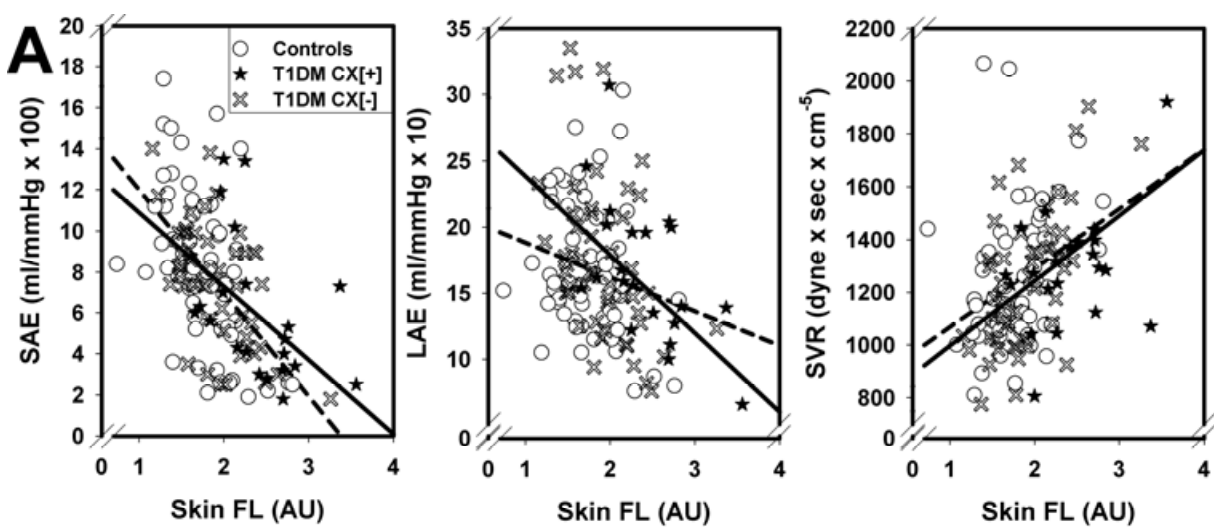
**Table 1 ?**

	Controls	Diabetes without vascular complications	Diabetes with vascular complications	P for trend
N (F/M)	60 (34/26)	50 (25/25)	19 (6/13)	–
Age (years)	36 ± 13	34 ± 13	43 ± 15	–
Duration of diabetes (years)		16 ± 13#	31 ± 11	–
BMI (kg/m <sup>2</sup> )	26.1 ± 4.8	25.7 ± 3.5#	30.3 ± 6.0†	0.0005
MAP (mmHg)	88 ± 14	88 ± 9#	99 ± 15†	0.001
Glucose (mM)	4.8 ± 0.5	10.2 ± 4.6†	11.5 ± 4.9†	0.0001
HbA <sub>1c</sub> (%)	5.1 ± 0.3	7.5 ± 1.1†	8.0 ± 1.1†	0.0001
HbA <sub>1c</sub> (mmol/mol)	32 ± 3	58 ± 12†	64 ± 12†	0.0001
Total cholesterol (mM)	5.1 ± 0.9	4.6 ± 1.3†	4.4 ± 0.8†	0.004
Triglycerides (mM)	1.1 (0.8, 1.3)	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	–
HDL-cholesterol (mM)	1.4 ± 0.4	1.4 ± 0.3	1.3 ± 0.4	–
LDL-cholesterol (mM)	3.1 ± 0.8	2.7 ± 1.1	2.6 ± 0.6	0.04
c-glomerular filtration rate (GFR) (ml/min)	112 (93, 130)	111 (94, 129)	116 (89, 127)	–
Estimated glucose disposal rate (mg/kg.min)	10.6 ± 1.7	8.8 ± 1.9#†	6.0 ± 1.9†	0.0001
Serum creatinine (mM)	0.08 (0.07, 0.11)	0.08 (0.08, 0.09)	0.09 (0.09, 0.16)	–
Urine albumin/creatinine (mg/mmol)	0.4 (0.3, 0.7)	0.4 (0.3, 0.7)#	2.3 (0.6, 8.5)†	0.01
WCC (10 <sup>9</sup> /l)	6.1 ± 1.4	5.9 ± 1.8	5.9 ± 2.2	–
Erythrocyte sedimentation rate (mm/h)	5 (5, 10)	5 (5, 7)#	13 (7, 15)†	0.002
Hs-C-reactive protein (mg/l)	0.97 (0.32, 1.87)	1.16 (0.54, 2.02)	2.00 (0.86, 6.04)†	0.04
Low molecular weight fluorophores (AU)*	3.3 (2.9, 3.8)	3.7 (3.2, 4.7)	4.1 (3.3, 4.4)†	0.02
Red blood cell lysate Hb-advanced glycation end-products (AU)*	0.14 ± 0.05	0.13 ± 0.03#	0.19 ± 0.09†	0.008
Globin haemoglobin-advanced glycation end-products (AU)*	2.21 ± 0.12	2.27 ± 0.19#	2.39 ± 0.20†	0.003
Smokers (%)	14	18	0	–
Lipid lowering drugs (%)	3	15#†	52†	
Blood pressure drugs (%)	6	17#	81†	

Data shown are mean ± SD or median (Q1, Q4). #P < 0.05 vs. DM CX[+], †P < 0.05 vs. controls.

\*Low molecular weight fluorophores and haemoglobin-advanced glycation end-products (AGEs) were analysed in a representative subset of subjects (n = 41 controls and n = 40 Type 1 diabetes subjects; n = 26 diabetes without vascular complications and n = 14 diabetes with vascular complications).





**B**

		SAE	LAE	SVR
Anterior Pole	Control	$r=-0.42$ ; $p=0.001$	$r=-0.26$ ; $p=0.04$	$r=0.42$ ; $p=0.001$
Lens Capsule FL	T1DM	$r=-0.45$ ; $p=0.001$	$r=-0.31$ ; $p=0.01$	$r=0.37$ ; $p=0.002$
Posterior Pole	Control	$r=-0.38$ ; $p=0.003$	$r=-0.15$ ; $p=ns$	$r=0.44$ ; $p=0.0004$
Lens Capsule FL	T1DM	$r=-0.45$ ; $p=0.001$	$r=-0.40$ ; $p=0.0008$	$r=0.42$ ; $p=0.0003$
Corneal FL	Control	$r=-0.17$ ; $p=ns$	$r=-0.25$ ; $p=0.05$	$r=0.19$ ; $p=ns$
	T1DM	$r=-0.36$ ; $p=0.003$	$r=-0.39$ ; $p=0.001$	$r=0.26$ ; $p=0.03$

